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Performance comparison of calibrated fourier transformation infrared spectroscopy (FTIR) and automated beer analyzer in quality control studies

Fourier transformation infrared spectroscopy (FTIR) as to its potential in quantifying beer parameters was investigated, aiming to figure out the feasibility for its application in practice compared to a commonly used automated beer analyzer. For this purpose, a FTIR system including a database was set up and calibrated for the most important beer parameters, such as original wort, pH, alcohol content, specific gravity, extract (real and apparent), fermentation, caloric value, and refraction index. A set of in total 329 beers marketed in Europe was analyzed with an FTIR system and a commercially available automated beer analyzer. A statistical evaluation revealed no significant differences of the analytical performance of both systems. Highest correlation coefficients R^2 (close to 1) were found for all parameters, except for the pH (0.901). A certain limit was observed in analyzing the pH parameter via FTIR, but was still sufficient for a precise quantification. The analysis of the parameters via FTIR remained stable over a period of 80 days. The use of antifoaming agents affected the FTIR spectra and, therefore, cannot be recommended. When testing the FTIR system in interlaboratory studies, a standardized sample preparation is highly recommended to obtain reproducible spectra. The results concluded that a well calibrated FTIR system can replace less practicable automated beer analyzers in quantifying quality relevant beer ingredients.

Descriptors: FTIR, quantitative analysis, beer analyzer, beer parameters, quality control

1 Introduction

Alcohol content, original wort, extract, specific gravity and pH are the most important quality characteristics of beer. To date, these parameters are determined with automated beer analyzers mostly for the quality control of beers. These analyzers apply, for example, a densiometer to determine the specific gravity or a sensor for the alcohol content. The other parameters such as original wort and extract are calculated from these measurements by acknowledged formulae [1]. Thus, determining beer parameters via beer analyzers means to apply at least three different analytical steps for a capacious evaluation of beer quality.

Another way of analyzing these parameters can be found in Fourier transformation infrared spectroscopy (FTIR). In recent years, this method has become an efficient and flexibly manageable technology, especially in routine analysis and quality assurance [2]. It has been widely and successfully applied for the analysis of, e.g., wine, milk and other environmental constitutes [2–8]. Recently, FTIR has found its way to quality controlling of beer. Duarte et al. started to introduce FTIR as a potential quality control tool and investigated 50 beer samples to obtain rapid information about factors on beer production. By spectral analysis, the authors were able to separate beers according to their alcohol content, but could not obtain in depth information on other components than ethanol [9]. Llario et al. investigated two set of samples from the Spanish market, one (24 samples) for building and validating the FTIR model and the other one (21 samples) for evaluating the model's robustness [10]. They made a rough classification of beers according to original extract and alcohol content. The authors favored FTIR to an automatic reference analyzer. Lachenmeier recently applied this methodology for the rapid quality control of beers and spirit drinks [11]. The author considered FTIR as a tool for the screening of alcoholic beverages and the results obtained lied within legal requirements. The author acknowledged as well the advantages of FTIR against a reference analyzer, such as being faster and processing a higher quantity of samples, but pointed out a certain lack of accuracy for a quantitative analysis.

These works investigated the possibility of FTIR as a general tool for the analysis of beers by screening parameters for, e. g., food controlling purposes. It was our purpose to scrutinize FTIR as to its potential in routine analysis and quality control of beers by obtaining precise quantitative information on beer parameters. In this respect, several requirements compared to commonly used automated beer analyzers should be fulfilled before such a system can be implemented in practice. First, beer parameters must be determined with the highest accuracy possible, regardless to legally constituted standard values. For the brewer, this is of utmost importance because decisions must be made about the correct adjustment of quality important beer ingredients. Second, the method should be economical (low acquisition and running costs), robust, user friendly and fast, and third, the entire range of beer samples should be covered, including those with a span in the alcohol content (from alcohol free to beers with more than 8.5 % v/v alcohol such as Bock beer).

We therefore analyzed quantitatively a wide range of samples with a calibrated FTIR system and an automated beer analyzer acknowledged in brewing industry and in food control. The sample range included all different sorts of marketed top and bottom fermented beers (also variations such as shandy, etc.). The differences between these two methods were evaluated by inferential statistics. Our work also emphasized on a standardized sample preparation for the FTIR system, the influence of anti-foaming agents on IR spectra, and the repeatability and stability of the FTIR analysis.

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Tables and figures see Appendix

We aimed further to calibrate the FTIR system so that it can be applied as an officially accredited analytical system, as it is the case for an automated beer analyzer.

2 Materials and Methods

Course of action

In total 329 beers all sorts of (first set of samples, acquired from breweries all over Europe) were analyzed in parallel by both analytical systems (FTIR spectroscopy and beer analyzer). The spectra obtained via FTIR were included into a database, which was calibrated by partial least square (PLS) regression. The calibration was examined and verified in terms of stability by applying a second set of samples (in total 31) to the FTIR system. The differences of both analytical methods were figured out via inferential statistics, for which the second set of samples was also applied to the beer analyzer. These steps served to examine the precision of the FTIR system. Subsequent steps were to observe the instrument's repeatability and stability in analyzing beer parameters, as well as the influence of defoamers on IR spectra.

Reference beer analysis and beer parameters

As the reference analysis served the acknowledged method according to MEBAK [1], c.f. EBC-method [12], using an automated SCABA Beer Analyzer, model 5610, from Foss GmbH, Rellingen (Germany). The SCABA analyzer included three different analytical systems: a densiometer for determining the specific gravity (S_L 20/20 °C), a ceramic sensor for analyzing the alcohol content (in % w/w and % v/v), and a pH meter. From these measurements, the following parameters were calculated according to Balling formulae [1]: the original wort, apparent and real extract and fermentation (all in % w/w and % w/v), the caloric value in kilocalories and kilojoule (both related to 100 ml), and the refraction index. The reference analysis provided in total 15 different measured results per sample.

