APPLICATION OF VISIBLE/NEAR INFRARED SPECTROSCOPY TO ASSESS THE GRAPE INFECTION AT THE WINERY

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Introduction

With the grapes consignment at the winery ends the viticulture phase and starts the vinification phase. This is a crucial phase and great attention must be paid to avoid that a wrong operation compromises the wine quality, especially in large scale companies such as cooperatives where the number of the members is very large.

The first process the grapes undergo at the winery is the visual inspection by the operators to define the phytosanitary state of the bunches. This phase is a critical point of the winemaking process as the grapes shouldn't show clear microbial infections (for example from Botrytis cinerea) in order to not decrease the quality of the final product.

The role of the operators at this stage is crucial because, depending on the quality of the grapes, the wineries often impose a price penalty which decreases the value of the raw material and imposes a remarkable reduction of the winegrowers' incomes.

The lack of an objective judgment could cause disagreements between members and wineries. Therefore, it's important, mainly in big companies, to develop standardized methods for an objective and quick evaluation (without slowing down the different logistical movements during the grape consignment) of the qualitative characteristics and the health status of the grapes in order to optimize the selection phase before starting the winemaking process.

Experimentation

The Department of Agricultural and Environmental Sciences (DiSAA) of the Università degli Studi di Milano, in collaboration with Cantine Settesoli (Menfi, AG, Italy), has investigated the applicability of an optical system performing spectroscopic measurements in the visible near infrared (vis/NIR) region to estimate the grape infection at the consignment to the winery. Spectroscopy is a rapid and non-destructive technique requiring minimal sample processing before analysis, coupled with chemometric methods, seems to be one of the most powerful analytical tool applicable to food products (Guidetti et al. 2012).

Materials and methods

Sampling

The experimental activity, which took place during the harvest season 2015 at Cantine Settesoli, was carried out to test the applicability of vis/NIR and NIR spectroscopy in order to quantify the qualitative status of grapes bunches directly at the check point station in winery.
The vis/NIR analysis allows to obtain information on the structural composition of sample through the interaction with the electromagnetic radiation (light) which induces a variation of vibrational energy in molecules (Figure 1) (Nicolai et al., 2007).

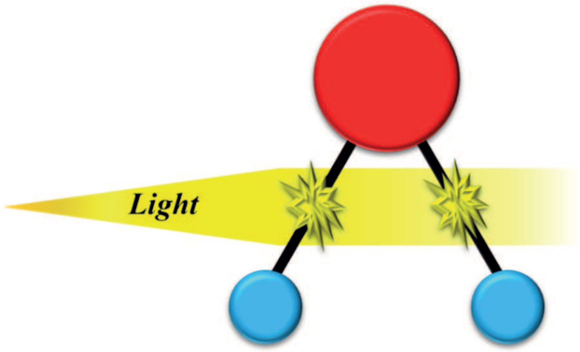

Fig. 1. Interaction of the electromagnetic waves (light) with water molecule (H_2O).

In this case, the spectrophotometer measures a material's reflection at specific wavelengths (400-1600 nm); the result is a reflection spectrum which will be analyzed with complex mathematical-statistical techniques (chemometrics). Reflectance spectroscopy is the study of light as a function of wavelength reflected or scattered from a solid, a liquid or a gas. The photons enter in contact with the sample and some are reflected by the surface, some pass through the sample and some are absorbed by the sample. The photons reflected from the sample's surface or refracted through particles which compose the matter may be detected and measured (Kortüm 2012).

During the analytical phase, red (Nero d'Avola, Alicante, Syrah) and white grapes (Grillo, Chardonnay, Viognier, Inzolia) were examined in lab-scale condition.

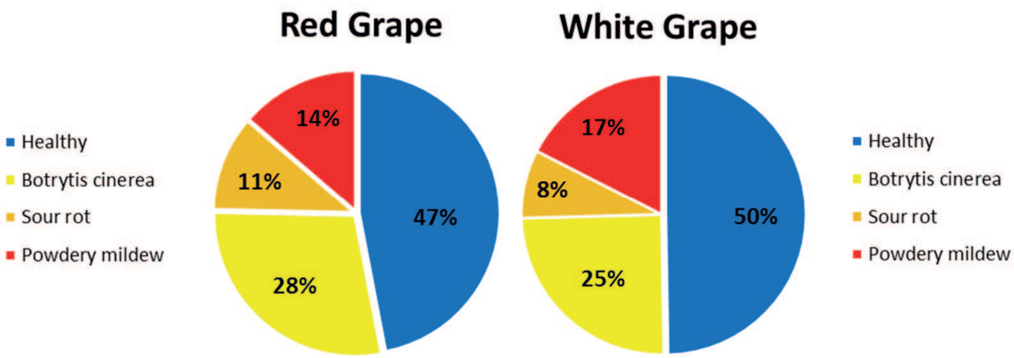

Fig. 2. Percentage of red and white grapes healthy and infected by Botrytis cinerea, powdery mildew and sour rot.

