

Determination of Alcohol and Extract Concentration in Beer Samples Using a Combined Method of Near-Infrared (NIR) Spectroscopy and Refractometry

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A new approach of combination of near-infrared (NIR) spectroscopy and refractometry was developed in this work to determine the concentration of alcohol and real extract in various beer samples. A partial least-squares (PLS) regression, as multivariate calibration method, was used to evaluate the correlation between the data of spectroscopy/refractometry and alcohol/extract concentration. This multivariate combination of spectroscopy and refractometry enhanced the precision in the determination of alcohol, compared to single spectroscopy measurements, due to the effect of high extract concentration on the spectral data, especially of nonalcoholic beer samples. For NIR calibration, two mathematical pretreatments (first-order derivation and linear baseline correction) were applied to eliminate light scattering effects. A sample grouping of the refractometry data was also applied to increase the accuracy of the determined concentration. The root mean squared errors of validation (RMSEV) of the validation process concerning alcohol and extract concentration were 0.23 Mas% (method A), 0.12 Mas% (method B), and 0.19 Mas% (method C) and 0.11 Mas% (method A), 0.11 Mas% (method B), and 0.11 Mas% (method C), respectively.

KEYWORDS: NIR spectroscopy; refractometry; PLS; alcohol; real extract; beer; quantification; multivariate calibration; scatter correction

INTRODUCTION

Well over 450 constituents have been characterized in beer. Together, all of these constituents produce the character of beer (*1*). Investigations, based on the threshold values and flavor units, outline the significance of constituents to the overall beer flavor (*2, 3*). According to these investigations, changes in alcohol or sugar produce a decisive change in flavor. In addition to the influence on the flavor, the amount of alcohol and real extract is also important for economic reasons, particularly in relation to the taxes imposed. Focused on customer needs as well as economic reasons, the quantitative determination of alcohol and sugar is an inalienable procedure in the quality control of beer. For years the quantitative determination of alcohol and real extract was based upon a distillation method. According to refs *4* and *5*, the distillation method is regarded as the official reference method. The following disadvantages speak against the distillation method as a routine analytic method: first, it is a time-consuming method; second, it has a restricted degree of automation; and, third, a degree of operational variation is incorporated in the system.

Near-infrared (NIR) spectroscopy is a nondestructive method based upon molecular overtone and combination vibrations (*6–8*). Throughout the sector of the agricultural and food industry,

several applications proclaim NIR spectroscopy as a powerful tool for qualitative (e.g., discriminating coffee (*9*) or alcoholic beverages (*10*)) as well as quantitative (e.g., juices (*11–13*), vinegar (*14*), or rice wine (*15*)) analysis (*16, 17*). The specific character of the NIR spectroscopy certainly yields to several quantitative applications in beer analysis (for alcohol and real extract concentration by the majority). A univariate model based on the measurement of the ethanol absorbance maximum at 1693 nm above a baseline, established between 1657 and 1720 nm, has been developed by Galligani et al. (*18*). Gallignani et al. also proposed in a different work a derivative FT-IR procedure for the direct determination of alcohol in all types of alcoholic beverages (beer, wine, rum, and whiskey, for example) (*19*). Another univariate study uses the difference between the absorption bands at 1692 and 1719 nm as the alcohol reference (*20*). In addition to the aforementioned univariate models, different multivariate models for quantitative purposes in beer using NIR technology (*21–23*) and/or MIR technology (*24, 25*) have been applied. **Table 1** presents an overview on previously published investigations on quantitative determination of beer constituents using IR technology. Mendes et al. even proposed that FT-NIR spectroscopy presents better results than gas chromatography in evaluating the alcohol content of beverages (*26*).

Refractometry is the method of measuring a substance's refractive index and is based on the bending of a ray of light

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Table 1. Overview of Investigations on the Quantitative Determination of Real Extract, Original Extract, and/or Alcohol in Beer Samples by Use of Infrared Spectroscopy

reference	spectral technique	spectral region	parameter	accuracy
Norgaard et al. (23)	NIR-PLS	na	original extract	RMSEP 0.1–0.2% w/w
Galligani et al. (18)	NIR, univariate	1657–1720 nm	alcohol	RSD 0.02% v/v (regular bers) RSD 2% v/v (low-alcohol beers)
Galligani et al. (19)	NIR, derivative univariate	1680–1703 nm	alcohol	na
Llario et al. (25)	MIR-PLS	3050–800 cm ⁻¹	real extract original extract alcohol	RMSEP 0.10% w/w RMSEP 0.20% w/w RMSEP 0.12% v/v
Inon et al. (21)	NIR-PLS	1662–1684 nm 1667–1686 nm 1677–1742 nm	real extract original extract alcohol	RMSEP 0.15/0.14% w/w RMSEP 0.28/0.22% w/w RMSEP 0.08/0.09% v/v
Inon et al. (24)	NIR and MIR-PLS	2220–2345 nm 850–1201 cm ⁻¹	real extract original extract alcohol	RMSEP 0.10% w/w RMSEP 0.19% w/w RMSEP 0.12% v/v

when passing from one medium into another. Common applications of refractometry in the agricultural and food industry are the measurement of the dissolved sugar in beverages called Oechsle (predominant in wine) or Brix (predominant in fruit juices or soft drinks). There are several studies published outlining the application of refractometry for the quantitative determination of real extract, original extract, and alcohol (27–29). Fundamental investigations by Schild and Irrgang in 1956/1957 proposed the use of the refractive index and the specific gravity (30, 31) to determine extract and alcohol concentration. These investigations also illustrate the three-dimensional problem of measuring the refractive index in beer.