FTIR spectroscopy

For the FTIR analysis, a spectrometer FT 120 Winescan from FOSS GmbH, Rellingen (Germany) was used in the following specification: fully deionized water for the zero point and cleaning solution (plus sodium hypochloride); equalizer solution for the internal calibration (aqueous alcoholic solution); CaF cuvette with 36 µm thickness; IR lamp: glowing metal wire; HeNe laser with 632 nm; measurement temperature at 40 °C; software used for the calibration: FT 120 software, version 2.2.2 (included the database for the scans). All measurements were repeated twice.

Calibration of the FTIR system

A successful measurement of beer parameters via FTIR necessitates a diversified database, including reference spectra of nearly all existing different sorts of beers. The use of such a database is enhanced by the quantity and quality of the contained spectra. Table 1 gives an overview of the sorts of beers considered for these experiments (first set of samples).

Sample preparation and measurements

A standardized sample preparation procedure is necessary for building-up the calibrated database and to guarantee reproducible spectra [13], as well as a correct comparison of the measurements obtained by both analytical ways. First, beer bottles were opened up at room temperature. The samples afterward were poured into aliquots and shaken until visible CO_2-formation took place. After the foam disintegrated (foam that clung on the bottle wall was re-dissolved by gentle shaking), the samples were directly and simultaneously measured with both analytical systems without further pretreatment. To avoid losses of beer ingredients, extra care was taken that the entire foam remained in solution. Any kind of filters or antifoam substances for the FTIR analysis had not been used to exclude falsification of measurements – only for the analysis with SCABA, each sample was filtered twice using folded cellulose filters according to EBC standard (Whatman® Schleicher & Schuell). The measurements were repeated twice. Attention was paid on the sample matrix to be kept as natural as possible during the experiments.

3 Results and Discussion

FTIR calibration

After the first set of samples had been measured with the FT 120 system, the spectra were recorded and the results obtained were compared to those of the SCABA reference analysis. To detect and to assign the scans to a specific beer parameter, the partial least square (PLS) regression model was used. The smallest distribution of the quadratic deviation was considered, so that the software for the PLS-calibration searches always for the best conformity of the analytical results. Figure 1 shows the scans of all beer samples (grey curves) used for calibrating the database. The scans represent the absorption of each beer sample at a specific wave length, converted into the wave number. The magnitude of each scan equals to the absorption strength at a specific wave number (values are not given in the diagram). In addition, it is exemplarily depicted the correlation with the original wort parameter (% w/w), black curve, against the wave number. It can be seen from several bands (areas between two different wave numbers) that the correlation *r* is either +1 or –1. The closer the correlation lies at either values, the better the relation is between the characteristics of the spectrum and the reference values of the SCABA analysis. As either values had been reached, the correlation observed was found useful for a significant differentiation of the parameters. Not the entire spectrum correlates with the parameters to be calibrated but certain areas. This allows for the FT 120 software to choose between areas of analysis, showing the advantage of a broad recording of the IR spectrum.

In Table 2, the data of the calibration of all 15 beer parameters are summarized. The first three columns relate to the initial set of data used for the calibration of each parameter. All parameters were calibrated independently from each other, which can be seen from the number (*N*) of the spectra. The difference of *N* to the initial set (*N* = 329) regards the amount of samples considered as outliers, which were eliminated for each parameter individually. Here, all parameters show a perfect fit of the specific regression (RSQ = 1), except for the apparent and real fermentation and the pH. This might not be of concern for the first mentioned parameters as their values can be calculated from the other parameters. The RSQ-value of 0.95 for the pH, however, indicates that a certain limit is reached for the analysis via the FTIR system, which shall be discussed later on. From the RSQ-values below 1.0, it can be deduced that none of these parameters were calculated with the help of other already calibrated parameters. This should be emphasized because it is possible to calculate, e.g., the real and apparent fermentation parameters from other calibrated values showing RSQ-values of 1.0 via the Balling formulae without having even measured these two parameters [1]. It should be mentioned that a perfect fit (RSQ of 1) can of course always be obtained by erasing outliers excessively. Therefore, the quality of the calibration should in general be verified with an independent controlling set, for which the second set of samples served. In Table 3, the sorts of beers used for this purpose are described, showing the wide range of samples used. The number N (= 31) sufficed to run in prior successfully an outlier test [14].

Comparison of calibrated FTIR and automated beer analyzer – inferential statistics

The analytical results of the control set were examined in terms of differences in the measurements of the calibrated FT 120 and the SCABA reference analysis. For the parameters original wort (% w/w and % w/v), alcohol (% w/w and % v/v), specific gravity, and pH, six measurements were obtained and evaluated. For each parameter, 31 paired measurements (x, y) were formed, where x stands for the SCABA and y for the FT 120 analysis. In the following, only the calculation for the original wort parameter (% w/w) shall be discussed exemplarily, for which the values are shown in Table 4.

Hypothesis testing was put forward to examine the viability of the null hypothesis (H_0) at 5 % probability of error. The null hypothesis is viable if the true mean value (μ_d) of all theoretically possibly paired differences (d) equals zero (for opposite considerations, the alternative hypothesis is: $\mu_d \neq 0$). As, however, the true mean value is not known, an estimation of the value – $(\sum d)/N = \bar{d}$ – must be used for the calculation.

Applying the David-test revealed that the normal distribution of the paired independent differences d, which are also independent from the sample matrix, can be assumed for all five confidence intervals (90-, 95-, 97.5-, 99-, and 99.5 %) [15]. Prior to this test, outliers had been eliminated according to Schlötel – e.g. value entry for d of sample no. 14 in Table 4. Both the normal distribution and the elimination of outliers are basic requirements for the following evaluation via inferential statistics.