Sampling was done considering healthy grapes and naturally infected bunches by Botrytis cinerea, powdery mildew and sour rot. For the experimentation were used 2559 red and white grapes bunches in healthy and infected conditions, as shown in Figure 2.

Instrumentation

The spectral acquisitions were obtained from grape samples using the Corona Process system (Zeiss, Germania). The device was built for the application to food industry to realize analysis in vis/NIR region in reflectance mode.

Thanks to its compact dimensions (size 40 x 30 x 30 cm, weight about 15 kg), the instrument may be easily positioned into any technological process. The optics, spectrometer, electronics and referencing are all located in the same housing, therefore this measuring system has excellent long term stability and does not require frequent external calibration. The spectrophotometer is protected against shock and vibrations and analyses are reliable in all process environments. This device is therefore particularly efficient for application during the grape selection process at the check point station.

Measurements were performed at a variable auto-focused distance (80-600 mm) between sensor and samples (Figure 3). The fast and automatic measuring system allows to identify and quantify the portions of the bunches that exhibit fungal attack symptoms. The operator can define the product's acceptability threshold and obtain an objective indicator for grapes batches classification into the winery.

Fig. 3. Grapes bunches spectra acquisition method.

Data processing

The spectra were employed to calibrate a classification model by using a statistical tool for multivariate analysis. The pretreatment of vis/NIR and NIR raw data is the first step of model calculation. Data pretreatment helps to correct unwanted systematic sample-to-sample variation in measured spectral data.

It was afterwards performed a qualitative analysis of data by visual analysis of spectra.

The following step was a classification analysis using the Partial least Squares Discriminant Analysis (PLS-DA), particularly suitable for vis/NIR data. PLS regression technique generalizes and combines features from principal component analysis and multiple regression, it’s particularly useful when a prediction of dependent variables (X) from independent variables

(Y) set is needed (Wold et al., 2001). So, in the future application phase the PLS-DA classification algorithm will express the classified spectra as healthy and infected. For this reason, in function of a specific number of acquisitions, the percentage of total infection was referred to all measurements performed for each single wagon of grapes bunches (for example, if 100 spectra were acquired for each wagon and 75 spectra are classified as healthy and 25 as infected, the percentage of infection will be estimated at 25%).

Results and discussion

Qualitative analysis

Qualitative analysis was focused on spectra interpretation and PCA analysis. Figure 4 shows the average spectra acquired from healthy and infected (by Botrytis cinerea, powdery mildew and sour rot) grapes (red 4a, white 4b), in order to draw attention to the spectral differences. In visible (400-750 nm) and vis/NIR (600-950 nm) region no relevant spectral differences between healthy and infected grapes can be noticed. Regarding the NIR region (950-1600 nm), it's possible to highlight a variation of the reflection of about 10%. Considering these differences, it is presumably possible to train the instrument for a recognition of only healthy grape spectra.

