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Analytical Methods

Mid infrared spectroscopy and multivariate analysis: A tool to discriminate between organic and non-organic wines grown in Australia

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ABSTRACT

The combination of mid infrared (MIR) spectroscopy and multivariate analysis was explored as a tool to classify commercial wines sourced from organic (ORG) and non-organic (NORG) production systems. Commercial ORG ($n = 57$) and NORG ($n = 115$) red and white wine samples from 13 growing regions in Australia were analysed using a MIR spectrophotometer. Discriminant models based on MIR spectra were developed using principal component analysis (PCA), discriminant partial least squares (DPLS) regression and linear discriminant analysis (LDA). Overall, the LDA models based on the PCA scores correctly classified on average, more than 75% of the wine samples while the DPLS models correctly classified more than 85% of the wines belonging to ORG and NORG production systems, respectively. These results showed that MIR combined with discriminant techniques might be a suitable method that can be easily implemented by the wine industry to classify wines produced under organic systems.

1. Introduction

Several new organic farming techniques have been developed in recent years with the aim of improving the quality of plant and animal products, meeting the safety standards required by consumers, and reducing impact on the environment (Rembiałkowska, 2007; Watson, Walker, & Stockdale, 2008). However, as reflected in the relentless media coverage of organic farming and food, expert opinion differs widely and the direction of future studies on organic production is still under dispute (Rembiałkowska, 2007; Watson et al., 2008; Weller & Bowling, 2007). A diverse number of published studies report different topics related with different organic production systems, including the quality of crops produced using organic techniques, the comparison between contaminants and health-promoting components and the sensory characteristics of the food produced from organic systems (Watson et al., 2008). In recent years, research has demonstrated the effect of organic systems on storage and yield of different crops (Rembiałkowska, 2007), the involvement of secondary plant metabolites that can act as toxicants (Schulzováa, Hajslov, Botek, & Peroutka, 2007), the beneficial effects of organic systems on soil characteristics (Katsvairo, Wright, Marois, & Rich, 2007), the reduction of disease and pest infestations when converting a farm production technique or system from conventional to organic (Topp, Stockdale, Watson, & Rees, 2007), the improvement on the use of resources (e.g. nutrients, energy and water) within organic farming systems (Martin, Lynch, & Frick, 2007) and the effect on animal welfare and production (Kelly et al., 2007; Spoolder, 2007; Zollitsch, 2007).

The organic industry in Australia has internationally recognised and accredited certification and auditing programs verifying audit trail and production and handling compliance to recognised organic standards. Although organic wine is produced commercially, at present there is no recognised system available to easily track and monitor organic wine composition and authenticity in the wine industry. Conventional methods used to authenticate beverages involve time consuming, laborious and costly procedures, including chemical analysis, microscopy analysis and DNA testing (Arhurst & Dennis, 1996; Cordella, Moussa, Martel, Sbirrazzuoli, & Lizzani-Cuvelier, 2002). Therefore a rapid and inexpensive method based on spectroscopy for the certification process would be ideal in order to monitor whether wine characteristics conform to specification. Moreover, the application of multivariate data analysis techniques like principal component (PCA) or discriminant analysis opens the possibility to unravel and interpret the spectral properties of the sample and allow qualitative analysis of the samples, such as discrimination or classification (Cordella et al., 2002; Downey, 1998; Munck, Norgaard, Engelsen, Bro, & Andersson, 1998; Siebert, 2001). One of the advantages of the spectroscopic technology is not only to assess chemical structures through the analysis of the molecular bonds in the infrared spectrum, but also to build a characteristic spectrum that represents the finger print of the sample (Downey, 1998).

Currently, determination of food authenticity is one of the most important issues in food quality control and safety. The authenticity of wine is regulated by strict guidelines laid down by the

