Enhanced NIR Calibration for Wort Fermentability Using Orthogonal Signal Correction

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ABSTRACT

J. Inst. Brew. 109(1), 16-26, 2003

An enhanced method for the calibration of Near Infra Red (NIR) reflectance spectra to wort fermentability is proposed using a signal pre-processing algorithm called orthogonal signal correction (OSC). Pre-processing NIR spectra prior to partial least squares Project to Latent Structures (PLS) regression modelling is becoming commonplace in multivariate calibration. A set of twenty wort samples subjected to a replicated 2² factorial design with a centre point and nine production samples were used to construct multivariate prediction models. The experimental design factors were the mash tun saccharification temperature and time used to purposely provide a sample set with significant leverage in the fermentability responses. Calibration PLS models for both wort apparent degree of fermentation (ADF) and final attenuation apparent extract (Final AE) values with and without OSC corrected spectra were compared demonstrating significant improvements in prediction capability with the prior ($Q^2 = 0.90$) versus $Q^2 = 0.28$). The OSC algorithm removed almost 60% of the variance in the NIR spectra, which was independent or orthogonal to the fermentability measures. By cleaning up the spectra, the standard errors of prediction (SEP) for ADF and Final AE were improved by 50 and 90%, respectively, illustrating not only the enhancement in calibration but also the aptness for process control applications. Various model validation tests, including an external validation example and random response permutation, verify the validity of the models using OSC. Furthermore, interpretation of the important wavelengths related to wort fermentability is provided and demonstrates that some key wavelengths are related to both carbohydrate overtones as well as nitrogen functional groups. The application of OSC prior to developing calibration models with NIR demonstrates promising results for brewers interested in real time control of wort fermentability.

Key words: Near Infra Red, orthogonal signal correction, projection to latent structures regression, wort fermentability.

INTRODUCTION

Fermentability control in brewing is paramount to final product consistency. If wort is too fermentable, the final beer will become thin due to the excess water required to standardize the product's alcohol content to required levels in the package. Quite often, measures such as fermentable

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Publication no. G-2003-0227-132 © 2003 The Institute & Guild of Brewing sugar profiles or laboratory scale fermentations are collected on wort; however, the time to obtain results is typically a few days and making inference on how the process is running to design targets is not real time. This lack of real time feedback can be due to the batching of analyses on complex measurements such as High Performance Liquid Chromatography (HPLC) or the time to complete tests such as a rapid fermentability, which is typically 24 hours²². Nonetheless, real time feedback for process control is lacking in the traditional brewing environment that would provide brewers the capability for process corrections such as modifications to the saccharification steps in the mash tun³².

The use of Near Infra Red (NIR) technology is becoming more prevalent in brewing and other industries to provide quick and accurate quality control measures. Many brewing related quality characteristics measured using NIR techniques have been investigated. Some examples include the measurement of barley and malt moisture, nitrogen, and amino acid content^{2,18,38}, whole hop cone moistures, α and β acids, and oils²³, wort extract content^{24,31}, and fermentable sugars and free α -amino nitrogen^{8,10,24,29,32,34}. Both Halsey²⁴ and Sjoholm et al.³¹ studied calibration models for fermentability and comment on moderate success. Halsey²⁴ incorporated multiple linear regression (MLR) on selected wavelengths after first and second differentiation of the spectra. In this study, only a few wavelengths were selected for the regression model and it is questionable what information in the NIR spectra was naively removed. Sjöholm et al.³¹ incorporated PLS modelling with an argument on the assumption of a bilinear relationship between wort fermentability measures and NIR spectra is conveyed through the wort fermentable carbohydrate profile. The application of multivariate calibration techniques such as PLS²⁵ has generally gained acceptance over MLR or Principal Component Regression (PCR). In situations where specific wavelengths have been assigned to certain functional groups, some researchers opt to using MLR¹⁸; however, due to potential collinearity issues the MLR approach is not recommended.

Pre-processing spectra prior to calibration to remove systematic noise is commonplace. The purpose of preprocessing algorithms for NIR spectra is to remove systematic variation in the spectra such as base-line variation and multiplicative scatter effects. The most common approaches include differentiation and signal correction. Differentiation approaches are subjected to removing information relative to the analytes of interest. Some of the more popular signal correction approaches include Savitzky-Golay smoothing³⁰, multiple signal correction¹⁹, and

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baseline correction⁷. These methods suffer from the drawback that information in the spectra related to the analytes of interest for calibration can be removed³⁹.