With the help of the differences (d), the measured parameters of different beers can be compared to each other. For the hypothesis testing, a two-tailed test of significance was chosen to test whether μ_d equals zero. This test is based on test-statistics in which the sampling distribution pertains to a t-distribution, where $\hat{t}$ is the term to be examined (Eq. 1). According to Gosset, the null hypothesis can be rejected on 100 % α-level when $\hat{t}$ is greater or equal than the t-variable ($\hat{t}_{v;a}$) [16]. The latter in turn is dependent on the degree of freedom ($v = N–1$) and the probability of error (α).

$$\hat{t} = \frac{\left|\frac{\sum d}{N}\right|}{\sqrt{\frac{\sum d^2 - \frac{(\sum d)^2}{N}}{N(N-1)}}} = \frac{|\bar{d}|}{s_{\bar{d}}} \qquad \text{(Equation 1)}$$

Eq. 1 is based on eq. 2 [16]:

$$t = \frac{\bar{d} - \mu_d}{\frac{s}{\sqrt{N}}} \qquad \text{(Equation 2)}$$

where t is the variable of the t-function, $\bar{d}$ the mean value of the sample (estimated), μ_d the true mean value, s the estimated value of the true standard deviation σ, and N the number of samples. The denominator refers to the standard error of the mean value ($\bar{d}$).

When presuming that the null hypothesis is true ($\mu_d = 0$), then the numerator of Equation 1 contains just the mean value ($\bar{d}$). As the two-tailed test is used, the numerator is set to the absolute value. Computing the data from table 4 by Equation 1, with the degree of freedom $v = 31–1 = 30$, results in $\hat{t} = 0.069/0.041 = 1.676$ (for the calculation, the value entries in Table 4 were rounded and the outlier was included).

The 95 % confidence level for the true mean value (μ_d) is given by the equation: $\mu_d = \bar{d} \pm t_{n-1;0,05} \cdot s_{\bar{d}}$ (the denominator of Eq. 1). It can clearly be seen that $\hat{t}$ is smaller compared to the t-variable (2.042) of the t-distribution with a degree of freedom $v = 30$, at 5 %-level. With $\hat{t} = 1.676 < 2.042 \approx t_{\infty,0.05}$, it is proven that the null hypothesis cannot be rejected. The 95 % confidence interval of $-0.015 \leq \mu_d \leq 0.153$ shows at first that μ_d always includes the null, and secondly that the interval is narrow, which means that the precision of both methods is high. Therefore, the analysis of the original wort parameter by either analytical methods does not differ significantly. Table 5 shows the results for all examined parameters, for which $\hat{t}$ is always smaller than the value of the t-variable distribution. Therefore, the measured parameters do not differ in dependence of both analytical methods.

Figure 2 shows further the two analytical methods plotted against each other for each parameter as well as the calculated slope and the multiple regression correlation coefficient R^2. For each parameter, the line through the origin could be calculated as the linear smoothing function, which can be deduced from R^2. As R^2 is close to 1.0, no offset exists between the two measurement principals. Strong correlations for beer parameters such as ethanol, density, original gravity and lactic acid ($R^2 = 0.97–0.98$) could already be observed but not for other parameters such as the pH ($R^2 = 0.705$) [11]. Our correlation found for the pH was 0.901, which, although relatively high, is still lower compared to the correlation of the other parameters. This might be explained due to the fact that the pH value cannot be analyzed faultlessly by either analytical methods.

The problem lies in degassing the beer properly. For our samples, the pH values varied between 4 and 5. Below pH 4, the entire carbon dioxide in beer is dissolved as gas [17], and thus, does not have an influence anymore on the pH figure. At pH values greater than 4, a difference between carbonized and degassed beer can be recognized. In average, the pH value of a carbonized beer is about 0.3 units lower than those of a degassed beer. The maximum difference of the pH values determined with both the FT 120 and the SCABA system is at 0.1 units, which can be attributed to degassing problems. It should be added that independently from the degassing method, a CO_2 residual concentration of 2 g/l still remains in the beer sample [18].

Hydronium cations as the most relevant ions in the pH measurement cannot be detected by the FT 120 system. The system can only detect dissociated COO^- acidic parts. It is, therefore, not possible for the system yet to differentiate between dissociated and un-dissociated acidic parts. Depending on the solubility of carbon dioxide, the pH varies maximal between 3½–5. Therefore, all beers have nearly similar pH values (around 4.5), which makes it difficult for the FT 120 to identify different pH characteristics in the spectra. Nevertheless, the analysis of the pH by either methods does not differ significantly, but there is still potential left for a better differentiation of the beer samples to analyze more precisely the pH with the FT 120 system.

Repeatability of the FTIR analysis

To make further statements about the quality of the FTIR system, the calibration was finally examined by auditing the measurements as to their repeatability (r) according to DIN ISO 5725 [19]. In general, such an examination includes an interlaboratory study. Due to the exclusiveness of the FT 120 system, such a study could not be performed, but every other step was followed accordingly. To re-enforce the significance of the calculation and for comparison, the repeatability of the SCABA analysis was also calculated with the same method. In proceeding, a bottom and a top fermented beer were measured ten times simultaneously on both systems. The calculation of *r* is demonstrated again exemplarily for the original wort parameter (% w/w) analyzed by the FT 120 system (Table 6).

According to the norm, the repeatability deviation (s_r) is calculated as follows (Eq. 3):

$$s_r = \sqrt{\frac{1}{N-m}\sum_{j=1}^{m}(n_j - 1)\cdot s_j^{\,2}} \qquad \text{(Equation 3)}$$

where *m* is the amount of the different samples (top and bottom fermented beers), *N* the total number of measurements (= 20), n_j the amount of the measurements for one sample individually (= 10), and s_j^2 the variance of the measurements of the sample *j*.

Running the calculation with the measurements given in Table 6 results in $s_r = 0.00505$. With s_r, the repeatability can be calculated: $r = s_r \times 2.83 = 0.0143$.

