FTIR Spectroscopy for Grape and Wine Analysis

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FTIR SPECTROSCOPY
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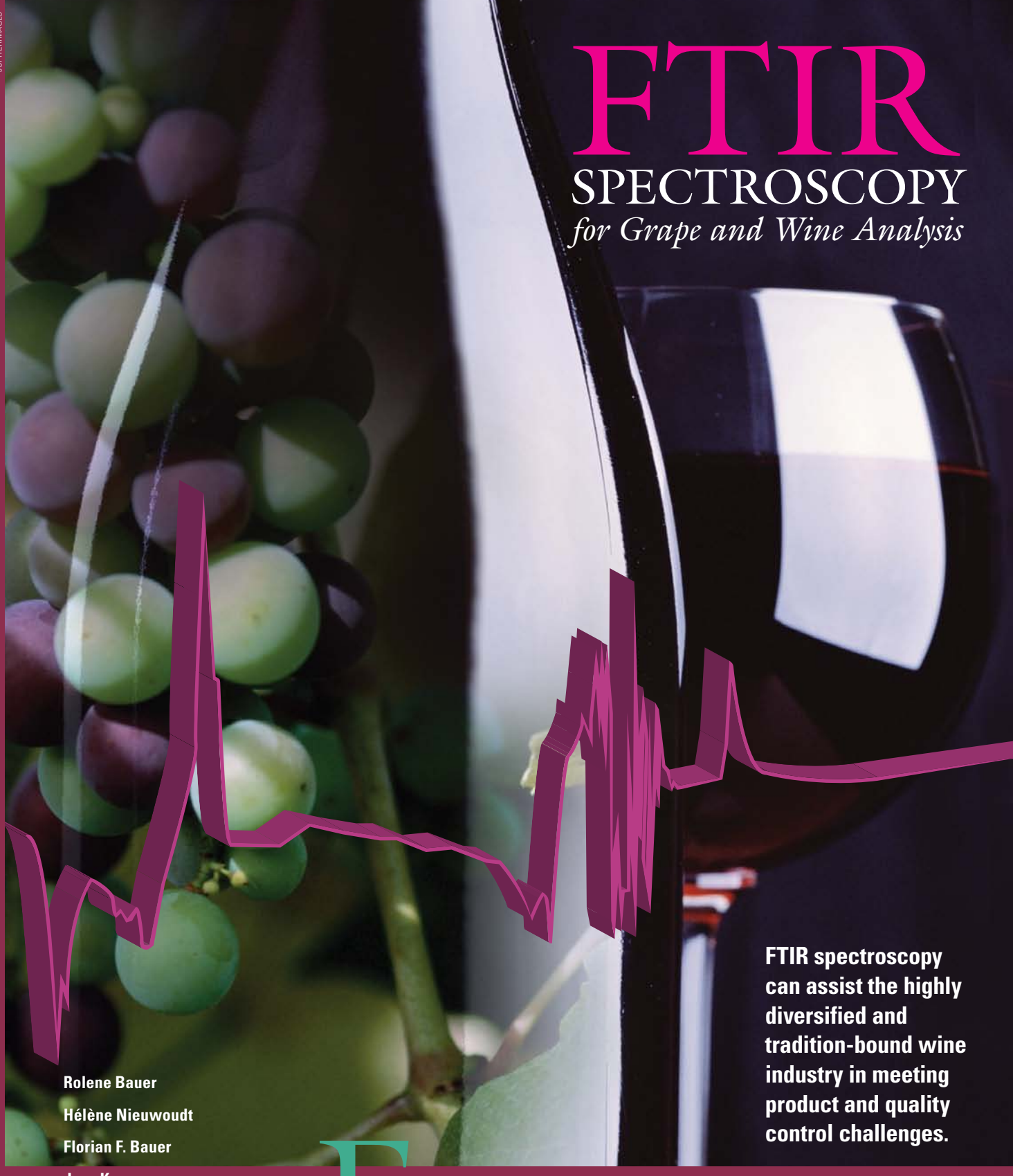

FTIR spectroscopy can assist the highly diversified and tradition-bound wine industry in meeting product and quality control challenges.

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FTIR spectroscopy is a nondestructive technique that provides structural information on molecular features of a large range of compounds. Its main advantages are speed, a high degree of automation, medium resolution, and cost-effectiveness. Recent improvements in instrumentation together with advances in fiber optics and chemometrics have provided an analytical tool that is suitable for routine qualitative analysis and process control in many industries.

