Application of NIR Technique for Food Safety Evaluation During the Grapes Delivery in the Cellar

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Abstract
The oenological sector is increasingly moving towards an improved quality of wines.
To meet this requirement can be useful, at least in large cellars, use of instruments potentially capable of providing real-time information on the grapes or the first products resulting from their processing. Knowing in real time the health status of grapes might allow the grapes to split into homogeneous groups and consequently choose the treatments to be carried out in winemaking.
The aim of this work is to verify the potential use of NIR technique (Near Infrared Spectroscopy) for the detection of some fungal diseases in grapes conferred in the cellar.
The research was set to three years. In the first two years the assessments were made in the laboratory on samples of grapes from the same vineyard, which differ only in a different level of fungal diseases (Botrytis cinerea and Uncinula necator). In the third year the tests were conducted in a large cellar with 2360 hectares of vineyards distributed in the province of Forlì-Cesena (Italy).
The laboratory evaluations on samples of grapes from the same vineyard were positives and they allowed to distinguish between the diseases and intensities of infection.
An equally positive response was not found in the cellar, where similar state of health did not provide comparable results. This result is probably caused by different environmental conditions of the vineyards (soil, orography, etc.) and it limits the application of NIR.

Keywords: Grapes, NIR spectrometry, food quality, diseases

Introduction

The Italian wine sector is increasingly moving towards better quality wines that also involves lower middle standards, produced in large wineries, representing over 60% of italian production.

The grapes in large cellars are subjected mostly to two tests: the sugar content and acidity.

Sometimes you can also have a visual analysis on health status, but this is a subjective outcome and as such it can be considered as purely indicative result. The assessment of the health state is undoubtedly one of the most important evaluations to differentiate the quality production and it would deserve a more secure determination.

An alternative methodology would be NIR spectroscopy, which has gained approval in oenology (Cozzolino, 2003, Liu, 2008) and other agroindustrial sectors. In these sectors, the NIR technique is considered easy to apply and rapid in supplying results and, most importantly, requires no sample preparation. This means it can be applied directly on the

production lines, providing data in real time, and great potential can be foreseen in the horticultural sector, where this non-destructive technique could be applied to characterize the quality standards of samples (Katayama et al., 1996; Williams and Norris, 1987, Caprara et al., 2009).

The aim of this research is to determine if NIR spectroscopy is an efficient method of quality evaluation for health status of grapes delivered to the cellar.

Materials and Methods

Laboratory tests

Laboratory tests were conducted over two years, 2006 and 2007, and samples were taken from farms in the area of Faenza (Emilia Romagna region).

The varieties Sauvignon and Chardonnais have been selected and several product samples were collected. The samples were differentiated by disease (Botrytis cinerea and Uncinula necator) and infection level. The following classes were established:

- 0, healthy product 100%
- 33, healthy product 66%, diseased product 33%
- 66, healthy product 33%, healthy product 66%, diseased product 33%
- 100, healthy product 0%, diseased product 100%

Three replicates were considered for each sample.

The grapes were pressed using a small manual press and the must was then filtered through a membrane of paper and collected in a glass beaker.

The spectrometer was a Bruker Optics Matrix-F, used with a probe for liquid samples in the NIR wavelength from 0.7 to 2.5 μ.

The probe was immersed in the beaker containing the sample, avoiding contact with the bottom of the container, and the data were sent and processed by a computer.

