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Comparison of two immersion probes coupled with visible/near infrared spectroscopy to assess the must infection at the grape receiving area

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ABSTRACT

The aim of this work was to investigate the applicability of visible/near infrared (vis/NIR) spectroscopy comparing two immersion probes to evaluate the phytosanitary status of must at the grape receiving area to support wineries for an objective quantification of grape infection level.

The experimentation was conducted on white and red varieties (Vitis vinifera L.), employing grapes naturally infected with the major wine grape diseases. A total of 159 musts were product and analysed by using a vis/NIR spectrophotometer coupled with two kind of immersion probes: a reflectance probe and a transmittance one. Classification analysis (Partial Least Squares – Discriminant Analysis, PLS-DA) was performed on musts spectra to test the performance of the spectrophotometer, combined with the two probes, to classify healthy and infected samples.

Considering three different spectral range analysed (430–1000 nm; 1000–1650 nm; 430–1650 nm), the results obtained from PLS-DA models, in cross-validation, gave values of correctly classified samples (accuracy, %) between 52.5% and 90.4%, and ranged from 68.4% to 84.3% for reflectance and transmittance probes respectively.

The optical system was tested in controlled laboratory conditions, simulating the desirable final use of device. A future real scale application could be envisaged inside the analysis tank at the grape receiving area, after setting the operative conditions to perform the measurements directly coupled with the traditional and common quality analysis (soluble solid content and acidity) performed on grape musts sampled at the grape consignment.

1. Introduction

Grape berries are susceptible to infection by a range of microorganisms that affect grape in field, causing a loss of yield, and influence wine quality during winemaking process. Wine made from grapes affected by bunch rot has typically mouldy or fungal aromas and flavours, and grapevine pathogens affect wine quality through the degradation of grape phenolics and the production of extracellular polysaccharides (Scott et al., 2010; Steel et al., 2013). Moreover, it must be considered that these quality aspects are heavy critical points. Especially nowadays that customers' awareness is constantly increasing and they ask for good quality products at competitive prices.

Since management of these diseases in the vineyard is unable to solve the problem, wineries should provide selection at check point station entering the winery. Currently, the phytosanitary status control of grapes is commonly performed by a visual assessment by the operator or utilizing several chemical analyses in innovative wineries (Ribéreau-Gayon et al., 2006). These methods result, in the first case, subjective and liable to assessment errors while in the second case are limited by the lack of laboratories and technologies in most wineries (Porep et al., 2014a, 2014b). Nevertheless, the chemical analysis is often expensive, time consuming, destroys the samples and generally highlights only one or a few information which reduces the chances to optimize the production. Hence, there is a strong need for developing a rapid, cost-effective, and reliable health-monitoring sensor that would facilitate advancements in agriculture (Sankaran et al., 2010), in particular for the grape phytosanitary status assessment, possibly directly at the receiving area.

Based on these requirements, wineres to be competitive must find good compromises between preserving the wine quality exposing to major losses; or enhance the risk of moving down the quality of wine accepting the consignments of partially infected grapes to preserve the

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produced quantity of wine reducing the losses. Therefore, to maximize the quantity of good quality wine, a computerized decision-support system is needed to help the control of grapes phytosanitary status at the check point station entering the winery.

The visible near infrared (vis/NIR) and near infrared (NIR) spectroscopy has proved to be one of the most efficient and advanced tools for monitoring and controlling processes and product quality in food industry (Guidetti et al., 2010), thanks to well known advantages (Elmasry et al., 2012; Guidetti et al., 2012; Dale et al., 2013).

A statistical analysis is also required to extract the useful information about quality attributes which are hidden in the vis/NIR and NIR spectra (McGlone et al., 2002; Dambergs et al., 2004). Chemometric techniques are an essential tool to process and to model data sets in order to extract the highest information contained in spectra (Guidetti et al., 2010).

NIRs spectroscopy has been proposed like reliable measure for rapid evaluation of phytosanitary status of wine grapes and must. On grape must Porep et al. (2014a) used NIRs spectroscopy to determine the ergosterol content as a new target marker for fungal grape rot infection; the objective of that work was to reduce the time of chemical analysis and optimize the wine making process. Dambergs et al. (2005) investigated the potential of NIR spectroscopy as a tool for detection of powdery mildew in homogenised grapes. On grape bunches, Beghi et al. (2017) studied the feasibility of NIR spectroscopy application to classify grape samples based on the infection by Botrytis cinerea, powdery mildew and sour rot in order to replace the subjective visual evaluation performed currently on the wagon at the check point station entering the winery. Nevertheless, further studies are needed to find alternative computerized decision-support solutions easily adaptable and usable to identify the infection degree of wine grape entering the winery.