According to the references above, the multivariate combination of the specific NIR spectroscopy and the sensitive and reliable refractometry shall yield precise results for the parameters alcohol and real extract. A major advantage of a combined method of NIR spectroscopy and refractometry would be the use of one robust measurement device with one flow cell and a very small sample volume. This combination would also enable an additional specification of novel mixtures of beverages compared to commercial analysis methods, especially for soft drink or fruit juice based matrices. However, the results of this study may open a wide range of applications in process control or in laboratory routine for the quantification of real extract and alcohol in beer or other beverages. To the best of our knowledge, there are no previous investigations published using this combination for the quantitative determination of alcohol and real extract in beer.

MATERIALS AND METHODS

Samples. The sample set used for this investigation consists of 78 samples involving nonalcoholic beer (alcohol concentration between 0.05 and 0.5 vol %), draft beer, beer mixtures, regular beer, and bock beer. Of these, 28 samples were used for model building (calibration set) and 20 samples were used for testing the obtained model. An additional 30 samples (3 brands and 10 duplicates per brand) were used for testing the repeatability of the calibration (validation set). Additional ethanol standards with defined concentrations were used for NIR measurements to evaluate an appropriate pretreatment method.

The reference value of the alcohol and the extract concentration of the beer samples were measured with an Alcozyler Plus Beer Analyzing System (Anton Paar GmbH, Graz, Austria). An overview and the characteristics of the samples used in this investigation are listed in **Table 2**.

Multivariate Calibration. The most common statistical methods for solving multivariate problems are multiple linear regression (MLR)

models, principal component regression (PCR) models, and partial least-squares (PLS) models. A MLR model is based on the multiple linear coherence of a dependent variable y and of one or more independent variables x . The PCR model is based on the MLR model extended by a principal component analysis (PCA). The PLS model involves, compared to the PCR and MLR models, the dependent as well as independent variables in the model-finding process (32, 33).

For the characterization of the obtained calibration models, various numbers of coefficients are computable. In this work, the following coefficients have been chosen to evaluate the quality of the obtained models. The root mean squared error of calibration (RMSEC) and the relative root mean squared error of calibration (RRMSEC) are measures to quantify the difference between a value obtained by a model and a value obtained by a reference method and are defined as

$$\text{RMSE} = \sqrt{\frac{\sum_{i=1}^n (y_i - \hat{y}_i)^2}{n}}$$

where \hat{y}_i represents the concentration of the constituents obtained by the model, y_i the concentration obtained by the reference method, and n the number of samples in the calculated set.

To compare the obtained methodology against the reference, different quality indicators were also given: the coefficient of correlation (r_{xy}) and the systematic error (bias).

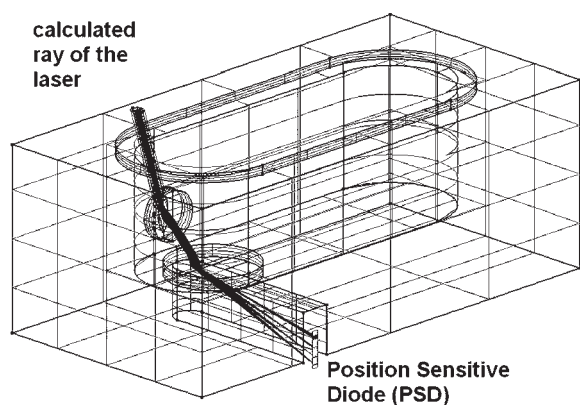
Validation. Validation is the process of checking if something satisfies a certain criterion. To validate the correctness of the obtained model, the root mean squared error of validation (RMSEV) and the relative root mean squared error of validation (RRMSEV) were calculated for a sample set different from the one used for model building (validation set). An additional measure, the residual prediction deviation (RPD), was calculated to evaluate the performance of the multivariate model. The RPD value is defined as the ratio between the standard deviation of the population (SD) and the standard error of validation (SEV). As a measure of accuracy and repeatability, the standard deviation was also calculated. Further information about validation procedures can be found in ref 34

NIR Analysis. A grating NIR spectrometer, developed during research and development investigations, was used for these NIR measurements. A more complete description and the performance of the developed NIR spectrometer can be found in ref 35. The NIR spectra were measured in a flow cell with a 10 mm path length and tempered at 20 ± 0.01 °C to avoid the influence of temperature changes in the NIR spectra (36–38). Before sample measurement, the cell was flushed with approximately 400 mL of the sample, to avoid sample carry-over. The spectra were recorded in the spectral range of 1100–1220 nm (according to the second overtone of CH, CH₂, and CH₃), by accumulating five scans with a resolution of 4 nm and water as reference. For each sample, a total of three spectra were recorded (determination in triplicate).