The repeatability describes the variation of experimental outcomes carried out under the same conditions. The values for both analytical systems are listed in table 7. The *r*-values of the FT 120 are smaller than those of the SCABA instrument for all four parameters, which makes the FT 120 system more advanced. Compared to MEBAK guidelines, all FT 120 *r*-values lie within margins [1]. Some of the *r*-values as to MEBAK are above, e.g. between 0.04–0.06 for the original wort parameter, which is nearly three times higher than for the FT 120 system. This confirms the practicability of the FT 120 system during routine beer analysis. However, these values strictly taken are only valid within either systems and, hence, more laboratories should participate in repeatability studies to obtain more meaningful *r*-values for the FT 120 system. The handling of the system is simple, and therefore, repeatability studies carried out in external laboratories should be running nearly identical. It should further be noted that it is possible to increase the number of the multiple measured different samples to simulate an interlaboratory test.

Stability of the FTIR analysis

The stability of the results was also randomly examined with the help of a pilot sample (bottom fermented Bavarian pale ale in bottle, originating from the same filling as the investigated samples). The analysis was repeated 10 times over a period of 80 days. The pilot sample was the first sample analyzed at the day of the experiments after the FT 120 system had been set to the initiation point according to the manual – the standardization procedure was followed either after 100 measurements, or when the procedure for setting the initiation point brought up an error message. Figure 3 shows the course of some of the parameters and the days when the FT 120 machine was calibrated (after 42, 58, and 76 days).

In Table 8, the repeatability deviation s_r for some beer parameters measured with the FT 120 system are listed. It should be noted that the values given do not represent the repeatability deviation according to DIN ISO 5725 as they were not measured within a short interval. However, a comparison still makes sense in order to verify the quality and stability of the measurements. Compared to the values shown in Table 7, they are higher but still lie within in the same range. The fact that the s_r-values registered after a longer period (e.g. 78 and 80 days) are low compared to those obtained after a shorter period (16 and 36 days), favors the good stability of the FT 120 system. Considering that also natural aging effects take place in the sample such as oxidation processes, another positive argument for the system's high measurement stability is given.

Antifoaming test

In beer analysis, antifoaming agents are frequently used during sample preparation to disintegrate foam so that no ingredients to be determined go astray (e.g., alpha-acids). Also, the time until the foam reverts back to liquid could be spared, should there be no effect of such agents. In general, the use of defoamers in beer analysis should be avoided as these substances can have a strong influence on the beer matrix. Therefore, an antifoaming test was carried out to observe whether the use of such agents incurs changes in IR spectra of parameters. In total 11 samples the same type of (Lager) were prepared and subjected to different amounts of 1-octanol and a silicon-antifoaming emulsions.

The samples were analyzed for the parameters with both the FT 120 system (repeated twice) and the SCABA beer analyzer as shown in Tables 9 and 10. For the FTIR system, a provisional calibration sufficed to examine changes in the spectra caused by antifoaming substances. The analytical data were evaluated in terms of correlation (R^2) as to the antifoaming agents. The R^2 values parameters that are close to 1 are underlined. From the results, it can be deduced that the defoamers did not influence the entire IR spectrum (e.g., the alcohol content in % v/v) but single bands specific for certain parameters. The specific gravity, for example, was not influenced by either agents, but others were, such as the real and apparent extract. Therefore, the use of antifoaming substances for the analysis of beers via the FT 120 system is not recommendable.

4 Conclusion

In this work, Fourier transformation infrared spectroscopy (FTIR) has been evaluated as an alternative to a commonly used automated beer analyzer for the purpose to quantify beer ingredients and by that, to investigate the feasibility of FTIR in routine analysis and quality control of beers. Due to the huge variety of calibrated samples, a successful quantitative determination of parameters involving literally all sorts of beers could be achieved. Herein, only beers marketed in Europe were considered representatively, but the FT 120 system could be adjusted to beers brewed elsewhere. The results have shown that the system is viable to the reference analyzer in analyzing beers. Regarding repeatability, the FT 120 even advanced the SCABA beer analyzer. Low repeatability deviations observed still after a longer period during a stability test proved the robustness of the system. Furthermore, the FT 120 is easier to operate and is notedly faster in running the analysis. The acquisition costs are comparable to those of an automated analyzer, but with lowered running costs for the FTIR system as only one measuring principle has to be calibrated. In this respect, calibration solutions used, e.g., for a pH-meter are omitted, as are costly filters applied during sample preparation for the SCABA analysis. The FT 120 allowed for analyzing each parameter individually within one process, whereas with an automated beer analyzer, some of the parameters can only be determined indirectly via calculation from others. As the use of different chemicals, e.g. defoamers, during sample preparation can affect the analysis of ingredients via FTIR, a coherent preparation procedure to be followed in, for example, interlaboratory studies is highly recommended. Conclusively, the FTIR is applicable for routine analysis of beers once the system is decently calibrated. Future investigations concern the improvement of the calibration, especially in terms of the pH value, and widening the range of analytically measurable raw materials.

