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Identification of Spectral Regions for the Quantification of Red Wine Tannins with Fourier Transform Mid-Infrared Spectroscopy

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Accomplishment of fast tannin measurements is receiving increased interest as tannins are important for the mouthfeel and color properties of red wines. Fourier transform mid-infrared spectroscopy allows fast measurement of different wine components, but quantification of tannins is difficult due to interferences from spectral responses of other wine components. Four different variable selection tools were investigated for the identification of the most important spectral regions which would allow quantification of tannins from the spectra using partial least-squares regression. The study included the development of a new variable selection tool, iterative backward elimination of changeable size intervals PLS. The spectral regions identified by the different variable selection methods were not identical, but all included two regions (1485−1425 and 1060−995 cm^{-1}), which therefore were concluded to be particularly important for tannin quantification. The spectral regions identified from the variable selection methods were used to develop calibration models. All four variable selection methods identified regions that allowed an improved quantitative prediction of tannins (RMSEP = 69−79 mg of CE/L; $r = 0.93-0.94$) as compared to a calibration model developed using all variables (RMSEP = 115 mg of CE/L; $r = 0.87$). Only minor differences in the performance of the variable selection methods were observed.

KEYWORDS: FT-MIR spectroscopy; tannins; red wine; variable selection; partial least-squares regression

INTRODUCTION

Tannins are the most abundant group of phenolic compounds typically found in red wines (*1*), and the tannins play an important role in the mouthfeel properties and color stability of red wines (*2–4*). According to their chemical structure, tannins in wines are commonly classified as either condensed tannins or hydrolyzable tannins (**Figure 1**). Condensed tannins originate primarily from the skins and seeds of grapes and are oligomers or polymers of flavan-3-ol subunits (termed catechins), whereas hydrolyzable tannins mainly originate from oak (and thus occur in wines that have been aged in oak barrels) and are gallic acid and/or ellagic acid esters of glucose (*5, 6*).

Tannins have the ability to precipitate with proteins present in saliva. This interaction is presumed to be responsible for the astringent sensation of red wines (*2*). The ability to precipitate with proteins has been used for the quantitative analysis of tannins with bovine serum albumin (BSA) (*7, 8*). Tannin concentrations measured by protein precipitation have been found to correlate particularly well with the perceived astringency of red wines (*4*), and tannin analysis by protein precipitation has been recommended within winery settings (*9*). As reviewed elsewhere, several other principles for tannin analysis in wines have been reported, for example, precipitation by methyl cellulose, HPLC, and various colorimetric assays (*6, 10*). These types of methods are all slow, and the time requirement for accomplishing these tannin analyses currently represents a major obstacle for the implementation of such tannin analysis in the array of routine wine quality control measurements at wineries. Due to the increasingly recognized importance of tannins and hence tannin measurement in relation to red wine quality, a significant need thus exists for more rapid analytical techniques for quantification of tannins.

Employment of Fourier transform mid-infrared (FT-MIR) spectroscopy has recently emerged as a possible solution for rapid measurement of wine tannins (*11, 12*). FT-MIR has already found use in the industry for the analysis of several other important components in wine, including ethanol, organic acids, and sugars (*13, 14*). Interference between the characteristic absorption bands of major wine components and tannins poses a problem for direct quantification of tannins in wines by infrared spectroscopy. This problem has been overcome by sample purification using solid phase extraction (*11*), but again this strategy is not feasible for

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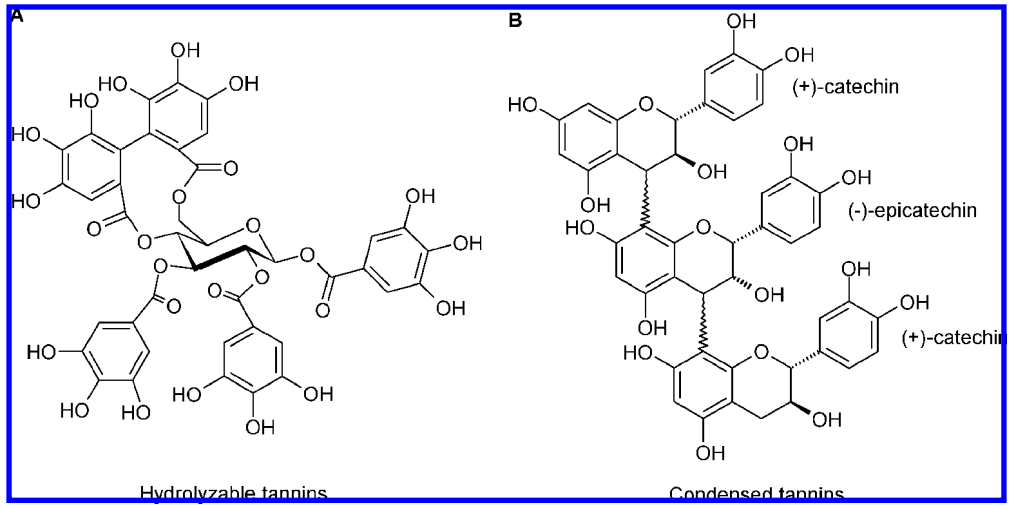

