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The Use of Fourier Transform Infrared (FTIR) Spectroscopy and Artificial Neural Networks (ANNs) to Assess Wine Quality

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Abstract

The aim of this study was to develop a simple method to assess wine quality from its Fourier Transform Infrared Spectroscopy (FTIR) spectrum with minimal or no sample preparation. FTIR spectral data of selected wine samples, grape variety, wine barrel type, wine type and production year were correlated with total phenolic content, total and volatile acidity and alcohol content using Artificial Neural Networks (ANNs). A total of 20 (2 whites and 18 reds) different wines used in this study came from three different states across Australia; New South Wales, Victoria and South Australia.

FTIR spectroscopy proved to be a promising technique that provides a rapid and accurate method in the quality assessment of wine. A plot of the values predicted by the validated ANN models showed excellent correlation with the experimentally measured values for acetic acid concentration, alcohol content, total phenols, and total acidity (r=0.898-0.942).

Keywords: Fourier transform infrared spectroscopy; Artificial neural network; Wine quality assessment; Polyphenolic content

Introduction

It is known that moderate consumption of red wine and fruit juices can reduce the risk for cardiovascular disease [1,2]. The protective effects of wine have been attributed to polyphenols that are efficient scavengers of free radicals and breakers of lipid peroxidative chain reactions [3]. One of the most fascinating observation is the 'French paradox', a term first coined by Dr. Serge Renauld, in the 1980s [4,5] that refers to the fact that French population have an incredible low coronary heart disease death rates despite high intake of dietary cholesterol and saturated fat [5,6]. Numerous studies have documented the health benefits of red wine consumption, including anti-oxidative, anti-carcinogenic, anti-inflammatory, anti-cardiovascular and antibacterial properties [7,8]. Grapes and wines are rich in a large number of polyphenolic compounds, belonging to non-flavonoids, flavonoids and phenolic-protein-polysaccharide complexes, which possess high antioxidant activity and are believed to reduce and prevent oxidative stress related diseases [9,10], as well as several organic acids, such as tartaric and malic acids, which have antimicrobial effects, especially at the low pH of wine [11,12]. The amount of these potentially beneficial compounds present in red wine usually varies depending on the variety of grapes and the vinification process used [13]. The major polyphenolic compounds found in red wine contribute significantly to the major organoleptic qualities such as mouth feel, taste and colour, and therefore play an important role in the overall quality of the wine. It is believed that wine quality variations are related to both the origin (structure related) and quantity (concentration factor) of the polyphenols present in red wine [14]. Polyphenolics in wine are responsible for varietal and flavor characteristics of red wines such as color and tannin characteristics [15]. Phenolics also affect the sensory characteristics of wines, contributing to bitterness and astringency. Astringency and bitterness are produced primarily by flavonoids that are extracted from the skins and seeds of grapes. The types and amounts of different phenolic compounds present have been used as broad indicators of wine quality and good correlation has been obtained between several aspects of the phenolic content and assessed quality of red wine [16]. However, it would be useful to be able to perform a single measurement that is representative of a wine's composition universally correlates with perceived wine quality [17].

The development of more effective and efficient methods to assess grape and wine quality is important to the wine industry. It is desirable that these methods require minimal sample preparation and are able to produce rapid results, preferably providing information on multiple parameters simultaneously. The aim of this study was to develop a simple and rapid method based on Fourier Transform Infrared Spectroscopy (FTIR) spectroscopy combined with Artificial Neural Network data modeling to assess multiple quality indicators of wine samples. Using non-destructive FTIR spectroscopy with a horizontal Attenuated Total Reflectance (ATR) accessory enables wine spectra to be obtained using relatively small volumes (around 0.5 to 1 mL) of wine with minimal sample preparation and reagent consumption. Mid-IR spectrometry has been previously used in the analysis of a number of foods, including wine. The typical analytes that have been measured using this technique are ethanol, pH, organic acids, sugars and glycerol [17,18]. However, only a few studies have applied FTIR to analyse selected polyphenolic compounds such as tannins [19] and anthocyanins [20] and total antioxidant capacity [21]. The use of Artificial Neural Networks (ANNs), as a non-linear statistical data modelling tool was chosen to correlate FTIR spectra of selected wine samples, grape variety, wine barrel (oak type), wine type (red or white) and production year with total phenolic content, total and volatile acidity, and alcohol content. The aim of this modelling was to: (1) successfully predict the overall polyphenolic content of a wine sample from its FTIR spectra; and (2) to determine how parameters such as alcohol content, pH etc. affect the overall polyphenolic content in red wine. This type of information should enable winemakers to better optimise the concentration of polyphenolic compounds during the winemaking process.

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Wine sample	State/ Region	Type of Wine	Varietal Composition	Year	Alcohol Content	Type of Oak
1	VIC	Red	Cabernet	2008	14.5%	French
2	VIC	Red	Cabernet	2006	14%	French
3	VIC	Red	Cab. Sauvignon 31% Merlot 30% Cab. Franc 24% Malbec 15%	2008	15.6%	
4	VIC	Red	Shiraz Cabernet	2007	15%	French
5	VIC	Red	Shiraz	2008	15%	French
6	NSW	Red	Shiraz	2008	13.0%	Unknown
7	VIC	White	Semillon	2009	14%	French
8	VIC	Red	Cab. Sauvignon 60% Cab. Franc 20% Merlot 20%	2008	13.3%	French
9	VIC	White	Riesling	2008	13%	Tank
10	VIC	Red	Shiraz	2008	14%	American
11	SA	Red	Shiraz	2009	14%	French American
12	SA	Red	Shiraz 96% Petit Verdot 4%	2008	14%	American
13	VIC	Red	Shiraz	2010	13.5%	French
14	VIC/ SA	Red	Shiraz Merlot	2009	13.5%	Unknown
15	VIC	Red	Cabernet Merlot	2009		
16	VIC	Red	Shiraz	2010	15%	American
17	VIC	Red	Shiraz	2001	15%	American
18	VIC	Red	Shiraz	2008	15.5%	American
19	VIC	Red	Shiraz 87% Malbec 7% Cab. Sauvignon 6%	2008	14%	American
20	VIC	Red	Cabernet Sauvignon	2008	15%	American

Table 1: Wine samples collected from different wineries with the information found on the label.