Table 2. Beer Samples and Reference Values of Alcohol and Real Extract

calibration set				validation set			
no.	beer samples	alcohol [Mas%]	extract [Mas%]	no.	beer samples	alcohol [Mas%]	extract [Mas%]
1	nonalcoholic beer	0.26	4.50	29	nonalcoholic beer	0.36	3.94
2	nonalcoholic beer	0.33	4.37	30	nonalcoholic beer	0.37	5.69
3	nonalcoholic beer	0.35	5.93	31	nonalcoholic beer	0.37	4.12
4	nonalcoholic beer	0.36	7.19	32	nonalcoholic beer	0.38	5.74
5	nonalcoholic beer	0.37	5.94	33	draft beer/beer mixture	2.22	3.11
6	draft beer/beer mixture	1.94	1.91	34	draft beer/beer mixture	2.22	2.87
7	draft beer/beer mixture	1.95	2.15	35	draft beer/beer mixture	2.33	2.79
8	draft beer/beer mixture	1.98	4.91	36	draft beer/beer mixture	2.34	2.72
9	draft beer/beer mixture	1.96	4.95	37	draft beer/beer mixture	2.46	2.71
10	draft beer/beer mixture	2.27	2.59	38	regular beer	4.12	3.77
11	draft beer/beer mixture	2.38	2.88	39	regular beer	3.83	3.92
12	draft beer/beer mixture	2.61	2.21	40	regular beer	3.92	3.53
13	draft beer/beer mixture	2.81	4.00	41	regular beer	3.76	3.85
14	regular beer	3.76	3.97	42	regular beer	4.10	4.18
15	regular beer	3.80	3.64	43	regular beer	3.93	4.19
16	regular beer	3.85	4.02	44	bock beer	5.51	5.47
17	regular beer	3.86	3.81	45	bock beer	5.54	7.48
18	regular beer	3.89	4.45	46	bock beer	5.55	5.51
19	regular beer	3.95	4.19	47	bock beer	5.61	7.49
20	regular beer	4.00	4.03	48	bock beer	5.91	7.42
21	regular beer	4.10	4.81	49–58 ^a	regular beer	4.10	4.73
22	regular beer	4.14	4.73	59–68 ^a	regular beer	3.90	4.07
23	regular beer	4.12	4.10	69–78 ^a	bock beer	5.94	4.88
24	bock beer	5.41	7.52				
25	bock beer	5.37	6.74				
26	bock beer	5.57	5.34				
27	bock beer	5.94	4.97				
28	bock beer	6.40	6.34				
	min	0.26	1.91		min	0.36	2.71
	max	6.40	7.52		max	5.91	7.49
	mean	3.13	4.51		mean	3.24	4.53
	SD	1.80	1.45		SD	1.90	1.59

^a Samples used to determine the repeatability of the model.

**Figure 1.** Tempered cell for the measurement of the refractive index.

Refractometric Analysis. The refraction indices were collected on laser-based refractometer test equipment, consisting of a tempered flow cell, a 600 nm laser diode, and a position sensitive detector (PSD) comprising two silicon–PIN diodes. A simulation of the refracted laser rays in the cell is pictured in **Figure 1**. The refractometer test equipment was validated in a previous work (not published) using sucrose standards with defined refraction indices. This validation process is not part of this publication. Only the characteristics of the validation will be published to verify the performance of the refractometer test equipment used: standard deviation, five sucrose standards measured 10 times, $SD \leq 0.00001$; RMSEV, five sucrose standards in comparison with the refraction indices of the literature (39), $RMSEV \leq 0.00002$.

Further information about validation processes using defined standards can be found in ref 34.

Before sample measurement, the cell was flushed with approximately 400 mL of the sample to avoid sample carry-over and afterward tempered at 20 ± 0.01 °C. Every measurement of the refraction index was performed 10 times and averaged using the median. For each sample, a total of three refraction indices were measured (determination in triplicate).

All recorded data of each sample were averaged (median), converted to JCAMP-DX (Joint Committee on Atomic and Molecular Physical data – Data Exchange) format, and transferred to The Unscrambler (version 9.1, CAMO Software AS, Oslo Norway) for multivariate analysis.

RESULTS AND DISCUSSION

To build the multivariate model of refractometry and NIR spectroscopy, appropriate pretreatment techniques have to be applied to the data of NIR spectroscopy and refractometry to evaluate the quantification as a single measurement.

NIR Data. Rayleigh scattering and Mie scattering are processes in which electromagnetic radiation is scattered by particles, bubbles, droplets, or even density fluctuation. Several publications have already discussed light scattering and the effect on spectral measurements in vinegar (40) or grape must (41). In this investigation the light scattering yields a spectral setoff and a spectral shift as a function of the wavelength (**Figure 2a**). Mathematical pretreatments such as first- or second-order derivation, extended multiplicative signal correction (EMSC), standard normal variate (SNV) transformation, or linear baseline correction are able to eliminate the spectral shift. Investigations using EMSC

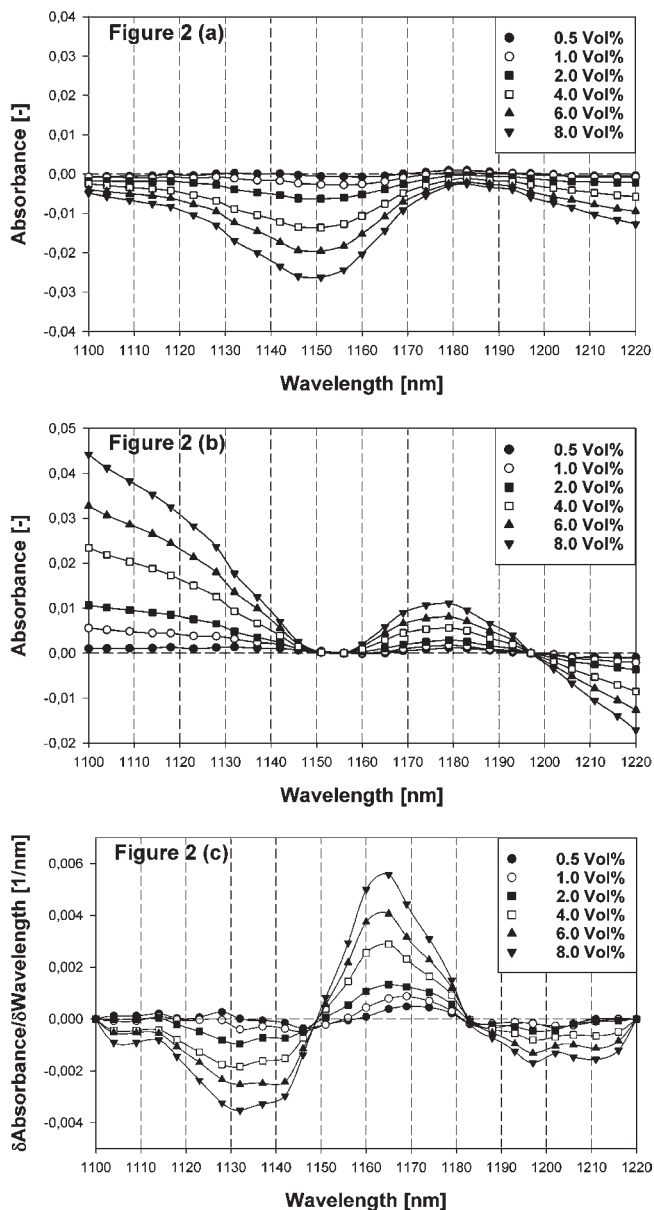


Figure 2. NIR spectra of ethanol standards: original data (a); linear baseline correction (b); first-order derivation (c), water as reference.

