Near infrared monitoring of wine fermentation

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Introduction

The term process analytical technology (PAT) describes the field of process analysis and measurement technologies that has expanded to include several physical, chemical, mathematical and other analytical tools used to characterise chemical and biological process.[1, 2] This approach has become a key process for monitoring parameters in the pharmaceutical industry. It has led to an emphasis on measurement methods to characterise products with properties beyond the information that is provided by traditional laboratory based analytical techniques.[1, 2] Similarly, the modern wine industry needs tools for process control and quality assessment in order to better manage fermentation. During wine fermentation, it is important to measure both substrate and product concentrations; however, the analysis of these compounds by traditional methods is time consuming.[3] The use of NIR spectroscopy and chemometrics potentially provides an ideal solution to accurately and rapidly monitor changes in wine during fermentation without the need for traditional chemical analysis.

Materials and Methods

Samples

Samples were sourced from fermentation trials conducted during four successive harvests. Samples from several pilot scale fermentors, grape varieties and treatments (i.e. different temperatures and yeasts) were collected at different fermentation points. Grapes were machine harvested into bins holding approximately 400 kg each and transported to the Roseworthy Hickinbotham Wine Science Laboratory at the Waite Campus of the University of Adelaide. The grapes were then de-stemmed, crushed, and pumped into small-scale temperature controlled fermentation tanks (two randomly-selected bins per tank). The 2001 fermentations were conducted in two different types of fermenters, namely rotary (Vinomatic, 900 L) and stationary (Potter, 1100 L). The fermenters were then inoculated with *Saccharomyces cerevisae* (EC1118, Lalvin, Canada). For each type of fermenter, the alcoholic fermentation was carried out at two different temperatures (18 and 25°C). The 2002 fermentations were also conducted in both rotary and stationary fermenters as described for the 2001 trial.[3,4] The six stationary fermenters were inoculated with *Saccharomyces bayanus* (AWR1375) from the Australian Wine Research Institute culture collection. Alcoholic fermentation was carried out at two different temperatures (20 and 28°C). In the case of the six rotary fermenters, two different strains of yeast were used namely *Saccharomyces cerevisae* (AWR838) and *Saccharomyces bayanus* (AWR1375) but the fermentations were carried out at one temperature (20°C). The 2003 fermentations were conducted using the stationary fermenters described above inoculated with *Saccharomyces cerevisae* and alcoholic fermentation was conducted at one temperature (20°C). A total of 652 samples (ferments) were obtained (Cabernet Sauvignon 2001 = 182, Cabernet Sauvignon 2002 = 186, Shiraz 2001 = 144, Shiraz 2003 = 140) and used for both spectroscopic and chemometric analyses.[3, 4]

Visible and near infrared spectroscopy

Ferments were scanned on the day of sampling in transmission mode (400–2500 nm) using a scanning monochromator FOSS NIRSystems6500 (FOSS NIRSystems, Silver Springs, MD, USA). Spectral data collection was made using Vision version 1.0 (FOSS NIRSystems, Silver Springs, MD, USA). Samples were centrifuged, and then temperature equilibrated at 33°C for two minutes in a rectangular cuvette with a 1 mm pathlength before being scanned.[3,4]

Data analysis and interpretation

Spectra were exported from Vision version 1.0 in NSAS format to The Unscrambler version 9.1 (CAMO ASA, Oslo, Norway) for chemometric analysis. Principal component analysis (PCA) was used to reduce the dimensionality of the data to a small number of components, to examine any possible grouping of samples according to the time course of the fermentation, and to identify any outliers.[5, 6] Full internal cross-validation (leave-one-out) was used to validate the PCA models. The number of principal components (PCs) used in the PCA models was selected by the PRESS function (predicted residual error sum of squares) and no attempts were made to increase the number of PCs in order to avoid overfitting of the models.

D. Cozzolino, W. Cynkar and N. Shah (2012). Near infrared monitoring of wine fermentation, in:
Proceedings of the15th International Conference on Near Infrared Spectroscopy,
Edited by M. Manley, C.M. McGoverin, D.B. Thomas and G. Downey, Cape Town, South Africa, pp. 187-189.