Materials and Methods

Wine samples

A total of 20 different wines (2 whites and 18 reds) used in this study came from three different states across Australia; New South Wales, Victoria and South Australia. The wines selected varied considerably in regards to growing region, varietal composition, year, alcohol content and type of oak used (Table 1).

Total acidity

Total acidity (free acid) or titratable acidity of the wine samples, expressed the concentration of tartaric acid (g/L), was determined titrimetrically using a standard 0.05M NaOH solution with phenolphthalein indicator. The color of the end point was a stable gray for red wines and a faint pink color for white wines. In order to overcome the problem associated of the intense color of the red wines, samples were diluted by 1 in 75 using distilled water prior to the titration. All pH and total acidity determinations were replicated twice.

Volatile acidity

The volatile acidity, expressed as the amount of acetic acid (g/L) was determined spectrophotometrically using an enzymatic analysis kit (Vint essential Laboratories, Dromana, Australia) with absorbance measurements made at a wavelength of 340 nm.

Total phenolic content

The total phenolic concentration was determined according to the Folin-Ciocalteu colorimetric method. Samples of wine (1.00 mL) were first diluted with distilled water (4.00 mL). An aliquot (0.200 mL) of the diluted wine sample and 1.00 mL of Folin-Ciocalteu reagent (Sigma Chemicals Co, St. Louis, MO, USA), were added to a 20.00 mL volumetric flask. Exactly after 1 minute, 4.00 mL of sodium carbonate (20% w/v) (Merck, Vic, Australia) was added and the solution was made to a total volume of 20.00 mL using distilled water. Finally, the mixture was allowed to stand at room temperature in the dark for 30 min after which time the absorbance of the solution was measured at 750 nm. The total polyphenolic concentration was calculated from a calibration curve using Gallic acid (Aldrich Chemical Co (WI, USA) as a standard (1-6 mg/L). Polyphenolic concentration was expressed in grams of Gallic acid equivalents per Litre (GAE g/L) and was an average of three measurements.

Apparatus

UV-Vis spectra were collected using a UV-Vis single beam spectrometer (Mini 1240, Shimadzu). The IR spectra of the wine samples were examined over the range of 400-4000 cm^{-1} using a Bruker Equinox 55 FT-IR spectrometer equipped with a horizontal Attenuated Total Reflectance (ATR) device with a diamond crystal. Spectra were recorded using OPUS software (Bruker Optik, Germany) by averaging 100 scans for each spectrum with resolutions of 2 cm^{-1}. Background spectra were obtained and subtracted from each sample IR spectra. Small quantities of untreated wine samples were smeared directly onto the ATR diamond crystal using a disposable pipette. In order to avoid the strong interference due to the presence of water and alcohol in each wine sample, a 12% (v/v) ethanol spectrum was subtracted from each wine spectrum. Statistical Neural Networks 4.0 F (Stat Soft Inc., Tulsa,

OK, USA) was used to model the spectral data and develop predictive ANN models.

Pre-processing the FTIR data

The FTIR spectra were sampled between 400-4000 cm^{-1} and obtained spectra were smoothed to reduce the noise and improve signal-to noise ratio using the Moving Average method by counting the average of 20 data sets in every tenth wavelength record [22]. Significant signal noise in the spectra would degrade the signal-to-noise ratio and resolution of the spectra and hence reduce the accuracy and precision of a model. The resulting 185 spectral intensities for each wine sample, together with variety of grape, wine barrel (oak type), and production year were used as ANN inputs and experimentally measured total phenolic content, total acidity, volatile acidity (acetic acid) and pH, were used as ANN outputs.

Artificial Neural networks (ANNs)

ANNs are biologically inspired computational model designed to simulate the way in which the human brain processes information [23]. ANNs are composed of many individual processing units or artificial neurons which are extensively inter-connected with connection weights to form a network. They collect their knowledge by detecting patterns and relationships in inputs and outputs and learn from experience from previously seen data, rather than using a pre-designed equation in a model. Artificial neurons are typically organized into an input layer, one or more hidden layers, and an output (prediction) layer. They function by linking the input neurons (i.e. spectral intensity/peak) to output neurons (i.e. measured wine quality indicator), through a set of connections with adjustable strengths (weights). The standard supervised network architectures (multilayer perceptrons and radial basis functions) are models in which connection weights and the number of hidden neurons are adjustable parameters that are optimized during the learning phase. This is performed using the training and validation sets of compounds. Training is performed iteratively, such that as it progresses, the ANN will generate a more accurate output and establish a (linear or non-linear) relationship between input and output data. Following this, true predictive ability of the model can be tested using an independent set of compounds, validation set.