and SNV as preprocessing methods have shown unsatisfying results. The EMSC method is based on the calculation of a scatter spectrum, and every spectrum is corrected by this scatter spectrum (42), whereas the SNV transformation centers each spectrum and scales it by its own standard deviation (42, 43). A pretreatment method using the scatter spectrum (EMSC) or the standard deviation (SNV) to scale the spectral data might be an inappropriate technique for quantitative purposes. The investigations of Inon et al. (21) have shown unsuccessful results using EMSC and SNV transformation as preprocessing methods for quantitative NIR spectroscopy. Therefore, first-order derivation and linear baseline correction were applied in this investigation as pretreatment methods. Both pretreatments, the linear baseline correction as well as the first-order derivation, were applied on ethanol standards to evaluate the parameters of the technique. Figure 2 shows the NIR spectra of the ethanol standards before the pretreatment (a), after the linear baseline correction (b), and after the first-order derivation (c). Both techniques are able to eliminate the spectral shift.

On the basis of these pretreatments, three methods were developed to determine the concentration of ethanol in the NIR

spectra using the ethanol standards and the beer samples of the calibration set: method A, first-order derivation, absorbance at different wavelengths as reference of the alcohol concentration; method B, linear baseline correction, absorbance at different wavelengths as reference of the alcohol concentration; method C, linear baseline correction, area beneath NIR spectra as reference of the alcohol concentration.

The selection of the most informative parameters was performed through interactive trial and error processes, taking RMSEC and the coefficient of correlation as measures of performance. Table 3 shows the characterization of the three most precise models, applied to the ethanol standards as well as the beer samples of the calibration set. All three methods proved to be useable for the prediction of the alcohol concentration in ethanol standards as well as in real beer samples. No systematic error occurred in any of the methods (bias almost 0). According to Table 3 the prediction of alcohol in the ethanol standards is slightly more precise than in the beer samples. Especially the RMSEC of the beer samples is nearly twice the RMSEC of the ethanol standards. One of the reasons is probably the additional failure of the measured alcohol concentration in the beer samples (failure of reference method). Compared to the beer samples, the ethanol standards are produced via volumetric measurement using volumetric pipets and flasks. Another reason for the differences in performance is the much more complex beer matrix. Especially the prediction of the beer samples with low alcohol concentration (nonalcoholic beer) are slightly overestimated (predictions of the nonalcoholic samples are placed above the regression line in Figure 3). This overestimation is probably due to the higher extract with respect to the ratio alcohol/extract (< 0.1), which is different from the ratios in draft, regular, and bock beer (approximately 1.0). According to this, the performance of the model is highly influenced by the failure of the determination in nonalcoholic beer. Table 3 also presents the characteristics of the NIR calibration excluding nonalcoholic beer. Compared to the NIR calibration including nonalcoholic beer, the failure of the calibration excluding nonalcoholic beer is nearly 50% less.

Refractometer Data. As described above, the measurement of the refraction index is in the case of beer at least a three-dimensional problem, largely influenced by the variables alcohol concentration and extract concentration. Schild et al. already proposed these influences of alcohol and extract on the refraction index (30, 31). A common way to take this influence of alcohol into account is the additional measurement of a second parameter (e.g., the specific weight).

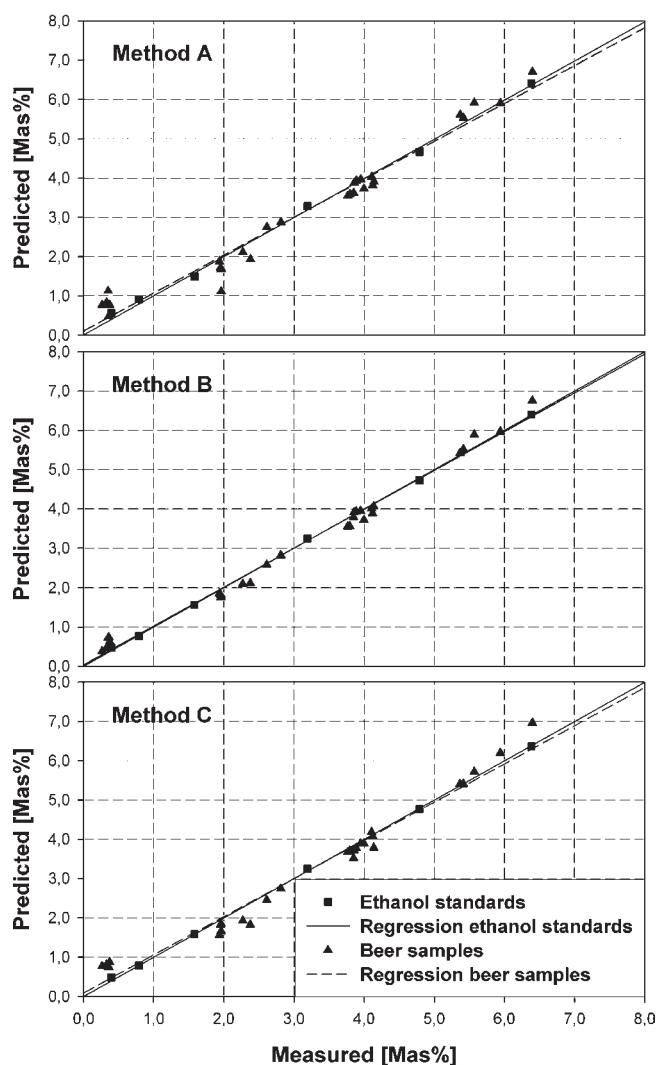
This investigation uses the influence of alcohol on the refraction index for a special sample grouping. The samples are grouped by their alcohol concentration, respectively grouped by their brand: nonalcoholic beer, draft beer and beer mixture, regular beer, and bock beer. Figure 4 visualizes the correlation of extract concentration and the refraction index including the sample grouping regarding the alcohol concentration. These arrangements produce much better results instead of an overall correlation (Table 4).