Figure 1. Examples of chemical structures of the two different classes of tannins: hydrolyzable tannins (**A**) and condensed tannins (**B**).

accomplishing rapid tannin analyses in industrial wine production. An alternative way is to identify the characteristic spectral regions of tannins, which do not suffer from this interference, and in turn use this identification to develop calibration models that then allow the rapid quantitative assessment of tannins by FT-MIR. A number of tools to identify important spectral regions for improving partial least-squares (PLS) calibrations are available and include synergy interval PLS (*15*), backward interval PLS (*16*), and genetic algorithm PLS (*17*). In brief, some of the main features of the methods are the following: synergy interval PLS finds the combination of up to four spectral intervals, which leads to the best PLS model; backward interval PLS eliminates the most noninformative regions of the spectra iteratively; and, finally, the genetic algorithm PLS finds the best combinations of spectral intervals using an evolutionary approach. One particular drawback of these present methods is that they all require predefined interval sizes, which may lead to identification of spectral intervals covering both noninformative and informative regions.

This study was undertaken to assess and compare different variable selection methods for the identification of important spectral regions for the quantification of red wine tannins by FT-MIR spectroscopy and the method of PLS regression. Furthermore, we wanted to evaluate the applicability a new variable selection method involving an iterative backward elimination of changeable size intervals.

MATERIALS AND METHODS

Materials. Chemicals for tannin analysis, including BSA (fraction V powder), tartaric acid, potassium tartrate, sodium dodecyl sulfate (SDS), triethanolamine (TEA), ferric chloride hexahydrate, and (+)-catechin hydrate, were all of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO). Commercial tannin extracts from grapes (tannin grape) and oak wood (Tannivin Superb) were purchased from Erbslöh Geisenheim AG (Geisenheim, Germany). One hundred and twenty-eight commercial red wines were purchased from local shops in Denmark. The wines were selected to represent a wide range of different vintages (11 vintages ranging from 1996 to 2006), grape varieties (covering at least 30 different varieties), and production countries (16 different countries).

Mid-Infrared Spectra. Spectra in the mid-infrared range were measured by Fourier transform interferometry on a Winescan Auto spectrometer (FOSS, Hillerød, Denmark) equipped with a liquid flow system and a 37 μm calcium fluoride cuvette, thermostated at 40 °C. Transmission infrared spectra of 1060 data points in the range between 5012 and 926 cm^{-1} of all wines were measured in triplicate. The

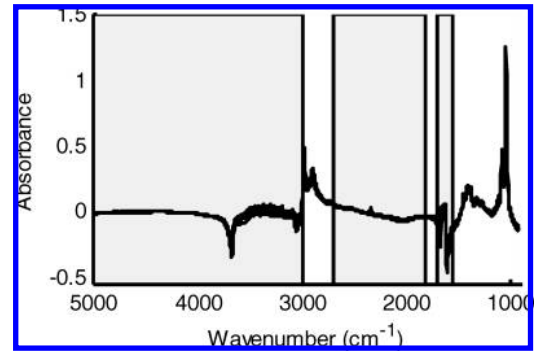

Figure 2. FT-MIR spectra of the 128 commercial wines (5012−926 cm^{-1}). The noninformative and noisy parts (specified by the gray rectangles) of the spectra are discarded to retain only the "good range" spectral regions.

noninformative and noisy parts of the full spectra were removed to give the "good range" region of 265 data points in the following regions: 2969−2699, 1812−1716, and 1577−933 cm^{-1} (**Figure 2**).

