Characterization of wine fermentations using fiber optic-mediated UV-VIS-SWNIR spectroscopy

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Abstract

Spectroscopy is widely used in biological sciences, being applied to liquids, pastes, powders, films, fibers, gases and surfaces. It makes it possible to characterize proteins, peptides, lipids, membranes and carbohydrates in pharmaceuticals, foods, plants or animal tissues. It can also provide detailed information about the structure and mechanism of action of molecules.

In this work we explore the use of fiber optics UV-VIS-SWNIR spectroscopy to characterize grape must fermentations of 114 differents *Saccharomyces cerevisiae* strains. Within the range of wavelengths used (200-1200 nm) two spectral regions were analyzed in large detail: LWUV-VIS and VIS-SWNIR. The collection comprises 114 strains (among which almost 40 are sequenced strains), between industrial strains used for winemaking, brewing, bakery, distillery (sake, cachaça) and ethanol production, natural isolates obtained in winemaking environments, and also strains from particular environments (e.g. pathogenic strains, isolates from insects, fruits and oak exudates). Results show that fiber optics UV-VIS-SWNIR spectroscopy is a robust technique for characterize different wine fermentations, being able to characterize and differentiate the fermentation of different strains of S. cerevisiae based on their origins, by each spectroscopic fingerprint.

This technique associated with other physico-chemical information can contributes to create an information system capable of providing detailed information about physical processes that will aid both scientists and engineers to study and develop new biotechnological products.

1. Materials and Methods

1.1. Sample preparation

Individual fermentations were carried out in 100 mL wine (cv. Loureiro) must for each of the 114 strains, and the growth rate, CO_2 release and glucose concentration were followed throughout fermentation. When glucose concentration was below 5 g/L, samples were collected, immediately frozen and stored for fiber optics spectroscopy analysis. From the results obtained with the 114 strains, and in order to evaluate the reproducibility of the method, a smaller subset of 28 strains was chosen and further fermentations (in triplicate) were performed as previously described.

1.2. Spectroscopy

Figure 1 shows a scheme of the experimental setup. Transmittance fiber optics UV-VIS-SWNIR spectroscopy was used to record the spectra between 200 and 1200 nm, using a highly sensitive scientific-grade spectrometer (Ocean Optics, QE65000) for maximum resolution (1). The procedure was performed in a special probe container designed to isolate the environmental light and maintain the probe horizontally, to prevent the deposition of debris in the mirrored surface. The following experimental procedure was performed: spectra were obtained at room temperature after light sources stabilization (20 min); dark spectra were recorded and measurements were taken with linear and electric dark correction. Light spectra were statistically monitored, assessing the reproducibility of the light source by regular light measurements. Twenty spectra replicates were recorded for each fermentation.
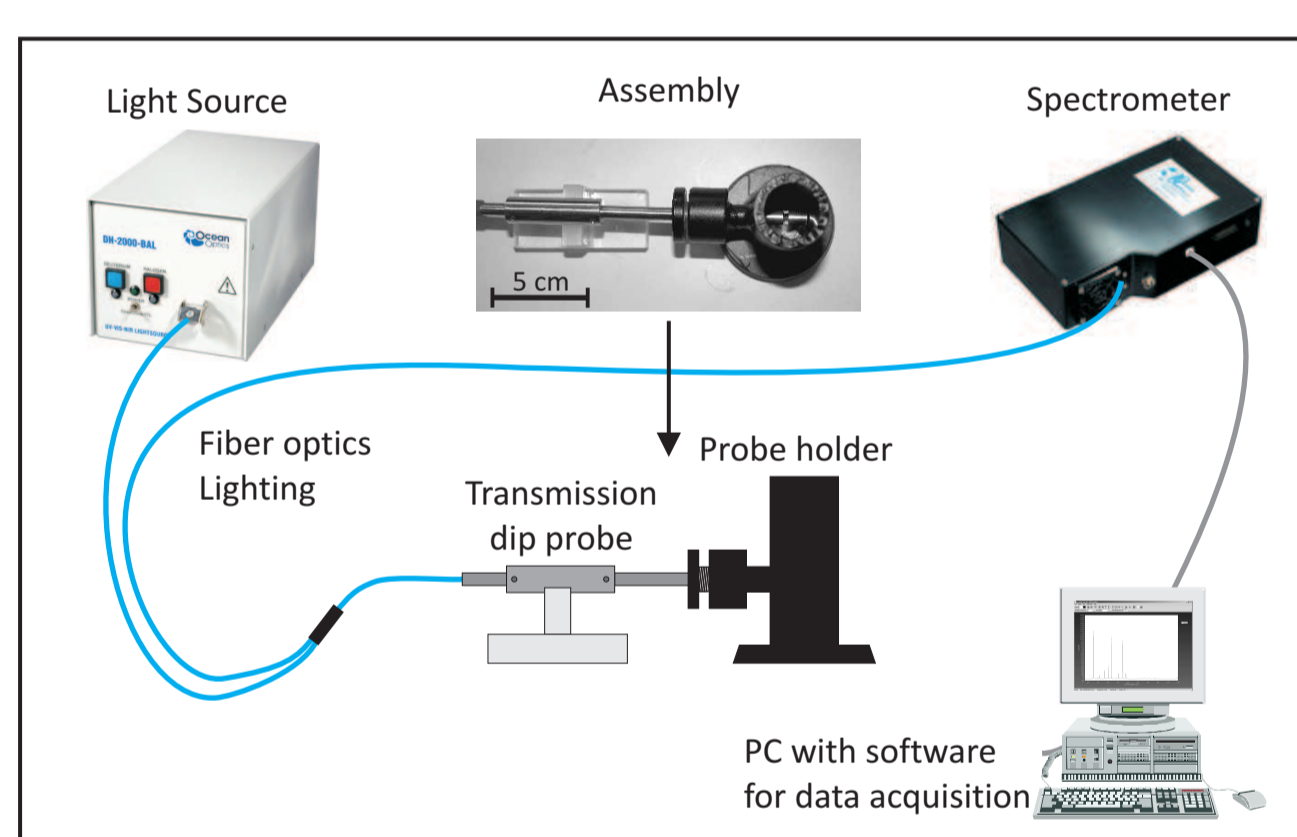

