Quantitative Analysis by Near-Infrared Spectroscopy of Compounds Relevant in Bioethanol Production

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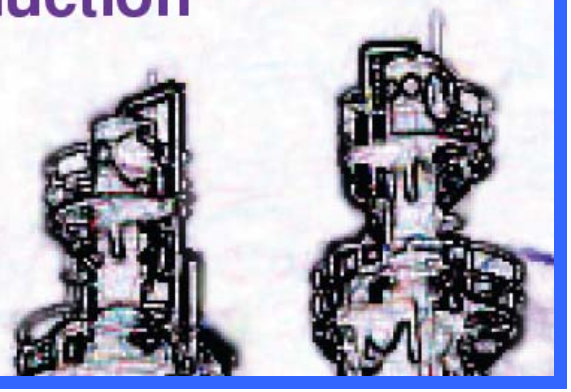

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1. Introduction

Near-infrared (NIR) spectroscopy was applied to bioethanol fermentations with

- High sample variability from batch to batch due to changes in feedstock and enzymatic pretreatment
- Multi-constituent substrates
- Minimal sample preparation for rapid, nondestructive analysis

Objectives

- Quantify relevant compounds: glucose, ethanol, glycerol, lactic acid, fructose, maltose, arabinose
- Develop PLS regression models based on NIR absorbance data
- Select important variables by a Genetic Algorithm (GA)
- Optimize the PLS models' complexity and estimate its prediction performance for new cases by "rdCV"

2. Experimental

Process Steps

Wheat/rye/corn → enzymatic pretreatment → enzymatic starch degradation → fermentation by yeast → ethanol containing **mash** → separate ethanol by distillation → **stillage** remains as residue

Sample Preparation

- Centrifugation to remove solids
- Stepwise addition of known amounts of the compound under investigation (for calibration)
- Determination of reference concentrations (g/L) by HPLC with refractive index detector

NIR Absorbance Data

1100-2300 nm at 5 nm intervals, AOTF-NIR spectrometer *Brimrose Luminar 5030*, fiber-optic transflectance probe.
1^{st} derivative Savitzky-Golay results in 235 *x*-variables; variable reduction by GA [1,2] to 15 variables (different variables for each compound)