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responsible national authorities, which may include official sensory evaluation, chemical analysis and examination of the records kept by the wine producer (Arhurst & Dennis, 1996; Cordella et al., 2002; Cozzolino, Smyth, & Gishen, 2003). Using multivariate statistical techniques, wine identification or classification, mainly in terms of variety and geographical region of origin, has received increasing attention during the past 10 years (Cordella et al., 2002; Cozzolino et al., 2003; Munck et al., 1998). Vibrational spectroscopy in the infrared region (IR) has been applied to rapid qualitative analyses for a wide variety of products (Downey, 1998; Kelly & Downey, 2005). The use of mid infrared (MIR) spectroscopy has been proposed and implemented for routine analysis of a large number of wine compositional parameters such as alcohol concentration, volatile acidity, pH, titratable acidity, lactic acid, glucose plus fructose, acetic acid, tannins and polyphenols (Bevin, Fergusson, Perry, Janik, & Cozzolino, 2006; Boulet, Williams, & Doco, 2007; Edelmann, Diewok, Schuster, & Lendl, 2001; Moreira & Santos, 2004; Patz, Blieke, Ristow, & Dietrich, 2004). No reports were found in the literature about the discrimination of wines using MIR spectroscopy according to their origin in terms of organic production.

This study aims to investigate the use of mid infrared (MIR) and multivariate data analysis techniques to classify organic and non-organic wines produced in Australia.

2. Materials and methods

2.1. Wine samples

One hundred and seventy two ($n = 172$) commercial wine samples were sourced from eight different wine varieties (Cabernet Sauvignon, Shiraz, Pinot Noir, Merlot, Riesling, Chardonnay, Sauvignon Blanc and Verdelho) and thirteen regions of Australia (Barossa valley, Hunter valley, Yarra valley, Margaret River, Riverland, McLaren Vale, Clare, Canberra, Adelaide Hills, Coonawarra, Padthaway; Langhorne Creek and Tasmania) collected from commercial retailers, organic producers and the Australian Wine Research Analytical Services were used. Australian grown commercial ORG ($n = 57$) and NORG ($n = 115$) wine samples analysed showed a diversity of chemical and sensory characteristics due to different regions and ages, ranging in vintage from 2000 to 2008. Samples were analysed for alcohol concentration, specific gravity, pH, titratable acidity, glucose plus fructose and volatile acidity using standard methods (Iland, Ewart, Sitters, Markides, & Bruer, 2000).

2.2. Instrumentation

Samples taken from freshly opened commercial bottles of wine were scanned in transmission in a multispec system Bacchus/Multispec System, Thermo Nicolet, Avatar 380 (Bacchus, 2007) in the mid infrared (MIR) region (400–4000 cm^{-1}). Replicate bottles from an individual commercial label were used for scanning. Bacchus acquisition software was used for spectra collection and instrument diagnostics (Quant, version 4, 2001).

2.3. Data processing and statistical analysis

Spectra were exported from Bacchus acquisition software in Grams format (Thermo Galactic) and imported into The Unscrambler® software (Camo, Norway) for principal component analysis (PCA) and partial least squares (PLS) discriminant analysis. Principal component analysis (PCA) was used for reducing the dimensionality of the data to a small number of components, to examine the possible grouping of samples according to the origin (ORG and NORG) and to visualise the presence of outliers (Naes, Isaksson, Fearn, & Davies, 2002; Otto, 1999). Discriminant models were developed using discriminant partial least squares (DPLS) regression technique as described elsewhere (Naes et al., 2002). In this technique, each sample in the calibration set is assigned a dummy variable as a reference value, which is an arbitrary number designating whether the sample belongs to a particular group or not; in this case samples of organic wines (ORG) were assigned a numeric value 1 and non-organic wines (NORG) 2 and the cut-off was set to be 0.5. Linear discriminant analysis (LDA) is a supervised classification technique based on the determination of linear discriminant functions, which maximise the ratio of between class variance and minimise the ratio of within class variance (Berrueta, Alonso-Salces, & Herberger, 2007). In LDA, classes are supposed to follow a multivariate normal distribution and be linearly separated (Otto, 1999). The criterion of LDA for selection of latent variables is maximal differentiation between the categories and minimal variance within the categories (Naes et al., 2002; Otto, 1999). The method produces a number of orthogonal linear discriminant functions, equal to the number of categories minus one, that allow the samples to be classified in one or another category (Naes et al., 2002; Otto, 1999). LDA was carried out using JMP software (version 5.01, SAS Institute Inc., Cary, NC, USA) on the PCA sample scores on the principal components (PCs) 1–3 that gave the highest level of separation (high variance) in the PCA models developed. The PCA, LDA and DPLS calibration models were developed using full cross validation (CV) and it was used to validate the classification models. The number of latent variables used was automatically selected by the software using the PRESS function (predicted residual error sum-of-squares) (Naes et al., 2002; Otto, 1999).