Although FTIR spectroscopy is widely applied in the food industry, acceptance of this technology in the grape and wine industry has been relatively slow and mainly

restricted to large wineries. Wine making demands constant product monitoring and requires process control from the start of grape ripening until bottling of the finished product. Direct spectroscopic measurement is well suited in this context because it allows for rapid and simultaneous analysis of several compounds with minimal sample preparation and reagent consumption. Commercially available FTIR instrumentation with versatile and innovative software, designed specifically for grape and wine analysis, has recently received much attention. FTIR spectroscopy is based on the principle that functional groups within a sample will vibrate upon exposure to IR radiation. The distinction between NIR and mid-IR techniques is diminishing, and the methods are seen as complementary, with each spectral region providing unique advantages (*1*, *2*).

Spectral acquisition with multicomponent FT-mid-IR has received much attention because of its accuracy, precision, and speed.

IR techniques

Most compounds with covalent bonds absorb specific frequencies of radiation in the IR region of the electromagnetic spectrum. Only asymmetric bonds with a dipole moment that changes as a function of time are capable of absorbing IR radiation, and absorptions characteristic of each type of bond are found in specific small sections of the vibrational IR region. The vibrational IR region is at wavelengths ~2500–25,000 nm, corresponding to wavenumbers of 4000–400 cm^{-1}. The simplest types of vibrational motion in a molecular bond are the stretching and bending modes, which give rise to fundamental absorptions whereby vibrational transitions in molecules are induced, resulting in transitions from the ground state to the lowest-energy excited state.

Although fundamental absorptions or vibrations are visualized as strong bands or peaks, an IR spectrum is complicated by the presence of three types of bands: weak overtone, combination, and difference bands. Overtones result from molecules being excited from the ground state to higher-energy states and correspond to multiples of the frequency of the fundamental absorption. Combination bands result from frequencies that are vibrationally coupled, and difference bands result from the difference between two interacting bands. No two molecules of different structure have exactly the same IR absorption pattern; thus, the IR spectrum of a pure sample can be described as a molecular fingerprint characteristic of a specific chemical or biochemical substance.

The NIR spectral region lies between the visible and IR regions of the electromagnetic spectrum and corresponds to 14,000–4000 cm^{-1}. Although the mid-IR part of the spectrum contains many sharp peaks and is very information-rich, NIR spectra consist of overtones and combination bands. The predominant NIR spectral vibration feature is C–H-associated stretching; this manifests as both C–H combination bands and C–H overtones and occurs four times between 14,500 and 3300 cm^{-1}. The closer one approaches the fundamental region, the more detailed is the vibrational information.

The main advantages of NIR spectroscopy are low absorptivity and low reflectivity. Low absorptivity allows energy to pass easily through samples without rapid attenuation. Therefore, sample path lengths can be much longer than for mid-IR analysis (up to 10 cm), permitting analysis of most samples without the need for sample pretreatment. Low reflectivity allows NIR energy to penetrate beneath the surface of even visually opaque samples. By contrast, the high reflectivity of UV or visible light precludes analysis of intact grape berries.

Even though advanced IR technology has been welcomed enthusiastically (*3*) and has led to an almost explosive flurry of applications in quantitative analysis, one critical factor is still problematic—accuracy. The reliability of the analytical results is directly proportional to the extent to which the analytical volumes sampled adequately represent the lots from which they were taken, be they loads, drums, tanks, vats, reactors, and so forth. Because sampling rates of $1{:}10^6$ are typical and far from trivial, representative sampling is critical.

Multivariate statistical techniques

Much of the modeling of analytical data from wine has been conducted in a univariate manner, typically by examining the response of a single, isolated variable on the overall matrix and ignoring the effects of chemical or physical interactions among the large numbers of constituents present in the sample. Since the introduction of modern analytical instrumentation capable of multivariate responses, food quality control is now often based on indirect, full-spectrum measurements of the chemical and physical properties of a sample. As a result, extremely large data sets are generated in which essential information may not be readily evident. Univariate methods, such as the analysis of variance, are of limited use in complex data analysis. Multivariate analysis, also known as chemometrics, provides a means of quantifying constituents that are involved in complex matrix interactions without eliminating matrix interferences. The use of chemometrics for calibration, validation, and comparison of main components of wine as determined by IR is indispensable.

Qualitative and quantitative IR spectroscopic methods typically require multivariate calibration algorithms to model the spectral response to chemical or physical properties of the calibration samples. Measured frequencies often have to be preprocessed through a series of mathematical procedures, including various forms of scaling, corrections, and FT, to yield a suitable absorbance spectrum. Vibrational bands (peaks) are generally overlapping and may often appear nonspecific and poorly resolved. The approaches and methodology in chemometrics are described elsewhere (*4*–*8*).