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5 References

1. Miedaner, H.: Würze und Bier (Chapter Two). In: Brautechnische Analysenmethoden, Methodensammlung der Mitteleuropäischen Analysenkommission (MEBAK), 4th ed., MEBAK, Freising, Germany, 2002, Vol. 2, pp. 72-84.
2. Garcia-Jares, C. M.; Medina, B.: Application of multivariate calibration to the simultaneous routine determination of ethanol, glycerol, fructose, glucose, and total residual sugars in botrytized-grape sweet wines by means of near-infrared reflectance spectroscopy, Fresen. J. Anal. Chem., 357 (1997), pp. 86-91.
3. Luginbuhl, W.; Liniger, A.: Infrared analysis of raw milk: PLS calibrations of casein, whey protein, true and crude protein content with native calibration samples, Milchwiss.-Milk Sci. Int., **61** (2006), pp. 22-25.
4. Patz, C.-D.; David, A.; Thente, K.; Kurbel, P.; Dietrich, H.: Wine analysis with FTIR spectrometry, Wein-Wiss., **54** (2-3) (1999), pp. 80-87.
5. Provenzano, M. R.; Senesi, N.: Differential scanning calorimetry of river aquatic fulvic acids and their metal complexes, Fresen. Environ. Bull., **7** (7-8) (1998), pp. 423-428.
6. Sommerlade, R.; Ekici, P.; Parlar, H.: Gas phase reaction of selected isothiocyanates with OH radicals using a smog chamber-mass analyzer system, Atmos. Env., **18** (2006), pp. 3306-3315.
7. Kimmel, L.; Coelhan, M.; Leupold, G.; Vetter, W.; Parlar, H.: FTIR Spectroscopic characterization of chlorinated camphenes and cornenes in technical toxaphene, Environ. Sci. Technol., **34** (14) (2000), pp. 3041-3045.
8. Sommerlade, R.; Parlar, H.; Wrobel, D.; Kochs, P.: Product analysis and kinetics of the gas-phase reactions of selected organosilicon compounds with OH radicals using a smog chamber-mass spectrometer system, Environ. Sci. Technol., **27** (12) (1993), pp. 2435-2440.
9. Duarte, I. F.; Barros, A.; Almeida, C.; Spraul, M.; Gil, A. M.: Multivariate analysis of NMR and FTIR data as a potential tool for the quality control of beer, J. Agric. Food Chem., **52** (2004), pp. 1031-1038.
10. Llario, R.; Inon, F. A.; Garrigues, S.; de la Guardia, M.: Determination of quality parameters of beers by the use of attenuated total reflectance-Fourier transform infrared spectroscopy, Talanta, **69** (2) (2006), pp. 469-480.
11. Lachenmeier, D. W.: Rapid quality control of spirit drinks and beer using multivariate data analysis of Fourier transform infrared spectra, Food Chem. 2006, In Press, Corrected Proof, Available online.
12. EBC Analysis Committee. Section 9, Beer: Method 9.2.2, Alcohol in beer by catalytic combustion; Method 9.4, Original, real and apparent extract and original gravity of beer; Method 9.43.2, specific gravity of beer using a density meter. In: European Brewery Convention; EBC Analysis Committee, Ed.; Analytica-EBC, Fachverlag Hans Carl, Nürnberg, Germany, 1998.
13. Titze, J.; Ilberg, V.; Jacob, F.; Parlar, H.: A comparison of the EBC 9.8 method with a common applied HPLC analysis for determining bitterness in beer, Mschr. Brauwissenschaft, **12** (2005), pp. 18-24.
14. Schlötel, E.: Quality in the Federal Republic of Germany. In: Juran's quality control handbook, 35D.1-35D.8; Juran, J. M.; Gryna, F. M., Eds., New York: McGraw-Hill, 1988.
15. David, H. A.; Hartley, H. O.; Pearson, E. S.: The distribution of the ratio, in a single normal sample, of range to standard deviation, Biometrika, **41** (3-4) (1954), pp. 482-493.
16. Gosset, W. S.: The probable error of a mean, Biometrika, **6** (1908), pp. 1-25.
17. Benes, R.; Trinkel, M.: Messung des pH Wertes in carbonisiertem Bier, Brauwelt, **45** (5) (2005), pp. 1485-1488.
18. Blüml, S.: Manual of Filling Technology. The theory and practice of filling liquid products, Behr, 1st ed., 2005.
19. Deutsches Institut für Normung e. V. (DIN): ISO 5725 (1981) Precision of test methods – Determination and reproducibility by interlaboratory tests.

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Appendix

Table 1 **The sorts of beers (first set of samples) used for the calibration of the FTIR-database**

	Wheat beer [a]	Pilsener [b]	Export beer [b]	Pale ale [b]	Bock beer [b]	Others [c]	∑
Amount	99.0	84.0	57.0	51.0	8.0	30.0	329
Share (%)	30.1	25.6	17.3	15.5	2.4	9.1	100

[a] Brewed top-fermented. [b] Brewed bottom-fermented. [c] Beers from local breweries (lager, shandy, etc.)

Table 2 **Calibration data for the beer parameters of the first set of samples**

Parameter(Unit)	*N* [a]	MEAN [b]	SD [c]	SEC [d]	RSQ [e]	SECV [f]
Original wort (% w/w)	237	11.9461	1.6358	0.0419	1.00	0.0473
Original wort (% w/v)	236	12.5128	1.7936	0.0446	1.00	0.05
Alcohol (% w/w)	236	4.0873	0.5986	0.018	1.00	0.0204
Alcohol (% v/v)	236	5.2119	0.7675	0.0239	1.00	0.027
Apparent extract (% w/w)	222	2.1886	0.6205	0.018	1.00	0.0206
Apparent extract (% w/v)	223	2.2149	0.6347	0.0183	1.00	0.0211
Specific gravity	220	1.0085	0.0024	0.0001	1.00	0.0001
Real extract (% w/w)	224	4.0655	0.7249	0.0193	1.00	0.021
Real extract (% w/v)	225	4.0984	0.7403	0.0215	1.00	0.0228
Apparent fermentation (%)	225	82.6391	3.3171	0.4184	0.98	0.4578
Real fermentation (%)	224	67.5777	2.7327	0.3692	0.98	0.392
Caloric value (kcal/100 ml)	236	45.1017	6.439	0.1625	1.00	0.1809
Caloric value (kJ/100 ml)	234	180.9368	25.2409	0.6269	1.00	0.6995
Refraction index	235	37.6371	3.4066	0.1073	1.00	0.1141
pH-value	234	4.4615	0.1776	0.0378	0.95	0.0418

[a] No. of samples. [b] Mean value of the calibrated parameter over *N*-samples. [c] Standard deviation of the parameter. [d] Standard error of calibration. [e] Coefficient of determination. [f] Standard error of cross validation.

Table 3 **Selected beer samples (second set) used for testing the quality of the calibrated FT 120 system**

	Wheat beer[a]	Pilsener[b]	Export beer [b]	Pale ale [b]	Bock beer[b]	Others [b,c]	∑
Amount	10	4	4	8	3	2	31
Alcohol free	2	2	0	5	0	0	4
Light beer	6	0	0	2	0	0	8
Share (%)	32.3	12.9	12.9	25.8	9.7	6.4	100

[a] Brewed top-fermented. [b] Brewed bottom-fermented. [c] Beers from local breweries (lager, shandy, etc.)