Figure 1. Experimental Setup

1.3. Spectral Analysis

Robust mean scattering correction
The collected reflectance spectra were smoothed by using a Savisky-Golay filter and log-transformed (absorbance) prior to any exploratory data analysis procedure. Afterwards, the spectra was pre-processed using a modified multiplicative scatter correction algorithm (2,3,4).

Singular value decomposition
Singular value decomposition (SVD) was used to decompose the correct spectra in order to determine the number of relevant components to use in the PCA. (5).

Multivariate regression methods
Partial least squares regression (PLSR) is a multivariate regression method, which is used to relate multivariate data set X to a reference value y (6).

$$y = X \cdot b + e \qquad (1)$$

where, b represents the regression coefficient and e the error. Typically, X is a low-cost and high-output multivariate method, such as UV-VIS-SWNIR measurements, whereas y are often time-consuming and expensive reference methods, such as metabolomic data. PLSR was used to interpret the relationship between the two dataset, allowing the prediction of y in order to use spectroscopy as a software sensor. . PLSR algorithms maximise the covariance between y and X (7).

1.4. Chemical Analysis

Fermentation samples were analyzed for their concentration in: tartaric acid; malic acid; fructose; succinic acid; glycerol; acetic acid and ethanol; using HPLC(20µl of sample; Time: 35 min; Column: Ion exclusion; Detector: RI at 40ºC; Mobile Phase: 2.5mM H2SO4; Temperature: 60ºC)

References

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2. Results and Discussion

In order to evaluate whether UV-VIS-SWNIR spectroscopy is able to distinguish *S. cerevisiae* strains from different sources, 114 isolates of this species were submitted to spectral analysis.