Tannin Analysis. Tannin concentrations of all wines were measured in duplicate using a slightly modified method of Harbertson et al. (*7*). Briefly, the method relies on tannins being precipitated with BSA, redissolved and measured by a color reaction with ferric chloride. Prior to analysis, wines were diluted in a model wine solution of 12% v/v ethanol containing 5 g/L of tartaric acid, which had been adjusted to a pH value of 3.3 with NaOH. Modifications to the original method were as follows: The precipitation step was conducted for 30 min instead of 15 min, the centrifugation speed for forming the tannin−protein pellet was increased from 13500*g* to 14000*g*, and finally the SDS/TEA buffer volume for redissolving the tannin−protein pellet was increased from 0.875 to 1.5 mL to allow background measurement (A^{BG}) on a 1 mL sample, which was then reacted with 0.125 mL of iron chloride (11.4 mM $FeCl_3$ in 11.4 mM aqueous HCl), and the absorbance was measured after 10 min (A^{FeCl3}). Dilutions of the sample in the model wine solutions were carried out to give a tannin response (calculated as $1.125A^{FeCl3}$ minus A^{BG}) between 0.3 and 0.75, which was defined as the valid range of the assay (*18*). Tannins were reported in milligrams of catechin equivalents (CE) per liter from a linear standard curve of the color reaction between catechin and ferric chloride [absorbance = 0.006258 × (concentration of catechin in mg/L); $r = 0.9997$].

Spiking Experiments. Separate solutions with 2 g/L oak tannin, (+)-catechin, and grape tannin respectively dissolved in 20% v/v aqueous ethanol were prepared, and the FT-MIR spectra of the solutions were measured. The spectral characteristics of the three products were determined as the difference between the FT-MIR absorbance spectra of the solutions and the FT-MIR absorbance spectra of the aqueous ethanol solution. A red wine (Cabernet

Sauvignon, Chile, 2005) was spiked with different levels (0, 0.1, 0.2, 0.5, 1.0, 1.5, and 2.0 g/L) of grape tannin and analyzed by FT-MIR spectroscopy. For each spiking level, the dose−response signal was evaluated as the difference between the FT-MIR absorbance spectrum of the spiked wine sample and the unspiked wine. The wine was analyzed to have a tannin level of 298 mg of CE/L, and the grape tannin powder contained 355 mg of CE/g of tannin powder.

Model Development. Multivariate calibration models were developed in MATLAB R14 (MathWorks, Natick, MA) using the PLS toolbox 4.02 (Eigenvector Research, Natick, MA). The infrared absorbance spectra were mean centered, and calibration models for measurement of the determined components were developed with PLS regression using cross-validation in nine segments. The triplicate spectra were included in the models to make it possible for the model to compensate for replicate variations between the spectra. The optimal number of latent variables in each model was determined from the minimum root-mean-square error of cross validation (RMSECV), allowing a maximum of 10 latent variables. The first 81 wines were used for developing the calibration models both for the "good range" region (265 data points), the fingerprint region from 1577 to 933 cm^{-1} (168 variables), the expected main region for phenolics from 1157 to 1577 cm^{-1} (110 variables), and for spectral regions of the reduced spectrum identified by different variable selection methods described below. The ability of the developed calibration models to predict the tannin concentration in wines was evaluated using an independent validation set of 47 wines to calculate the correlation coefficient between the measured and predicted values and the root-mean-square error of prediction (RMSEP). The wines used for validation were analyzed at a different point of time from the calibration wines to ensure independence of the validation set.

Variable Selection Methods. Four different variable selection methods were used to develop calibration models from the calibration set (81 wines) with segmented cross-validation in 9 segments, allowing up to 10 latent variables. The prediction performance of the calibration models was evaluated from the external validation set (47 wines). The following variable selection methods were evaluated: backward interval PLS (*16*) (bi-PLS; using 17 intervals and up to 10 latent variables), synergy interval PLS (*15*) (si-PLS; using 17 intervals, 4 regions, and up to 10 latent variables), genetic algorithm PLS (*17*) (GA-PLS; using a window size of 15 and up to 10 latent variables), and iterative backward elimination of changeable size intervals PLS (IBECSI-PLS, as described below) and compared with models developed using manually selected spectral intervals. The predictive performances of the models were compared pairwise from a F test of the RMSEP values: $F(n_1,n_2) = \mathrm{RMSEP}_1^2/\mathrm{RMSEP}_2^2$, on a $p < 0.05$ level (*19*).