Multivariate Calibration. For consideration, the aforementioned influence of high extract with respect to high alcohol/extract ratios on NIR spectroscopy as well as the sample grouping by alcohol concentration, the obtained data of NIR spectroscopy and refractometry were combined in a multivariate matrix and solved by a multivariate method called PLS method. This multivariate matrix consists of two x variables (data of NIR spectroscopy and refractometry) and two y variables (alcohol and extract concentration). A PLS2 model for at least two variables and two PCs were used for solving the problem respectively fitting the model. An overview of the obtained model and the performance of the multivariate calibration are listed in Table 5. In comparison

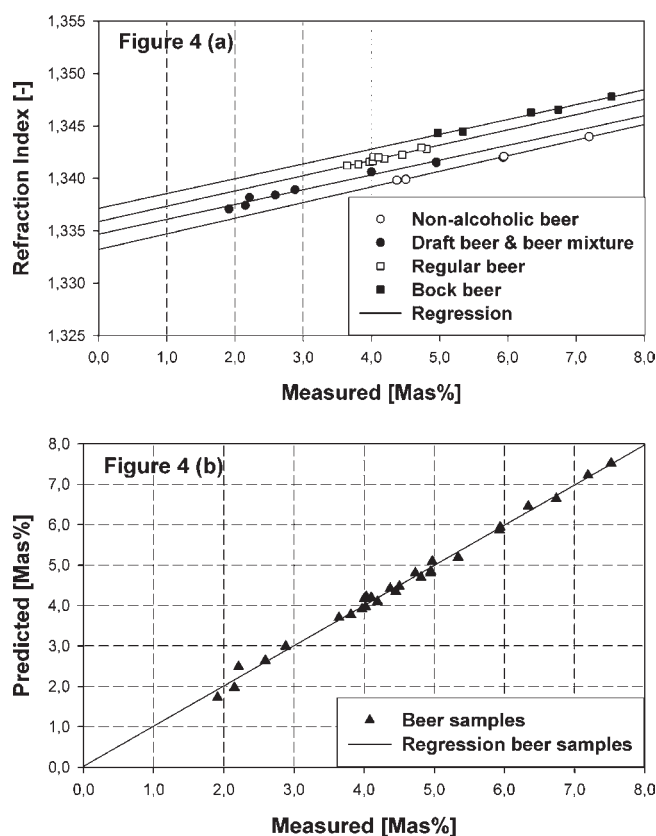
Table 3. Characteristics of NIR Calibration^a

	ethanol standards			beer samples (including nonalcoholic beer)			beer samples (excluding nonalcoholic beer)		
	A	B	C	A	B	C	A	B	C
RMSEC [Mas%]	0.11	0.05	0.06	0.30	0.18	0.25	0.18	0.11	0.13
RRMSEC (%)	4.49	2.04	2.45	9.58	4.37	6.07	5.75	3.51	4.15
r_{xy}	0.9990	0.9998	0.9997	0.9825	0.9939	0.9858	0.9825	0.9939	0.9858
bias	-8×10^{-8}	-3×10^{-8}	-1×10^{-7}	1×10^{-7}	2×10^{-8}	-1×10^{-7}	1×10^{-7}	3×10^{-8}	-1×10^{-7}

^a RMSEC, RRMSEC, coefficient of correlation, and systematic error regarding the concentration of alcohol of methods A, B, and C for ethanol standards and beer samples (calibration set).

**Figure 3.** Predicted versus measured alcohol concentration [Mas%] of NIR calibration model, methods A, B, and C.

of the NIR calibration and the multivariate calibration with regard to the alcohol concentration, the results of the multivariate model (Table 5) are better than the results of NIR spectroscopy excluding nonalcoholic beer but more precise than the NIR spectroscopy including nonalcoholic beer (Table 3). The influence of high extract concentrations on the prediction of alcohol by NIR spectroscopy can obviously be corrected by taking the extract concentration of the refractometric measurement in the model. Galligani et al. also proposed the negative interference of sugars in the determination of ethanol in the MIR range between 1052 and 1040 cm^{-1} (9505 and 9615 nm) (44) and in the NIR range between 1300 and 1800 nm (19). However, the precise alcohol determination of nonalcoholic beer samples is a major

**Figure 4.** Refractometer calibration of the beer samples (calibration set): (a) measured versus refraction index of beer samples and sample grouping; (b) measured versus predicted extract concentration [Mas%].

innovation of the multivariate combination of this study. Figure 5 provides a score plot of the multivariate model and visualizes the difference between varieties. A score plot involves the projection of the data onto the PCs in two dimensions. The samples were clustered in the multivariate model process by their variation according to the aforementioned sample grouping.

The results of the extract determination by only refractometry are similar to the results of the combination of NIR spectroscopy and refractometry. Obviously, the spectral data of the NIR technique do not contribute any additional information to the multivariate model concerning the determination of real extract. Even though high extract concentrations obviously influence the NIR spectroscopic measurement, the quantification of the real extract can not be enhanced by the spectral data. This is probably explained by the definition of the real extract as a sum parameter, which describes the total amount of nonvolatile constituents in beer such as inorganic salts, sugars, amino acids, nucleotides, proteins, and polysaccharides (1). The refraction index can reflect the sum of constituents and especially changes of constituents in novel beverages much better than NIR spectroscopy.