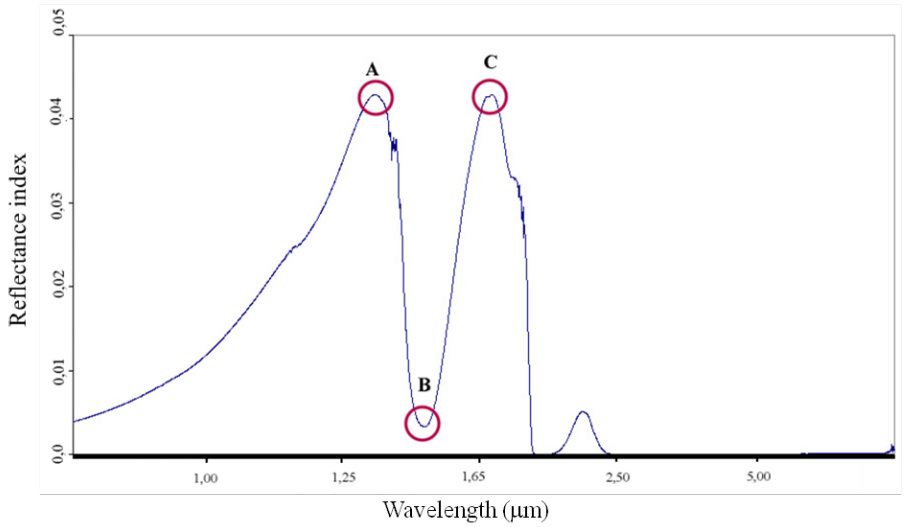

Figure 1. Spectrometric curve acquired with the NIR spectrometer (an example).

The NIR readings were evaluated by introducing an evaluation index calculated on the basis of equation 1 that utilizes characterizing points of the spectrometric curves (Caprara et al., 2009), in order to free the analysis from the absolute values of the individual readings (Fig. 1):

$$I = \frac{A - B}{B} \quad (1)$$

The data were analyzed using STATGRAPHICS software (StatPoint Inc., VA, USA). Analysis of variance (ANOVA) was performed on the data, considering storage time as main factor of analysis. The method used to discriminate among the means is Fisher's 95% least significant difference (LSD) procedure.

Tests in the cellar

The second part of the trial was conducted in 2008 at the "Cantina Sociale di Forli" on a day at the end of the harvesting period.

The analysis was performed on Trebbiano grape that is the most representative vine for the winery in question.

The samples were collected with a beaker from the tap of refractometric station where usually the tests were carried out.

For each sample were known the receiving area of the grapes, pH, Brix and health status assessed visually.

The evaluation was performed with the NIR with a probe for liquids with the same procedures adopted in the laboratory and using the same evaluation index.

The parameters considered as main factors of the statistical analysis are:

- health status (healthy product, diseased product)
- pH (higher values than average –lower values than average)
- ° Brix (higher values than average –lower values than average)
- Place of production (Valley – Hill)

The analysis was performed by comparing the evaluation index with the health status of the grapes and subsequently a multiple analysis of variance was performed considering as factors the health status coupled with one of the other three parameters (pH, ° Brix, place of production)

Analysis of the results and discussion

Laboratory tests

Laboratory tests, conducted under controlled conditions, gave indications of some interest.

Different samples from the same vineyard and with the same level of health showed the same absorption spectra.

In the presence of fungal diseases a lowering of maximum peak always occur, proportional to the level of infestation (Fig.2).

Statistical analysis done on the evaluation index revealed significant differences between the levels of infection and the diseases (Table 1).

Table 1. Multiple analysis of variance and mean values of evaluation index for the Chardonnais must in laboratory tests.

Effects		P-Value (observations = 33)	
Main effects	Grape deseases	0.0000	
	Level of infection	0.0000	
Interactions		0.0004	
Grape deseases	Level of infection	Evaluation index	
		Mean ± SE	Homogeneous groups*
Botrytis	100	9.82 ± 0.14	A
	66	10.13 ± 0.14	A
	33	10.83 ± 0.14	B
	0	11.69 ± 0.14	C
Oidio	100	10.89 ± 0.10	A
	66	10.84 ± 0.10	A
	33	11.00 ± 0.10	B
	0	11.54 ± 0.10	C

*Within each cultivar, means designated by the same homogeneous group letter were not significantly different based on Fisher's 95% LSD method.