3. Method

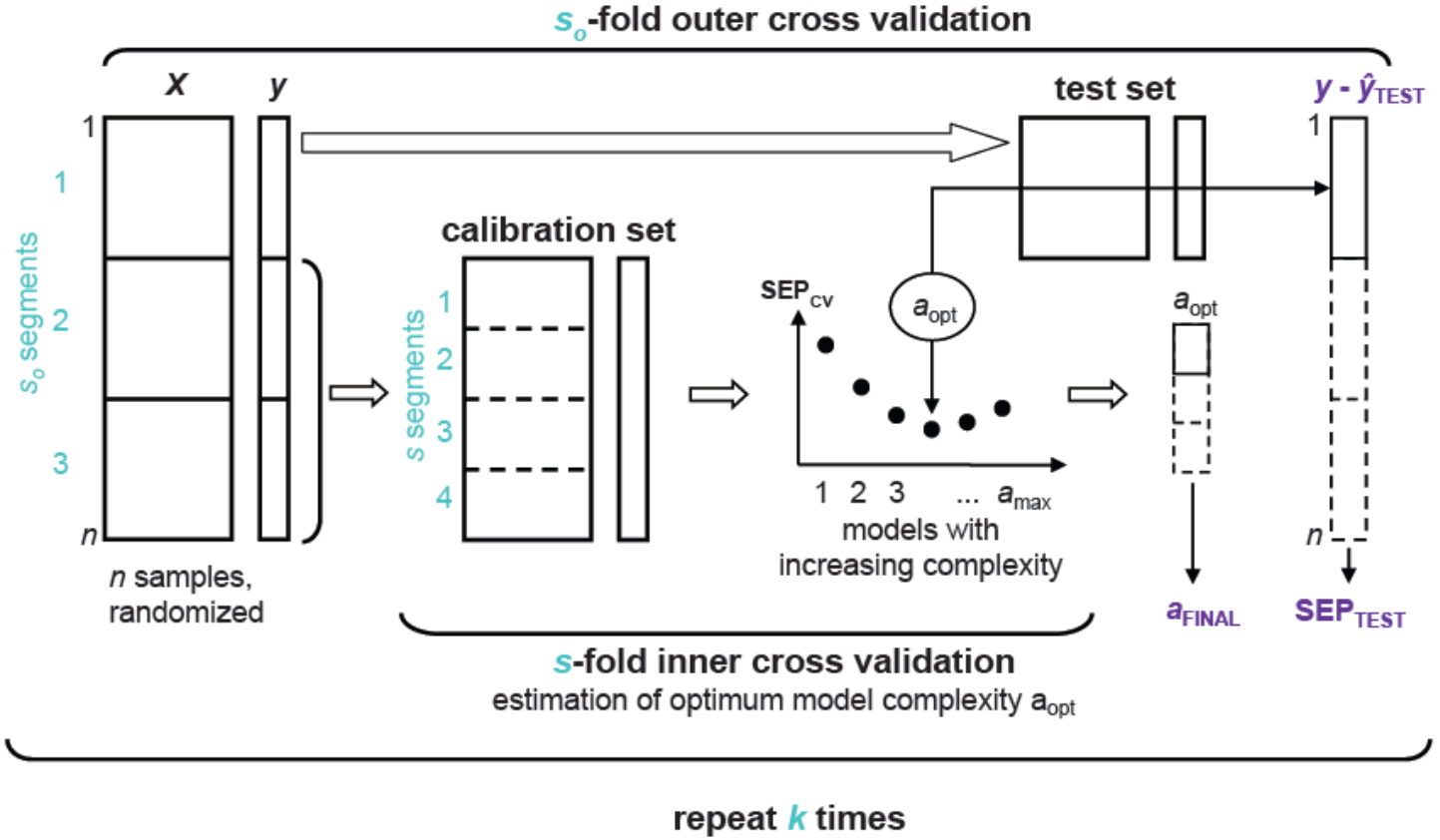
Repeated Double Cross Validation rdCV

- The data set is randomly partitioned into s_o segments: s_o - 1 segments for calibration, 1 segment as test set.
- A PLS model is derived from the calibration set with optimum number of PLS components estimated by *s*-fold inner cross validation.
- Application of PLS model to test set results in n/s_o predicted values $\hat{y}_i$.
- Systematic variation gives a $\hat{y}$ for each object.
- The whole process is repeated *k* (e.g. 100) times.
- Finally, $k \cdot n$ values $\hat{y}$ are available.

Implementation in R

rdCV is available as function `mvr_dcv` in new package *chemometrics* [3,4] developed in R [5,6].

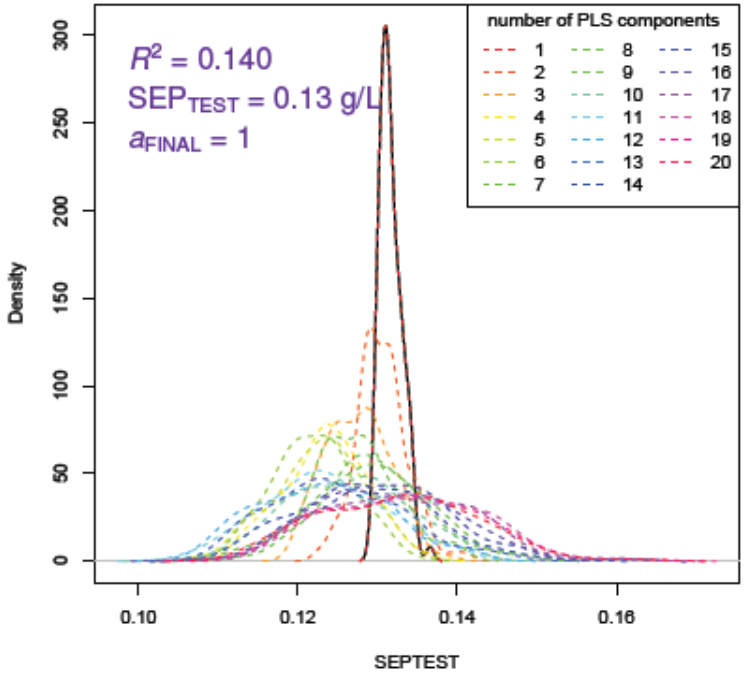
Scheme of repeated double cross validation with s_o = 3 segments in the outer loop and s = 4 segments in the inner loop. The process is repeated k times.