Results and Discussion

Principal component analysis (PCA) was applied to explain the variance-covariance structure through a few linear combinations of the original variables. The first three principal components (PCs) in this study explained more than the 98% of the variance related to the visible (Vis) and NIR spectra. Figure 1, obtained from 2002 Cabernet Sauvignon ferments, shows the time course changes in the PC scores throughout wine fermentation (arrow). Similar patterns were observed in all fermentation experiments over the three vintages although different grape varieties were used. Changes to both colour (total anthocyanins) and polyphenol compounds were evident along the first two PCs. The highest loading along PC1, which explained more than 80% of the variation, was around 540 nm. This spectral region is dominated by colour pigments, principally grape-derived pigments (anthocyanins) characteristic of red wines, and is an indication of the transformations that occur during the first stages of fermentation of wine grapes.[3,4] No other spectral regions were observed to have a big effect on PC1. PC2 explained 15% of the variation in the dataset and the loadings plot showed a large negative correlation with the Vis region around 540 nm, particularly in the Shiraz samples, and high positive correlation with the 2200–2300 nm spectral region.[3,4] We therefore conclude that PC2 explained the conversion of sugars into alcohol, as denoted by high loadings at wavelengths around 1800 nm (O-H and C-H tones), and at wavelengths around 2200–2300 nm related to ethanol. PC3 explained 3% of the total variation in the dataset. The highest loadings for PC3 were observed around 2200–2300 nm and were associated with ethanol, tannins and pigmented tannins (PP).[3,4] Juice samples had positive PC1 and negative PC2 scores (labelled as grape juice; Figure 1); fermentation caused PC2 values to increase (samples labelled as wine; Figure 1). The PC1 and PC2 score values of wine samples decreased after fermentation. Changes in the scores and loadings related to the time course of the fermentation are an indication of the changes that commonly take place during red wine fermentation.[3,4] In this study, the combination of spectroscopy and chemometrics as an analytical tool gave the advantage of rapid monitoring of the changes occurring during red wine fermentation without the need for quantitative data. It is well-known that, in contrast to quantitative analysis, a qualitative analysis compares spectra and looks for similarities and differences. Thus, in order to identify the spectrum of an unknown substance one computes spectral distances (scores) to define if the spectrum of the unknown sample is the same as the spectrum in the library. This holistic approach could provide an opportunity to produce a more consistent product by the wine making process in order to meet quality specifications.

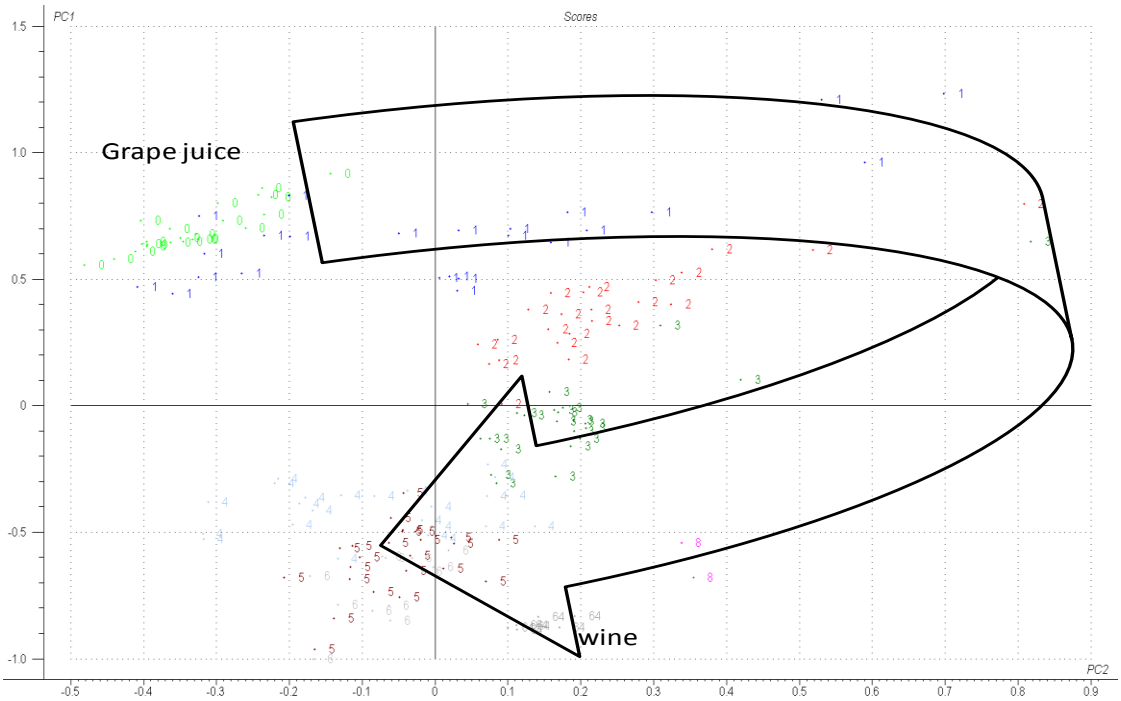

Figure 1. Principal component score plot of the time course of fermentation for Cabernet Sauvignon grapes.

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Conclusion

Near infrared spectroscopy combined with chemometric analysis has the potential to significantly reduce the analytical time and cost required to monitor red wine fermentation. Although the NIR technique is essentially qualitative (i.e. no quantification of any compositional variable was made), it was possible to identify changes that occurred during fermentation. Finally, NIR spectroscopic data was useful for classifying the progress of fermentation, independent of variables such as grape variety, yeast strain, and temperature.

Acknowledgements

The authors thank the Orlando Wyndham and Southcorp wine companies for providing the grapes used in the fermentation trials. This project is supported by Australia's grapegrowers and winemakers through their investment body the Grape and Wine Research and Development Corporation.

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