Iterative Backward Elimination of Changeable Size Intervals PLS. A new variable selection method, "iterative backward elimination of changeable size intervals PLS" (IBECSI-PLS), was developed using the PLS toolbox 4.02 (Eigenvector Research). The IBECSI-PLS method works by an iterative elimination of intervals of changeable sizes from the spectra, by minimizing the RMSECV of the PLS model (**Figure 3**). The intervals were found by the following routine: The spectra were divided into a number of equally sized intervals (here 20 intervals), and PLS regression models with each of the intervals left out were calculated. The center point of the interval, which gave the lowest RMSECV when left out, was set as the starting point for the region to be eliminated (step 1 in **Figure 3**). The region to be eliminated was then stepwise expanded one data point at a time in the direction (left or right) causing the lowest RMSECV(step 2 in **Figure 3**) and repeated until a local minimum of RMSECV was found and the region was eliminated (step 3 in **Figure 3**). Steps 1−3 were repeated for the reduced spectra, and the routine was repeated until an optimal region could be identified (step 4 in **Figure 3**)—as discussed under Results and Discussion.

RESULTS AND DISCUSSION

Tannins in Wines. The concentration of tannins in the 128 commercial wines ranged from 92 to 1060 mg of CE/L (**Table 1**) and covered the most typical values of tannin concentrations in red wines reported by others using the same analytical method (*4, 7, 11, 12, 20, 21*). However, in some cases tannin levels have been reported as high as 1655 mg of CE/L in commercial wines (*12*). For the development of the calibration models for the quantification of tannins by FT-MIR spectroscopy using the protein precipitation data as reference, samples were split into a calibration set (81 wines) and a validation set (47 wines) with similar standard deviations and comparable ranges of the tannin levels (**Table 1**).

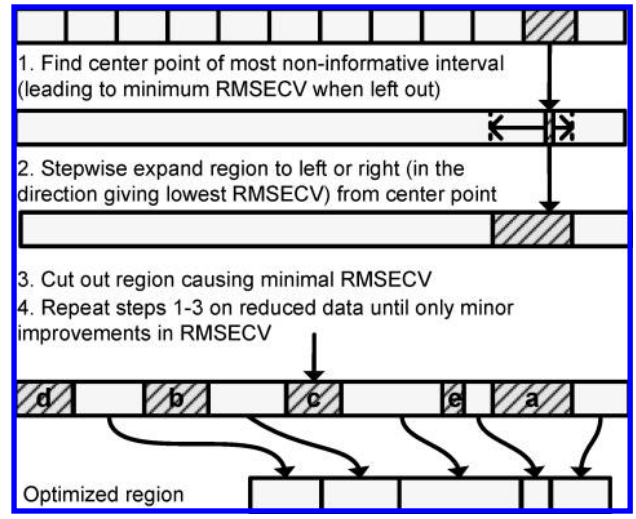

Figure 3. Overview of IBECSI-PLS for variable selection.

Table 1. Descriptive Statistics of the Red Wine Samples Used for Calibration and Validation

		tannin concentration[a]		
sample	N^b	range	mean	SD
all samples	128	92–1060	456	181
calibration	81	112–1060	472	180
validation	47	92–830	429	181

[a] Tannin concentration in mg of CE/L. [b] Number of samples.

Spectral Features of Tannins. The spectral response of a commercial grape tannin product at different levels in the FT-MIR spectrum of a selected red wine (Cabernet Sauvignon, 2005) was investigated (**Figure 4**). Although the absorbance values of the tannin signals were very low compared to the wine spectra (**Figure 2**), there was a systematic spectral dose−response effect of the added grape tannins, which was particularly evident in the regions 2969−2699 and 1577−1060 cm^{-1} (**Figure 4**). On the other hand, small or no distinct grape tannin signals were evident in the regions from 1812 to 1716 cm^{-1} and from 1060 and 933 cm^{-1}. The occurrences of the small negative absorbances observed in some regions were primarily ascribed to small drifts in the FT-MIR spectra with time. The most prominent signals for the grape tannins were: two major peaks at 1520 and 1445 cm^{-1} in the typical region of aromatic ring stretches (*22*) (**Table 2**), a peak at 1285 cm^{-1}, corresponding to the C−O stretch of the pyran derived part of flavonoid based tannins (*5*), and several peaks between 1400 and 1050 cm^{-1}, in the overlapping regions of OH stretch and deformations of phenols and CH deformations in aromatic compounds (*22*).

