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Exploring the applicability of MIR spectroscopy to detect early indications of wine fermentation problems

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Abstract

In this study we explore the applicability of MIR technology to detect early indications of wine fermentation problems. An oenologist could improve the chances of a vinification process finishing optimally if anomalies are detected early. A comparative analysis of three fermentations with artificial musts was performed; one of normal behaviour, one subject to a temperature gradient, and the third deficient in assimilable nitrogen. We tracked each fermentation through changes in spectra in addition to changes in must composition. It was easier to detect anomalous behaviour by monitoring wine metabolite concentrations than through direct spectra analysis, nevertheless, calibrations needed to be derived from fermenting must samples and so cost more. All measured compounds (glucose, fructose, ethanol, glycerol, succinic and acetic acids) exhibited behavioural changes at 30 h of fermentation in nitrogen deficient musts. Temperature deviations were reflected in the anomalous behaviour of ethanol, glycerol, succinic acid and acetic acid.

Keywords: Artificial must; Nitrogen deficiency; Problematic fermentations; Stuck; Sluggish; Temperature

1. Introduction

Stuck and sluggish alcoholic fermentations still occur frequently today and pose the wine industry considerable problems. In a sluggish or stuck fermentation, yeast sugar consumption slows or comes to a complete halt. Recovery depends principally on how early problems are identified. Frequently, a sluggish or stuck fermentation results from multiple combinations over time of factors including excessive initial sugar contents, ethanol toxicity, limited nutrients, short-chained fatty acid toxicity, extreme temperatures and nitrogen deficiency.

Temperature extremes inhibit the rate of fermentation. Like ethanol, temperature directly affects membrane fluidity and therefore nutrient transport. Temperature also has an influence on the yeasts' capacity to assimilate amino acids during alcoholic fermentation. Amino acids are consumed rapidly at the beginning of the fermentation at a rate directly proportional to temperature. At temperatures above 30 °C, the risk of the accelerating fermentation getting stuck early on also increases, because the effect is amplified when the sugar content is high (Ribereau-Gayon, 1999).

Quantitatively, nitrogen is the second most abundant nutrient in winemaking fermentations, and is another important risk factor. Ammonium and amino acids are the only assimilable sources of nitrogen in the must. Assimilable nitrogen determines yeast population and the fermentation rate (Varela, Pizarro, & Agosin, 2004). Therefore, a lack of assimilable nitrogen will cause fermentation problems.

Controlling the outcome of a winemaking fermentation is only possible if timely and accurate measurements of decisive parameters are available. While standard instruments, such as temperature and pressure gauges, are useful for tracking basic must conditions, advanced instrumentation is needed to detect change in nutrient levels as a fermentation progresses.

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Infrared spectroscopy offers a quick alternative to conventional methods of analysis. According to Christy, Ozaki, and Gregoriou (2001) the energy levels of most molecular vibrations are found in the mid-IR region of the spectrum (4000–400 cm^{-1}). For organic structure analysis it is the most informative region. MIR has been successfully applied, for instance, in monitoring industrial wine fermentations (Urtubia, Pérez-Correa, Meurens, & Agosin, 2004). The authors developed IR calibrations to analyse fermenting must at every stage of fermentation. Calibration models were obtained using partial least squares (PLS) and proved effective for analyzing Cabernet–Sauvignon fermentations for glucose, fructose, glycerol, ethanol, as well as the most abundant organic acids found in wine. Other MIR applications developed include the classification of dried extracts from red wines according to origin (Picque, Cattenoz, & Corrieu, 2001), and laboratory fermentations using artificial musts (Fayolle, Picque, Perret, Latrille, & Corrieu, 1996). In the latter, the authors studied the effect of temperature and calibration methods on MIR accuracy to measure must concentrations of glucose, fructose, glycerol and ethanol, and found that sample temperature variations proved detrimental to calibration accuracy. Elsewhere, Blanco, Peinado, and Mas (2004) developed effective PLS calibrations for measuring glucose, ethanol, glycerol, biomass and acidity using near infrared (NIR) spectroscopy. Calibration and validation samples came from laboratory fermentations and from artificial mixtures.

