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Real time monitoring of multiple components in wine fermentation using an on-line auto-calibration Raman spectroscopy

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ABSTRACT

In this paper, the novel technique for monitoring time-related changes that occur during wine fermentation, associated with auto-calibration Fourier transform Raman spectroscopy (FT-RS) and chemometric data analysis, was investigated. The auto-calibration system, which is composed of a reference optical path and measurement optical path, is first presented in this paper. The reference optical path was used to eliminate the frequency shift and intensity shift of the Raman spectra induced by the ambient temperature. The micro-fermentation trials conducted in our laboratory during 2013 were monitored by the auto-calibration FT-RS and by high-performance liquid chromatography (HPLC). Principal component analysis (PCA) and Partial least square (PLS) were applied to the Raman spectra to determine the relationship between the spectra and the concentration of sugar or alcohol during the fermentation process. The results from the PLS-PCA and HPLC showed good correlations and were obtained between predicted and actual sample values for sugar (0.995), ethanol (0.9999), and glycerol (0.98). The experimental results showed that the auto-calibration FT-RS is useful to identify crucial information about the quality of the final product in agreement with the chemical analyses during wine fermentation.

1. Introduction

Wine fermentation is the process by which yeast converts sugars to ethanol and carbon dioxide. To reduce stuck and sluggish fermentation, as well as to ensure the quality and productivity of wine products at all stages of the fermentation process, effective fermentation monitoring, which can rapidly provide real time information, is important due to the rapid pace of development in the wine industry. During wine fermentation it is important to measure both the substate and quality concentration (e.g., sugars, ethanol and glycerol) and to evaluate other quality characteristics of the final product [1]. However, the analysis of these compounds by traditional methods requires sample preparation and specific analytical equipment and is time-consuming [2]. Therefore, the real time monitoring and control of fermentation processes are necessary to increase productivity, efficiency, and reproducibility.

Electronic sensor technologies are already commonly used to monitor critical process parameters (e.g., temperature, pH and dissolved oxygen) to achieve process control. However, many of these methods require that a sample be withdrawn from the fermenter to obtain the desired information. This approach has a time delay, lack the robustness of simple procedures and handling issues. One single sensor cannot realize simultaneous detection of multiple components. Therefore, the development of a rapid and low-cost detection method for multiple components is the most promising direction.

Among innovative methods, infrared spectroscopy (in both the near and mid infrared regions), which is an alternative to conventional chemical analyses for real time monitoring of various products and processes, represents a fast method. Absorption spectra in the infrared range can be related to the main chemical components of food, such as proteins, carbohydrates, fats and water [3]. Infrared spectroscopic techniques have already been employed in many applications, both in the laboratory and in on-line control in industrial plants [4–7]. Infrared spectroscopy is used as innovative systems for wine quality assessment and alcoholic fermentation control in the wine industry. However, infrared spectra peaks are broad and overlapping, making the interpretation of the spectra difficult and the strong interference from water can make sample preparation in aqueous system difficult.

Raman spectroscopy is unique among spectroscopy techniques because liquid, gas or solid samples can be analyzed directly and rapidly without any sample preparation. Raman spectroscopy offers several advantages over chromatography and infrared spectroscopy in the analysis of water-rich and multi-component

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samples [8–11]. Raman spectroscopy can meet the demand of automation and continuous measurement due to its speed and on-line capabilities. However, the characteristics of the Raman spectra of the substance, such as frequency and power, will change with the ambient temperature and the time of use [12]. Raman spectrometers for scientific research use a laser with a stable frequency and power as the excitation light source and have strict requirements regarding the operating ambient. A laser with stable frequency and power is expensive, which hinders the popularization of the Raman spectrometer. Therefore, the objective of the present study was to develop a Raman spectrum detection system and a method for eliminating effects on the detected Raman spectra caused by changes in system performance resulting from changes in the ambient factors and key components.

In this paper, an auto-calibration FT-RS, composed of a reference optical path and measurement optical path, was first investigated and used to monitor wine ferments. HPLC was used to determine the sugar, ethanol and glycerol concentrations in the fermentation products. The data obtained from Raman spectroscopy and HPLC were modeled to identify the critical points during fermentation.

2. Materials and methods

2.1. Micro-fermentation trials and sampling

Two micro-fermentation trials were conducted using stainless steel vessels (capacity 320 L). Each trial was conducted during the 2013 vintage, using grapes from Changli Vineyards. The fermentation trials were performed at room temperature (17–23 °C) under similar and standardized conditions, using active dry yeast inoculums. In the micro-fermentation trial, the probe of the auto-calibration FT-RS was place at the centre of the vessel and detected the Raman spectra of the samples at subsequent times during wine fermentation.

A total of 85 samples (juice and ferments) were collected from micro-fermentation trials. 35 samples were obtained from fermentation trial 1 and analyzed from the grape crushing to the end of alcoholic fermentation (from 0 to a maximum about 9 days) by the auto-calibration FT-RS system. Fifty samples were obtained from fermentation trial 2 and used to establish the calibration model.