Figure 2 (a) presents relevant scores plot in the 2 PC's for LWUV-VIS absorbance of the 114 strains, totalizing 99.1 % of spectral variance with discriminant power (PC1 (88,1 %) and PC2 (11,0 %). From the results obtained, 28 strains were chosen as being the most heterogeneous, aiming at further evaluation of the method's reproducibility. Figure 2(b) shows relevant scores plot in the 2 PC's for LWUV-VIS absorbance of 28 strains selected from 114 original strains, with three replicates each (A,B and C).
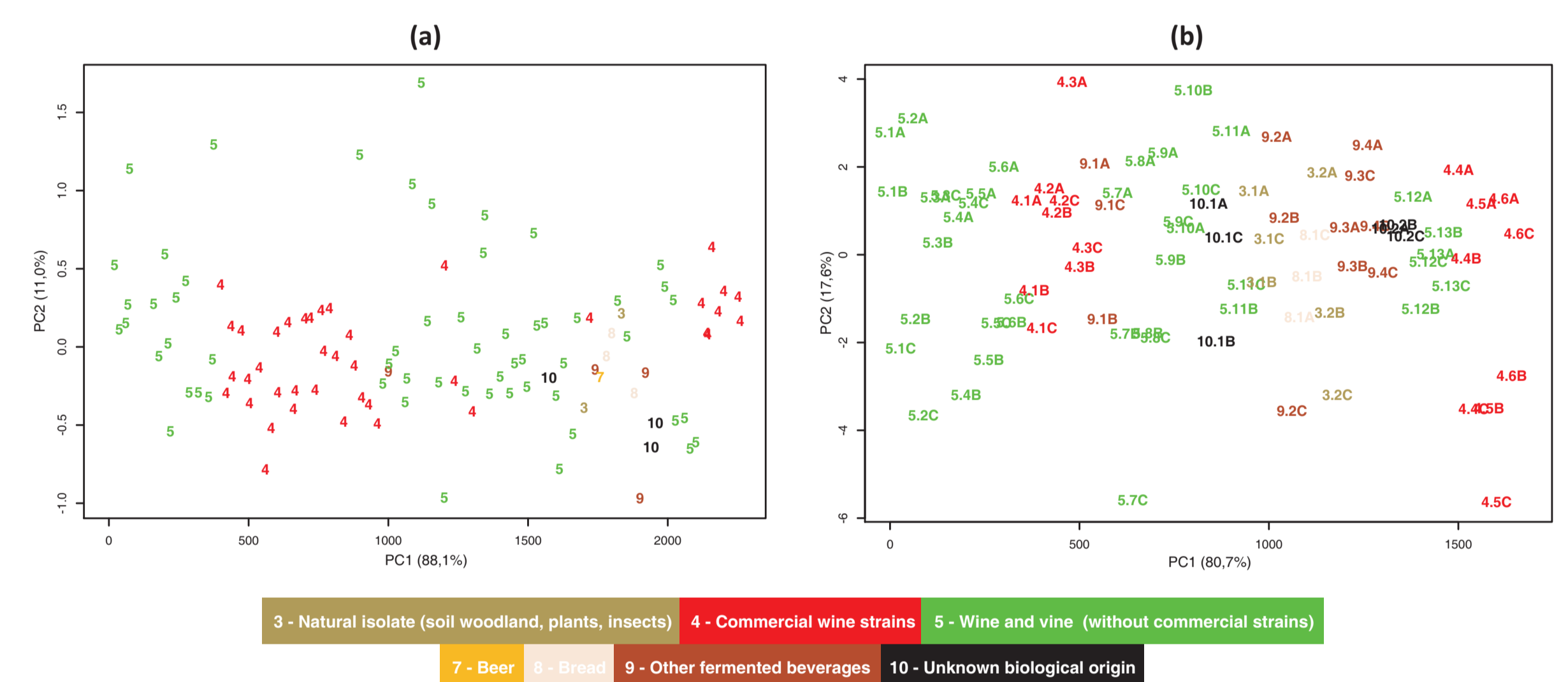

Figure 2. Spectra PCA analysis: (a) Absorbance LWUV-VIS Gabriel Plot (114 strains); and (b) Absorbance LWUV-VIS Gabriel Plot (28 strains with three replicates each, A, B and C).

Figures 2 (a) and (b) agree in the distinction of groups of strains from different sources. PC1 segregates the samples by spectral intensity into two big groups: i) wine and vine (without commercial strains) and commercial wine strains. PC2 allows discriminating the other strains' groups (Figures 2 (a) and (b) and replicas - A, B an C - in Figure 2(b)). Also, a comparison between Figures 2 (a) and (b) shows that the global disposition of the strains in the PCA plot is maintained, which is a clear sign of a good reproducibility.

PLS-R calibrations were performed in order to relate spectral signatures with the chemical composition of the final fermentation products. The calibrations were tested for robustness and PLS-R regression results are presented in Figure 3. These results were obtained from the spectral analysis of 28 strains and the corresponding chemical analysis of samples for the VIS-SWNIR light source (the one presenting the best results).

