Effects of variety and region on near infrared reflectance spectroscopic analysis of quality parameters in red wine grapes

M.B. Esler,[a,c] M. Gishen,[a,c] I.L. Francis,[a,c] R.G. Dambergs,[a] A. Kambouris,[b] W.U. Cynkar[a,c] and D.R. Boehm[a]

[a] *The Australian Wine Research Institute, PO Box 197, Glen Osmond, SA 5064, Australia*

[b] *BRL Hardy Limited, PO Box 238, Berri, SA 5343, Australia*

[c] *The Cooperative Research Centre for Viticulture, PO Box 154, Glen Osmond, SA 5064, Australia*

Introduction

The wine industry requires objective measures of red wine grape quality to determine optimal harvest time, allocate freshly harvested grapes to winery process streams for particular red wine products and determine quality-based payment to grape growers. The practical requirement that these analyses also be rapid and inexpensive currently restricts them to the measurement of TSS (total soluble solids, mainly sugars, in °Brix) by refractometry or hydrometry and pH and acid content by potentiometry and titration. These parameters do not, however, provide comprehensive compositional characterisation for the purpose of winemaking.

Total anthocyanins in red wine grapes

The total concentration of anthocyanin pigments in red wine grapes is believed to be an indicator of potential wine quality and price.[1] However, routine analysis for total anthocyanins is not considered as a practical option by the wine industry because of the high cost and slow turnaround time of this multi-step wet chemical laboratory analysis. The analysis requires homogenisation of about 100 g of grapes, a one-hour solvent extraction of the homogenate, centrifugation of the extract, pH adjustment with three-hour equilibration of the supernatant and, finally, spectrophotometric analysis at 520 nm. Recent work by this group[2,3] has established the capability of near infrared (NIR) spectroscopy to provide rapid and accurate measurement of total anthocyanins as well as the simultaneous measurement of TSS and pH in red wine grapes. For the present study, the only sample processing step still required is homogenisation of the grapes.

Sample collection

In the five weeks leading up to the 1999 harvest, approximately 150 samples of red wine grapes from the single region of the Riverland were collected and then stored frozen. In the lead up to the 2000 harvest, a further 750 samples were collected from commercial vineyards managed by four different wine companies in several different growing regions. Only four varieties of grape account for the over-

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whelming majority, 98%, of the samples. These were Shiraz (45%), Cabernet Sauvignon (43%), Merlot (7%) and Grenache (3%), approximately representing the relative proportions in which these varieties are grown in Australia.[4] The remaining 2% of samples consisted of the less common grape varieties (Ruby Cabernet, Cabernet Franc, Mataro, Malbec, Petit Verdot and Cinsaut). Similarly, not all regions were sampled to the same extent, the relative proportions being Riverland: 25%, Barossa Valley: 22%, Padthaway: 13%, McLaren Vale: 13%, Langhorne Creek: 11%, Wrattonbully: 9%, Coonawarra: 5% and Mildura, Adelaide Hills and Robe about 1% each. In all, the sample set spans two vintages (1999 and 2000), ten distinct geographical winegrowing regions in South Australia and ten of the varieties of red grape cultivated commercially in Australia.

Analysis

The samples were thawed in batches of about 20, homogenised and scanned in diffuse reflectance mode on a FossNIRSystems 6500 spectrometer and immediately subjected to laboratory analysis the same day using the traditional methods for total anthocyanins, TSS and pH. All of the NIR scanning and the reference method analysis were completed in a period of six months.

Calibrations

All calibrations were generated using partial least squares (PLS) in *The Unscrambler 7.5* (CAMO ASA, Norway). In nearly all cases the calibration initially obtained by the PLS method was improved by using Martens' uncertainty test[5] (or 'jack-knifing') to select the optimal set of wavelengths for inclusion in the calibration. This improvement was manifested as either reduced standard error of cross-validation (*SECV*) or reduced number of PLS factors, or both. The optimal total anthocyanin calibrations used two to four PLS factors and the pH calibrations four to five factors. The calibrations for total anthocyanin and pH required 2nd-derivative preprocessing of the spectra. The TSS calibration used raw reflectance spectra and five PLS factors.