Table 4. Characteristics of Refractometer Calibration^a

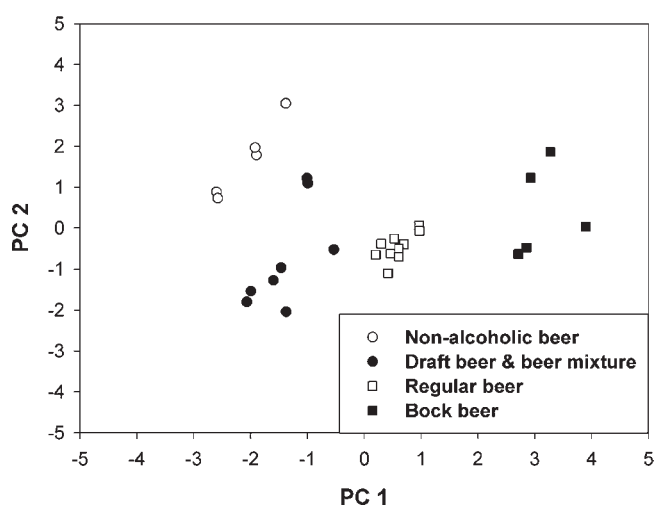
	sample grouping					no sample grouping
	nonalcoholic beer	draft beer and beer mixture	regular beer	bock beer	mean value	overall regression
RMSEC [Mas%]	0.04	0.16	0.09	0.10	0.10	0.64
RRMSEC (%)	0.89	3.55	2.00	2.22	2.16	14.20
r_{xy}	0.9992	0.9899	0.9681	0.9929	0.987525	0.8901
bias	-9.54×10^{-8}	-1.79×10^{-7}	-2.15×10^{-7}	-2.86×10^{-7}	-1.94×10^{-7}	$-4.26E+00$

^a RMSEC, RRMSEC, coefficient of correlation, and systematic error of the regressions regarding the concentration of real extract for nonalcoholic beer, draft beer and beer mixture, regular beer, bock beer, and overall.

Table 5. Characteristics of Multivariate Calibration^a

	alcohol			real extract		
	A	B	C	A	B	C
RMSEC [Mas%]	0.20	0.10	0.15	0.10	0.10	0.10
RRMSEC (%)	6.39	3.19	4.79	2.22	2.22	2.22
r_{xy}	0.9915	0.9970	0.9950	0.9966	0.9966	0.9966
bias	5.44×10^{-2}	1.90×10^{-2}	4.04×10^{-2}	3.75×10^{-5}	3.75×10^{-5}	3.75×10^{-5}

^a RMSEC, RRMSEC, coefficient of correlation, and systematic error of the regressions regarding the concentration of real extract and alcohol for methods A, B, and C.

**Figure 5.** Score plot of the PLS method based on method B.

Validation. Investigations concerning the correctness of the obtained multivariate model illustrate only trivial differences between the characteristics of the calibration (Table 5) and validation (Table 6) procedure. This discrepancy is negligibly small and illustrates the good performance of the model also on unknown samples. Another value to evaluate the performance on a model is the residual predictive deviation (RPD) value. The RPD values of methods A, B, and C are 8.13, 15.58, and 9.84 with respect to the prediction of the alcohol concentration and 14.19 with respect to the prediction of the extract concentration. If the standard error of validation (SEV) is large compared to the spread of that compound in all samples (SD), a relatively small RPD is calculated, thereby demonstrating that the multivariate calibration model is not robust. In contrast, relatively high RPD values indicate models having greater power in prediction. Generally, an RPD of > 10 could be considered very appropriate for prediction purposes (45). Obviously the performance of the multivariate model in predicting unknown samples is very high, especially method B. Additional investigations concerning the accuracy of the obtained multivariate models show a very high reproducibility of the measurement with a standard deviation of < 0.05 Mas%.

In summary, both applied mathematical pretreatments are able to eliminate the light scattering effects in the spectral data.

Table 6. Characteristics of Multivariate Validation^a

	alcohol			real extract		
	A	B	C	A	B	C
RMSEV [Mas%]	0.23	0.12	0.19	0.11	0.11	0.11
RRMSEV (%)	7.35	3.83	6.07	2.44	2.44	2.44
RPD	8.13	15.58	9.84	14.19	14.19	14.19
SD [Mas%]	0.05	0.03	0.04	0.01	0.01	0.01

^a RMSEV, RRMSEV, RPD, and standard deviation regarding the concentration of real extract and alcohol for methods A, B, and C.

Comparison of the two pretreatments reveals that the results of method B (linear baseline correction) are slightly better than the results of method A (first derivation correction). Methods A and B use the absorbance of different wavelengths to correlate the alcohol concentration. Galligani et al. (19) corrected the negative interference of sugars on the quantification of alcohol by determining the concentrations of fructose, glucose, sucrose, and alcohol at 1400, 1680, and 1703 nm in a univariate model. For the determination of alcohol in beers having high alcohol/extract ratios, it is necessary to determine the extract content and to apply a multivariate calibration similar to the model of Galligani et al. (19). A special sample grouping of the refraction indices combined with the spectral data in a multivariate model can obviously correct the influence. This study demonstrates a novel combination of NIR spectroscopy and refractometry as an accurate and reliable method with a high repeatability to determine the concentration of alcohol and extract in different beer samples over a wide range of brands. Previously published investigations of NIR analysis of beer samples are presented in Table 1. The results of these studies are comparable to those reported in this work, especially to those of method B. Method B provides RMSEV values of 0.12 and 0.11 for alcohol with respect to real extract and standard deviation values of 0.03 and 0.01 for alcohol with respect to real extract, which are better than those reported by Norgaard et al. (23) concerning RMSEP/RMSEV and better than those reported by Llarío et al. (25) and Inon et al. (21, 24). None of the investigations presented in Table 1 provides a model that comprises a wider range of beer samples related to the alcohol concentration than this study (min, 0.26 Mas%; max, 6.40 Mas%). Further developments on a NIR spectrometer and refractometer with a focus on the determination of alcohol and extract should enhance the performance once again.