3. Results and discussion

3.1. Chemical analysis

Table 1 shows the descriptive statistics for the chemical constituents analysed in both ORG and NORG wines. No statistically significant differences were observed in the chemical composition of the wines due to the system of production. Overall the chemical composition of the wine samples was considered to be typical for Australian table wines (Godden & Gishen, 2005).

No visual differences related to the system of production were observed between the MIR spectra of wine samples analysed (data not shown). It was observed that water and ethanol absorption peaks dominate the spectrum, with CO stretch for primary alcohols

Table 1
Descriptive statistics for chemical composition of commercial wine samples labelled as organic and non-organic (red and white wines).

	Non organic		Organic	
	Mean	SD	Mean	SD
Red wines				
Ethanol (%)	12.6	0.76	12.2	1.0
Specific gravity	0.99	0.001	0.99	0.004
pH	3.44	0.06	3.4	0.04
TA	6.69	0.62	6.5	0.45
G + F (g/L)	1.51	2.0	1.07	1.67
VA (g/L)	0.85	0.54	0.66	0.07
White wines				
Ethanol (%)	11.22	0.42	10.98	0.24
Specific gravity	0.993	0.008	0.993	0.008
pH	3.26	0.07	3.18	0.07
TA	7.24	0.34	7.01	0.27
G + F (g/L)	3.27	2.42	3.02	0.12
VA (g/L)	0.53	0.04	0.42	0.007

TA: titratable acidity, VA: volatile acidity, G + F: glucose plus fructose, SD: standard deviation.

and glycerol at 1087 and 1050 cm^{-1}. Absorption peaks related to CH stretch was observed at 2850–2960 cm^{-1} associated with ethanol. The region between 1288 and 1457 cm^{-1} is very complex and contains information relating to C=O stretching for aldehydes, carboxylic acids, proteins and esters, around 1457–1427 cm^{-1} C=C, CH_2 and CH_3 (Bevin et al., 2006; Boulet et al., 2007; Edelmann et al., 2001; Moreira & Santos, 2004; Patz et al., 2004; Sivakesava, Irudayaraj, & Demirci, 2001).

3.2. PCA analysis

PCA was performed on the MIR spectra to examine qualitative differences within the set of red and white wines related to the two production systems analysed. Fig. 1 shows the PCA score plot (PC1 vs. PC2) derived from the MIR spectra of the red wine samples. Generally, a separation was observed between the samples by system of production (ORG vs. NORG), however some samples did overlap. To investigate the basis for the observed spectral separation in the set of red wines related to the production system, the PCA eigenvectors were analysed (see Fig. 2). The first two principal components (PC) account for more than 97% of the variation in the MIR spectra of the red wine samples analysed. PC1 explained 93% of the total variance in the samples, and the highest loadings were observed around 1045–1080 cm^{-1} related to the C—OH of ethanol, glycerol and sugars (glucose and fructose), at 1268 cm^{-1} related with aromatic groups associated with phenolic compounds, and between 1419 and 1454 cm^{-1} related with CO=O and C=C, C—H_2, C–H_3 associated with organic acids and aldehydes (Bevin et al., 2006; Boulet et al., 2007; Edelmann et al., 2001; Moreira & Santos, 2004; Patz et al., 2004). The PCA score plot (see Fig. 3) and eigenvectors for the MIR of the white wines analysed showed similar separation between ORG and NORG compared to those observed in the set of red wines.