A more novel approach is proposed called Orthogonal Signal Correction³⁹ (OSC). The elegance of the OSC approach is that noise in the **X** block, the spectral data set, is removed that is near or exactly orthogonal to the information in the **Y** block, analytical data set. With this filtering, noise irrelevant to analyte concentrations is removed prior to developing calibration models with the desire to improve prediction capabilities. In the case of NIR spectra where the vector containing individual wavelength responses is typically much larger than the number of samples used for calibration, exact orthogonal solutions are obtained by the algorithm and guarantee removal of noise independent of the analyte concentrations²¹.

Other methods of signal correction aimed at removal of noise in the **X** block independent of the **Y** block have been recently developed. These include Direct Orthogonal Signal Correction^{1,17,37}, Modified OSC¹⁶, Net Analyte Preprocessing²⁰, and Orthogonal Projection to Latent Structures OPLS³⁶. A detailed comparison of these methods is reported³³. Another novel approach considered neural networks to pre-process data to remove temperature effects and demonstrated significant improvements in prediction errors¹⁵. This paper will only consider evaluating the original OSC³⁹ algorithm to demonstrate the improvements in calibration with this signal pre-processing.

The scope of this paper will be to evaluate the improvement in calibration between data sets with and without OSC and to provide an interpretation of the important wavelengths as they relate to wort fermentability. A comparison to the work of others^{24,31} will be provided to demonstrate improvements in this calibration approach.

MATERIALS AND METHODS

Wort samples. Twenty wort samples were produced according to a 2^2 factorial design with centre point replicated four times. The experimental factors were saccharification rest temperature and time. The details of the design are provided in Table I. The centre point is not provided here; however, that information is irrelevant and it is common knowledge that these factors affect wort fermentability.

Nine extra wort samples not part of the test design but within the experimental design space given above were also included in the initial calibration data set. Wort samples were collected at the wort cooler, split for both NIR and forced fermentability tests. All samples were analysed within 24 hours of being produced.

Forced fermentability tests. A solid yeast cake (*Saccharomyces cerevisiae*) was prepared by vacuum filtration with 3 litres of yeast. A 500 mL wort sample was collected and equilibrated to 20°C–22°C. The wort density was mea-

TABLE I. Details of the mash tun saccharification experimental design.

Factor	Units	Low	High
Saccharification rest temperature	°C	-1.5	+1.5
Saccharification rest time	min	-5	+5

sured using an Anton Paar DMA5000 (Anton Paar, Austria) and converted to °Plato original gravity (°P_{og}) following the ASBC methods of Analysis⁵. A 350 mL aliquot of the wort sample was transferred into a 600 mL beaker with 55 g of yeast cake. Antifoam (Dow Corning, USA) was added and the sample was stirred for 18 hours (the yeast was completely suspended in solution). During the 18 hour fermentation, the temperature was maintained between 18°C-22°C. The beer was poured from the beaker, centrifuged and decanted from the remaining yeast. The sample was degassed following the ASBC Methods of Analysis³. The beer density was measured using an Anton Paar DMA 5000 (Anton Paar, Austria) and converted to apparent extract (Final AE) following the ASBC methods of Analysis⁴. The apparent degree of fermentation (ADF) was calculated using the following equation:

$$ADF = \frac{[^{\circ}P_{og} - Final AE]}{^{\circ}P_{og}} * 100\%.$$

For each sample, the bivariate responses of ADF and Final AE were recorded.

NIR – Analysis. The NIR analysis was performed using a NIRSystems 6500 scanning instrument (FOSS NIR-Systems Inc., Silver Spring, MD) configured for reflectance. Samples were scanned at wavelengths from 400–2498 nm at 2 nm segments using a cell with a 10 mm path length. Each spectra was obtained by averaging 25 scans/sample, the data was expressed as absorbance (Abs = log 1/R, where R = reflectance). Data was collected using WINISI on a PC and the exported to a commadelimited file for statistical analysis. The spectrum of each sample consisted of 1050 absorbance points. Samples were scanned on an as is basis with no sample preparation at ambient temperature (approx. 20°C). The spectra of all twenty-nine samples were collected into a data matrix of size 29×1050 .