LITERATURE CITED

- (1) Briggs, D. E.; Boulton, C. A.; Brookes, P. A.; Stevens, R. Chemical and physical properties of beer. In *Brewing Science and Practice*; Woodhead Publishing: Abington Cambridge, U.K., 2004; pp 662–715.
- (2) Meilgaard, M. C. Flavor chemistry of beer. Flavor and threshold of 239 aroma volatiles. *MBAA Tech. Q.* **1975**, *12*, 107–117.
- (3) Meilgaard, M. C. Flavor chemistry of beer. Flavor interaction between principal volatiles. *MBAA Tech. Q.* **1975**, *12*, 107–117.
- (4) European Brewery Convention (EBC). Alcohol in beer by distillation. In *Analytica-EBC*; Hans Carl Fachverlag: Nürnberg, Germany, 2009; Chapter 9.2.1
- (5) Mitteleuropäische Brautechnischen Analysenkommission (MEBAK). Destillationsanalyse (Referenzmethode – EBC-Methode). In *Brautechnische Analysenmethoden Band 2*, 4th ed.; Selbstverlag der MEBAK: Freising, Germany, 2002; p 76.
- (6) Osborne, B. G. Near-infrared spectroscopy in food analysis. In *Encyclopedia of Analytical Chemistry*, 1st ed.; Meyers, R. A., Ed.; Wiley: New York, 2000.
- (7) Murray, I.; Williams, P. C. Chemical principals of near-infrared technology. In *Near-Infrared Technology in the Agricultural and Food Industries*; Williams, P. C., Norris, K. H., Eds.; American Association of Cereal Chemists: St. Paul, MN, 1987; pp 17–34.
- (8) Molt, K. 50 Jahre IR-Spektroskopie. *Nachr. Chem.* **2002**, *50*, 1232–1236.
- (9) Downey, G.; Boussion, J. Authentication of coffee bean variety by near-infrared reflectance spectroscopy of dried extract. *J. Sci. Food Agric.* **1996**, *71*, 41–49.
- (10) Jones, G. R.; Deakin, A. G.; Spencer, J. W. Chromatic signatures of broadband optical spectra for liquor discrimination. *Meas. Sci. Technol.* **2009**, *20*, 1–10.
- (11) Li, W.; Goovaerts, P.; Meurens, M. Quantitative analysis of individual sugars and acids in orange juices by near-infrared spectroscopy of dry extract. *J. Agric. Food Chem.* **1996**, *44*, 2252–2259.
- (12) Rodriguez-Saona, L. E.; Fry, F. S.; McLaughlin, M. A.; Calvey, E. M. Rapid analysis of sugars in fruit juices by FT-NIR spectroscopy. *Carbohydr. Res.* **2001**, *336*, 63–74.
- (13) León, L.; Kelly, J. D.; Downey, G. Detection of apple juice adulteration using near-infrared transreflectance spectroscopy. *Appl. Spectrosc.* **2005**, *59*, 593–599.
- (14) Saiz-Abajo, M. J.; Gonzalez-Saiz, J. M.; Pizarro, C. Prediction of organic acids and other quality parameters of wine vinegar by near-infrared spectroscopy. A feasibility study. *Food Chem.* **2006**, *99*, 615–621.
- (15) Niu, X.; Shen, F.; Yu, Y.; Yan, Z.; Xu, K.; Yu, H.; Ying, Y. Analysis of sugars in Chinese rice wine by fourier transform near-infrared spectroscopy with partial least-squares regression. *J. Agric. Food Chem.* **2008**, *56*, 7271–7278.
- (16) Osborne, B.; Fearn, T.; Hindle, P. T. Applications of near infrared spectroscopy in food and beverage analysis. In *Practical NIR Spectroscopy with Applications in Food and Beverage Analysis*, 2nd ed.; Wiley: New York, 1993; pp 145–199.
- (17) Williams, P.; Norris, K. In *Near-Infrared Technology in the Agricultural and Food Industries*, 2nd ed.; American Association of Cereal Chemists: St. Paul, MN, 2001.
- (18) Galignani, M.; Garrigues, S.; de la Guardia, M. Stopped-flow near-infrared spectrometric determination of ethanol and maltose in beers. *Anal. Chim. Acta* **1994**, *296*, 155–161.
- (19) Galignani, M.; Garrigues, S.; de la Guardia, M. Direct determination of ethanol in all types of alcoholic beverages by near-infrared derivative spectrometry. *Analyst* **1993**, *118*, 1167–1173.
- (20) Engelhard, S.; Kumke, M. U.; Löhmannsröben, H. G. Examples of the application of optical process and quality sensing (OPQS) to beer brewing and polyurethane foaming processes. *Anal. Bioanal. Chem.* **2006**, *384*, 1107–1112.
- (21) Inon, F. A.; Llarío, R.; Garrigues, S.; de la Guardia, M. Development of a PLS based method for determination of the quality of beers by use of NIR spectral ranges and sample-introduction considerations. *Anal. Bioanal. Chem.* **2005**, *382*, 1549–1561.
- (22) Westad, F.; Martens, H. Variable selection in near infrared spectroscopy based on significance testing in partial least squares regression. *J. Near Infrared Spectrosc.* **2000**, *8*, 117–124.
- (23) Norgaard, L.; Saudland, A.; Wagner, J.; Nielsen, J. P.; Munck, L.; Engelsen, S. Interval partial least-squares regression (iPLS): a comparative chemometric study with an example from near-infrared spectroscopy. *Appl. Spectrosc.* **2000**, *54*, 413–419.
- (24) Inon, F. A.; Garrigues, S.; de la Guardia, M. Combination of mid- and near-infrared spectroscopy for the determination of the quality properties of beers. *Anal. Chim. Acta* **2006**, *571*, 167–174.