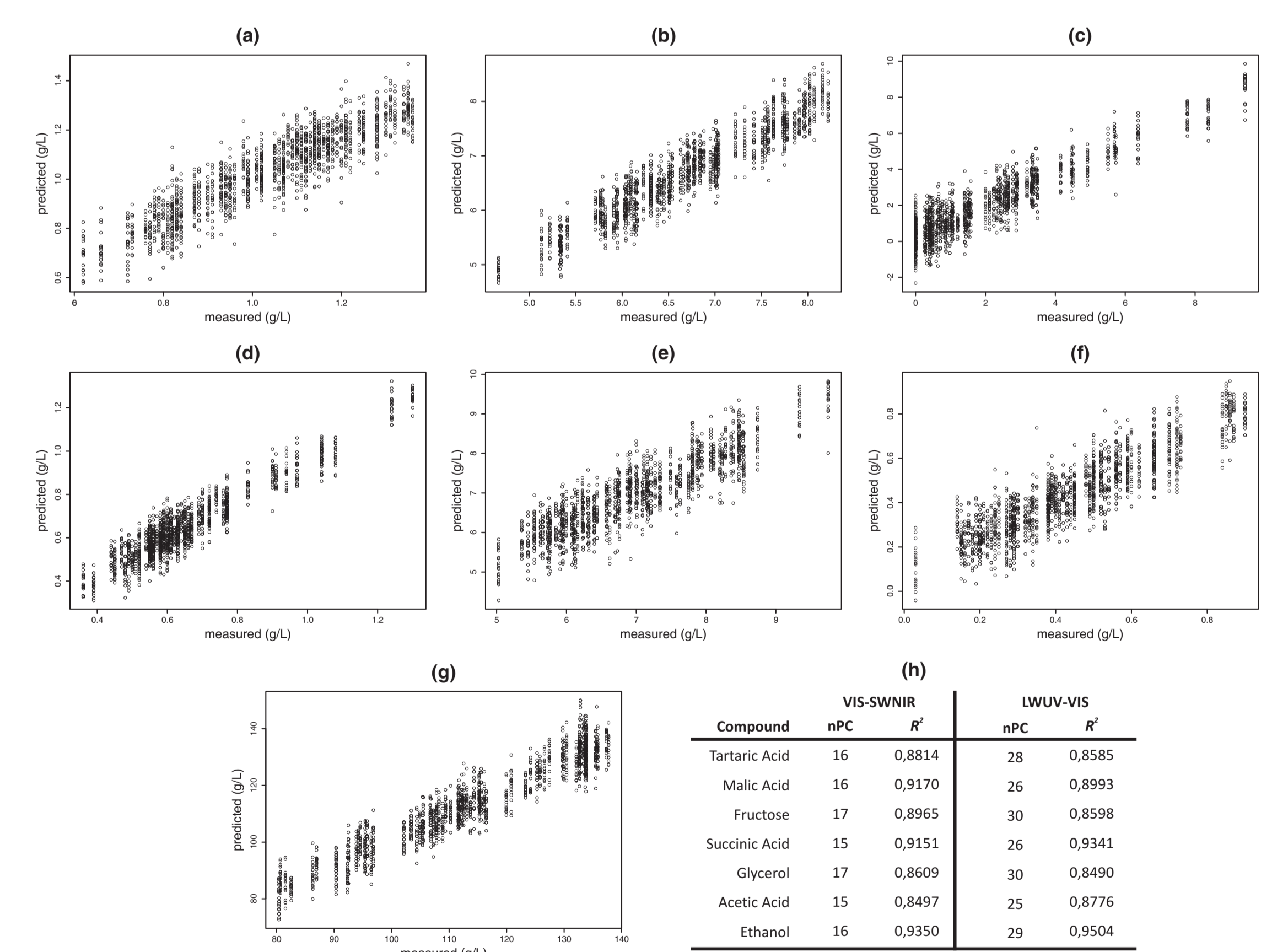| Compound	VIS-SWNIR nPC	VIS-SWNIR R^2	LWUV-VIS nPC	LWUV-VIS R^2
Tartaric Acid	16	0,8814	28	0,8585
Malic Acid	16	0,9170	26	0,8993
Fructose	17	0,8965	30	0,8598
Succinic Acid	15	0,9151	26	0,9341
Glycerol	17	0,8609	30	0,8490
Acetic Acid	15	0,8497	25	0,8776
Ethanol	16	0,9350	29	0,9504

Figure 3. PLS-R Calibrations for VIS-SWNIR (28 strains): (a) tartaric acid; (b) malic acid; (c) fructose; (d) succinic acid; (e) glycerol; (f) acetic acid; (g) ethanol and (h) partial least squares regression model.

A comparison of the correlation factors (R^2) and of the number of components (ncomp) allows to conclude that VIS-SWNIR calibrations are better than the calibrations in LWUV-VIS spectra. Furthermore, VIS-SWNIR wavelengths allowed to obtain robust calibrations with less number of spectral decompositions. In all LWUV-VIS PLS-R regressions 25 to 35 components were needed, while a lower number (15 to 17) was necessary when using the VIS-SWNIR region. This apparent discrepancy possibly suggests that the LWUV-VIS region contains less information regarding the chemical compounds under analysis.

3. Conclusions

This work demonstrates that after appropriate preprocessing and signal classification, fiber optics UV-VIS-SWNIR spectroscopy is a robust technique for the quantification of wine primary fermentaion products. It has also been shown that it is possible to use this technique to distinguishfermentations carried out by different *S. cerevisiae* strains, based on their main fermentation metabolites.

Multivariate methods such as the combination of the two spectral regions using n-way and multiblock PLS-R (e.g. LW UV-VIS + VIS-SWNIR spectra) as well as using wavelets or Fourier transformation for compressing and modelling the spectra may provide better interpretation of spectra variance between strains, thus improving the quality of predicted data.