Notation. All matrices will be denoted in bold face capital letters. NIR spectral data matrices will be denoted as **X** whereas fermentability response data matrices will be denoted as **Y**. Sample information was stored row-wise in these matrices. Small bold and underlined characters will be used for column vectors and row vectors will be expressed as transposed vectors, e.g. \mathbf{p}' . For the sake of consistency, other notation given by Wold et al.³⁸ will be followed. The term R²Y and Q² represent the percent variance explained and predicted, respectively, by the model on the fermentability measures. The term R²X represents the percent variance used in the **X** space to predict the fermentability responses.

Statistical model development. All statistical calculations and modelling was done using SIMCAP+ v10 software [Umetrics, Kinnelon, NJ]. The NIR spectra and fermentability measures, ADF and Final AE, were centred and scaled to unit variance prior to any calibration work. This is standard practice in multivariate analysis²⁸. Prior to development of models, a principal component analysis (PCA) model was constructed on the standardized NIR data to identify potential outliers. A Distance to the Model in the **X** block (DMODX) versus Hotelling's T² plot was constructed and any observations falling outside both rejection regions were omitted. Significant regions were defined as the 99th percentile of the null distributions. For the DMODX region, the significant region was determined using the F-distribution²⁶ and for Hotelling's T^2 the significance region was determined using the distribution Beta distribution³⁵.

For orthogonal signal correction, the algorithm is provided in the Appendix. The corrected NIR spectra block, $X_{\text{osc,A}}$ is simply determined by removing A successive orthogonal components calculated from the OSC algorithm, i.e.:

$$\mathbf{X}_{\text{osc,A}} = \mathbf{X}_0 - \sum_{a=1}^{A} \underline{\mathbf{t}}_{\text{osc,a}} \underline{\mathbf{p}}_{\text{osc}}^{t}$$

Based on recommendations by practitioners³⁹ only two orthogonal components were removed to clean up noise due to baseline and multiplicative effects. The PLS models for non corrected, denoted as \mathbf{X}_{non} , and corrected spectra, $\mathbf{X}_{osc,A}$, were developed as follows:

$$\mathbf{X}_{\text{non}} = \mathbf{T}\mathbf{P}^{t} + \mathbf{E} \qquad \mathbf{X}_{\text{osc,A}} = \mathbf{T}\mathbf{P}^{t} + \mathbf{E}$$
$$\mathbf{Y}_{\text{non}} = \mathbf{U}\mathbf{Q}^{t} + \mathbf{F} \qquad \mathbf{Y}_{\text{osc,A}} = \mathbf{U}\mathbf{Q}^{t} + \mathbf{F}$$

Model development was carried out using the method of internal cross validation to determine the number of components in the PLS models. This technique removes a subset of the observations from the model, fits a model with the remaining data, uses the removed **X**-data to predict the respective **Y** results, and compares the predicted results to the actual. This procedure is replicated a number of times such that all observations are removed at least once. A comparison of prediction error to the total sums of squares is done using the PRESS statistic^{13,27} and is used to determine the number of components to be used in the PLS model. This approach has been demonstrated to be effective in PLS model development9. Due to speculated optimistic results of cross validation with OSC40 Random Response Permutation Validation (RRPV)¹¹ was used to further validate the OSC model. This technique consists of randomly permuting the rows of Y, keeping the rows of X intact, develop a PLS model using cross validation with the shuffled Y matrix, record R²Y and Q² results, and repeat this process a number of times, say 100. This bootstrapping-type approach can be used to compare the model's record R²Y and Q² results to the distribution of results obtained by RRPV. If the model's R²Y and Q² results are significantly lower than what was observed in the original model, then one can feel confident about the model's validity for predicting new observations¹⁴. It does not, however, provide model developers with the confidence obtained by external validation with new samples.