- (25) Llarío, R.; Inon, F. A.; Garrigues, S.; de la Guardia, M. Determination of quality parameters of beers by the use of attenuated total reflectance FT-MIR. *Talanta* **2006**, *69*, 469–480.
- (26) Mendes, L. S.; Oliveira, F. C. C.; Suarez, P. A. Z.; Rubim, J. C. Determination of ethanol in fuel ethanol and beverages by Fourier transform (FT)-near infrared and FT-Raman spectrometries. *Anal. Chim. Acta* **2003**, *493*, 219–231.
- (27) Weyh, H.; Hagen, W. Determination of the original gravity of draught beer. Development of a formula for the refractometer method. *Brauwelt* **1992**, *132*, 51–53.
- (28) Hagen, W.; Weyh, H. Refraktometrische Stammwürzebestimmung mit Nomogrammen und Formeln. *Brauwelt* **1995**, *135*, 816–822.
- (29) Weyh, H.; Hagen, W. A uniform beer analysis for the European Community (EC). *Brauwissenschaft* **1975**, *28*, 293–300.
- (30) Schild, E.; Irrgang, G. Zur refraktometrischen Bieranalyse Die Entwicklung von Berechnungsformeln für Alkohol und wirklichen Extrakt aus der Refraktionszahl und dem spezifischen Gewicht eines Bieres Teil A: Theoretischer Teil. *Brauwissenschaft* **1956**, *9*, 314–323.
- (31) Schild, E.; Irrgang, G. Zur refraktometrischen Bieranalyse Die Entwicklung von Berechnungsformeln für Alkohol und wirklichen Extrakt aus der Refraktionszahl und dem spezifischen Gewicht eines Bieres Teil B: Praktischer Teil. *Brauwissenschaft* **1957**, *10*, 19–24.
- (32) Kessler, W. Multivariate regressionsmethoden. In *Multivariate Datenanalyse für die Pharma-, Bio- und Prozessanalytik*, 1st ed.; Wiley-VCH Verlag: Weinheim, Germany, 2007; pp 89–151.
- (33) Martens, H.; Naes, T. Methods for calibration. In *Multivariate Calibration*, 1st ed.; Wiley: Chichester, U.K., 1991; pp 73–236.
- (34) Kromidas, S. Die Validierungsparameter (oder nach ISO 17025: Verfahrensmerkmale). In *Validierung in der Analytik*, 1st ed.; Wiley-VCH Verlag: Weinheim, Germany, 1999; pp 47–196.
- (35) Kron, A.; Schäfer, T.; Klug, K. I.; Rädle, M. Development of a robust low-cost VIS/NIR-spectrometer. Unpublished Material.
- (36) Cozzolino, D.; Liua, L.; Cynkar, W. U.; Damberg, R. G.; Janik, L.; Colby, C. B.; Gishen, M. Effect of temperature variation on the visible and near infrared spectra of wine and the consequences on the partial least square calibrations developed to measure chemical composition. *Anal. Chim. Acta* **2007**, *588*, 224–230.
- (37) Arnold, S. A.; Gaensakoo, R.; Harvey, L. M.; McNeil, B. Use of at-line and in-situ near-infrared spectroscopy to monitor biomass in an industrial fed-batch *Escherichia coli* process. *Biotechnol. Bioeng.* **2002**, *80*, 405–413.
- (38) Harthun, S.; Matischak, K.; Friedl, P. Determination of recombinant protein in animal cell culture supernatant by near-infrared spectroscopy. *Anal. Biochem.* **1997**, *251*, 73–78.
- (39) International Commission for Uniform Methods of Sugar Analysis (ICUMSA). Specifications and Standards SPS3 – Refractometry and Tables. In *ICUMSA Methods Book*; ICUMSA Publications Department: Norwich, U.K., 2000.
- (40) Casale, M.; Saiz Abajo, M. J.; Gonzalez Saiz, J. M.; Pizarro, C.; Forina, M. Study of the aging and oxidation processes of vinegar samples from different origins during storage by near-infrared spectroscopy. *Anal. Chim. Acta* **2006**, *557*, 360–366.
- (41) Preys, S.; Roger, J. M.; Boulet, J. C. Robust calibration using orthogonal projection and experimental design. Application to the correction of the light scattering effect on turbid NIR spectra. *Chemom. Intell. Lab. Syst.* **2008**, *91*, 28–33.
- (42) Dhanoa, M. S.; Lister, S. J.; Sanderson, R.; Barnes, R. J. The link between multiplicative scatter correction (MSC) and standard normal variate (SNV) transformations of NIR spectra. *J. Near Infrared Spectrosc.* **1994**, *2*, 43–47.
- (43) Barnes, R. J.; Dhanoa, M. S.; Lister, S. J. Correction to the description of standard normal variate (SNV) and de-trend (DT)

transformation in practical spectroscopy with applications in food and beverage analysis. *J. Near Infrared Spectrosc.* **1993**, *1*, 185–186.

- (44) Galignani, M.; Garrigues, S.; de la Guardia, M. Derivative Fourier transform infrared spectrometric determination of ethanol in alcoholic beverages. *Anal. Chim. Acta* **1994**, *287*, 275–283.
- (45) Williams, P. C. Variables affecting near-infrared reflectance spectroscopic analysis. In *Near-Infrared Technology in the Agricultural and*

Food Industries; Williams, P. C., Norris, K. H., Eds.; American Association of Cereal Chemists: St. Paul, MN, 1987; pp 143–167.

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