To obtain the best calibration model, fifty samples (juice and ferments) with different ethanol, sugar and glycerol concentrations (ranging from 5% to 38% v/v for ethanol, from 1 to 50 g/L for sugar and from 1% to 20% v/v for glycerol) were prepared to produce calibration curves. Absolute ethanol and glucose were locally purchased as commercial products and were used without further purification. Standard solutions at several concentrations were obtained by diluting the juice and ferments. They are randomly separated for calibration (80%) and validation (20%). The sets spanned the entire range of values, so there was no need to perform an arbitrary assignment of samples.

2.2. HPLC determination of fermentation parameters (sugar, ethanol and glycerol)

In our experiments, the sugar, ethanol and glycerol concentrations of the samples were determined by HPLC according to the method of López and Gómez [13] (Merck Hitachi, Japan). Before sample detection, all of the samples were filtered through a membrane filter with a pore size 0.45 μm. Then, the analysis was performed via isocratic elution using 0.01 N sulfuric acid at a flow rate of 0.7 mL/min. The column temperature was 60 °C, and the injection volume was 20 μL. The analysis was performed in triplicate. The HPLC results were used as reference data in the Raman analysis.

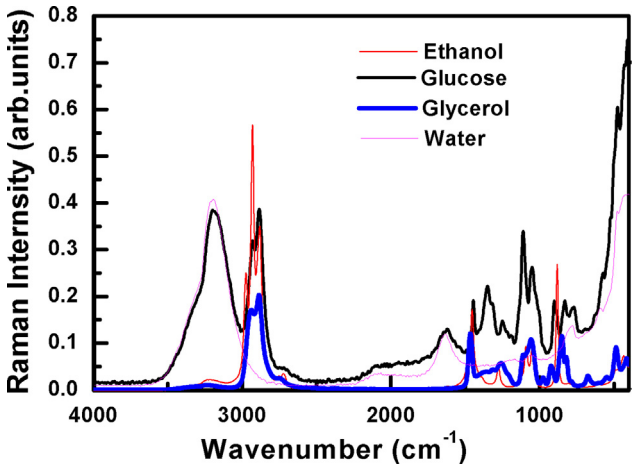

Fig. 1. Raman spectra of ethanol (10% v/v), glucose (25 g/L), water and glycerol (20% v/v).

2.3. FT-Raman measurements

Auto-calibration FT-Raman spectra were obtained using a Bruker MultiRAM system (Bremen, Germany) with a 1064 nm laser source and a liquid nitrogen cooled Ge detector. The probe of the FT-RS was sealed in a filter tube (as shown in Fig. 2), and placed at the centre of the vessels. The filter tube eliminates the effects of scatter caused by impure samples during wine fermentation.

Spectral data were collected over the range 400–4000 cm^{-1} at a resolution of 8 cm^{-1} under fixed measurement parameters (laser power: 500 mW, average of two measurements, 256 scans). The OPUS software package provided by Bruker Optics was used for spectral acquisition, instrument control and preliminary file manipulation. A full factorial experimental design was used to determine the most appropriate acquisition parameters, such as the number of accumulations, the acquisition time and the laser power.

Each sample measurement was repeated twice, and the measurement with the largest deviation with respect to the average was eliminated. The Raman spectra of the aqueous solutions of ethanol (10% v/v), sugar (25 g/L), glycerol (20% v/v) and water are shown in Fig. 1. These spectra provide evidence of the unique spectral features of the key fermentation constituents, showing component-specific peaks that allow for quantitative analysis and modeling. The intense bands at approximately 3200 cm^{-1} and 1640 cm^{-1} correspond to the O—H stretching vibration of water. The bands at approximately 2878, 2929 and 2972 cm^{-1} correspond to the $—CH_2$ and $—CH_3$ stretching vibration of ethanol. The band at 1454 cm^{-1} corresponds to the $—CH_2$ and $—CH_3$ stretching vibration of ethanol, the band at 1096 cm^{-1} corresponds to the CH_3 rocking vibration of ethanol, the band at 1054 cm^{-1} corresponds to the C—O stretching vibration of ethanol and the band at 883 cm^{-1} corresponds to the C—C stretching vibration of ethanol [7,8]. The peaks of sugar are generally broader, especially the O—H bending vibration at approximately 1300–1400 cm^{-1}, the band at 1462 cm^{-1} corresponding to $—CH_2$ bending vibration and the various C—C or C—O bending modes near 1130 and 1050–1080 cm^{-1} [7]. For glycerol, the peaks are similar to those of ethanol and sugar. There is generally little interference between water, sugar and ethanol; therefore this region cannot be neglected in quantitative analysis.

2.4. Data processing

Spectral pre-processing is the first and key step in multivariate analysis because appropriate data treatment is required to develop