Table 4 **Original wort (% w/w) measurements sorted in paired samples (x, y) of the second set of samples used for running inferential statistics (no units included)**

Beer sample No. (N)	x [a]	y [b]	d (x-y) [c]	d^2
1	4.70	4.76	–0.063	0.004
2	10.82	10.85	–0.028	0.001
3	7.17	7.63	–0.458	0.210
4	13.51	13.56	–0.051	0.003
5	11.00	11.05	–0.048	0.002
6	13.06	13.06	0.006	0.000
7	12.53	12.56	–0.031	0.001
8	3.84	3.90	–0.055	0.003
9	5.00	5.00	–0.001	0.000
10	12.55	12.63	–0.075	0.006
11	7.08	7.48	–0.394	0.155
12	4.87	4.89	–0.021	0.000
13	7.36	7.99	–0.628	0.394
14	11.01	10.21	0.805*	0.648
15	7.26	7.54	–0.278	0.077
16	9.16	9.23	–0.069	0.005
17	11.11	11.12	–0.016	0.000
18	7.84	7.95	–0.109	0.012
19	13.23	13.31	–0.078	0.006
20	12.18	12.24	–0.060	0.004
21	6.95	6.97	–0.026	0.001
22	7.86	7.92	–0.063	0.004
23	11.80	11.80	0.000	0.000
24	18.52	18.60	-0.078	0.006
25	7.92	7.83	0.095	0.009
26	7.52	7.56	–0.040	0.002
27	16.67	16.81	–0.135	0.018
28	4.84	5.23	–0.393	0.154
29	7.63	7.58	0.053	0.003
30	7.34	7.34	0.002	0.000
31	11.48	11.39	0.090	0.008
$N = 31$	-	-	$\sum d = –2.147$	$\sum d^2 = 1.736$

[a] value from SCABA analysis. [b] value from the FT 120 analysis. [c] The difference of the value entries for both analytical systems.
* Outlier.

Table 5 **Results derived from inferential statistics for each parameter**

Parameter	N	$\hat{t}$ [a]	t_N-1,0.05 [b]	Confidence interval (95 %)
Original wort (% w/w)	30	1.676	2.045	$-0.015 \leq \mu_d \leq 0.153$
Original wort (% w/v)	29	0.173	2.048	$-0.042 \leq \mu_d \leq 0.050$
Alcohol (% w/w)	31	0.814	2.042	$-0.039 \leq \mu_d \leq 0.091$
Alcohol (% v/v)	31	1.147	2.042	$-0.039 \leq \mu_d \leq 0.088$
Specific gravity	31	1.010	2.042	$-3.216 \times 10^{-4} \leq \mu_d \leq 2.278 \times 10^{-4}$
pH-value	31	0.284	2.042	$-0.043 \leq \mu_d \leq 0.057$

[a] Examined term in t-statistics. [b] t-variable ((16)).

Table 6 **Original wort parameter measured ten times in one bottom and one top fermented beer sample with the FT 120 system and the calculated repeatability deviation (s_r)**

	Original wort (% w/w)	
Measurement	Bottom fermented beer (pale ale)	Top fermented beer (wheat beer)
1	11.37	12.80
2	11.38	12.80
3	11.37	12.80
4	11.38	12.79
5	11.38	12.80
6	11.38	12.79
7	11.38	12.79
8	11.38	12.79
9	11.38	12.79
10	11.37	12.80
Mean value	11.377	12.795
s_r	0.00483	0.00527

Table 7 **Repeatability (r) and repeatability deviation (s_r) for both analytical methods**

	FT 120		SCABA	
Parameter	s_r	r	s_r	r
Original wort (% w/w)	0.00505	0,0143	0.03365	0.0952
Alcohol (% v/v)	0.00454	0.0128	0.02100	0.0594
Specific gravity	1.783×10^{-5}	5.046×10^{-5}	2.634×10^{-5}	7.454×10^{-5}
pH	0.0113	0.0319	0.0218	0.0617

Table 8 **Selected beer parameters (original wort, alcohol, specific gravity, and pH) determined over a longer period for stability testing of FT 120 system**

Days	Original wort (% w/w)	Alcohol (% v/v)	Specific gravity	pH
0	11.88	5.48	1.00602	4.34
8	11.86	5.48	1.00603	4.34
16	11.87	5.48	1.00602	4.35
36	11.83	5.48	1.00597	4.37
36	11.83	5.48	1.00598	4.37
44	11.88	5.49	1.00603	4.36
58	11.83	5.47	1.00602	4.31
59	11.81	5.48	1.00599	4.35
73	11.82	5.47	1.00597	4.32
78	11.86	5.48	1.00601	4.31
80	11.82	5.48	1.00598	4.34
Mean value	11.8445	5.4791	1.0060	4.3418
s_r [a]	0.0258	0.0054	2.401×10^{-5}	0.0214

[a] repeatability deviation.

Table 9 **Correlation coefficient R^2 of FT 120 analysis with the silicon-antifoaming emulsion**

Sample-No. [a]	Alcohol (% v/v)	Alcohol (% w/w)	Apparent extract (% w/w)	Original wort (% w/w)	pH	Real extract (% w/w)	Specific gravity
1 (0)	5.38	4.24	1.48	11.47	4.54	3.35	1.004854
1 (0)	5.38	4.25	1.50	11.51	4.55	3.36	1.005051
2 (1)	5.40	4.26	1.49	11.54	4.52	3.37	1.005036
2 (1)	5.37	4.22	1.48	11.44	4.52	3.36	1.004841
3 (2)	5.41	4.26	1.51	11.49	4.50	3.38	1.004899
3 (2)	5.40	4.25	1.51	11.50	4.53	3.39	1.004814
4 (3)	5.40	4.25	1.51	11.48	4.50	3.39	1.004874
4 (3)	5.40	4.25	1.52	11.54	4.50	3.41	1.005225
5 (4)	5.40	4.24	1.53	11.48	4.49	3.42	1.005497
5 (4)	5.40	4.25	1.53	11.82	4.50	3.44	1.005335
6 (5)	5.39	4.23	1.54	11.51	4.48	3.45	1.005159
6 (5)	5.40	4.24	1.54	11.50	4.46	3.44	1.005261
R^2	0.26	0.01	0.90	0.07	0.83	0.90	0.44

[a] In brackets: amount of silicon-antifoaming drops.