For the prediction of new samples, signal correction of new spectra, say $\underline{\mathbf{x}}_{new}$, is easily obtained by removal of the A OSC components. First, the new signal is standardized to create $\underline{\mathbf{x}}_{new,0}$. Then, using weight, $\underline{\mathbf{w}}_{osc,a}$, and loading vectors, $\mathbf{p}_{osc,a}$, from the OSC algorithm, the signal corrected new spectra is determined as

$$\underline{\mathbf{x}}_{\text{new,osc}} = \left[\mathbf{I} - \sum_{a=1}^{A} \underline{\mathbf{p}}_{\text{osc,a}} \underline{\mathbf{w}}_{\text{osc,a}}^{t}\right] \underline{\mathbf{x}}_{\text{new,0}},$$





Fig. 2. Three-dimensional PCA model of DMODX (ordinate) versus T-Squared (abscissa) plot illustrating one outlier existed in the original data set.

where I represents the identity matrix. The corrected spectra, $\underline{\mathbf{x}}_{new,osc}$, is then fed into the PLS model for prediction.

To understand the important wavelengths associated with the prediction of wort fermentability measures, Variable's Influence in Projection¹² (VIP) plots can be constructed for the spectra to determine key regions associated with fermentability.

RESULTS

The NIR reflectance spectra are graphed (Fig. 1). Visual inspection does not demonstrate any significant anomalies. However, a PCA analysis of the twenty-nine NIR spectra revealed that one outlier existed. The PCA model consisted of three components explaining over 95% of the variability in the PCA model. The DMODX versus Hotelling's T-Squared statistic illustrates how the outlier falls outside the statistical norm of both the PCA subspace region and the orthogonal complement (Fig. 2). This observation was removed prior to any PLS modelling and is justified on the basis that the starting point of the OSC algorithm for the score vector, $\mathbf{\underline{t}}_{start}$, is the major eigenvector of the standardized NIR data matrix, \mathbf{X}_0 (see appendix). By keeping this vector in the calibration data set, the OSC score and loading vectors, $\underline{\mathbf{t}}_a$ and $\underline{\mathbf{p}}_a$, could be negatively influenced.

PLS results with no signal pre-processing. PLS regression was applied to the spectra and the observed fermentability results (Table II). Only 1 PLS component was extracted through cross validation, using 69.6% of the variation in the **X**-space to explain 36.6% of the variation

TABLE II. Results of PLS without OSC.

Dim	R ² X %	Eigenval	R ² Y %	Q ² %
1	69.6	20.173	36.6	28.3

in the **Y**-space. However, the model's total prediction capability is 28.3%. In general, models capable of predicting over 50% of the observed variance in the y-variables are considered good. The prediction capability of these models is less than adequate (Figs. 3 and 4).

PLS results with OSC. The OSC corrected spectra reveals some interesting patterns visually (Fig. 5). For example, as the OSC corrected signal becomes higher in value towards the region of 1300 nms and greater, the less fermentable the wort would be. In fact, for the signal that appears the highest in the 1300-2500 nm range, the wort ADF result was approximately 73% whereas those signals towards the lower end ranged in the 79-81% ADF range. Discussions provided later will demonstrate that this subset of wavelengths contains important variables for the PLS model. The OSC model parameters are provided (Table III). Both components are orthogonal to the Y-space as indicated by the ninety-degree angle. The OSC corrected spectra only contains 41.5% of the original variance in the X-block clearly demonstrating the amount of noise filtered out.

The PLS algorithm extracted only 1 component, verified through cross validation, yet was capable of predicting over 90% of the total cumulative observed variance

TABLE III.	OSC pa	rameters
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Angle Component in degrees		Remaining SS in %	Eigenvalue
1	90.00	53.75	12.9502
2	90.00	41.54	3.41769

TABLE IV. Results of PLS with OSC.

Dim	R ² X %	Eigenval	R ² Y %	Q ² %
1	79.3	22.2	91.5	90.0



Fig. 3. Observed (ordinate) versus predicted (abscissa) plot for ADF without OSC.



Fig. 4. Observed (ordinate) versus predicted (abscissa) plot for Final AE without OSC.

(Table IV). These prediction capabilities are considered to be quite good by chemometric standards. To illustrate the prediction capabilities for ADF and Final AE, observed versus predicted plots are provided (Figs. 6 and 7) as well as R^2Y and Q^2 statistics (Table V). A comparison of the Standard Errors of Calibration (SEC) and Standard Errors of Prediction (SEP) results from both models and results from previous research is also provided (Table VI).

Validation trials. Random response permutation trials

TABLE V. Comparison of R² and Q² statistics with and without OSC.