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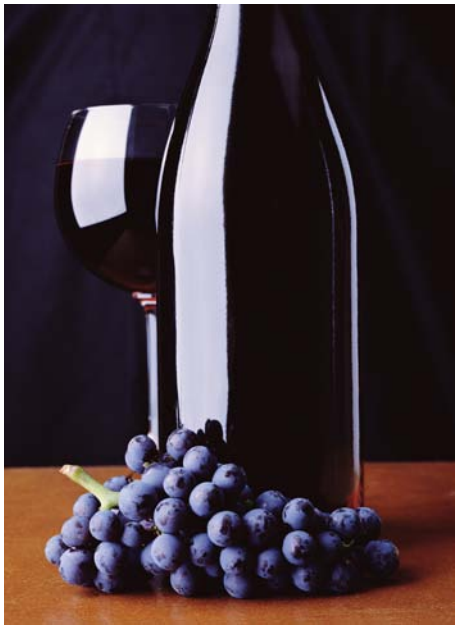
Methods based on multiple linear regression (MLR) relate variations in the selected response (y) to the variations of several linearly independent predictors (x) with the purpose of predicting the dependent response variable (*5*). The regression coefficient expresses the link between variation in the predictors and variation in the response. The squared multiple correlation coefficient R^2 is used as a goodness-of-fit measure in the sense that the closer the value is to 1, the better is the prediction for a given validation data set. Both R^2 and the root mean square error of prediction (RMSEP) are often used to validate a model (*5*). RMSEP is a measurement of the average difference between predicted and reference response values at the validation or prediction stage, and it is expressed in the same units as the original response values.



The most commonly used regression technique in chemometrics is partial least squares regression (PLSR) (*5*, *7*). The technique is based on so-called bilinear projection, meaning that two sets of variables (x, y) are linked to each other by means of linear projection models. Instead of MLR being applied to the full set of regressors, the information carried by the original x variables is projected onto a smaller set of latent, uncorrelated variables called PLS components. Because the y data are actively used in estimation of these latent variables in the x space, the first PLS components will always be the most relevant for predicting y.

PLS components point out main associations between x and y internally while optimizing the effective interrelationships between x and y data for optimal prediction characteristics. In multivariate calibration, a biased regression technique such as PLSR is preferred to classical MLR, because MLR gives rise to high variance when regressors are highly correlated. Another weakness associated with MLR is the assumption that errors exist only for y. PLSR can establish whether a new test sample is similar enough to those used in training. If not, the model should not be used for prediction, which minimizes the likelihood of applying a regression model to an outlier sample.

Principal components analysis (PCA), which is the bilinear modeling method most often used, gives an overview of the main information in a single multidimensional data table x (*7*). The information carried by the original x variables is projected onto a smaller set of latent variables called principal components. Unlike PLS components, principal components are calculated without taking information about y into account (there are no y data for a PCA). Because principal components are orthogonal to one another, each principal component can be interpreted independently. The scores are used to study the interobject relationships in terms of similarities as well as differences, and complementary loadings are also used to detect and interpret interrelationships among different variables. When PCA is carried out on x variables, the resultant principal components can be used to predict x in a variant of MLR known as principal component regression, in which some of the MLR problems have been eliminated.

Disjoint PCA modeling by soft independent modeling of class analogy (SIMCA) focuses on classifying objects into disjointed data classes based on the similarities among members of the same data class (a natural group or cluster of objects). A new sample will be rejected if it is not similar enough to other members of the class (*7*). SIMCA classification also enables identification and interpretation of outlier samples or samples that are poorly predicted by calibration models.

Instrumentation

In IR techniques, absorption intensity is typically plotted against wavenumber or wavelength (*1*). Two types of IR spectrophotometers are commonly used: dispersive and modern FT instrumentation. A dispersive instrument measures spectral points sequentially and records a spectrum in the desired frequency domain, whereas the optical pathway of FT instrumentation produces a complex time-dependent response, called an interferogram, that is essentially the variation of the absorbed IR intensity as a function of time (*1*). The conventional frequency-domain IR spectrum is obtained by mathematically performing the FT on the interferogram. Compared with the dispersive device, the FTIR instrument is faster and produces spectra with better S/N, which, conveniently, can be manipulated digitally.