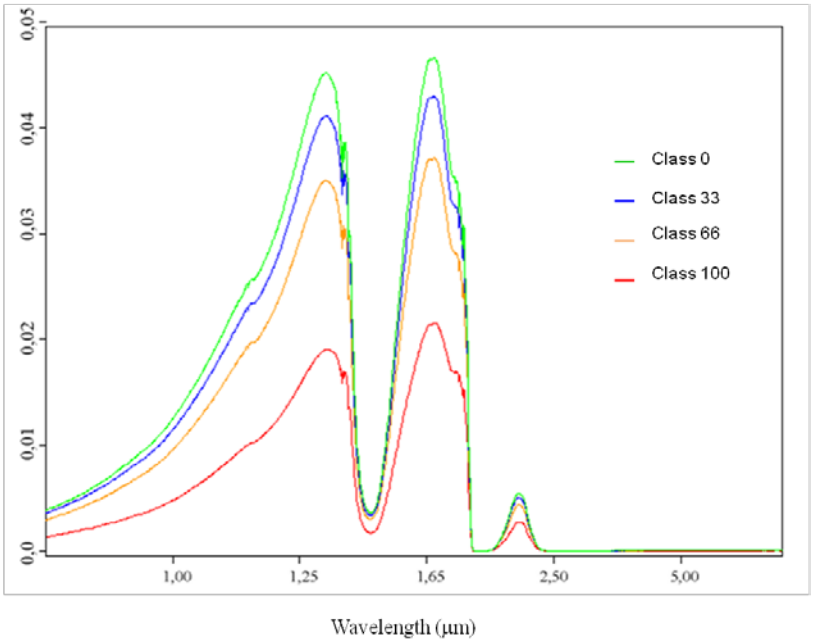

Figure 2. Spectrometric curve acquired with the NIR spectrometer for the Chardonnais must in laboratory tests. The curves show four classes of infection level for botrytis (0 - healthy product 100%; 100 - diseased product 100%)

Tests in the cellar

The 2008 weather conditions not adversely affected the health of grapes with maximum levels of infection reported mainly by botrytis (10%).

Table 2. Analysis of variance of evaluation index for tests in cellar.

Effects		Mean ± StE	P-Value (observations = 21)
Grape deseas	No	14.14 ± 0.39	0.70
	Yes	9.93 ± 0.37	
Place of production	Valley	9.89 ± 0.29	0.31
	Hill	10.59 ± 0.60	
° Brix	> 10.6	10.50 ± 0.36	0.09
	≤ 10.6	9.60 ± 0.34	
pH	> 3.6	10.30 ± 0.40	0.39
	≤ 3.6	9.83 ± 0.35	

The evaluation of the spectrometric curves, which in laboratory tests have shown considerable significance, not shows in trials in the cellar no difference between test samples with disease and healthy ones. This can not be attributed to the low level of found infection but it is due to the high variability of the samples. Indeed, even considering separately the two groups of samples (healthy and diseased), variability still remains high.

Performing statistical analysis, the evaluation index shows no significant difference (Table 2).

This behavior is repeated with a multiple analysis of variance considering as factors the health status coupled with one of the other three parameters: pH, ° Brix, place of production (Table 3). The only parameter that has a tendency to highlight differences ($p = 0.09$) is ° Brix.

Table 3. Multiple analysis of variance of evaluation index for tests in cellar.

Main Effects	P-Value (observations = 21)
Grape deseas	0.53
Place of production	0.27
Grape deseas	1.00
° Brix	0.09
Grape deseas	0.91
pH	0.45

Conclusions

The similarity of spectrometric curves recorded on the must with NIR has enabled a comparative evaluation by establishing an evaluation index obtained with two characteristic points of the curves.

Laboratory evaluations carried out on different grapes coming from homogeneous areas showed the ability of instrumentation to detect the presence of the disease and the level of infection.

The application in a real situation with heterogeneous grapes for place of production and mode of cultivation it has not the same capability to identify the diseased musts.

The analysis of correlations between the defined evaluation index and other characteristic parameters such as pH, ° Brix and place of production, shows no statistically significant differences in the same way.

The uncertainty in the results obtained in the cellar is due to the heterogeneity of samples from grapes grown in different climatic and pedological environments with cultivation techniques that are not always similar.

This result confirms the findings in other applications of NIR on fresh food, where the actual application of technology requires a very complex preparation which provides for the systematic acquisition of a large number of measurements on samples from fairly homogeneous areas of production.

In the case of grapes the issue proves even more complex because the variability may be higher due to various factors such as crop variety, clone, rootstock, etc., that make the masses of product to evaluate the more heterogeneous samples.

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