Response	Withou	ut OSC	With	OSC
	R ² Y%	Q ² %	R ² Y%	Q ² %
ADF	30.6	21.9	83.6	80.7
Final AE	40.6	32.1	99.5	99.3

were replicated 100 times for both fermentability measures. The observed R^2Y and Q^2 results, shown to the far upper right in the plots, are much greater than all 100 RRPV trials (Figs. 8 and 9). If there were some overlap, say 5% of the RRPV values greater than the observed results, the model's validity could be questionable¹¹. The results of RRPV trials also suggest that these models are not being driven by spurious correlations simply due to the fact that 1050 predictor variables are used. Simple probability arguments can be made that with these many predictors, the chances of seeing significant correlation coefficients between dependent and predictor variables is high.

In an attempt to externally validate this model with limited calibration samples, six observations were removed from the data set, OSC was applied again to the X-block subtracting two components, a PLS model was developed, and the removed spectra was then filtered using the OSC



Fig. 5. OSC corrected log(1/R) spectra of the twenty-eight calibration samples.

model developed. Once the filtered NIR spectra were obtained, it was fed into the PLS model for predicting results. The observed versus predicted plot illustrate that these external samples were well modelled (Fig. 10).

VIP results & OSC-PLS regression coefficients. The important wavelengths in the NIR spectrum from the OSC-PLS model can be grouped into three general regions with VIP scores greater than 1.0: 1) between 560 and 640 nms, 2) between 1340 and 1500 nms, and 3) greater than 1520 (Fig. 11). The first region corresponds to an electronic transition and not an IR overtone. The VIP

correlation is strongest at approximately 640 nm, which corresponds to the absorbance of red light. The absorbance of red light will increase as the blue-green appearance of the wort increases. This could lend itself to a quick and simple method using ultra violet-visible absorbance spectroscopy. A survey of all NIR methods on wort fermentability^{24,31,32,34} finds that no calibration models used wavelengths less than 1200 nms; however, it is hard to ignore the significant VIP scores in this study with OSC and it is recommended that future research consider this region.



Fig. 6. Observed (ordinate) versus predicted (abscissa) plot for ADF with OSC.



Fig. 7. Observed (ordinate) versus predicted (abscissa) plot for Final AE with OSC.



Fig. 8. RRPV trials for ADF. A represent R^2 results and the represent Q^2 results. The ordinate represents the RRPV R^2 and Q^2 trials. The abscissa represents the correlation coefficient between original order of the ADF data and the shuffled ADF data. The model's result is indicated at the upper right. The R^2 and Q^2 intercepts are -0.05 and -0.18, respectively. The results of the 100 RRPV trials being less than the model's scores and low R^2 and Q^2 intercepts illustrate the validity of the model.

TABLE VI. Summary of both models standard error of calibration (SEC) and standard error of prediction (SEP) along with previous work of Sjöholm et al.³¹ and Halsey²⁴.

	Withou	ut OSC	With	With OSC Sjöholm et al. Halse		Sjöholm et al.		lsey	
Response	SEC	SEP	SEC	SEP	SEC	SEP	SEC	SEP	
ADF (%)	1.427	1.484	0.698	0.744	1.034	1.106	1.6	1.5	
Final AE (°Plato)	0.285	0.299	0.027	0.030	n/a	n/a	n/a	n/a	

The NIR spectra for the second and third regions have wavelengths that correspond to alkane, alkene, alcohol, amide, and amine functional groups. Specifically, the third region, which spans a large spectrum, is probably related to amino acids and complex longer chain carbohydrates, i.e. non-fermentable sugars. Halsey²⁴ reported a spectral trough at 1664 nms common to dextrins and fermentable sugars and suggested that discrimination between fermentable and non-fermentable sugars falls in 1410 and 1970 nm wavelengths. Sojholm et al.³¹ found that the key pre-



Fig. 9. RRPV trials for Final AE. A represent R^2 results and the represent Q^2 results. The ordinate represents the RRPV R^2 and Q^2 trials. The abscissa represents the correlation coefficient between original order of the Final AE data and the shuffled Final AE data. The model's result is indicated at the upper right. The R^2 and Q^2 intercepts are -0.08 and -0.19, respectively. The results of the 100 RRPV trials being less than the model's scores and low R^2 and Q^2 intercepts illustrate the validity of the model.