Table 10 **Correlation coefficient R^2 of FT 120 analysis with the 1-octanol antifoaming solution**

Sample-No. [a]	Alcohol (% v/v)	Alcohol (% w/w)	Apparent extract (% w/w)	Original wort (% w/w)	pH	Real extract (%)	Specific gravity
1 (0)	5.38	4.24	1.48	11.47	4.54	3.35	1.004854
1 (0)	5.38	4.25	1.50	11.51	4.55	3.36	1.005051
7 (1)	5.38	4.25	1.48	11.48	4.53	3.34	1.004961
7 (1)	5.38	4.25	1.47	11.45	4.52	3.33	1.0048.91
8 (2)	5.39	4.25	1.47	11.49	4.53	3.32	1.004624
8 (2)	5.37	4.23	1.46	11.44	4.52	3.31	1.004560
9 (3)	5.36	4.22	1.45	11.43	4.51	3.31	1.004749
9 (3)	5.38	4.23	1.43	11.44	4.52	3.28	1.005184
10 (4)	5.38	4.23	1.43	11.42	4.54	3.27	1.004880
10 (4)	5.36	4.22	1.43	11.36	4.55	3.26	1.004696
11 (5)	5.36	4.21	1.41	11.36	4.53	3.24	1.0048.59
11 (5)	5.36	4.20	1.42	11.37	4.55	3.25	1.004963
R^2	0.46	0.72	0.92	0.81	0.04	0.95	0.01

[a] In brackets: amount of 1-octanol antifoaming drops.

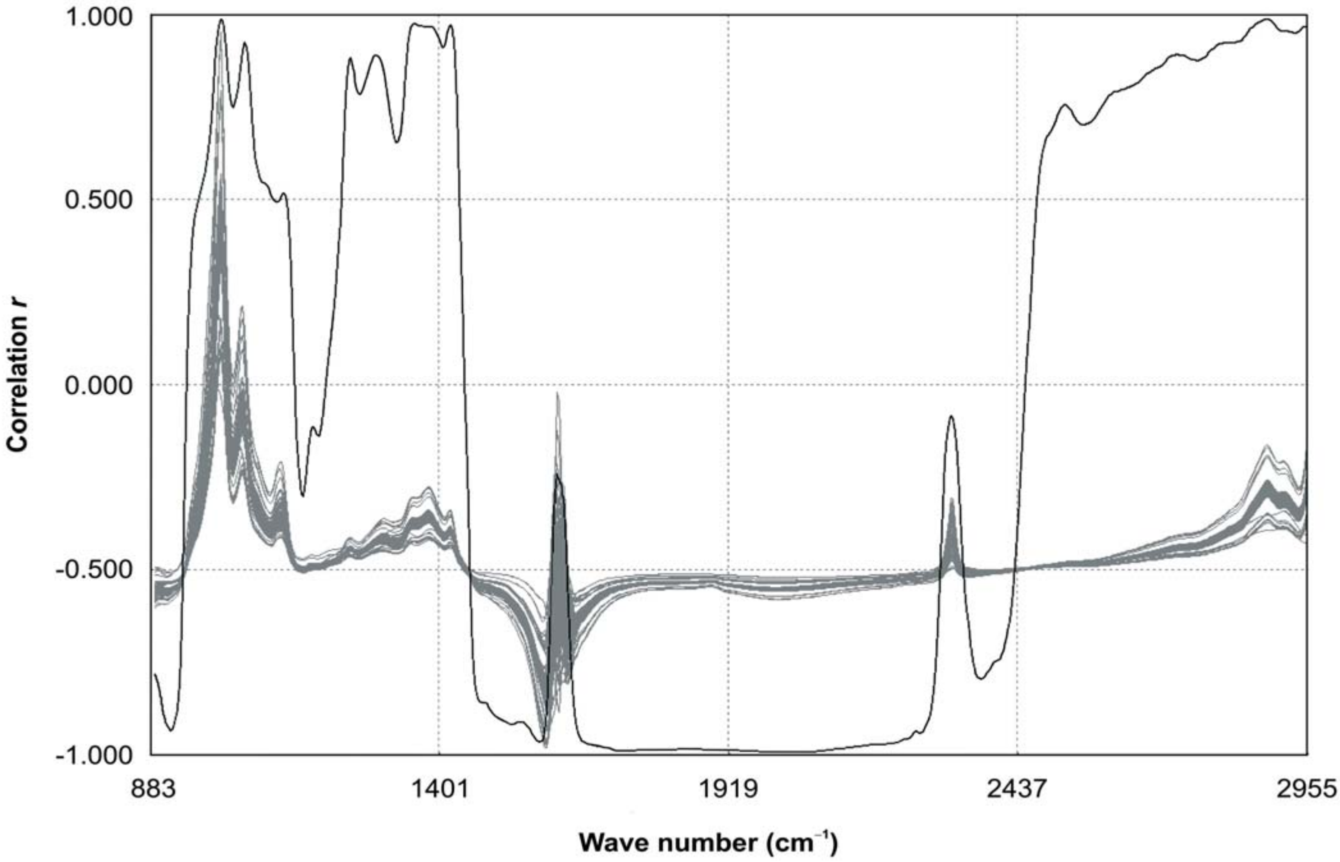

Figure 1 IR scans of the first set of beer samples (marked in grey) and exemplarily the correlation *r* with the original wort parameter (% w/w), black curve, plotted against the wave number