Network training and design

Averaged spectral intensities, variety of grape (Merlot, Cabernet, Shiraz, Semillon, Sauvignon, Malbec), wine barrel (French oak, American oak, mixed, tank, unknown), and production year were used as inputs and calculated concentrations of total phenols expressed as Gallic Acid Equivalents (GAE g/L), acetic acid concentration (g/L), total acidity (g/L), alcohol content (%), and measured pH were used as outputs in the ANN. The most straightforward approach was used to build the ANN model. Input/output data sets were were automatically randomised into training (60%), testing (20%) and validation (20%) subsets. The training set was used for learning and to fit network parameters (weights) while the testing set was used to optimise the network topology and avoid over fitting. An external validation subset was used to assess the predictive performance (generalization) of a developed neural network. The extent of training was monitored internally by the ANN program and was stopped when the Root Mean Square (RMS) error failed to improve during training cycles and the testing RMS error started to increase. A number of networks with different topologies were trained and tested to determine the optimum topology for the dopamine receptor data and back-propagation Multi Layer Perceptron (MLP) with one hidden layer selected due to its superiority in network performance. In contrast to linear statistical techniques, there is no known method for the automatic determination of an optimal network structure to fit a specific dataset [24]. As a result, training algorithms are run a number of times through automated network searches so that the best networks could be selected.

Selection of input variables

Sensitivity analysis was used for feature selection to identify the most important inputs that are directly correlated to total phenolic content, total and volatile acidity, pH and alcohol content by determining how sensitive a model is to changes in the input values. Sensitivity is defined as the ratio of error of a retrained optimum model, which does not contain the information of a specific molecular descriptor, to the error of the optimum model that includes the information from the molecular descriptor [25]. Since ANNs compute the output as a sum of nonlinear transformations of linear combinations of the inputs, sensitivity shows the percentage contribution of a corresponding input to the output value and reveals the effect that a change in that particular input has on output. Hence, inputs with low sensitivity are considered to only have a minimal contribution to the model being analysed so were eliminated from subsequent models.

Molecular descriptors with sensitivities less than one were sequentially removed after each automated network run until models that contained only molecular descriptors of relative importance for assessed wine quality parameters were developed.

Results and Discussion

Fourier Transform Infrared (FT-IR) spectroscopy is a non-destructive analytical technique that provides structural information on molecular features of a large range of compounds.

In general, no two wine samples will show exactly the same IR absorption pattern, thus a fingerprint IR spectrum can be produced, unique to each wine. However, the common constituents of wine (i.e. water and ethanol) produce IR absorption bands, which may disguise the characteristic IR vibrations of phenols. This is due to the fact that water, ethanol and organic acids absorb in the same MIR region as phenols. To eliminate this interference, a 12% (v/v) ethanol in water spectrum was subtracted from the wine spectra used in this work.

All wine samples including those from the Bendigo region, NSW and wines ranging over different vintages gave rise to similar spectra patterns (Figure 1). Several absorption bands were identified including those within the region between 800-1750 cm^{-1} which are categorized as C=C-C aromatic ring stretches (1580-1615 cm^{-1}; 1450-1510 cm^{-1}) while IR bands in the area from 820 to 760 cm^{-1} can be attributed

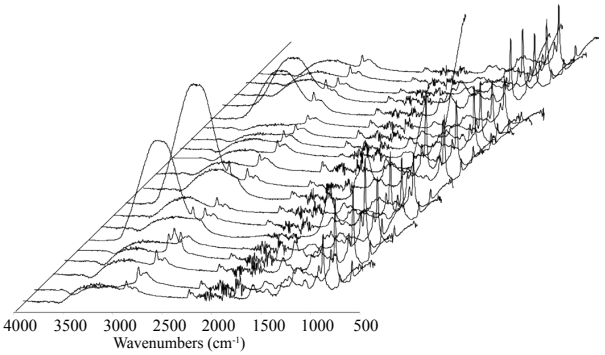

Figure 1: FT-MIR spectra for the different wines investigated.

Wine sample	Total acidity (g/L)	pH (±0.3)	Calculated acetic acid concentration (g/L)	Total phenols (GAE* g/L)
1	6.75	3.51	0.13	2.65
2	5.24	3.74	0.27	2.31
3	6.73	3.38	0.37	2.53
4	4.49	3.67	0.72	1.83
5	6.18	3.66	1.65	1.70
6	5.99	3.53	1.12	1.21
7	6.83	3.54	0.24	0.17
8	7.02	3.25	0.31	2.18
9	6.56	3.45	0.15	0.36
10	5.68	3.55	0.52	1.52
11	5.83	3.46	0.15	1.65
12	5.49	3.50	1.12	1.75
13	4.45	3.88	1.17	1.44
14	6.31	3.41	0.21	1.65
15	6.18	3.38	0.27	1.52
16	5.2	3.77	1.54	2.42
17	7.04	3.69	1.44	2.46
18	5.93	3.38	1.3	2.15
19	6.37	3.42	0.02	1.98
20	4.63	3.48	0.52	3.06

*GAE=Gallic acid equivalent

Table 2: Total acidity, pH, acetic acid concentration, and total phenolic concentration for the wine samples.

	Correlation				
	Training	Testing	Validation	Average	ANN architecture*
Acetic acid concentration (g/L)	0.960	0.998	0.924	0.898	25-1-1
Alcohol content	0.914	0.990	0.789	0.942	147-1-1
Total polyphenols (g/L GAE)	0.999	0.904	0.925	0.918	186-5-1
Total acidity (g/L)	0.954	0.999	0.801	0.898	143-1-1

*Number of inputs-*hidden neurones*-number of outputs

Table 3: Correlation data for the four developed ANN models.

to ring vibrations [26]. Furthermore, peaks between 670-900 cm^{-1} can be attributed to aromatic C-H out of plane (750-1000 cm^{-1}) and in plane bending (950-1225 cm^{-1}) [27]. The IR regions of significant importance to this study were from 1542 to 965 cm^{-1}, usually referred to as the "fingerprint" region and various IR bands, including those corresponding to the vibration of the C-O, C-C, C-H and C-N bonds, occurs in this region [1,28]. This area provides important information regarding organic compounds such as sugars, alcohols and organic acids present in the sample. The distinct absorbance peaks in the wave number regions 3626-2970 cm^{-1} and 1716-1543 cm^{-1}, are the result of the absorbance of water [29]. Other absorption bands of interest involved those at 1044 and 1085 cm^{-1}, which are indicative of an alcohol functional group. The 1382 cm^{-1} absorption band attributes to the O-H in plane deformation in polyphenols [3]. The cyclic nature of the ether was reflected by the peaks located at 1283-1247cm^{-1} range and as well at 1158 cm^{-1}, which was produced by the aromatic C-O bond stretching. The 1739 cm^{-1} absorption band may attributed to the carbonyl group, C=O of the galloyl unit on epicatechin gallate. The deformation vibration of the carbon–carbon bonds in the phenolic groups adsorb in the region of 1500-1400 cm^{-1} [2].