The relatively small spectral response from tannins in the wine spectra, combined with the known absorptions in the same spectral region as tannins of major wine components, such as ethanol and organic acids (*23*), complicates the use of infrared spectroscopy for the quantification of tannins in wine. Variable

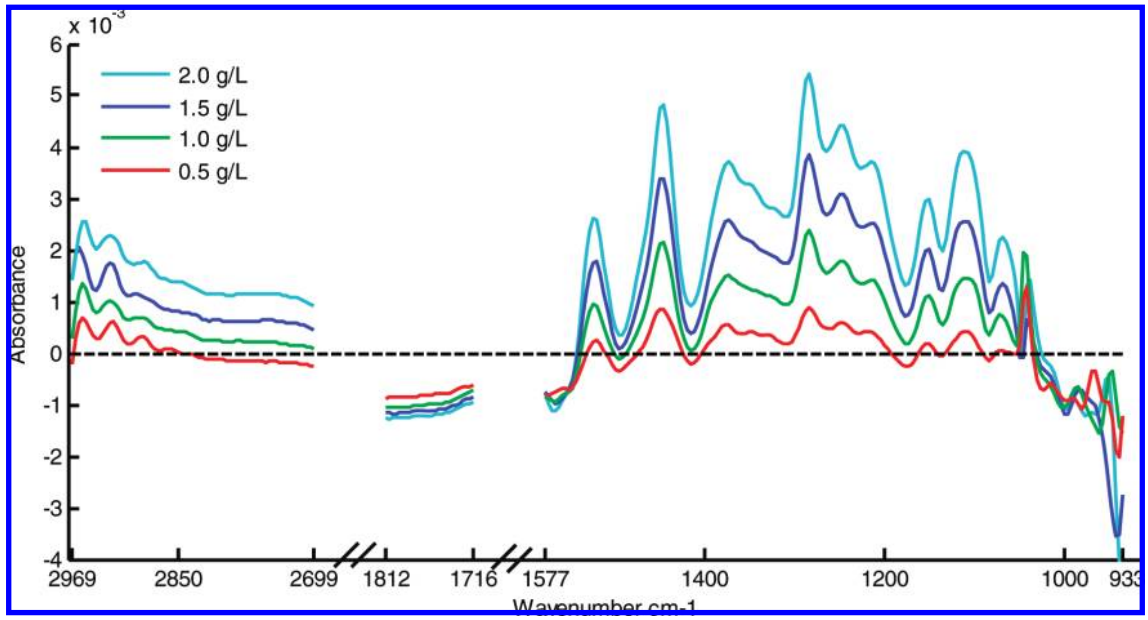

Figure 4. Spectral response of grape tannins to the spectra of a red wine at different concentrations (0.5, 1.0, 1.5, and 2.0 g/L) in the "good range" regions.

Table 2. Known Mid-Infrared Bands for Tannins in the Informative Regions (2996−2699, 1812−1716, and 1577−933 cm^{-1}) of the Spectrum (*5, 11, 22*)

functional group	group frequencies (cm^{-1})
aromatic overtones and combinations	2000–1700
C═O stretch of esters	1750–1740
C═C stretch of aromatic rings	1650–1430
C—O—H deformation of phenols	1390–1310
C—OH stretch of phenols	1340–1160
C—O stretch of flavonoid pyran ring	1285
C—H in-plane deformation of aromatic compounds	1270–1000

selection provides a way to remove interfering or noninformative regions of the infrared spectra, by which the models may be improved and in turn improve the accuracy of tannin measurement.