Results and discussion

Figure 1 illustrates the results for total anthocyanin analysis. Figures 1(a) and 1(b) illustrate the correlation of predicted against measured results for the optimal total anthocyanin calibration when all regions and grape varieties (of those sampled) are included. This is referred to as the 'global' calibration and is plotted in Figures 1(a) and 1(b), coded by region and grape variety, respectively. The *SECV* of this calibration, ± 0.14 mg g^{-1}, is equivalent to 12% of the mean total anthocyanin concentration and 6.7% of the observed range of concentrations in the 'global' population. This degree of measurement precision would be of only marginal use to commercial winemakers. In Figure 1(c) the focus of the calibration has been narrowed to include only one variety of grape, Cabernet Sauvignon, but still including all the regions from which that variety was sampled. This 'varietally-localised' calibration has a significantly improved *SECV* of ± 0.106 mg g^{-1}, (9.7% of the mean and 6.2% of the range for that population of samples). Similarly, in Figure 1(d) the calibration has been 'regionally localised' relative to the original 'global' calibration to focus only on a single region, in this case the Riverland, but includes all four varieties of grapes sampled from that region. The *SECV* is better again, ± 0.068 mg g^{-1}, (11% of the mean and 5.1% of the range for that population). Finally, Figure 1(e) illustrates the 'varietally and regionally localised' calibration for Riverland grown Cabernet Sauvignon grapes. The *SECV* of ± 0.054 mg g^{-1} represents 6.9% of the mean and 4.1% of the range of total anthocyanin concentrations for the population of Riverland grown Cabernet Sauvignon grapes and 4.7% of the mean and 2.6% of the range for the original 'global' population. This represents an improvement in measurement precision by a factor of almost three over that obtained in the initial 'global' calibration. This level of precision is likely to be of considerable use to commercial winemakers. The precision of the

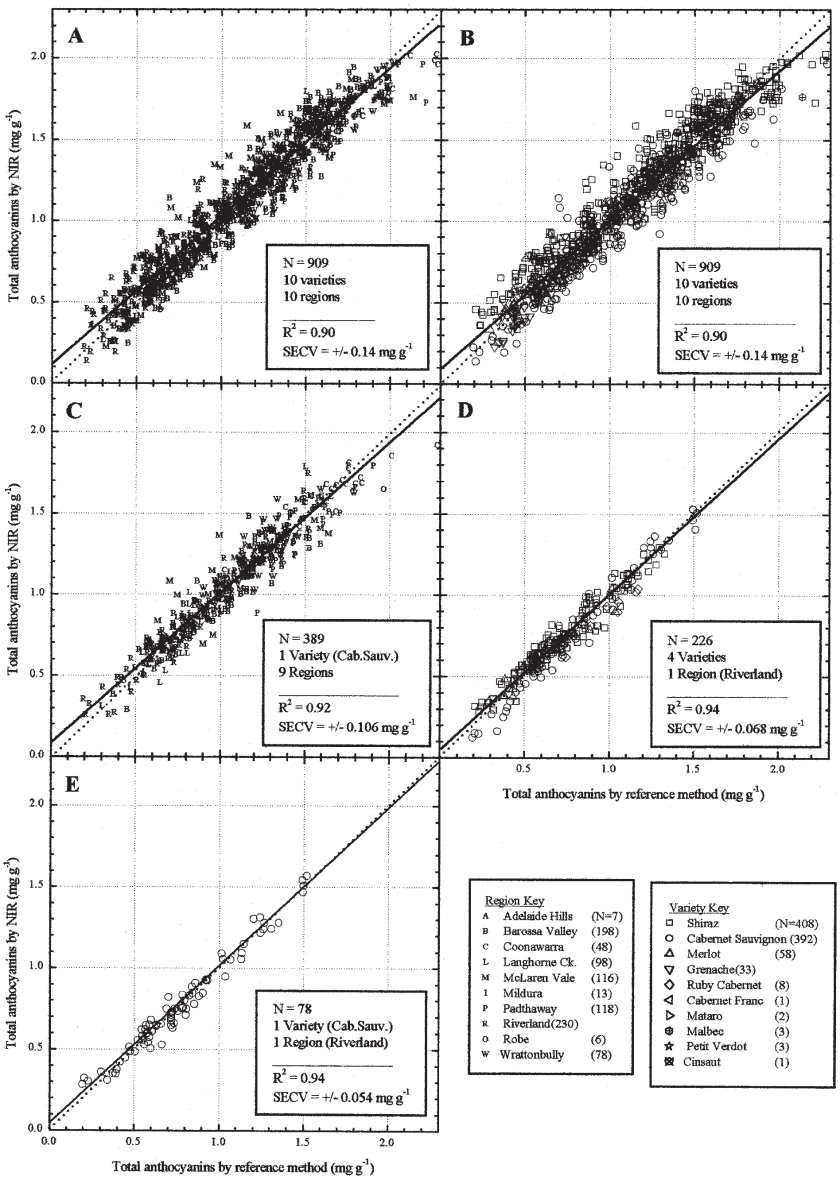

Figure 1. NIR calibrations for total anthocyanin concentration for a population of ~ 900 red wine grape samples and various of its sub-populations. (a) 'Global' calibration; 909 samples across ten growing regions, ten grape varieties and two seasons, plotted by region, (b) Same samples as (a), plotted by grape variety, (c) Subpopulation, localised to a single grape variety only, Cabernet Sauvignon; 389 samples across nine growing regions and two seasons, plotted by region, (d) Subpopulation, localised to a single growing region only, the Riverland; 226 samples across four grape varieties and two seasons, plotted by variety and (e) Subpopulation, localised for both a single grape variety, Cabernet Sauvignon, and a single growing region, the Riverland.