IR absorption due to the presence of sugar functional groups are within the range of 1200 and 950 cm^{-1}, more specifically the peaks observed at 1157, 1107, 1065, 1014 cm^{-1}. The stretch vibration of the C=O group is around 1700 cm^{-1}. Peaks at 1618 and 1407 cm^{-1} corresponds to symmetrical and asymmetrical stretching vibration for the carboxyl ion (COO-) indicating the existence of carboxylic acid, ester, or carbonyl groups [29]. Peaks located in the region of 1450 to 1410 cm^{-1} originate from symmetric stretching vibration of C-O, and those around 1500 cm^{-1} can be assigned to C-C stretching in rings [1].

In the same way, the IR peaks that are observed at approximately 1300 and 1150 cm^{-1} can be assigned to S=O stretch present in a sulfate group Peaks at 1350 and 1175 cm^{-1} for sulfonates and peaks in the area between 1000-750 cm^{-1} can be assigned to S-O [30].

Liquid sulfur dioxide shows IR bands at 530, 1142 and 1330 cm^{-1}. For the sulphuryl compounds such as sulphuric acid, the SO2 bend is in the range 500-600 cm^{-1} [31]. The IR absorbance at 1000 cm^{-1} may indicates the existence of PO_4^{3-} [32]. The presence of polyphenolic compounds is indicated by characteristic bands for gallic acid at 669, 763, 1025, 1100 and 1654 cm^{-1}, tannic acid at 669, 860, 1172, 1511 and 1627 cm^{-1} [4] (Figure 1).

Four separate nonlinear ANN models were developed to correlate the recorded IR spectra, variety of grape, wine barrel type and production year, with the experimentally measured total polyphenolic content, total and volatile acidity, pH and alcohol content in the selected wine samples (Table 2). The strength of the correlation between experimental and predicted data was assessed by the accuracy of the predicted values for the validation data sets (Table 3).

The developed ANN models confirmed that the characteristic IR bands, grape variety, year, wine type and wine barrel used for aging are directly related to the experimentally determined amounts of phenols, total acidity, acetic acid content, pH, and alcohol content. Good linearity (Table 3) of the ATR-FTIR method indicates that this technique can be used to assess the quality of wine accurately and specifically.

Total polyphenols

Not surprisingly the ANN model for the polyphenolic content (186-5-1) has the wine barrel as the most important input contribution in addition to the peaks at 1870,3740 2200 and 530-550 due to alcohol, carbonyl, alkyne, nitriles and vibrations in the fingerprint region. The composition of untreated wine samples consists of a complex mixture of various phenolic compounds and hence classification of each specific phenol by their spectral peak profile can be difficult. Never the less, the distinctive absorption bands within the finger print region of the IR spectrum can be assigned to particular functional groups present in several phenolic compounds. Moreover, wine consists of several components including water (80 to 90 percent), alcohol, sugar, carboxylic acids, tannins, polyphenols, amino acids, vitamin C, inorganic components and numerous fragrance ingredients [3]. Therefore, acknowledging their characteristic IR functional group absorption peak(s) can assist in more accurate determination of the phenolic compounds present.

Red wines are rich in polyphenolic substances, mainly flavanols and anthocyanins, which contribute to the sensory properties of wines, such as colour, taste, astringency and flavour [15,33]. Furthermore, the total phenol content directly correlates with the antioxidant activity of wines [34]. The antioxidant activity of wine polyphenols has been demonstrated in many studies and they act via various chemical pathways (i.e. as free radical terminators, singlet oxygen quenchers, and chelators of metal ions) [35,36]. Inhibition of low-density lipoprotein oxidation, inhibition of platelet aggregation, and anti-

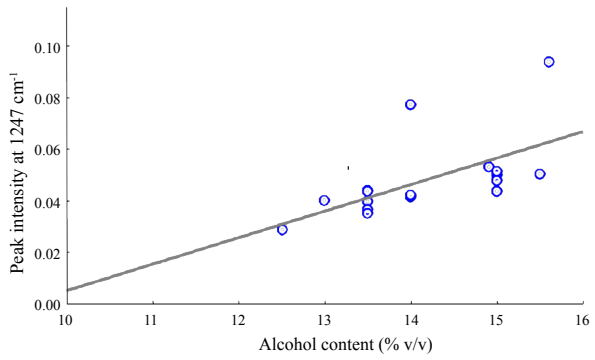

Figure 2: IR absorption at the characteristic peak for flavonoids at 1247 cm-1 (C-O stretching frequency of esters), versus alcohol content.