Iterative Backward Elimination of Changeable Size Intervals PLS. A new variable selection, IBECSI-PLS, which iteratively removes continuous regions from the spectra, was developed. As opposed to many other variable selection methods, the interval size of the eliminated region in IBECSI-PLS is found by a stepwise expansion of the region to be removed, which could be useful when informative and interfering spectral features are close. The IBECSI-PLS method was applied to the tannin data to remove the (differently sized) interfering or noninformative regions from the spectra. Due to the risk of overfitting the data by too extensive an elimination of variables, it is important to eliminate variables only while it gives a considerable decline in the model error and to validate the performance of the final model with independent samples. The optimal number of iterations was manually set to 11, because only minor declines in the model error were observed in the further iterations (**Figure 5B**). Additionally, only a few variables were removed per iteration in the further iterations (**Figure 5A**), also indicating that little further improvement was possible. The validation of the model performance with independent samples is discussed further below.

Spectral Regions for Tannin Quantification. The four variable selection methods for finding the best regions in the "good range" of the IR spectra were evaluated to see if the calibration models could be improved. The spectral regions identified either manually or by the variable selection methods are illustrated in **Figure 6** and compared with the spectral characteristics of red wine, oak tannin, (+)-catechin, and grape tannin. The spectral characteristics of oak tannin, grape tannin, and (+)-catechin were similar to the reported spectral characteristics of hydrolyzable tannins, grape tannin, and (+)-catechin (*5*). Although the regions identified by the four variable selection methods were not identical, two regions were selected by all four methods: the region from 1060 to 995 cm^{-1}, which was dominated by high absorption of the OH stretch in ethanol, and the region between 1485 and 1425 cm^{-1}, at which grape tannin gave a distinct absorption peak (**Figure 6**). Furthermore, all variable selection methods included wavelengths in the region from 2969 to 2699 cm^{-1} (**Figure 6**). The selected regions were, however, not the same for the different methods and, due to the lack of distinct peaks, thereby likely functioned as reference points in the spectra. Both bi-PLS and IBECSI-PLS retained wavelengths around 1750 cm^{-1}, with IBECSI-PLS retaining a much narrower region than bi-PLS. Oak tannins had a spectral response matching the C═O stretches of the ester group typically found in hydrolyzable tannins (**Figure 1**) and wavelengths around 1200 cm^{-1}, which matched some of the region of C-OH stretches of phenols (**Table 2**). Others have reported that the wavelength around 1285 cm^{-1} corresponds to the C—O stretch of the flavonoid pyran ring structure and may be used to distinguish condensed and hydrolyzable tannins (*5*). However, none of the variable selection methods retained the region around 1285 cm^{-1} for the quantitative analysis of tannins from the FT-MIR spectra of red wines. The elimination of this region for tannin quantification may be a consequence of the overlapping peak from (+)-catechin and the missing peak from oak tannins at 1285 cm^{-1} (**Figure 6**).

Measurement of Red Wine Tannins with Mid-Infrared Spectroscopy. The performances of the PLS models for measurement of wine tannins were evaluated from the prediction errors (RMSEP) and correlation coefficients (r_{val}) between the actual and predicted tannin concentrations of independent validation samples (**Table 3**). The results showed that the PLS

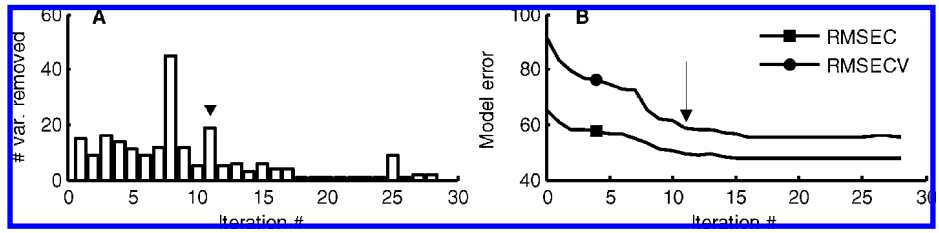

Figure 5. Variable selection by IBECSI-PLS results in iterative elimination of variables from the data (**A**), which attempts to minimize the model error (**B**). The arrow indicates optimal number of iterations.