In the present study, we explore the applicability of MIR spectroscopy to detect early indications of problematic fermentations by comparing a given normal fermentation and two problem fermentations; one that was severely nitrogen-starved and the other subjected to a steep temperature gradient.

2. Materials and methods

2.1. Fermentation conditions

In this work three batch cultures were analyzed under different conditions: a normal fermentation (F_N), a fermentation with a temperature gradient (F_T) and a nitrogen deficient fermentation (F_{ND}), All cultures were carried out with *Saccharomyces cerevisiae* Prise de Mousse EC1118 in medium MS300 (Salmon & Barre, 1998), which is an artificial must similar to standard grape juice. Assimilable nitrogen concentrations (ammonium and amino acids) were 300 mg N/L for F_N and F_T, and 50 mg N/L for F_{ND}.

Two reactors, one of 50 L, with a 35 L working volume (Bioengineering) and the other of 2 L, for F_T and F_{ND}, with a 1.5 L working volume (Bioflo IIc) were employed. Both were inoculated to get an initial density of 10^6 cell/mL. For the three cultures, pH was maintained at 3.5 by automatic control, and the initial temperature was 28 °C. This temperature was kept constant throughout the process in fermentations F_N and F_{ND}. In F_T, roughly emulating a cooling system failure and recovery, the initial temperature was 28 °C, but after 10 h it was raised linearly for 30 h to 41 °C, and then reduced abruptly to 28 °C.

3. Analytical techniques

Periodic measurements were taken to study the behaviour of each fermentation. Samples were analyzed using infrared spectroscopy and HPLC. Compounds measured were glucose, fructose, glycerol, ethanol and succinic and acetic acids. A high-performance carbohydrate cartridge was used for measuring glucose and fructose as described by the manufacturer (Waters Corporation, USA) while a Bio-Rad HPX-87H column was used for determining glycerol, ethanol and organic acid measurements. A Merck-Lachrom L-7490 refraction index detector was used for sugars, glycerol and ethanol while a Merck-Lachrom L-7450A diode array detector was used for organic acids.

4. Infrared equipment and calibration

Acquisition of sample spectra was performed with Fourier transform infrared (FTIR) Multispec equipment (module FTIR AVATAR 360 NICOLET) equipped with a DTGS KBr detector. Its spectral resolution was 0.5 cm^{-1}. Spectra were acquired at MIR range. To eliminate suspended solids, liquid samples were centrifuged prior to spectra acquisition. *Bacchus Acquisition* software was used to define measurement parameters. *Bacchus Quantification* and TQ Analyst v.6 software were used for spectra pre-processing and for multivariate calibrations. Bacchus Acquisition and Bacchus Quantification software packages were developed by CETIM, France (http://perso.wanadoo.fr/cetim2/gb/cetlab_index_gb.htlm) while TQ Analyst software was developed by Thermo Nicolet (http://www.thermonicolet.com).

After Blanco et al. (2004), we developed two calibrations to analyze artificial musts. First, using a factorial design, we prepared 75 samples of artificial mixtures (Statgraphics 4.0) whose compositions covered the entire concentration range commonly encountered in artificial fermenting musts (Salmon & Barre, 1998). For example, the glucose fermenting must composition ranged between 0 and 150 g/l, hence artificial mixture samples were 0, 20, 40, 75, 110, 150 and 180 g/l. Of the 75 samples prepared, 50 were used to develop the calibration. The remaining 25 artificial samples plus 10 samples taken from fermentations F_T and F_{ND} were used for validation. Both manual and automatic wavelength selection procedures were tested. Much lower prediction and calibration errors were obtained using manual selection based on known component absorption wavelengths (see section spectral analysis). By trial and error, we found the best choices were the first derivative for pre-treatment and PLS for regression. Bacchus software was used to develop and evaluate the calibrations.