The aim of this work is to evaluate a method for real-time assessment of phytosanitary status of the must sampled at the grape receiving area. Vis/NIR spectroscopy was applied comparing two immersion probes as a quick and reliable method to classify healthy and infected samples, to support wineries in the decision on grape destination and to provide an efficient tool for quality control and management.

2. Materials and methods

2.1. Sampling

The experimental activity took place during the harvest season 2015 (25th August/30th October 2015) at Cantine Settesoli (Menfi, AG, Italy, 37°36′14″N, 12°58′08″E). Sampling was performed on grape musts directly obtained during the grape consignment from each wagon containing individual varieties of white grape (Vitis vinifera cv. Ansonica, Cataratto, Fiano, Grecanico, Grillo, Inzolia, Trebbiano) and red grape varieties (Vitis vinifera cv. Anglicanico, Alicante, Cabernet Souvignon, Merlot, Nero d'Avola, Petit Verdot, Sangiovese, Syrah). The sampling was carried out on an industrial scale grape consignment operation, and therefore the number of samples for each variety is extremely variable (Table 1).

The experimentation was conducted using grape bunches, healthy and naturally infected with botrytis cinerea, powdery mildew (Erysiphe necator) and sour rot, the major wine grape diseases.

The grapes on wagon were visually inspected by winery experts and classified in healthy or infected; this is the only established method for the evaluation of the grape phytosanitary status applied in the studied winery. An on-line grape analyzer system composed by truck sampler (Maselli CC05, Maselli Misure, Italy) equipped with pump and mechanical shredder to pick and cut the bunches at different levels of consignment wagons (Fig. 1) was used to obtain the must samples. Each grape must samples, corresponding to about 2 dm^3, was then directed to a specific tank where it waits for the traditional and automated quality analysis (Maselli SA, Maselli Misure, Italy) (soluble solid content and acidity).

Spectral acquisitions were performed in the laboratory of the winery using the 2 dm^3 of grape must withdraw from the tank for each sample. Measurements were performed in reflection mode, on not filtered samples, and in transmission mode, on filtered samples, between 430 nm and 1650 nm.

2.2. Reference disease assessment

The reference measures are needed in order to define if the grape must samples are suitable for the winemaking process. Therefore, for each sample a direct visual inspection of the grape bunches on the wagon (before being crushed) was performed by operators to establish the phytosanitary status of grapes.

The visual inspection data are necessary to train the instrument to identify the real healthy and real infected must samples. Samples from each wagon were marked according to an arbitrary level of infection 0 e 1 (IL), as follows (Beghi et al., 2017):

- IL = 0 (IL0, infection level 0), corresponding to healthy tissues and bunches with small (< 2 mm in diameter) chlorotic spots with hyphae at the initial stage of development and with middle stage mycelial structures (rarely visible also with careful observation).
- IL = 1 (IL1, infection level 1), extended colonies (> 2 mm) with evident gray-whitish powdery appearance and brown senescent-necrotic lesion.

Bunches scored as IL0 were considered acceptable for the vinification process, while samples scored as IL1 were considered “rotted” and unacceptable for a high quality vinification and therefore downgraded by the winery. Subsequently the healthy and infected bunches were crushed to obtain the must samples for the spectral acquisitions.

2.3. Vis/NIR and NIR spectral acquisitions

The spectra collecting system is shown in Fig. 2. The system consists of four components: (i) a spectrometer, (ii) fibre optics (an incoming fibre and an outer Y-type fibre), (iii) a reflection/transmission probe and (iv) a personal computer. The spectral data were collected by a Zeiss MCS 600 spectrometer (Detectors were MSC 621VISII and MCS 611NIR 1.7, Carl Zeiss, Germany) in the vis/NIR region (430–1000 nm) and in the NIR region (1000–1650 nm). Both spectral ranges were also used joined to extract major information. Spectral acquisitions were performed without any sample preparation directly in the must using a Gladius reflection probe (*Hellma,* Germany) and subsequently the same samples were filtered (with *Sigma-Aldrich* separation filter papers) and analysed with an Excalibur transmission probe with specific optical path length (5 mm) (*Hellma,* Germany). The reflectance probe is composed by optical fibres which transport the light by the source at the sample (grape must), these fibres are positioned around the central fibre which deliver the reflected radiation from the sample to the spectrophotometer (Fig. 2b). The transmission probe is suitable for liquid clear solutions. The optical fibre spread the light radiation through the sample (filtered grape must), then the transmitted light is detected by the opposite side of the probe after a fixed optical path length (Fig. 2c).

As detailed in Table 1, spectral measurements were carried out on different varieties of grape for a total of 159 samples in the vis/NIR spectral range and 88 in the NIR range. Each spectral sample recorded resulted from an automatic average of 20 scans. The measurements performed on healthy and diseased musts (grape bunches affected by botrytis cinerea, powdery mildew and sour rot) allowed to obtain approximately the 60% of healthy samples and 40% of infected samples for each spectral range.