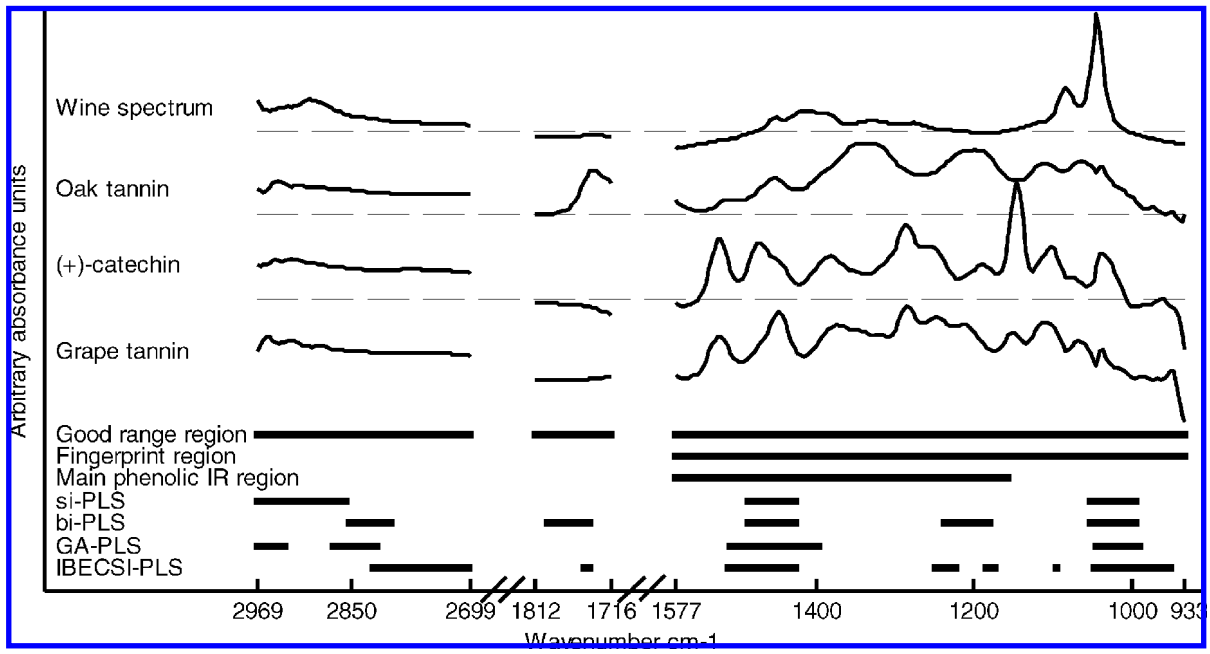

Figure 6. Identified spectral regions for tannin quantification obtained by different variable selection procedures in relation to the IR spectrum of a wine sample (scaled down 100 times) and the IR signals of oak tannin, (+)-catechin, and commercial grape tannin.

Table 3. Overview of Calibration and Validation Results for Quantification of Tannins in Red Wines from PLS Models Using the Spectral Regions Identified from Variable Selection Methods

selected variables	no. of variables	LV[a]	RMSEC[b]	RMSECV[b]	RMSEP[b,c]	r_{val}[d]
good range region	265	10	65	92	115 c	0.87
fingerprint region	168	10	69	91	92 bc	0.91
main phenolic region	110	10	54	75	88 b	0.90
si-PLS region	62	10	53	65	77 ab	0.93
bi-PLS region	78	10	53	65	69 a	0.94
GA-PLS region	70	9	55	69	79 ab	0.93
IBECSI-PLS region	97	10	49	59	75 ab	0.94

[a] Number of latent variables. [b] Root mean square error of calibration, cross-validation, and prediction, respectively, in mg of CE/L. [c] The same letters indicate no significant ($p < 0.05$) differences between the predictive abilities of the models. [d] Correlation coefficient between the measured and predicted tannin levels.

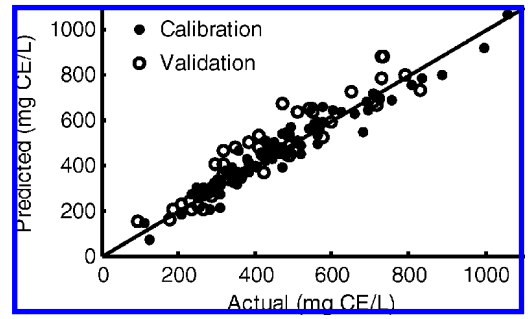

Figure 7. Measurement of red wine tannins (in mg of CE/L) by FT-MIR spectroscopy using the spectral region identified by IBECSI-PLS variable selection method.