Spectral acquisition with multicomponent analytical FT-mid-IR instrumentation, in particular the WineScan FT120 (FOSS Electric), has recently received much attention because of its impressive performance in terms of accuracy, precision, and speed of analysis (*9*–*12*). WineScan is equipped with a deuterated triglycine sulfate pyroelectric IR detector, and its calibration software, which is based on PCA and PLSR, has been designed specifically for grape and wine analysis. Measurements are carried out in liquid flow-through cells equipped with two CaF_2 windows mounted slightly nonparallel to minimize internal reflections. Each window has a 10-mm-diam circle with 2 mm thickness, providing an optical aperture of 88.54 mm^2 and a cell volume of 3.276 μL. A polyethylene terephthalate spacer provides a 37 μm optical path length. The interferogram is recorded with an optical resolution of 14 × 2000 cm^{-1} and averaging on the basis of 12 scans. The instrument is equipped with a HeNe laser (632 nm) and a silicon detector to track the position of the moving mirror in the interferometer. This laser determines not only the resolution but also the speed of the interferometer.

The regression algorithms incorporate absorptions at se-

Table 1. Validation of FTIR spectroscopy for German wine analysis.
(Adapted from Ref. 12.)

Parameter	Range	Mean	R^2	RMSEP
Alcohol (vol %)	7.4–14.0	11.4	0.9819	0.16
Alcohol (g/L)	58.7–110.7	90	0.9753	1.4
Sugars				
Before conversion	1.5–220.8	23.1	0.9978	1.7
After conversion	1.5–234.7	23.8	0.9983	1.3
Glucose	0.2–63.5	6.5	0.9957	0.7
Fructose	0.0–165.7	14.9	0.9983	1.1
Organic acids (g/L)				
Total acid	3.72–14.10	6.14	0.9734	0.25
pH	2.49–3.99	3.37	0.8344	0.12
Tartaric acid	0.80–3.30	2	0.4228	0.47
Malic acid	0.0–6.60	2.3	0.8110	0.63
Volatile acid	0.14–1.41	0.44	0.7680	0.09
Citric acid	0.0–2.30	0.32	0.4875	0.26
Glycerol (g/L)	5.20–27.80	7.85	0.9831	0.47
Total phenol (mg/L)	134–2260	570	0.9594	126
SO_2 (mg/L)				
Total (Tanner–Brunner)	32–588	120	0.7029	41
Total (automated photometry)	7–415	86	0.8431	33
Free	0–58	20	0.1196	12

lected wavenumbers or groups of wavenumbers (also referred to as filters) to generate a linear equation that best fits reference values in a data set. The most prominent wavenumbers, at which the correlation between the measured absorbance and the corresponding reference values for the analyte of interest is the highest, are selected by the software. Although the whole spectral range (929–5011 cm^{-1}) is stored for each calibration sample, filter selection is restricted to the ranges 965–1582, 1698–2006, and 2701–2971 cm^{-1}. The regions 1582–1692 and 2971–3627 cm^{-1} contain strong water absorption bands that prevent energy from passing through the cell. The region from 3627 cm^{-1} onward is eliminated because it contains very little useful information. It is possible to access the commercial algorithms and change critical parameters, such as slope, intercept, filters, and regression coefficients, to improve the accuracy of prediction.

Commercial wine samples can be scanned without sample preparation, although a filtration step is recommended to remove particulates. Samples containing CO_2 need to be degassed. NIR instrumentation used by the wine industry includes diode array spectrometers (25,000–9100 cm^{-1}) and the FOSS NIRSystems 6500 (25,000–4000 cm^{-1}). Spectrophotometers with a limited wavelength range are preferred because they are less expensive and more rugged than traditional scanning monochromator instruments.

Calibration and method validation

During calibration, a model is fitted to the available data in such a way that it describes the data as faithfully as possible. Validation establishes the accuracy and precision and thus how well a model will perform for future samples taken from the same population as the calibration samples (*7*). When several chemical components are to be determined, calibration requires selection of the most relevant spectral wavelengths first, followed by informed use of multivariate calibration, typically PLSR, to optimize the calibration equations for each of the different parameters.

An independent set of samples is required to validate the accuracy, precision, and robustness of the calibration model. In regression, validation allows for an estimation of the average predicted error in future predictions. The outcome of the validation stage is generally expressed by RMSEP[2]. Everything else being equal, the lowest RMSEP signifies the best prediction of model performance if validated on a test set. Cross-validation based on the training data set alone invariably leads to an estimate of the prediction performance that is overly optimistic and unrealistic (*7*).

Few published studies have focused on the validation of FT-mid-IR methodology for wines by comparing the predictions of wine components with standard reference methods (*9*, *12–14*). These studies typically were calibrated by PLSR. The model developed by Patz et al. allowed rapid simultaneous screening of wine and must (grape juice before and during fermentation) for components and physical parameters such as alcohol, relative density, extract, sugar-free extract, refraction, conductivity, glycerol, total phenols, reducing sugars, fructose, glucose, sucrose, pH, total acidity, individual organic acids, and total SO_2 (Table 1; *12*). Both R^2 statistics and RMSEP values were used to evaluate the calibration model.