3.3. Discrimination

Table 2 shows the classification results obtained by LDA applied to both red and white wines analysed separately. The results indicated that LDA models developed classify correctly 74% of red wine and 80% of the white wine samples belonging to ORG and NORG systems, respectively. Table 3 shows the DPLS classification results for both red and white wines. The correct classification rates obtained were 93% and 81% for white and red wines samples, respectively. The use of DPLS as a classification model gave better results compared with those obtained using LDA based on the PCA scores. It is important to note that wine samples used were commercially available bottles of wine and could not be completely verified in terms of their authenticity other than the claim made on the label by the producer. The Australian wine industry regulations stated that the manufacture and labelling of organic wine, whether relating to vineyard practices and/or manufacturing practices, is not specifically regulated in wine law (http://www.wineaustralia.com/australia) (Wine Australia, 2008). However, the certification for organic claims can be made by any of the many private organisations that perform this function. These organic organisations are authorised by the Australian Quarantine and Inspection Service (AQIS), primarily for the purpose of export certification of agricultural products, while additional overseas regulations, such as the USDA NOP (National Organic Program) which directly accredits such organisations as *Australian Certified Organic Pty Ltd* and other private and government certifiers, have in the main equivalent standards and certification requirements (albeit in the case of the USDA NOP no SO_2 is allowed in organic wines). It is therefore possible also that some of the variation in organic products might be related to the winemaking techniques used (e.g. less SO_2 addition) as well as different agronomic management practices at the vineyard that might explain the classification rates obtained, specially for the set of red wine samples. The highest correct classification rate was obtained with the set of white wines. This might be explained by differences in production methods e.g. the greater degree of extraction during the production of red wines and use of oak, compared with white wine, and by more distinctive differences in chemical and sensory characteristics in the set of white wines analysed, which might define the differences observed in the MIR spectra.

The combination of MIR and chemometric techniques presented in this study allowed good discrimination between samples produced under the two production systems ORG and NORG. Spectral

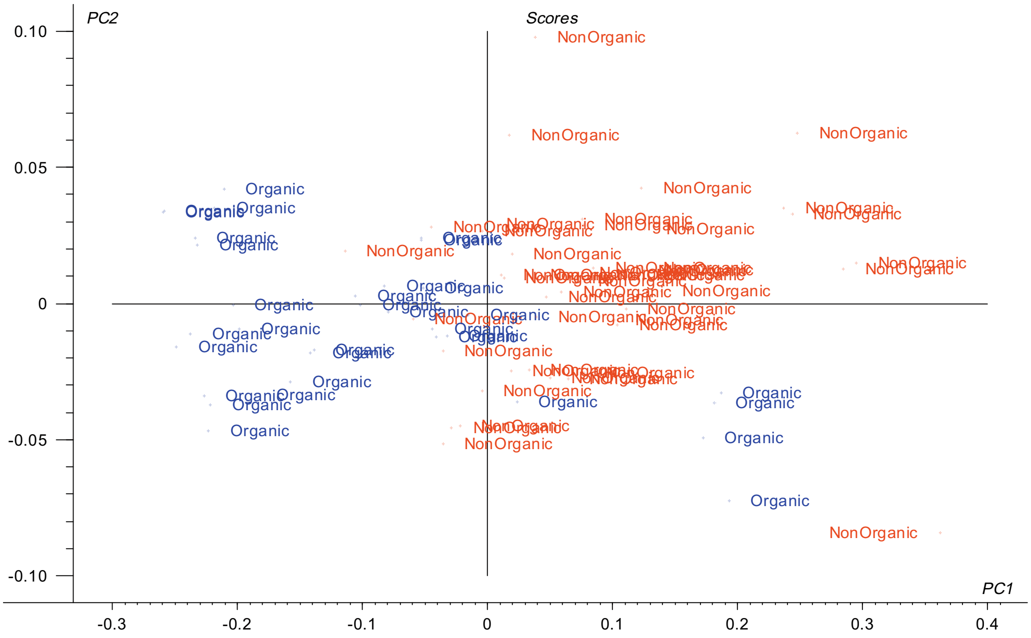

Fig. 1. Principal component score plot of red wine samples analysed using mid infrared spectroscopy, labelled as organic and non-organic.