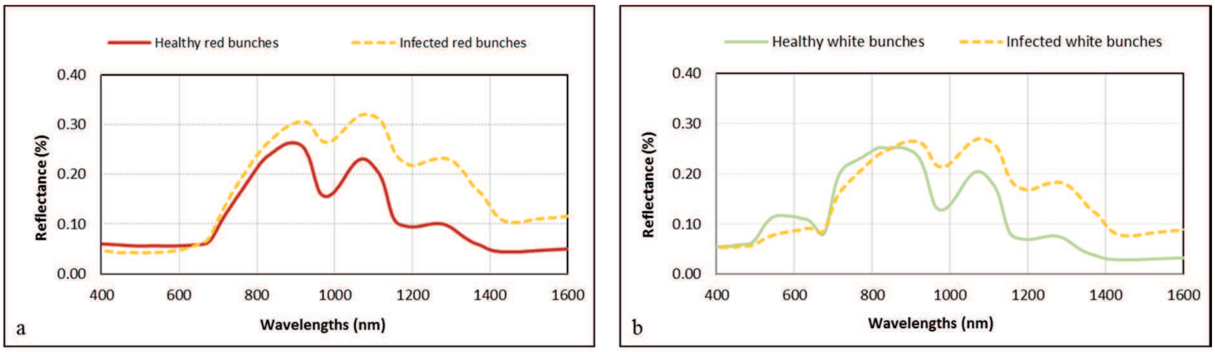

Fig. 4. Average spectra of healthy and infected grapes of red cultivars (a) and white cultivars (b).

Classification analysis

The spectral data were employed for the elaboration of PLS-DA classification models. This method allows to calibrate models considering the maximum separation between the classes of objects (Wold et al., 2001). So, the spectral data was used to set up models to classify the samples as healthy or infected.

In this work PLS-DA classification takes into consideration separately red and white grapes models, and a global model combining all the analysed bunches was also calculated.

Table 1 shows the results from the different samples datasets. For each PLS-DA model is reported the coefficient of determination R^2, useful as a measure of the predictive power of the dependent variable from the independent variables (Nagelkerke, N.J., 1991), and the amount of correctly (positive predictive value, PPV) and non-correctly (negative predictive value, NPV) classified samples.

All the analysed models present a high R^2 and a good quantity of correctly classified samples (in validation), these results determine a satisfactory performance level in the vis/NIR and NIR application to check the grapes phytosanitary status.

Table 1. PLS-DA classification models for red and white grape bunches.

Samples	N° of samples	Calibration			Cross-validation		
		R^2_{cal}	PPV_{cal}	NPV_{cal}	R^2_{cval}	PPV_{cval}	NPV_{cval}
Red grape	1375	0.62	1238	137	0.61	1235	140
White grape	1184	0.67	1085	99	0.66	1079	105
Total bunches	2559	0.63	2308	251	0.62	2301	258

It's important to emphasize that the level of correctly and non-correctly classified samples is a sum of data arising from several types of sample. So, for each classification model are present:

- Real healthy = healthy samples correctly classified as healthy by the instrument
- Real infected = infected samples correctly classified as infected by the instrument
- False positives = healthy samples erroneously classified as infected by the instrument
- False negatives = infected samples erroneously classified as healthy bunches

The analysis of false positives and false negatives samples (data not shown) is essential in choosing the model to be applied, because samples classified erroneously by the instrument can generate advantageous and disadvantaged situations against the winery or members. Therefore, these situations should be evaluated according to the strategies of the winery.

Conclusions

In this work, the vis/NIR spectroscopy was employed to investigate the possibility to perform a rapid grape infection assessment. Results are promising but further tests are desirable for a future real scale application.

Regarding the scale-up of the innovation, the optical system could be positioned on a conveyor belt between the grape reception area and the grape discharge hopper. With this solution, the measurement of the whole mass of grapes (transported in a thin layer on the belt) could be performed, resulting in an assessment of the infection degree of the total grape amount.

Finally, the automation of the grape assessment should be considered as a practical solution to solve the disagreements problems between members and wineries, making it objective the evaluation of the quality and the phytosanitary status of grape.

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Abstract

The aim of this work was to investigate the applicability of vis/NIR spectroscopy for rapid grape infection assessment in a view of a grape classification directly at the check point station entering the winery.

The experimentation was conducted on white and red wine varieties, using grape bunches naturally infected with Botrytis cinerea, powdery mildew and sour rot, the major grape diseases. The research applied a compact vis/NIR device (400-1600 nm) for analysis of flows and/or non-homogeneous product. The system is capable to perform measurements in reflection at a variable distance between sensor and sample of 80-600 mm. Spectral measurements were carried out on healthy and diseased bunches for a total of 2559 spectral acquisitions;

Qualitative (spectral analysis and Principal Component Analysis, PCA) and quantitative (Partial Least Squares – Discriminant Analysis, PLS-DA) analyses were applied on grape and must spectra in order to test the performance of vis/NIR device to classify healthy and infected samples.

The results obtained from PLS-DA models, in validation, gave a positive predictive value (PPV) of classification between 89.8% and 94.0% for grape. The results demonstrated that optical devices are capable to provide useful information for a better management of the vinification process.