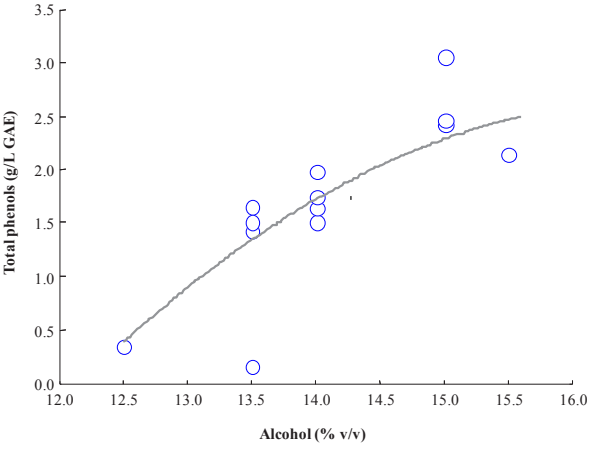

Figure 3: Plot of total phenolic content (GAE g/L) versus alcohol concentration (% v/v).

inflammatory properties by red wine phenolics [9,37] may be a major factor contributing to the health benefits of red wine and the decreased risk of cardiovascular diseases despite a high-fat diet in certain French populations [38]. The main component in red wine that is believed to provide cardiovascular protection is resveratrol (3,4,5-trihydroxy-trans-stilbene) and together with polyphenols are produced exclusively in the vine leaf epidermis and grape skin [39]. The resveratrol content of wine is related to the length of time the grape skins are present during the fermentation process. It was found that the synergistic contribution due to the presence of both phenolic and polyphenolic compounds, might be more important for antioxidant activity than any specific phenolic concentration [40]. Polyphenolic compounds are found in the skin and seeds of grapes. Red wines have a considerably higher amount of polyphenolic compounds than white wines, because the skins are removed earlier during white-wine production, lessening the amount that is extracted [6]. When wine is made, the alcohol produced by the fermentation process dissolves the polyphenols contained in the skin and seeds.

Based on Raman measurements of pure flavones and related compounds, the intense band at 1247 cm^{-1} can be attributed to flavonoid-type compounds [41]. The intense band at 1247 cm^{-1}, that could be assigned to bending of the OH groups coupled to C-H in plane bend in flavonoids, correlates well with the alcohol content found in a wine sample (Figure 2), but does not relate with total polyphenolic content, acidity and pH [16].

Furthermore, the total phenolic content increases as the alcohol content of the wine increases (Figure 3). Optimal antioxidant activity is achieved at an alcohol concentration of approximately 15% v/v. Above this range, however, antioxidant activity declines. Results show that the high alcohol content wines in the group (wine samples 4, 5, 16, 17, and 20), also have the highest overall antioxidant concentrations. However, wine sample 8, with a much lower alcohol content of 13.2% v/v had the highest antioxidant concentration of all of the wines in the group. This result however, may not be reliable as it was an outlier from the general trend and may have been subject to experimental error. Further testing of a wider range of wine samples with varying alcohol content is needed in order to confirm the reliability of this correlation. Moreover, the true antioxidant activity of a wine sample in vivo cannot be accurately determined unless physiological testing is also performed (Figure 3).

The phenolic composition of red wine depends on many factors, such as grape variety, wine maturation and ageing. During wine maturation and aging the polyphenolic components extracted from the grapes undergo various reactions, the most important being condensation reactions between anthocyanins and flavonoids, and reactions involving other wine components, such as acetaldehyde, pyruvate, hydroxycinnamic acids and vinyl phenols, which give rise to more stable oligomeric or polymeric pigments [42,43]. The rate and extent of these reactions is influenced by various parameters, including the initial concentration of reactant species, pH of the medium, temperature, ageing conditions, oxygen availability and the concentration of antioxidant agents such as sulphites [44-46]. It is also known that the effect of the type of wine barrel used is a contributing factor to the total phenolic content of red wine (Figure 4).

The aging of wines in wine barrels can potentially provide a modest contribution to the phenolic content of wine. The oak barrels contain gallotanins and ellagitannins which leach into the wine from the barrels resulting in an increase in the total tannin levels found in the wine [47]. For this reason, we need to consider the type of oak barrel used in wine storage as an important factor in determining the overall level of total phenolic compounds found in wine. Results indicate that variations in wine barrel oak type (i.e. French or American oak) will affect the amount of tannins extracted into the wine. (Figure 4) indicates that French oak based wines achieved a small yet higher content of total polyphenols than American oak based wines. According to studies, French oak contains an overall higher concentration of tannins than American oak [48]. For this reason several wineries opt for more

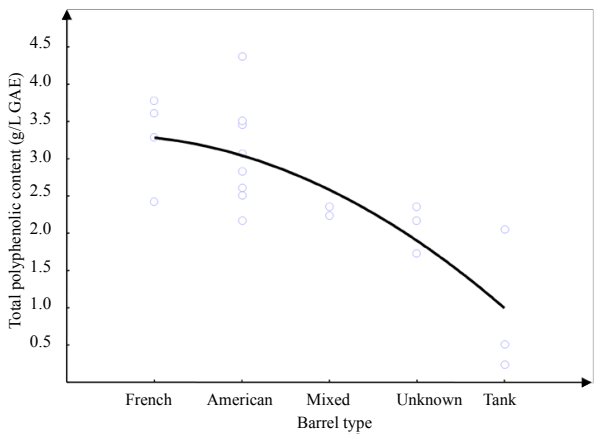

Figure 4: Total polyphenolic content as a function of wine barrel.