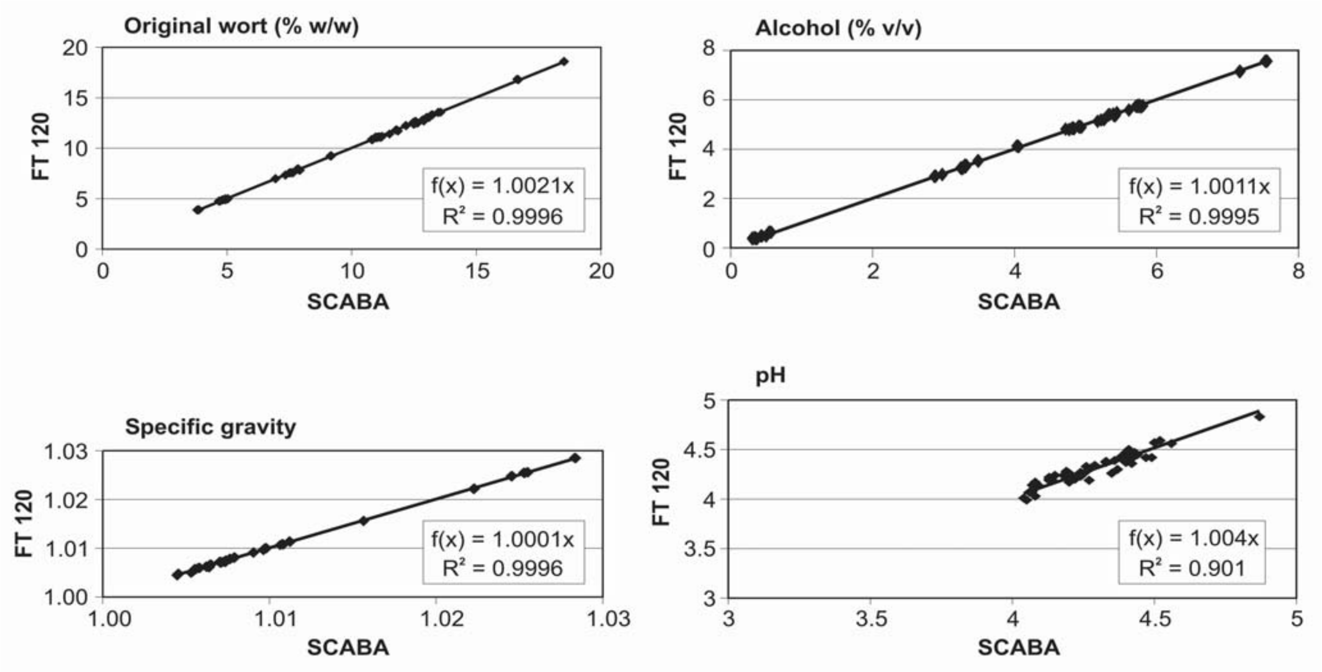

Figure 2 FTIR (using the FT 120 spectrometer) and SCABA reference analysis plotted against each other for each parameter (original wort, alcohol, specific gravity, and pH), including the slope and the correlation coefficient (R^2)

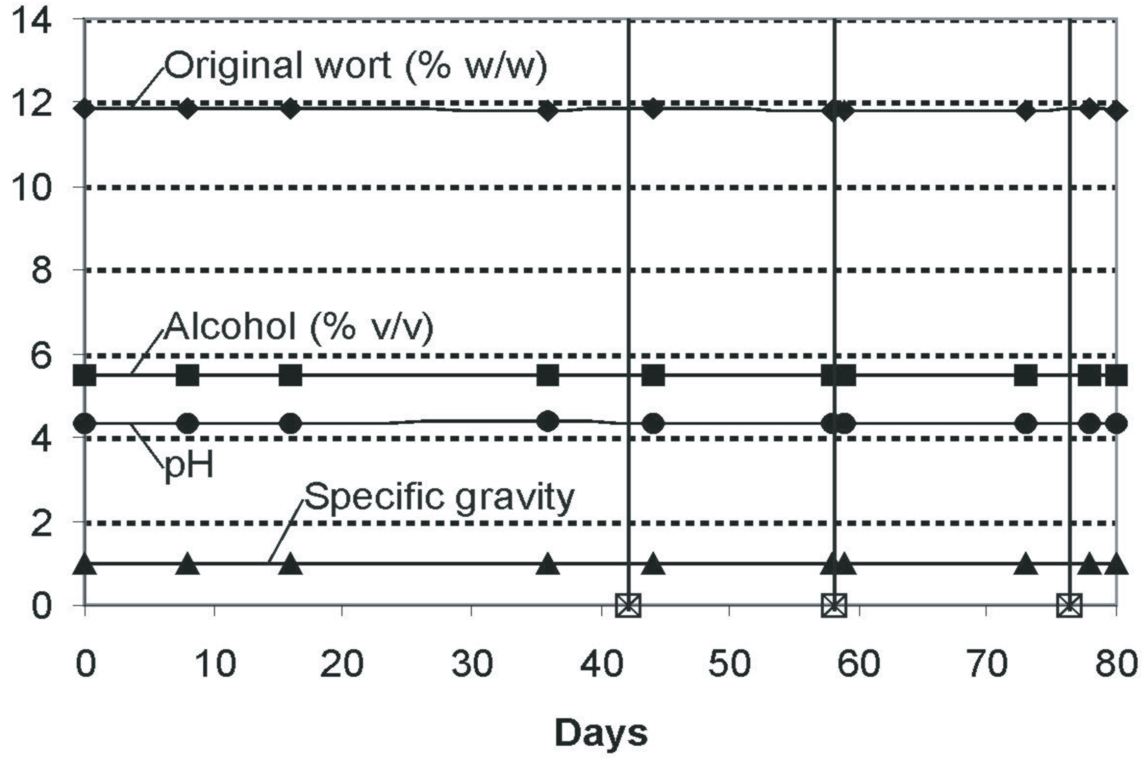

Figure 3 Parameter measured with the FT 120 spectrometer for observing the stability of the system (vertical lines show the times when the system was calibrated)