Because calibration assigns a response value, it introduces the need for quantitative classification of the spectra; qualitative classification is also needed to distinguish between adequate or deviating outlier samples (*15*). Such classifications arise from the presence of two different structures in a data set. One is related to the carrying over of systematic information from x to y, and it is identified by PLSR during calibration. The other structure is internal to the x space and can be identified by using cluster analysis. This internal structure is not related solely to the response structure, even though both are based on the spectral data.

During the building and validation of a PLSR model, samples that represent outlier spectral data (x) and samples that present abnormalities in the response (y) can be identified. When an established model is used on new spectra to make a prediction, samples that present spectral abnormalities can be detected. In other words, information is obtained not only about the spectra but also about the relationships between the spectra and the response. Therefore, the applicability of a PLSR model can be guaranteed only by ensuring that the samples used in the training set are representative of the calibration scenario and also of the situation pertaining to the future prediction application of the model. Esbensen deals in

depth with these critical modeling and validation issues (*7*).

Clearly, the evaluation of calibration models based on regression statistics alone often presents an overly optimistic view of the predictive abilities of the model. Ideally, a calibration model should allow for the identification and interpretation of outliers in the sample set and the identification of poorly predicted samples and sample types for which the model would not be suitable (*11*). Samples that at first may appear to be outliers may actually serve well as samples to be included in an augmented training data set. The key issue for a calibration data set is how representative it is with respect to the matrix and to future concentration ranges and data structure.

Table 2. NIR spectroscopy calibration statistics.

Parameter	Sample type	Range	R^2	RMSECV or RMSEP*	RPD[1]	Ref.
Alcohol						
Total alcohol (% v/v)	Grape spirits	93.7–96.5	0.969	0.08*	NA	24
Methanol (g/L)	Grape spirits	0.02–188.8	0.998	0.06*	NA	24
Acidity						
pH	Red grape homogenates	3.66–4.26	0.72	0.07	1.4	28
pH	Intact red grapes	NA	0.50	0.08	1.4	NA
Phenolic compounds						
Color (mg/g)	Red grape homogenates	0.38–1.72	0.90	0.10	4.2	28
Color (mg/g)	Intact red grapes	NA	0.50	0.14	1.9	NA
M3G (mg/L)	Red wine ferments	±183	0.81–0.94	17.5–31.5	2.5–4.3	32
Pigmented polymers (mg/L)	Red wine ferments	±21	0.82–0.94	3.2–26.8	2.1–5.8	32
Tannins as catechin (mg/L)	Red wine ferments	±318	0.7–0.97	49.1–118	1.8–3.0	32

[1]RPD is the ratio of the standard deviation of the calibration data to the standard error of prediction.

Because wine samples are highly variable due to origin, vintage, cultivar, viticulture (vine growing), viniculture (wine making), and wine style, apparent outliers may turn out to be legitimate representatives of the data. Nieuwoudt et al. evaluated PCA as a model for classifying the spectra of outlier samples (*11*). The authors showed that PCA, in combination with SIMCA, can be used to differentiate between outliers and samples that are poorly predicted by calibration models. Understanding the nature of any particular data structure (i.e., assigning status to the objects as training set, validation set, outliers, etc.) is very much a problem-dependent matter. In general, it is dangerous to rely on automated algorithmic approaches ("blindfold" calibration and validation).

Challenges

Four main challenges are associated with wine analysis by FTIR. First, the similarity among IR absorption profiles—wine samples are chemically very similar and so are many of its components that are routinely measured. The second challenge is interferences, due mainly to the dominating absorption of ethanol and water in the mid-IR region. Two wavenumber regions, 2970–3620 and 1550–1710 cm^{-1}, contribute considerable noise in spectra and are associated with the absorbance of water (*11*). Other interfering compounds may be present in high concentrations, such as reducing sugar and organic acids (*16*). Interferences are compensated for by multiple-wavenumber measurements and by subtraction of the water signal.

The third challenge relates to the presence of outlier samples. Because of the high degree of variation in wine samples, calibration requires a large number of samples with important dissimilarities among them. Fermentation and the maturation period have been identified as major sources of variation (*11*). The fourth challenge relates to concentration limitations—FTIR spectroscopy is not suitable for determining concentrations <0.2 g/L (*12*). The full potential for quality and production control has not been realized.