Fig. 10. External validation trials. \blacktriangle represent observed versus predicted results for model for 22 selected observations in the calibration model, \blacksquare represent observed versus predicted results of the six external samples predicted from the model with 22 observations.

dictors for the amount of extract fermented in a laboratory scale fermentation test fall in the 1500–1863 and 2050–2400 nm regions; however, the signs on their PLS regression coefficients do not all agree with these findings.

As for nitrogen sources, class II aromatic amino acids such as phenylalanine and tyrosine have been assigned to 1680 nm whereas glutamine, probably less important for fermentation, has been assigned to 2170 nm¹⁸. Assignments of N–H groups have been reported at 1528 nm and 2048 nm¹⁷ and a N–H stretch is assigned to 1982 nm⁶. This third region is key to predicting fermentability as manifested by the large VIP scores. The general pattern seems to be the higher the absorbance values are in this region, the less fermentable the wort is (Table VII). This has an intuitive appeal because one can argue that as the balance of wort carbohydrates consists of more maltodextrins as opposed to monomers, dimers, and trimers, the less fermentable it will be. Hence, interpretation of the regression coefficients in this region tends to support this hypothesis (Fig. 12).

The region between 900 nms and 1100 nms exhibit a low VIP score indicating that their relative contribution to



Fig. 11. VIP plot illustrating important wavelengths in the calibration model. Note that VIP scores greater than 1.0 are considered most influential in the model.

predicting wort fermentability is low or unimportant. It has been reported that simple sugars will absorb in this region¹⁰; however water also has a strong absorbance in this same range and it is clear that even with OSC corrected spectra, this region was not useful for calibration.

DISCUSSION

The prediction improvement in applying orthogonal signal correction to the model development is impressive. Without OSC, the overall prediction capability was $Q^2 = 28.3\%$ and the prediction capability is similar to what previous attempts reported^{23,30}. However, with OSC the overall prediction capability improved to $Q^2 = 90.0\%$ which is excellent and illustrates how OSC cleans up the noise in NIR spectra. In fact, about 60% of the noise in the NIR spectra was removed prior to calibration. The prediction capability of ADF was slightly lower than that of Final AE (Q^2 ADF = 80.7\% vs Q^2 Final AE = 99.3\%). The reason behind this difference may fall in the fact that ADF is also a function of the original gravity of the wort, of which no

predictive modelling was considered. Since all samples used in the calibration work came from production scale worts, variability in the original gravities may have influenced the calibration.

The VIP plot provides an explanation of the NIR spectra results and its relationship to fermentability. It appears that wavelengths greater than 1300 nms are key to the model's prediction capability and are probably related to complex carbohydrates. It is well known that as nonfermentable sugars such as maltotetraose and maltopentaose increase, the wort fermentability decreases. This phenomenon is the result of less saccharification probably driven by α -amylase, β -amylase, mash Ca⁺⁺ levels, saccharification time-temperature profiles, and the inexplicit interactions between these variables. The elegance of using NIR to predict fermentability falls in the brewhouse's ability to potentially control fermentability in real time. NIR technology requires very little training for operators. Real time monitoring can be incorporated into a Statistical Process Control (SPC) program in which predicted fermentability results can be charted against process targets,

TABLE VII. Pearson correlation coefficients of linear maltodextrins, fermentable sugars, and wort fermentability measures from the experimental trials. P-values are indicated in brackets below the correlation coefficients.

	Malto- heptaose	Malto- hexaose	Malto- pentaose	Malto- tetraose	Malto- triose	Maltose	Glucose	ADF
Malto-hexaose	.547 (.002)		·					-
Malto-pentaose	.338 (.068)	.916 (.000)						
Malto-tetraose	.489 (.006)	.842 (.000)	.874 (.000)					
Malto-triose	.035 (.856)	.224 (.235)	.332 (.073)	.432 (.017)				
Maltose	191 (.313)	.059 (.758)	.210 (.265)	.246 (.190)	.960 (.000)			
Glucose	.097 (.612)	.146 (.440)	.169 (.373)	.218 (.247)	.535 (.002)	.464 (.010)		
ADF	521 (.003)	625 (.000)	671 (.000)	729 (.000)	167 (.377)	054 (.777)	.138 (.466)	
Final AE	.259 (.167)	.383 (.037)	.516 (.003)	.618 (.000)	.425 (.019)	.370 (.044)	047 (.805)	831 (.000)



Fig. 12. OSC-PLS regression coefficients. Line above abscissa represents coefficients for Final AE. Line below abscissa represents coefficients for ADF.

and appropriate statistical rules can be applied to dictate process corrections.