4. Evaluation

Example: Lactic acid quantification in stillages by NIR, range: 0.06-0.63 g/L

Density distribution of 100 SEP_{TEST} values with increasing model complexity for 100 repetitions

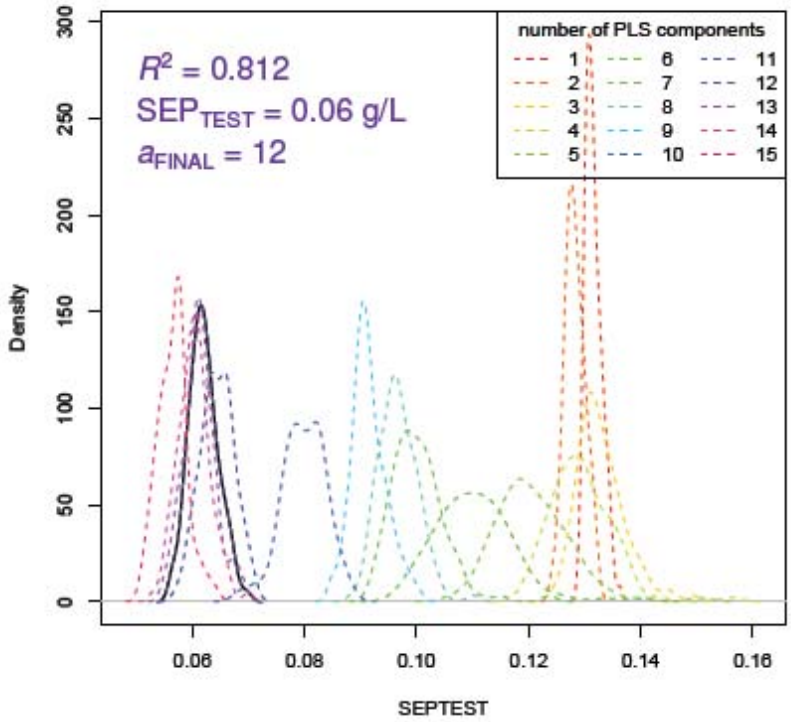


all 235 NIR variables

very low predictive performance

higher complexity → larger errors

broad error distributions



15 GA selected NIR variables

sound predictive performance

higher complexity → reduced errors

narrow error distributions

no overfitting

Performance criteria derived from rdCV:

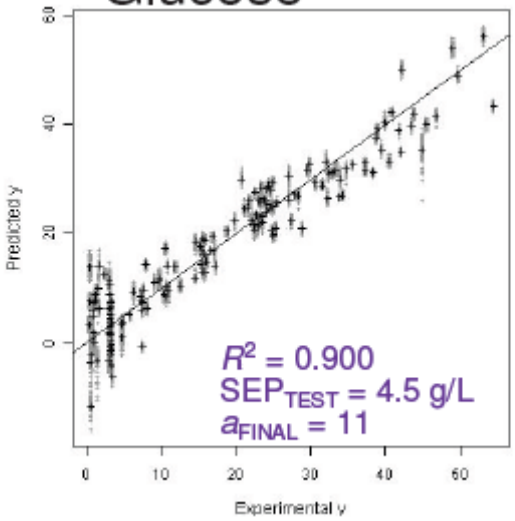
SEP_{TEST}	standard deviation of test set predicted errors y-$\hat{y}$ ($k \cdot n$ values $\hat{y}$ available)
a_{FINAL}	final optimum of $s \cdot k$ calculated numbers of PLS components (method: [7])