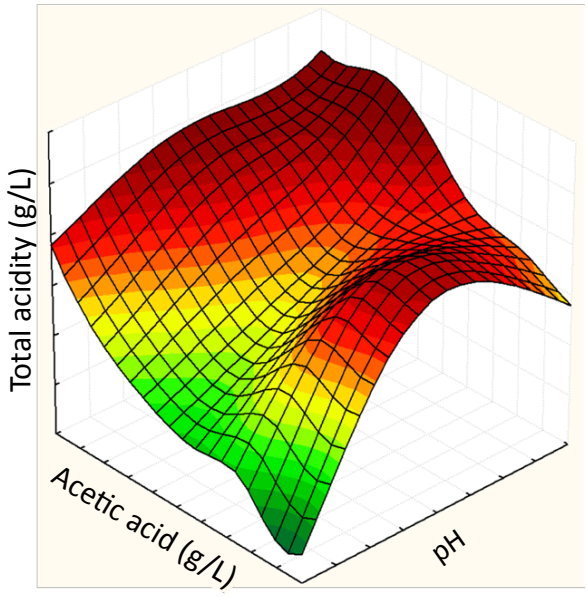

Figure 5: The relationship between acetic acid concentration (volatile acidity), pH and total acidity.

expensive French oak in order to help release phenols and tannins into the wine in order to improve flavor and structure. Wines aged in French and American oak both showed higher absorbance readings than wines that have been stored in fermenting tanks.

As expected, our results show that the total phenolic content was higher in the red wine samples. Furthermore, despite a spread in the amounts of the phenolic content present in the different wine varieties and vineyards, cabernet sauvignon wines had higher levels of phenolics compared to merlot wines.

Acetic acid content (Volatile acidity)

The ANN model correlating IR spectral bends with acetic acid content was the simplest with only 25 input descriptors, mostly related to carbonyl stretching vibration between 1650-1750 cm^{-1} and peaks in the fingerprint region (500-550 cm^{-1}) in addition to wine variety (red wine versus white wine) and type of the wine barrel used for aging. Volatile acids consist of a number of low molecular weight fatty acids [49] with acetic acid as the predominant acid. High levels of acetic acid are indicative of spoilage, mostly from *Acetobacter*. A small amount of acetic acid is normal, but should not exceed 0.3 g/L. Higher levels of acetic acid; especially 0.6-0.9 g/L is enough to provide a sensory indication of spoilage. At the level of 1.5 g/L the wine has essentially turned into vinegar. It can be seen from the 3D surface plot (Figure 1), that there is a correlation between pH, total acidity and acetic acid content, presenting optimal aspects for wine quality. As discussed earlier, in order to have a better quality wine, ideally the wine would have a higher content of total acidity and a lower acetic acid concentration. This can be seen at a pH of approximately 3.6 where spoilage is at a low (acetic acid < 0.75 g/L) yet total acidity is not affected. Therefore according to (Figure 1), a pH of 3.6 and a total acidity of 7.0 g/L would optimise wine quality in relation to these parameters. It is important to note the wines with a pH and total acidity in this region also have higher a phenolic content (and antioxidant capacity) than the other wines investigated. At higher pH levels, acetic acid content becomes more significant thereby affecting wine quality.

Titratable or total acidity

The most important descriptors that were included in the model for the total acidity were bends attributed the broad stretch of carboxylic acid O-H (2500-3000 cm^{-1}), carbonyl group stretching vibrations (1670-1820 cm^{-1}), mono substituted benzene ring bend (strong band at 690 cm^{-1}), nitrile (2210-2260 cm^{-1}), wine type and wine barrel (oak type). The total acidity of the wine is a measure of all types of acids present, i.e. inorganic acids, such as phosphoric acid, and organic acids where tartaric acid and malic acid are the predominate components, contributing to around 90% of the overall total acidity of the wine. There are also amino acids whose contribution to titratable acidity is not very well known. Often the total acidity is quantified simply as the measure of the total amount of tartaric acid present [49].

Organic acids (i.e. tartaric acid) make major contributions to the taste, feel and colour of the wine. More importantly their preservative properties also enhance wines microbiological and physicochemical stability. Red wines are stable at lower acidity, due to the presence of phenols which enhance acidity, balance the sweet taste of the alcohols and help to maintain stability throughout the aging process. Acidity also greatly influences the taste and colour of wine, and is important when assessing the general quality to the wine [50]. Grapes grown in warmer climates tend to have higher sugar content and lower acidity, compared with colder climate grapes. In general the total acidity of wine varies from 0.4% v/w to 1.0% w/v (4-10 g/L), but usually in red wines the acidity is from 0.6% w/v to 0.8% w/v (6–8 g/L). The measured total acidity of the wine samples in this work is within an acceptable range of 4.45 to 7.04 g/L. A total of 8 different wines had acetic acid levels greater than 0.5 g/L, with 2 of them above 1.5 g/L. A high level of spoilage is due to the concentration of acetic acid being anything above 1.5 g/L, but for good quality wine, the level should be less than 0.5 g/L [51].

Wine pH

As a wine quality parameter, pH is of equal or greater significance than the titratable total acidity. It affects wine colour, taste, oxidation-reduction potential, ratio of free to bound sulphur dioxide, and the extent of iron phosphate cloudiness present in the wine [52]. The pH and total acidity of a wine follows an inverse proportional relationship, where an increase in pH correlates to a lower total acidity. However, while there is a general relationship between total acidity and pH, these parameters are not directly related to each other. The association is regarded as an empirical relationship between the pH and the ratio of potassium bitartrate to total tartaric acid. Note that pH only measures the free hydrogen ions in solution, while titratable acidity measures the concentration of all of the available hydrogen ions, both those free in solution and those bound to undissociated acid molecules. Most of the wines samples in this study came from the central Victorian region, which is classified as a warm climate wine growing area. These wines are expected to have lower levels of acids and higher pH values, and are known for deep colour, low tannins and high levels of acid (which is unusual for a warm climate red grape). As the pH shifts to the lower end of the scale, the colour of the wine shifts to a more intense red form. With the taste of wine, pH values around 4.0 results in a flat taste while a pH of less than 3.0 results in a tart and sour taste. On average, the pH of red wine should not exceed 3.6 [53], with the optimum pH range for wine between 3.2-3.6. Although according to literature, there is no direct correlation proven between pH, volatile acidity and total acidity, there is a more general non-linear relationship between the total acidity, pH and volatile acidity, which can be seen in Figure 5 [54].

