

Quantitative analysis of alcohol, real extract, original gravity, nitrogen and polyphenols in beers using NIR spectroscopy

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This study was to develop a rapid and accurate NIR analysis method for determinations of alcohol, real extract, original gravity, total nitrogen and total polyphenols. Commercial European beers (110 samples) were used to create calibration models between EBC (European Brewing Committee) and NIR spectral data. The optimal correlation coefficients (r) were 0.94 to 0.98 and the corresponding CV% (coefficients of validation variation) were 4.29, 6.53, 4.50, 6.06 and 4.74 for NIR predictions of alcohol, real extract, original gravity, nitrogen and polyphenols, respectively. The stepwise MLR calibration proved to be a good choice for measurements of alcohol and original gravity, while PLS regression models seem to be better for the predictions of the real extract, nitrogen and polyphenols. Comparisons of results from MLR and PLS, demonstrate that MLR methods ($\log 1/R$) are better than those of PLS ($\log 1/R$) in calibration and prediction sets. The reflection mode is better than those of transmission in all above cases.

Keywords: Alcohol, beer, MLR, NIR spectroscopy, nitrogen, original gravity, PLS, polyphenols, real extract.

Introduction

Beer has become an extremely important commercial product. The most important analysis required for beer, at least from a financial aspect, is the determination of alcohol content, the original gravity and real extract. EBC (European Brewing Committee) methods involving distillation and specific gravity determinations enable comparisons for the purposes of scientific papers and discussion.

The EBC methods¹ as conventional laboratory analytical techniques of beers are relatively time-consuming and require expensive equipment. In contrast, NIR has three key advantages for these

measurements: little sample preparation is required, one scan can provide multiple parameters and sample presentation is extremely versatile—various modes of reflectance measurements can be made along with conventional transmission spectroscopy. Both of these approaches can also be effected by using remote fibre optics.²

It is only recently that software and instruments for the measurement of liquid samples and low concentration components have been developed to make a more sensitive and more accurate NIR analysis. In the case of NIR, analytical techniques have been shown to give the possibility of process control on-line purposes. Cavinato *et al.*³ used an optical-fibre

NIR instrument to monitor the alcoholic fermentation, Picque *et al.*⁴ reported monitoring of the alcoholic and the lactic acid fermentation, Ian B. Benson⁵ has shown that the use of NIR technology for alcohol and original gravity (OG) in beer is recognised as a rapid method.

The aim of this study was to develop NIR calibrations for alcohol, real extract, original gravity, total nitrogen and total polyphenols, as a rapid and accurate method. It could provide a tool for on-line services control during mashing.

Materials and methods

Beer samples

110 normal beers, collected from different countries in Europe, which had been analysed by using EBC methods in our laboratory. The average spectra of the total set of samples ($n = 70$) were used for calibration. The calibration equations were evaluated on 40 separate samples by both MLR and PLS analyses to predict versus EBC values.

Sample preparation

About 300 mL of beer sample were shaken at a temperature of 17 to 20°C, in a 500 mL flask closed by hand, to remove excess carbon dioxide. The beer was filtered through a dry filter paper in a covered funnel into a second flask for determination of alcohol, real extract, total nitrogen and total polyphenols by using EBC¹ and NIR methods.

NIR spectra were recorded in both reflection and transmission modes. In reflection mode, 800 μ L of each beer sample were taken by pipettes and deposited on a fibreglass filter Millipore AP40047 and dried at 50°C, 3 min by using NIRSystems (Perstorp

Analytical Co.). The absorbance data were recorded as $\log(1/R)$. In the transmission mode, a 1 mm quartz cuvette in which to fill each beer sample was used and then the spectrum was recorded as $\log(1/T)$. All NIR spectra were recorded every 2 nm from 1100 to 2500 nm.

NIR spectrometry

All NIR measurements were made with a Pacific Scientific spectrometer model 6250 working in a single beam mode in combination with an IBM computer model PS/2 using the NSAS software version 3.11 of NIRSystems.

The MLR and the PLS have been used to establish the calibration models between EBC reference data and NIR spectral data for each constituent. The MLR algorithm used is included in the NSAS 3.11 software package, whereas the PLS algorithm is applied by the software package Unscrambler 5.5 of CAMO A/S (Trondheim, Norway). The PLS method in Unscrambler is powerful for efficient detection.

The performances of the NIRS method calibration and prediction sets, were shown by Standard Error, Coefficient of Validation variation (CV%) and Correlation Coefficient (r).

Results and discussion

NIR performances of composition analysis

Table 1 shows the alcohol content, real extract, original gravity, total nitrogen and total polyphenols statistical references data from the EBC methods.

The optimal correlation coefficients (r) between both EBC input data and NIR output data were shown in 0.98, 0.94, 0.97, 0.95 and 0.96 for the de-

Table 1. Statistical EBC reference data from 110 commercial European beers.

Values	Alcohol (g/100g)	Real extract (g/100g)	Original gravity, °P	Nitrogen (ppm)	Polyphenols (ppm)
Min.	3.30	3.32	10.37	630	160
Max.	9.96	8.06	24.76	1190	290

Table 2. Comparison of the CV values between MLR and PLS for the alcohol, real extract and original gravity determination.

CV% of NIR from:	Reflectance $\log(1/R)$	Alcohol	Real extract	Original gravity
Calibration	MLR	4.09	5.40	4.04
	PLS	5.12	3.67	6.00
Prediction	MLR	4.29	6.65	4.50
	PLS	5.94	6.19	6.07

Table 3. Comparisons of CV values between the reflection and transmission modes for total nitrogen and total polyphenols determinations.

CV% of NIR from:	Calibration		Prediction	
	Nitrogen	Polyphenols	Nitrogen	Polyphenols
MLR; $\log 1/R$	6.80	5.54	8.97	6.21
$\log 1/T$	8.50	5.74	9.93	8.61
PLS; $\log 1/R$	3.66	3.72	6.06	4.74
$\log 1/T$	5.13	5.39	9.03	8.04

termination of alcohol, real extract, original gravity, total nitrogen and total polyphenols, respectively, in the calibration set.

The corresponding best coefficients of validation variation (CV%) were 4.29, 6.53, 4.50, 6.06 and 4.74 for NIR predictions of alcohol, real extract, original gravity, total nitrogen and total polyphenols, respectively.

Comparison of the CV% between the MLR and PLS for the alcohol, real extract and original gravity determination by using reflectance models

Four-term calibration MLR models have been estimated for the determination. The optimal data are from second derivatives of spectral transformation.

PLS belongs to the class of bilinear regression methods that could approximate complicated multivariate input data by a few bilinear PLS factors T , which account for most of the variance of all the variables and minimum prediction error.⁶ The optimal data were obtained 6, 7 and 5 factors for the de-

termination of alcohol, real extract and original gravity, respectively (Table 2).

The stepwise MLR calibration proved to be a good choice for the alcohol and original gravity measurements while PLS regression models seem to be better for the real extract predictions.

Comparison of CV values between the reflection and transmission modes for the total nitrogen and total polyphenol determinations in both MLR and PLS

Table 3 shows the reflection mode is better than those of transmission even in NIR and in PLS cases. The PLS regression models seem to be better for the nitrogen and total polyphenols predictions.

Conclusion

The results of the alcohol and original gravity with MLR calibration models are better than those obtained with PLS. In contrast, PLS models are better

for the real extract, total nitrogen and total polyphenols prediction.

The reflection mode ($\log 1/R$) is better than those of transmission ($\log 1/T$) even in all cases.

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