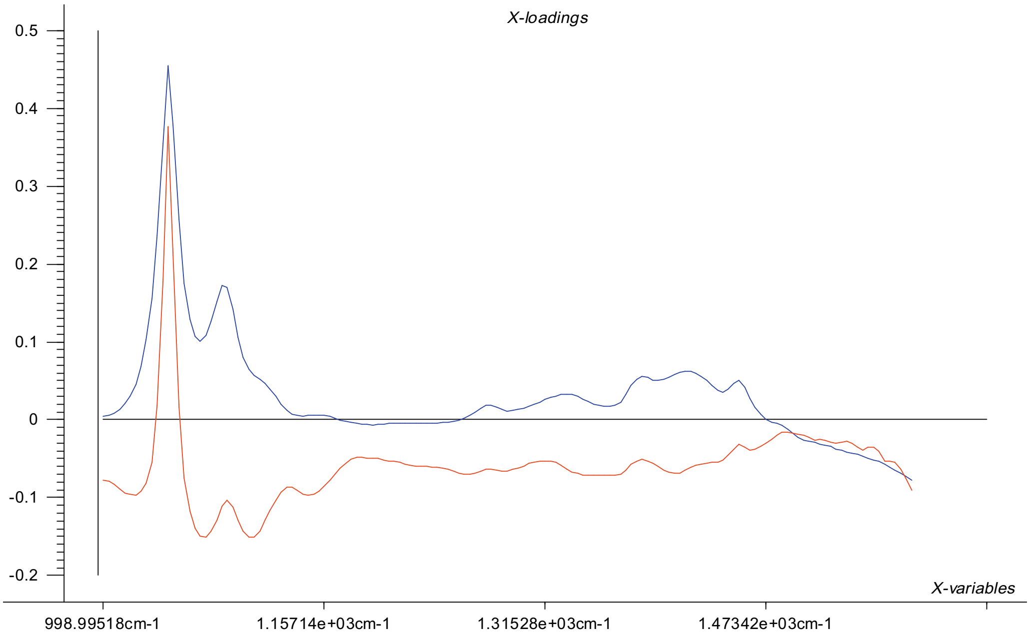

Fig. 2. Eigenvectors for the first two principal components of red wine samples analysed using mid infrared spectroscopy, labelled as organic and non-organic.

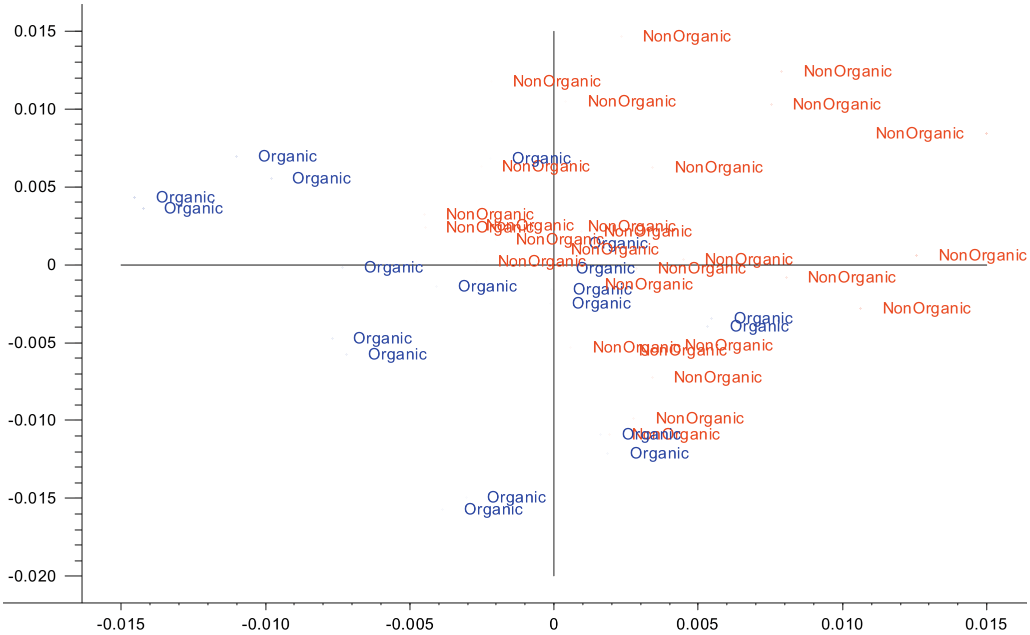

Fig. 3. Principal component score plot of white wine samples analysed using mid infrared spectroscopy, labelled as organic and non-organic.

Table 2
Linear discriminant classification rates of commercial red wine samples labelled as organic and non-organic.

	ORG	NORG
NORG	19 (28%)	49 (72%)
ORG	25 (75%)	8 (25%)

ORG: organic wines; NORG: non-organic wines. In brackets percent of correct classification.

Table 3
Discriminant partial least squares classification rates of commercial red and white wine samples labelled as organic and non-organic.

	White wines ($n = 47$)		Red wines ($n = 101$)	
	% Classification	Samples	% Classification	Samples
ORG	100%	21/21	73%	24/33
NORG	88%	23/26	85%	58/68

ORG: organic wines; NORG: non-organic wines, n = number of samples.