model using the main phenolic region was significantly better than the model using all variables in the "good range", decreasing the RMSEP values from 115 to 88 and increasing the correlation coefficients from 0.87 to 0.91 (**Table 3**). Improvements in the RMSEP values to between 69 and 79 mg of CE/L and the correlation coefficients to ~ 0.94 were obtained for the four variable selection methods. Although all models using the four variable selection methods were significantly better than using the "good range" region, only the bi-PLS model was statistically better than the model of the main phenolic region (**Table 3**). The differences in the RMSEP values between the four variable selection methods were relatively small and were not found to be statistically different from each other (**Table 3**). Recently it was shown that little or no improvement in the measurement of tannins by mid-infrared spectroscopy was obtained by variable selection, when the majority of the interfering substances were removed using solid phase extraction and evaporation (*11*). The considerable improvements by variable selection found in this study were ascribed to the presence of major interferences from other wine components in the infrared spectra.

Figure 7 shows the correlation between the actual and predicted levels for the model developed from the region identified by IBECSI-PLS. The repeatability of tannin levels (in percentage of the mean value) predicted from the triplicate

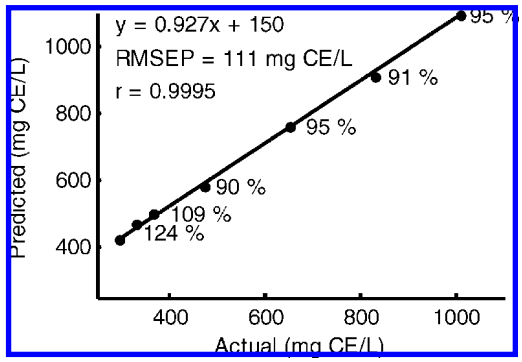

Figure 8. Prediction of tannin content by FT-MIR spectroscopy of a red wine (having 298 mg of CE/L tannin) spiked with 0, 0.1, 0.2, 0.5, 1.0, 1.5, and 2.0 g/L grape tannin corresponding to 0, 36, 71, 178, 355, 533, and 710 mg of CE/L. The recovered tannin amount (as a percentage of the added amount) is given next to the data points.

spectra of the validation samples was 3.5%. This was considerably higher than the repeatability of the tannin analysis reference method of 0.95% determined from duplicate measurements, but still acceptable. The prediction ability of the developed model (RMSEP = 75 mg of CE/L; r = 0.94; **Table 3**) was not as good as the model reported by Fernandez et al. (RMSEP = 51 mg of CE/L; r = 0.96), which, however, includes only a single grape cultivar and requires extensive sample purification (*11*). The prediction ability was similar to the reported values of Versari et al. (RMSECV = 63 mg of CE/L; r = 0.99), who also used FT-MIR spectroscopy, but their method was developed using a high number of latent variables for only 20 wines without any independent validation of the model (*12*). Skogerson et al. have recently shown that tannins can be measured by ultraviolet−visible (UV−vis) spectroscopy (RMSEP = 66 mg of CE/L; r = 0.93) in young Australian wines and fermenting juices (*20*). A similar accuracy (RMSEP = 75 mg of CE/L; r = 0.94) was found in our study with commercial wines covering a wide range of vintages, production countries, and grape cultivars using FT-MIR spectroscopy. The performance of the developed model was further tested for its ability to predict the tannin levels in a red wine spiked with different levels of grape tannin (**Figure 8**). Although the tannin content of the unspiked wine was predicted to be considerably higher (422 mg of CE/L) than the actual level (298 mg of CE/L), the spiked tannin levels gave a good linear response (r > 0.99) and acceptable recoveries for tannin levels higher than ~71 mg of CE/L.

The present study demonstrated that particularly important spectral regions could be identified almost equally well by the four variable selection methods: si-PLS, bi-PLS, GA-PLS, and IBECSI-PLS. The identified regions could be used to develop calibration models, which allowed the measurement of tannins in wines by FT-MIR spectroscopy. The results obtained demonstrate that FT-MIR spectroscopy (coupled with a proper calibration model) is a good option for the rapid quantification of tannins in red wines.

ABBREVIATIONS USED

BSA, bovine serum albumin; CE, (+)-catechin equivalents; LV, latent variables; PLS, partial least-squares; RMSEC, root-mean-square error of calibration; RMSECV, root-mean-square error of cross-validation; RMSEP, root-mean-square error of prediction; SD, standard deviation; SDS, sodium dodecyl sulfate; TEA, triethanolamine; UV−vis, ultraviolet−visible.

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