Alcohol content

Ethanol is produced mainly by *Saccharomyces cerevisiae* during alcoholic fermentation and is one of the main components of natural wine (6.5–16% v/v) and distillation products. Another source of ethanol in certain styles of wines is fortification with grape-derived spirits, usually distilled at ~96% v/v ethanol. A major contaminant in distilled spirits is methanol, which originates from microbial activity in the raw material used for distillation (*17*). Both ethanol and methanol are therefore routinely monitored during distillation. Fortification is not the only source of methanol in wine. Methanol may occur in trace amounts in natural wine, presumably resulting from enzymatic activity during wine making (*18*).

Clearly, a need exists for fast and reliable methods for determining ethanol and methanol content. Total alcohol is traditionally measured by densitometry, with the assumption that the major component is ethanol. Methanol is commonly measured by GC. Although the application of NIR in finished wine has been limited because of the lack of success in obtaining reliable calibrations, determination of alcohol with NIR is relatively simple (*19–21*). The OH bond in ethanol produces a NIR band that is easily distinguished from the OH band produced by water, and ethanol is easily distinguished from methanol (*22*, *23*). Grape spirits, which are a relatively simple matrix, are even more amenable to NIR calibration (*24*). PLSR models allow for almost perfect correlation between reference values and NIR spectra of grape spirits for both total alcohol

and methanol (Table 2). Mid-IR has also been successfully used to quantify total alcohol, because the data are generally easier to interpret than the corresponding NIR spectral regions (*12*).

Total soluble solids

The soluble solids in unfermented musts and wines are mainly sugars (90%), in particular glucose and fructose. Traditionally measured with a refractometer, they provide an indication of grape maturity and signal the optimal time for harvesting. Both NIR and mid-IR show potential as alternatives to refractometry.

MLR combined with PCA displayed a linear correlation between NIR spectra and the sugar content of must (*25*). Comparatively, mid-IR displayed superior predictive capability (*12*, *26*). Over a broad concentration range, almost perfect correlation was obtained between reference values and PLSR-modulated spectra representing total sugar (fructose and glucose; Table 1). Calibration of total sugars in dry wines may be complicated by the presence of high concentrations of organic acids with similar IR absorption bands (C=O, C–O, and OH). Better recovery is expected in sweeter wines because the sugar-to-organic-acid ratio is higher (*16*). Prediction was nevertheless compromised at >20 g/L total sugar (*12*). Sucrose absorbs strongly in the mid-IR, and although this sugar is not expected to be present in "still" wines, recently bottled sparkling wines may contain measurable amounts of unfermented sucrose. Because of the structural similarity between sucrose and its monosaccharides, glucose and fructose, separation of the respective spectral responses is complicated.

Acidity

The assessment of grape and grape juice quality usually includes an assessment of acidity (*27*). The main organic acids in wine are tartrate (~1.5–4.0 g/L), malate (~0–4.0 g/L), lactate (~0.1–3.0 g/L), acetate (>0.2 g/L measured as volatile acidity), citrate (~0–0.5 g/L), and in some cases, succinate (0–2 g/L). Thus, acidity is generally measured in terms of both total titratable acid and the pH of the wine.

A diode array spectroscopic instrument (25,000–9000 cm^{-1}) has been evaluated as an alternative method to rapidly predict pH in red grapes (*28*). The visible, NIR, and vis + NIR regions were compared by applying PLSR statistics. The possibility of extending NIR technology to intact berry samples was also explored in this study. NIR radiation easily passes through berry and liquid samples and may potentially permit the analysis of whole grape berries. However, calibration statistics obtained in the NIR region were less than satisfactory (Table 2). On the other hand, good results were obtained when homogenized grapes were analyzed in the visible region. The values obtained correspond well to the predictive capability of expensive scanning monochromator instruments (*29*). Comparative studies between NIR and mid-IR in the analysis of grape homogenates suggest that mid-IR has superior predictive capability for pH (*26*).

With various finished German wines, FT-mid-IR spectra displayed a linear correlation with both total acidity and pH as given by PLSR modulation (*12*). For individual organic acids, reasonable correlations were obtained for malate and volatile acidity. The calibration model did not allow for quantification of tartrate and citrate. On the other hand, close to 100% recovery has been obtained for tartrate when wine samples were spiked with different organic acids (*30*, *31*). Samples used for calibration in the cited recovery studies did not include late-harvest wines; in the German study, the wine samples covered a broad range of sugar concentrations.