5. Predicted vs. Experimental

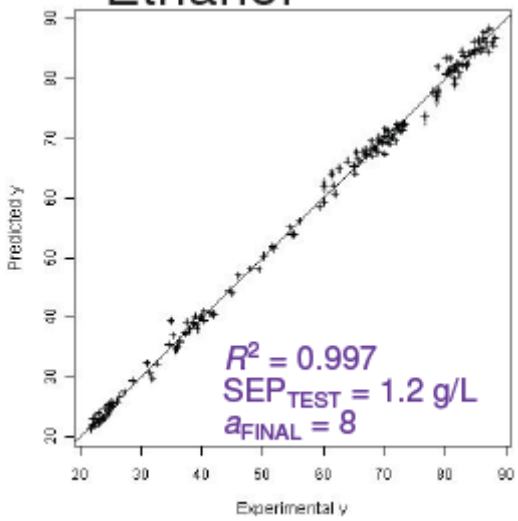
Compounds in Mashes

166 samples, 15 GA selected NIR variables
experimental/predicted *y* in g/L

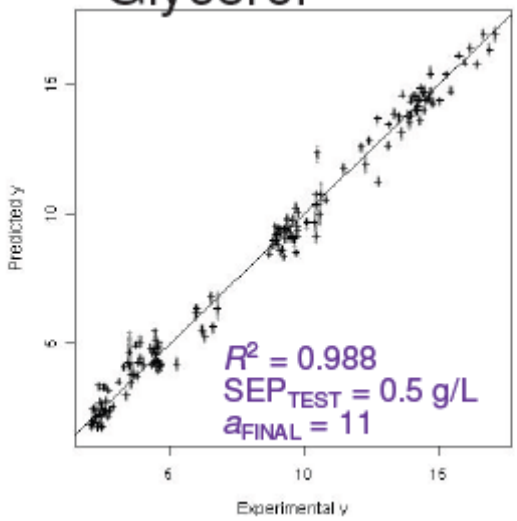
Glucose



Ethanol



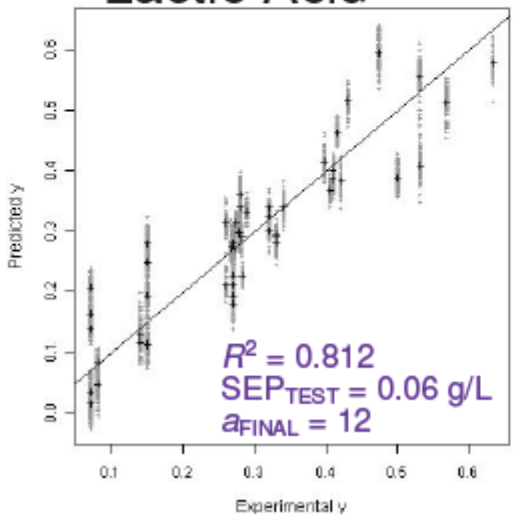
Glycerol



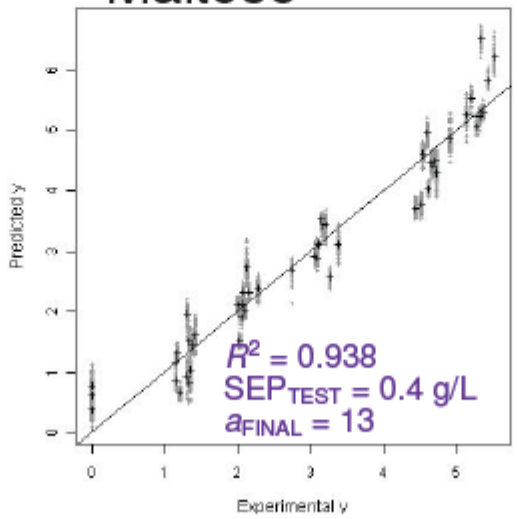
Compounds in Stillages

50 samples, 15 GA selected NIR variables
experimental/predicted *y* in g/L

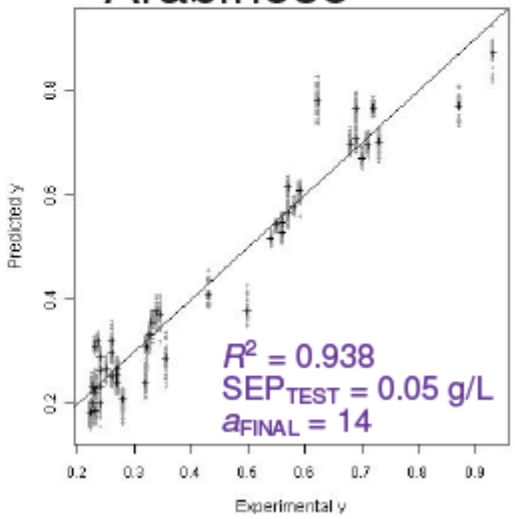
Lactic Acid



Maltose



Arabinose



6. Prediction Performances by rdCV

Compound	*n*	SEP_{TEST} NIR all	SEP_{TEST} NIR GA	Concentration range in g/L
Mashes				
glucose	166	5.6	4.5	0-54
ethanol	166	1.5	1.2	22-88
glycerol	166	0.7	0.5	2-17
Stillages				
glucose	50	4.0	1.7	0-24
ethanol	50	3.0	0.8	0-58
glycerol	50	1.7	0.6	3-14
lactic acid	50	0.1	0.1	0-1
fructose	50	0.7	0.5	0-6
maltose	50	0.8	0.4	0-6
arabinose	50	0.1	0.1	0-1

n	number of samples
SEP_{TEST}	standard deviation of 100·*n* prediction errors (g/L)
NIR all	all 235 NIR absorbance values available
NIR GA	15 GA selected NIR absorbance values

