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CHARACTERIZATION OF WINE FERMENTATIONS USING FIBER OPTIC LWUV-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 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 different *Saccharomyces cerevisiae* strains. 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.

MATERIALS AND METHODS

Sample preparation

Individual fermentations were carried out in 100 mL wine (cv. Loureiro) must for each of the 114 strains, and the growth rate, CO2 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.

Spectroscopy

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 for maximum resolution. 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.

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 (Gallager et al. 2005).

Singular value decompostion
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. (Baig and Rehman 2006).

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 (Geladi and Kowalsky 1986).

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

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. (Denham 1995).

Chemical Analysis

Fermentation samples were analyzed for their concentration in: tartaric acid; malic acid; fructose; succinic acid; glycerol; acetic acid and ethanol; using HPLC.

RESULTS AND CONCLUSIONS

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 1 (a) presents relevant scores plot in the 2 PC's for LWUV-VIS absorbance of the 114 strains. From the results obtained, 28 strains were chosen as being the most heterogeneous, aiming at further evaluation of the method's reproducibility. Figure 1(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).

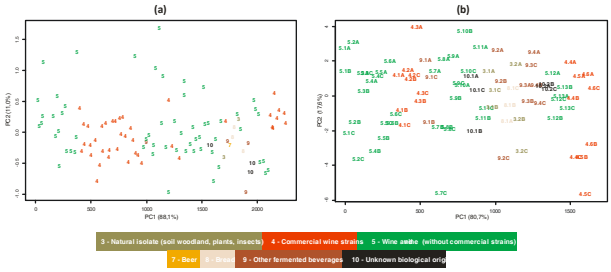

Figure 1: Spectra PCA analysis: (a) Absorbance LWUV-VIS Gabriel Plot (114 strains); and (b) Absorbance LWUV-VIS Gabriel Plot (28 strains with three replicates each, A, B and C).

Figures 1 (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 1 (a) and (b) and replicas - A, B an C - in Figure 1(b)).

PLS-R calibrations were performed in order to relate spectral signatures with the chemical composition of the final fermentation products. 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).
A comparison of the correlation factors (R2) 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. This apparent discrepancy possibly suggests that the LWUV-VIS region contains less information regarding the chemical compounds under analysis.
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.

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João S. Silva was born in Esposende, Portugal and went to the University of Minho, where she studied Chemical and Biological Engineering and obtained her MSc degree in 2007. He worked for two years in the MoNirtor project before start her PhD in Centre of Biological Engineering in 2009.
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