Organic acids and sugars have a carbonyl group in common and also share C–O and OH IR bands, unlike ethanol and glycerol. As a result, sugar may interfere with the quantification of organic acids. Analysis of individual organic acids may be further complicated by spectroscopic interferences arising from similarities between IR spectra of organic acids themselves, especially if these analytes are present in concentrations close to the detection limit. Interferences can be reduced by the careful choice of absorbance filters. The vibrational bands most relevant to the calibration of individual organic acids are the C=O stretch of carboxylic acid (±1720 cm^{-1}) for total acidity, tartrate, and malate; the OH bend of the carboxylic acid (±1400 cm^{-1}) for citrate; and the CH_3 bend (±1375 cm^{-1}) for volatile acidity (*31*). The C=O stretch is of less importance when calibrating for organic acids present in low concentrations (lactate, citrate, and volatile acidity) because interfering sugars display absorption bands in the same IR region.

Glycerol

Glycerol is a major component of wine (2–15 g/L), and its determination at various stages of the wine-making process provides quality control information. Patz et al. used mid-IR with PLSR to quantify glycerol from a variety of German wines (*12*). Nieuwoudt et al. developed a PLS-based glycerol calibration model for South African wine (reducing sugar content <30 g/L, alcohol content >8% v/v; *11*). The model provided satisfactory predictive ability and was suitable for quantification purposes.

Similarly, a calibration model for late-harvest wines (alcohol >11.6% v/v) was established with a prediction error of 0.65 g/L. The majority of variation (>85%) in glycerol content of different wines was correlated to the use of absorbance filters in the range 1229–929 cm^{-1}, which incorporates the C–O stretch bands of glycerol. PCA cluster analysis suggested a subdivision of samples according to alcohol and sugar content to obtain calibration models that display acceptable prediction accuracies. Furthermore, young wines and wines in different stages of fermentation displayed great variation when compared with bottled wine and should therefore be carefully monitored for prediction accuracy.

Phenolic compounds

Phenolic compounds contribute to both the color and the organoleptic properties of red wine, in particular mouth feel (*27*). The color in red wine mainly originates from grape-derived anthocyanins, such as malvidine-3-glucoside (M3G),

which are converted into pigmented polymers during fermentation and aging. Condensed flavanoid tannins, such as catechin and epicathecin, are mainly responsible for astringency. Nonvolatile phenolic acids are precursors of volatile phenols, which contribute to the aroma in wine. The structure of wine phenolics varies significantly as wine ages in the tank, the barrel, and the bottle.

Methods for determining phenolics are generally based on colorimetric or chromatographic techniques, in particular HPLC. These techniques require sample preparation and chemical manipulations that make them time-consuming, laborious, and costly, and these analyses are limited to simple and short polymeric molecules. Visible and NIR spectroscopies (25,000–4000 cm^{-1}) show promise for the simultaneous determination of M3G, pigmented polymers, and tannin in fermenting must and wine samples representing different red varieties and vintages (*32*). The specificity of cross-evaluated PLS calibrations is not optimal because of inconsistencies arising from the many color changes that occur during fermentation (Table 2). Color changes are governed by different oxidation, condensation, polymerization, and precipitation reactions. PCA revealed the spectral regions with the highest correlations: 540 nm (dominated by color pigments; *28*) and 2200–2300 nm (dominated by tannins; *33*). Although NIR allows for better color prediction than mid-IR, the best prediction was obtained when the spectral regions were combined (*23*, *26*). FTIR spectroscopy may potentially be extended to monitor fermentations qualitatively in terms of their phenolic content. If successful, FTIR should allow better process control during maturation of the finished wine (*23*).

Sulfur dioxide

Sulfite (<1 g/L) is routinely added to grape must and wine to restrict the growth of indigenous yeast and bacteria and to act as an antioxidant (*25*, *34*). At wine pH 3–4, sulfite predominates as "free SO_2", consisting mainly of HSO_3^- and a small proportion of $SO_2 \cdot H_2O$ and SO_3^{2-} present in equilibrium. Certain yeast strains produce significant amounts of SO_2 during alcoholic fermentation. Free SO_2, especially HSO_3^-, binds with carbonyl compounds in wine to form what is known as "bound SO_2" and has weak antimicrobial properties. The bound and free forms are routinely measured during all stages of the wine-making process, because of the fluctuating equilibrium between free and bound SO_2 and the difference in their properties.

Given the health risks associated with sulfites, the total amount of SO_2 in finished wines is strictly regulated. However, free SO_2 is generally maintained at >30 mg/L to prevent microbial spoilage. Correct SO_2 usage is clearly one of the most important practices in wine making, and fast, reliable methods for SO_2 quantification would be beneficial. Although little useful information is obtained for sulfides from the IR spectrum, S–O compounds display several strong bands in the region 1000–650 cm^{-1}, and S=O compounds display several characteristic stretches in the region 1375–1050 cm^{-1} (*1*). When sulfuric acid is added to wine, it entirely dissociates to sulfate ions with S=O absorption bands at 1150–1050 cm^{-1}; this region is particularly useful because no other functionality is measured here (*16*). As a result, recovery of sulfate ions present at <1 g/L is better than for other compounds at the same concentration levels (total sugars in dry wines).