Based on the results found in this study, it recommended that researchers interested in calibrating NIR to brewing type measures consider applying some form of signal pre-processing before applying multivariate calibration. The application of OSC appears to significantly enhance calibrations by removal of systematic variation in the spectra unrelated to the response variables.

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(Manuscript accepted for publication February 2003)

APPENDIX

Orthogonal Signal Correction Algorithm

- 1. Assume \mathbf{X}_0 and \mathbf{Y}_0 represent mean centered and scaled to unit variance data matrices of the NIR spectra and fermentability measures, respectively. Note \mathbf{X}_0 and \mathbf{Y}_0 arranged such that the spectra and fermentability results for one sample are placed row-wise, and the number of rows corresponds to the number of samples in calibration set.
- 2. Let a = 1.
- 3. Let $\mathbf{X}_{\text{osc,a-1}} = \mathbf{X}_0$.
- 4. Let $\underline{\mathbf{t}}_{\text{start}}$ be the score vector associated with the major eigenvector of $\mathbf{X}_{\text{osc,a-1}}$.
- 5. Project $\underline{\mathbf{t}}_{start}$ into the orthogonal complement of the column space of \mathbf{Y}_0 , $L^{\perp} \{Y_0\}$. This can be accomplished by $\underline{\mathbf{t}}_{new} = [\mathbf{I} \mathbf{Y}_0(\mathbf{Y}_0' \mathbf{Y}_0)^{-1} \mathbf{Y}_0'] \underline{\mathbf{t}}_{start}$.
- 6. Determine a least squares solution for $\underline{\mathbf{w}}_{osc,a}$, i.e.

$$\mathbf{X}_{\mathrm{osc},\mathrm{a-1}} \underline{\mathbf{w}}_{\mathrm{osc},\mathrm{a}} = \underline{\mathbf{t}}_{\mathrm{new}}$$

The solution involves the Moore-Penrose generalized inverse of $\mathbf{X}_{\text{osc},a-1}$ and is given as $\underline{\mathbf{w}}_{\text{osc},a} = \mathbf{X}_{\text{osc},a-1}^{-1} \underline{\mathbf{t}}_{\text{new}}$.

- 7. Calculate $\underline{\mathbf{t}}_{\text{osc,a}} = \mathbf{X}_{\text{osc,a-1}} \underline{\mathbf{w}}_{\text{osc,a}}$.
- 8. Check for convergence such that

$$\frac{\left|\underline{\mathbf{t}}_{\text{osc},a}-\underline{\mathbf{t}}_{\text{new}}\right\|}{\left\|\underline{\mathbf{t}}_{\text{osc},a}\right\|} < 10^{-6} \,.$$

If convergence is not attained, let $\underline{\mathbf{t}}_{start} = \underline{\mathbf{t}}_{osc,a}$ and go back to step 3; otherwise, proceed to step 9.

9. Compute loading vector $\mathbf{p}_{osc,a}$ using the following

$$\underline{\mathbf{p}}_{\mathrm{osc},\mathrm{a}}^{t} = \frac{\underline{\mathbf{t}}_{\mathrm{osc},\mathrm{a}}^{t} \mathbf{X}_{\mathrm{osc},\mathrm{a}-1}}{(\underline{\mathbf{t}}_{\mathrm{osc},\mathrm{a}}^{t} \underline{\mathbf{t}}_{\mathrm{osc},\mathrm{a}})}.$$

- 10. Subtract signal correction from $\mathbf{X}_{osc,a-1}$ to give $\mathbf{X}_{osc,a} = \mathbf{X}_{osc,a-1} \mathbf{\underline{t}}_{osc,a} \mathbf{\underline{p}}_{osc,a}^{t}$.
- 11. Let a = a + 1 and continue to extract as many components as desired. Go back to step 3.
- Once the desired number of components are extracted for signal correction, use X_{osc,A} for the PLS algorithm. X_{osc,A} can be determined as

$$\mathbf{X}_{\text{osc,A}} = \mathbf{X}_0 - \sum_{a=1}^{A} \underline{\mathbf{t}}_{\text{osc,a}} \underline{\mathbf{p}}_{\text{osc,a}}^t \,.$$