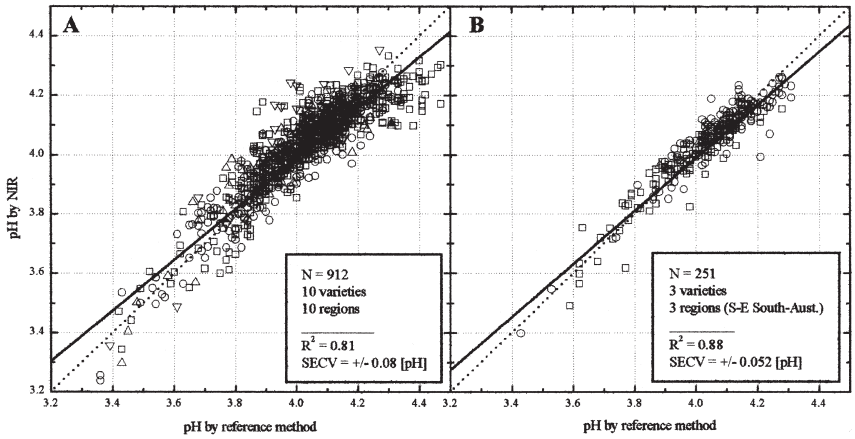

Figure 2. NIR calibrations for pH for a population of ~ 900 red wine grape samples and one of its subpopulations, (a) 'Global' calibration, 912 samples across ten growing regions, ten varieties and two seasons, plotted by variety and (b) Subpopulation, localised to a group of three closely related regions, across three varieties and one season.

reference method for total anthocyanins is estimated to be approximately ± 0.05 mg g^{-1}, so further improvement in the precision of the NIR analysis is unlikely to be seen.

In a similar manner, Figures 2(a) and 2(b) illustrate the improvement in pH measurement precision using NIR spectroscopy on moving from a 'global' to a more 'regionally- and varietally-localised' calibration. It has been suggested that NIR prediction of pH may be related to pH-induced shifts in anthocyanin chromophores and, indeed, much the same region of the spectrum is used for both pH and total anthocyanin prediction. Thus, the same type of improvement seen for both pH and total anthocyanins on going from a global to a more localised calibration is not so surprising. Both pH and total anthocyanin prediction by NIR may be sensitive to the same type of matrix effects due to differing regions and varieties.

In Figure 3, the 'global' calibration for TSS is illustrated, with an *SECV* of ± 0.33°Brix. No significant improvement was observed on 'localising' calibrations for TSS. This may indicate that the NIR measurement of TSS is less sensitive to matrix changes due to different varieties and regions than is the measurement of total anthocyanins and pH. Since the measurement precision of the reference method for TSS is estimated to be approximately ± 0.05°Brix, it is un-

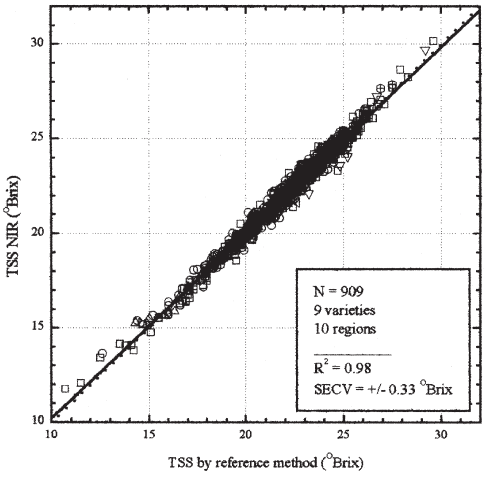

Figure 3. 'Global' calibration for TSS; 909 samples across ten growing regions, nine grape varieties and two seasons.

likely that the NIR prediction of TSS is limited by the reference method precision.

Conclusions

The important red wine grape quality parameters total anthocyanin concentration, TSS and pH can be measured rapidly by NIR spectroscopy. While large "global" calibrations across multiple regions, varieties and seasons provide useful degrees of measurement precision by NIR, calibrations further refined to particular regions, varieties and seasons provide significantly greater precision, at least for total anthocyanin concentration and pH. This study is continuing in 2001 when another ~ 2000 red wine grape samples from a broader range of Australian regions will be analysed, further elucidating regional varietal (and seasonal) effects on optimal calibration design. In addition, as the data set is now growing to an appropriately large size, it is intended to explore the application to it of Shenk's LOCAL algorithm[7] and of artificial neural networks (ANN) for further development of calibrations.

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