Conclusion

Parameters used to assess grape and wine quality such as total and volatile acidity, pH, alcohol, and antioxidant content were determined for each wine sample using conventional analytical techniques. Each of these parameters affects the overall quality of the wine, either contributing to taste, colour, protective and preservative properties, and potential health benefits.

The total acidity of the wines ranged from 4.45 to 7.04 g/L, pH from 3.25 to 3.88, acetic acid concentration from 0.02 to 1.54 g/L, and polyphenolic content from 0.17 to 3.06 g/L. Within the FTIR fingerprint region obtained for each wine sample, several absorption bands were identified as functional groups representative of those present in phenolic compounds. A plot of the values predicted by the validated ANN models showed excellent correlation with the experimentally measured values (r=0.898-0.942) for acetic acid concentration, alcohol content, total phenols, and total acidity. Experimental data collected from the wine samples showed that the phenolic content of the wines may be influenced by the type of oak used for aging the wines. Also there appeared to be a direct correlation between the alcohol content and the total phenolic content of the wines. FTIR spectroscopy proved to be a promising technique that provides a rapid method in the quality assessment of wine, for determining acetic acid concentration, alcohol content, total polyphenols, and total acidity. Moreover, this method uses small sample volumes (a few milliliters) with almost no sample preparation required.

References

1. Movasaghi Z, Rehman S, Rehman IU (2007) Raman spectroscopy of biological tissues. Applied Spectroscopy Reviews 42: 493-541.
2. Nakano Y, Takeshita K, Tsutsumi T (2001) Adsorption mechanism of hexavalent chromium by redox within condensed-tannin gel. Water Res 35: 496-500.
3. Ozacar M, Soykan C, Sengil IA (2006) Studies on synthesis, characterization, and metal adsorption of mimosa and valonia tannin resins. Journal of Applied Polymer Science 102: 786-797.
4. Kannan RRR, Rajasekaran A, Perumal A (2011) Fourier transform infrared spectroscopy analysis of sea grass polyphenols. Current Bioactive Compounds 7: 118-125.
5. Ferrieres J (2004) The French paradox: lessons for other countries. Heart 90: 107-111.
6. Kopp P (1998) Resveratrol, a phytoestrogen found in red wine. A possible explanation for the conundrum of the 'French paradox'? Eur J Endocrinol 138: 619-620.
7. Goldberg DM, Hahn SE, Parkes JG (1995) Beyond alcohol: beverage consumption and cardiovascular mortality. Clin Chim Acta 237: 155-187.
8. Just JR, Daeschel MA (2003) Antimicrobial effects of wine on Escherichia coli O157:H7 and Salmonella typhimurium in a model stomach system. Journal of Food Science 68: 285-290.
9. Frankel EN, Waterhouse AL, Teissedre LP (1995) Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. J Agric Food Chem 43: 890-894.
10. Soleas GJ, Diamandis EP, Goldberg DM (1997) Wine as a biological fluid: history, production, and role in disease prevention. J Clin Lab Anal 11: 287-313.
11. Ikigai H, Nakae T, Hara Y, Shimamura T (1993) Bactericidal catechins damage the lipid bilayer. Biochim Biophys Acta 1147: 132-136.
12. Jayaprakasha GK, Selvi T, Sakariah KK (2003) Antibacterial and antioxidant activities of grape (Vitis vinifera) seed extracts. Food Research International 36: 117-122.
13. Vaquero MJR, Alberto MR, de Nadra MCM (2007) Influence of phenolic compounds from wines on the growth of Listeria monocytogenes. Food Control 18: 587-593.
14. Kennedy JA, Matthews MA, Waterhouse AL (2000) Changes in grape seed polyphenols during fruit ripening. Phytochemistry 55: 77-85.
15. Robichaud JL, Noble AC (1990) Astringency and bitterness of selected phenolics in wine. Journal of the Science of Food and Agriculture 53: 343-353.
16. Canamares MV, Lombardi JR, Leona M (2009) Raman and surface enhanced raman spectra of 7-hydroxyflavone and 3',4'-dihydroxyflavone. e-Preservation Science 6: 81-88.
17. Bauer R, Nieuwoudt H, Bauer FF, Kossman J, Koch KR, et al. (2008) FTIR spectroscopy for grape and wine analysis. Anal Chem 80: 1371-1379.
18. Urtubia A, Perez-Correa JR, Pizarro P, Agosin E (2008) Exploring the applicability of MIR spectroscopy to detect early indications of wine fermentation problems. Food Control 19: 382-388.
19. Jensen JS, Egebo M, Meyer AS (2008) Identification of spectral regions for the quantification of red wine tannins with fourier transform mid-infrared spectroscopy. J Agric Food Chem 56: 3493-3499.
20. Versari A, Boulton RB, Parpinello GP (2006) Effect of spectral pre-processing methods on the evaluation of the color components of red wines using Fourier-transform infrared spectroscopy. Italian Journal of Food Science 4: 423-432.
21. Versari A, Parpinello GP, Scazzina F, Del Rio D (2010) Prediction of total antioxidant capacity of red wine by Fourier transform infrared spectroscopy. Food Control 21: 786-789.
22. Fuller WA (1996) Introduction to statistical time series. John Wiley & Sons, Inc., New York, USA.
23. Agatonovic-Kustrin S, Beresford R (2000) Basic concepts of artificial neural network (ANN) modeling and its application in pharmaceutical research. J Pharm Biomed Anal 22: 717-727.
24. Agatonovic-Kustrin S, Turner JV, Glass BD (2008) Molecular structural characteristics as determinants of estrogen receptor selectivity. J Pharm Biomed Anal 48: 369-375.
25. Turner JV, Maddalena DJ, Cutler DJ, Agatonovic-Kustrin S (2003) Multiple pharmacokinetic parameter prediction for a series of cephalosporins. J Pharm Sci 92: 552-559.
26. Lin-Vien D, Colthup NB, Fateley WG, Grasselli JG (1991) The handbook of Infrared and Raman characteristic frequencies of organic molecules. Academic Press, New York, USA.
27. Yadav BS, Tyagi SK, Seema (2006) Study of vibrational spectra of 4-methyl-3-nitrobenzaldehyde. Indian Journal of Pure & Applied Physics 44: 644-648.
28. Smith BC (1999) Infrared spectral interpretation: A systematic approach. 1st edition, CRC Press LLC, Florida, USA.
29. Zhang Y, Chen J, Lei Y, Zhou Q, Sun S, et al. (2010) Discrimination of different red wine by Fourier-transform infrared and two-dimensional infrared correlation spectroscopy. Journal of Molecular Structure 974: 144-150.
30. Flor S, Tripodi V, Contin M, Dobrecky C, Lucangioli S (2012) Spectroscopic approach of the association of heparin and its contaminant and related polysaccharides with polymers used in electrokinetic chromatography. Journal of Chemical and Pharmaceutical Research 4: 972-979.
31. Anderson A, Savoie R (1965) Raman spectrum of crystalline and liquid SO2. Canadian Journal of Chemistry 43: 2271-2278.
32. Yu D, Wu C, Kong Y, Xue N, Guo X, et al. (2007) Structural and catalytic investigation of mesoporous iron phosphate. J Phys Chem C 111: 14394-14399.
33. Eiro MJ, Heinonen M (2002) Anthocyanin color behavior and stability during storage: effect of intermolecular copigmentation. J Agric Food Chem 50: 7461-7466.
34. De Beer D, Harbertson JF, Kilmartin PA, Roginsky V, Barsukova T, et al. (2004) Phenolics: A comparison of diverse analytical methods. Am J Enol Vitic 55: 389-400.
35. Kinsella JE, Frankel EN, German B, Kanner J (1993) Possible mechanisms for the protective role of antioxidants in wine and plant foods. Food technology 47: 85-89.
36. Packer L, Hiramatsu M, Yoshikawa T (1999) Antioxidant food supplements in human health. Academic Press, London, UK.
37. Saito M, Hosoyama H, Artiga T, Kataoka S, Yamaji N (1998) Antiulcer activity of grape seed extract and procyanidins. J Agric Food Chem 46: 1460-1464.