'signatures' in the MIR result from the fundamental stretching, bending, and rotating vibrations of the sample molecules. The PLS loadings used for the discrimination model showed that there was no particular individual chemical parameter that explained the separation between wines, rather many chemical compounds could contribute to the discrimination between wines including phenolic compounds, volatile compounds or even non-volatile compounds related with sensory attributes. The ability of the MIR model to discriminate or identify wine samples is based on the vibrational responses of chemical bonds to MIR radiation. It is possible that the higher the variability between sample-types in those chemical entities, which respond in these regions of the spectrum, the better the accuracy of the model. This implies that it is not simply a few specific compounds, but rather the holistic compositional characteristics of the wine matrix provide the necessary information for discrimination among wines. Chemometric analyses together with spectroscopic methods are a very powerful tool for distinguishing groups of objects that have very similar properties, but have consistent overall differences.

In general, supervised classification techniques (e.g. DPLS and LDA) are used to test similar known authentic samples. This study has shown that the classification techniques applied were able to extract useful information from the MIR spectra to differentiate wines produced under organic and non-organic systems. Considering the difficulties associated with the conventional methods used to discriminate between organic and non-organic wines, this study demonstrated the potential of MIR spectroscopy as a rapid tool to be used in the wine industry.

4. Conclusions

Overall, the results of this study suggest that spectroscopic techniques might offer the possibility to analyse or trace wine for authenticity in regards to the system of production without the need for costly and laborious chemical and sensory analysis. However, in developing such models, it is known that the more properties (variables) used for classification, the more objects (samples) are needed to get a robust model. A MIR spectrum contains information about the chemical composition and physical state of the material under analysis, yielding structural information that constitutes the fingerprint of a sample. This holistic way of analysing and interpreting the information in the wine matrix using both spectroscopy and chemometrics may assist determine a paradigm shift in the way we produce wine as well as methods used to authenticate and trace the origin of wine. We believe that in the future a combination of rapid methods based on spectroscopy and chemometrics will be available in order to analyse wines to determine their origin with regard to production methods.

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References

Arhurst, P. R., & Dennis, M. J. (1996). *Food authentication*. London: Chapmann-Hall.

Bacchus Multispec System. (2007). *Users manual* (p. 74). Microdom, Divison Cetim.

Berrueta, L. A., Alonso-Salces, R. M., & Herberger, K. (2007). Supervised pattern recognition in food analysis. *Journal of Chromatography A, 1158*, 196–214.

Bevin, C. J., Fergusson, A. J., Perry, W. B., Janik, L. J., & Cozzolino, D. (2006). Development of a rapid "fingerprinting" system for wine authenticity by mid-infrared spectroscopy. *Journal of Agricultural and Food Chemistry, 54*, 9713–9718.

Boulet, J. C., Williams, P., & Doco, T. (2007). A Fourier transform infrared spectroscopy study of wine polysaccharides. *Carbohydrate Polymers, 69*, 79–85.

Cordella, Ch., Moussa, I., Martel, A.-C., Sbirrazzuoli, N., & Lizzani-Cuvelier, L. (2002). Recent developments in food characterisation and adulteration detection: Technique-oriented perspectives. *Journal of Agricultural and Food Chemistry, 50*, 1751–1764.

Cozzolino, D., Smyth, H. E., & Gishen, M. (2003). Feasibility study on the use of visible and near infrared spectroscopy to discriminate between white wine of different varietal origin. *Journal of Agricultural and Food Chemistry, 52*, 7701–7711.

Downey, G. (1998). Food and food ingredient authentication by mid-infrared spectroscopy and chemometrics. *Trends Analytical Chemistry, 17*, 418–424.

Edelmann, A., Diewok, J., Schuster, K. Ch., & Lendl, B. (2001). Rapid method for the discrimination of red wine cultivars based on mid-infrared spectroscopy of phenolic wine extracts. *Journal of Agricultural and Food Chemistry, 49*, 1139–1145.

Godden, P., & Gishen, M. (2005). Trends in the composition of Australian wine. *Australian New Zealand Wine Industry Journal, 20*, 21–46.