Mounting consumer demand for safe alternatives to chemical preservatives such as SO_2 has led to a decrease in the quantities added to must and wine, often <0.1 g/L (*35*–*38*). SO_2 concentrations in finished wines may therefore be close to the FT-mid-IR detection limit (0.2 g/L). It was therefore not unexpected to find that data validated against standard references methods allowed only for the qualitative screening of total SO_2 and not its quantification (*12*). Furthermore, reference results for free SO_2 displayed no correlation to spectral data. The low performance was most probably due to SO_2 concentrations in these wines being close to (total SO_2 <0.6 g/L) or below (free SO_2 <0.1 g/L) the detection limit of FT-mid-IR (Table 1).

Sensory properties

Many studies have focused on the contribution of volatile compounds to wine aroma (*39*). These compounds originate from the grape berry, the metabolism of yeast and bacteria, oak wood extraction, and chemical reactions during processing and storage. Traditionally, volatile compounds are analyzed by GC/MS, a technique that is expensive and time-consuming. Its success in predicting sensory characteristics has been limited because of the lack of knowledge regarding the aroma composition of wines. Recent developments in MS-based electronic-nose instruments (MS-eNose) allow headspace characterization of different food and beverage products (*40*).

Chemometrics combined with IR and MS-eNose make it possible to obtain a fingerprint of a sample matrix in terms of its volatile components (*41*, *42*). Still, such an approach does not readily link a particular characteristic, such as a sensory attribute, to one or more individual compounds. Although aromatic compounds are often present in concentrations be-

low the detection limit of FTIR, many nonvolatile compounds related to wine flavor are present at higher concentrations and offer possibilities for objective quality grading by FTIR (*23*). Nonvolatile compounds can also play a more indirect role in wine by reacting with volatiles, chemically, physically, or in a perceptual manner, to alter flavor and aroma (*43*). Furthermore, grape precursors such as glycoconjugates give rise to volatile components during wine making (*44*). FT-mid-IR has been successful in measuring individual glycoconjugates in Muscadet grapes (*45*). Glycoconjugates are also characteristic of a given grape variety. Quantification of such precursors could therefore be a useful index to determine not only grape quality but also optimal maturity of grapes and varietal origin.

Identification of microbial strains

Studies have shown that FTIR spectra can be used to identify and differentiate microorganisms down to the level of subspecies, strain, or even serotype (*46–49*). The technique produces a complex spectral fingerprint of intact microbial cells that reflects the total biochemical composition of the microorganism. The main bacterial contaminants in wine are acetic acid bacteria and lactic acid bacteria (LAB). LAB identified in wine represent the genera *Lactobacillus*, *Leuconostoc*, *Oenococcus*, and *Pediococcus* (*34*). Of these, only *Lactobacillus* species have been subjected to differentiation by FTIR spectroscopy (*50*, *51*). Although not applied to wine samples, the technique proved to be rapid, reliable, and reproducible when used with multivariate techniques. This methodology may be extended to yeast typing at the strain level and has already been successfully applied to differentiate among mixed populations of food-borne yeast strains, including *S. cerevisiae* (*52–54*).

Conclusions and future prospects

The use of analytical mid-IR and NIR spectroscopy in grape and wine analysis lags behind its use in other fields, but because of the quantitative increase and qualitative broadening of application in the industry in the past 5 years, this is rapidly changing. Generally, mid-IR spectral data are easier to interpret than corresponding information obtained in the UV–vis and NIR spectral regions. On the basis of calibration statistics, mid-IR appears to be superior for measuring pH, alcohol, and total suspended solids, and NIR and UV–vis are better at predicting color. FTIR technology could potentially be extended to allow rapid analysis of intact grapes while still on the vine with inexpensive handheld instruments (*55*).

Hyphenating FTIR with other separation tools, particularly CE, will most certainly allow for many new developments. Dynamic flow separation systems are expected to reduce complications linked to interferences during calibration. The separation ability of CE is extremely high, only microvolumes of sample are required, and FTIR is fully compatible with narrow capillaries. Wine making demands constant product monitoring and will undoubtedly benefit immensely from on-line analysis. Recent developments with on-line hyphenated flow cells aim at simultaneously fulfilling the specific demands posed by both the spectrometer and CE instrumentation (*56*). Such technologies promise to separate and identify a wide range of analytes at the picogram level in the near future.

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