38. Renaud S, de Lorgeril M (1992) Wine, alcohol, platelets, and the French paradox for coronary heart disease. Lancet 339: 1523-1526.

39. Liu BL, Zhang X, Zhang W, Zhen HN (2007) New enlightenment of French Paradox: resveratrol's potential for cancer chemoprevention and anti-cancer therapy. Cancer Biol Ther 6: 1833-1836.

40. Lin YT, Vattem D, Labbe RG, Shetty K (2005) Enhancement of antioxidant activity and inhibition of Helicobacter pylori by phenolic phytochemical-enriched alcoholic beverages. Process Biochemistry 40: 2059-2065.

41. Yamauchi S, Shibutani S, Doi S (2003) Characteristic raman bands for artocarpus heterophyllus heartwood. Journal of Wood Science 49: 466-468.

42. Bakker J, Timberlake CF (1997) Isolation, identification, and characterization of new color-stable anthocyanins occurring in some red wines. J Agric Food Chem 45: 35-43.

43. Monagas M, Nunez V, Bartolome B, Gomez-Cordoves C (2003) Anthocyanin-derived pigments in graciano, tempranillo, and cabernet sauvignon wines produced in Spain. Am J Enol Vitic 54: 163-169.

44. Schwarz M, Wabnitz TC, Winterhalter P (2003) Pathway leading to the formation of anthocyanin-vinylphenol adducts and related pigments in red wines. J Agric Food Chem 51: 3682-3687.

45. Romero C, Bakker J (2000) Anthocyanin and colour evolution during maturation of four port wines: Effect of pyruvic acid addition. Journal of the Science of Food and Agriculture 81: 252-260.

46. Romero C, Bakker J (2000) Effect of storage temperature and pyruvate on kinetics of anthocyanin degradation, vitisin A derivative formation, and color characteristics of model solutions. J Agric Food Chem 48: 2135-2141.

47. Waterhouse AL (2002) Wine phenolics. Ann N Y Acad Sci 957: 21-36.

48. Fernandez K, Agosin E (2007) Quantitative analysis of red wine tannins using Fourier-transform mid-infrared spectrometry. J Agric Food Chem 55: 7294-7300.

49. Ough CS, Amerine MA (1988) Methods of analysis of musts and wines. 2nd edition, John Wiley & Sons, New York, USA.

50. Mataix E, de Castro MDL (1999) Sequential determination of total and volatile acidity in wines based on a low injection-pervaporation approach. Analytica Chimica Acta 381: 23-28.

51. Zoecklein BW, Fugelsang KC, Gump BH, Nury FS (1999) Wine analysis and production. Chapman & Hall, New York, USA.

52. Peynaud E, Blouin J (1996) The taste of wine: The art science of wine appreciation. John Wiley and Sons, New York, USA.

53. Jackson RS (2000) Wine science: Principles and Applications. 3rd edition, Academic Press, San Diego, USA.

54. Boulton R (1980) The relationships between total acidity, titratable acidity and ph in wine. American Journal of Enology and Viticulture 31: 76-80.