Iland, P., Ewart, A., Sitters, J., Markides, A., & Bruer, N. (2000). *Techniques for chemical analysis and stability test of grapes juice and wine*. Adelaide, Australia: Patrick Il and Wine Promotions.

Katsvairo, T. W., Wright, D. L., Marois, J. J., & Rich, J. R. (2007). Transition from conventional farming to organic farming using Bahia grass. *Journal of the Science of Food and Agriculture, 87*, 2751–2756.

Kelly, H. R. C., Browning, H. M., Day, J. E. L., Martins, A., Pearce, G. P., Stopes, C., et al. (2007). Effect of breed type, housing and feeding system on performance of growing pigs managed under organic conditions. *Journal of the Science of Food and Agriculture, 87*, 2794–2800.

Kelly, J. F. D., & Downey, G. (2005). Detection of adulterants in apple juice using Fourier transform infrared spectroscopy and chemometrics. *Journal of Agricultural and Food Chemistry, 53*, 3281–3286.

Martin, R. C., Lynch, D. H., & Frick, B. (2007). Phosphorus status on Canadian organic farms. *Journal of the Science of Food and Agriculture, 87*, 2737–2740.

Moreira, J. L., & Santos, L. (2004). Spectroscopic interferences in Fourier transform infrared wine analysis. *Analytica Chimica Acta, 513*, 263–268.

Munck, L., Norgaard, L., Engelsen, S. B., Bro, R., & Andersson, C. A. (1998). Chemometrics in food science: A demonstration of the feasibility of a highly exploratory, inductive evaluation strategy of fundamental scientific significance. *Chemometrics and Intelligent Laboratory Systems, 44*, 31–60.

Naes, T., Isaksson, T., Fearn, T., & Davies, T. (2002). *A user-friendly guide to multivariate calibration and classification*. Chichester, UK: NIR Publications.

Otto, M. (1999). *Chemometrics: Statistics and computer application in analytical chemistry*. Chichester, UK: Wiley-VCH.

Patz, C.-D., Blieke, A., Ristow, R., & Dietrich, H. (2004). Application of FT-MIR spectrometry in wine analysis. *Analytica Chimica Acta, 513*, 81–89.

Rembiałkowska, E. (2007). Quality of plant products from organic agriculture. *Journal of the Science of Food and Agriculture, 87*, 2757–2762.

Schulzova, V., Hajṡlova, J., Botek, P., & Peroutka, R. (2007). Furanocoumarins in vegetables: Influence of farming system and other factors on levels of toxicants. *Journal of the Science of Food and Agriculture, 87*, 2763–2767.

Siebert, K. J. (2001). Chemometrics in brewing: A review. *Journal of the American Society of Brewing Chemists, 59*, 147–156.

Sivakesava, S., Irudayaraj, J., & Demirci, A. (2001). Monitoring a bioprocess for ethanol production using FT-MIR and FT–Raman spectroscopy. *Journal of Industrial Microbiology and Biotechnology, 16*, 185–190.

Spoolder, H. A. M. (2007). Animal welfare in organic farming systems. *Journal of the Science of Food and Agriculture, 87*, 2741–2746.

The Australian Wine and Brandy Corporation (2008). Wine law, making and labelling, organic wine. <http://www.wineaustralia.com/australia> via the INTERENT. Accessed September 2008.

The National Organic Program (2008). USDA. <www.ams.usda.gov/nop/>. Accessed September 2008.

Topp, C. F. E., Stockdale, E. A., Watson, Ch. A., & Rees, R. M. (2007). Estimating resource use efficiencies in organic agriculture: A review of budgeting approaches used. *Journal of the Science of Food and Agriculture, 87*, 2782–2790.

Watson, C. A., Walker, R. L., & Stockdale, E. A. (2008). Research in organic production systems – Past, present and future. *Journal of Agricultural Science (Camb), 146*, 1–19.

Weller, R. F., & Bowling, P. J. (2007). The importance of nutrient balance, cropping strategy and quality of dairy cow diets in sustainable organic systems. *Journal of the Science of Food and Agriculture, 87*, 2768–2773.

Wine Australia (2008). <http://www.wineaustralia.com/Australia>. Accessed September 2008.

Zollitsch, W. (2007). Challenges in the nutrition of organic pigs. *Journal of the Science of Food and Agriculture, 87*, 2747–2750.