7. Method Comparison

Results of repeated double cross validation (rdCV) are compared with 4-fold cross validation as implemented in software Unscrambler [8]. All data sets with 15 GA selected variables.

		rdCV		CV	
Compound	*n*	SEP_{TEST}	a_{FINAL}	SEP_{CV}	a_{CV}
Mashes					
glucose	166	4.5	11	5.2	8
ethanol	166	1.2	8	2.7	2
glycerol	166	0.5	11	1.0	4
Stillages					
glucose	50	1.7	13	2.3	5
ethanol	50	0.8	15	2.1	4
glycerol	50	0.6	15	0.8	10
lactic acid	50	0.1	12	0.1	10
fructose	50	0.5	12	0.6	4
maltose	50	0.4	13	0.5	6
arabinose	50	0.1	14	0.1	5

n number of samples

repeated double cross validation in R:

SEP_{TEST} standard deviation of $100 \cdot n$ test set predicted errors (g/L)

a_{FINAL} optimum number of PLS components

4-fold random cross validation in Unscrambler:

SEP_{CV} standard deviation of *n* CV predicted errors (g/L)

a_{CV} optimum number of PLS components

8. Conclusions

- Easily available **near-infrared spectroscopy** data are **very promising** for the quantification of diverse compounds in **highly variable substrates** of the bioethanol process. Samples included three different feedstock options (wheat, rye, and corn) and six different enzymatic pretreatments.
- **Variable selection** by Genetic Algorithm **improves prediction performance** for all PLS models.
- Repeated double cross validation offers a **sophisticated optimization strategy** for model complexity (number of PLS components). Furthermore, prediction performance can be reasonably estimated.
- In comparison, **4-fold cross validation** yields **higher prediction errors**, as the optimum number of PLS components is chosen more conservatively.
- Evaluation of prediction quality suggests that a higher number of PLS components does not necessarily imply overfitting.
- Implementation of repeated double cross validation **in software R is fast and easy** with typical computation times of 0.5 to 10 minutes.

9. References

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Abstract

In large industrial-scale processes such as bioethanol production, chemically undefined multiple substrates are present that can be highly variable from batch to batch. The complexity of the initial medium is varied by the type of feedstock (wheat, rye, and corn), the enzymatic pretreatment, as well as yeast fermentation itself. Compounds of interest are glucose, the nutrient for yeast fermentation, its fermentation product ethanol, as well as side-products such as lactic acid, acetic acid, and glycerol. Near-infrared (NIR) spectroscopy is well suited for rapid, non-destructive, multi-constituent analyses with minimal sample preparation directly in the fermentation broth.

The objective of this study is the development of PLS regression models for a prediction of concentrations of the above mentioned compounds in different bioethanol mashes by NIR spectroscopy [1]. We apply a genetic algorithm (GA) for variable selection and evaluate the models' prediction performance by a repeated double cross validation (rdCV). rdCV offers a strategy to estimate the optimum model complexity - that is the number of PLS components. Furthermore, rdCV allows a realistic estimation of the prediction errors and their variations for new cases, based on a large number of test set predicted values [2].

Variable selection by GA improved the prediction performance in all investigated cases. For centrifuged samples the standard deviations of the prediction errors (*SEP*) for test set data are about 2 gL^{-1} for glucose (0–55 gL^{-1}), 0.6 gL^{-1} for ethanol (0–56 gL^{-1} in stillage), and 0.2–1 gL^{-1} for the other compounds. These results are promising for a successful application of NIR spectroscopy and multivariate data evaluation in bioethanol production.

[1] Liebmann B., Friedl A., Varmuza K.: Anal. Chim. Acta, in press (2009)
[2] Varmuza K., Filzmoser P.: Introduction to multivariate statistical analysis in chemometrics. Francis & Taylor, CRC Press: Boca Raton, FL, USA, 2009.