Facing the Complexity of Grape Quality Management and Delivering an Highthroughput Device: VinePAT

Martins RC[1], Lopes VV[2] and Silva-Ferreira, AC[3]

[1] Molecular Biology and Ecology Research Center, University of Minho, Campus de Gualtar, Braga – Portugal, email: rui.martins@bio.uminho.pt

[2] Unidade de Modelação e Optimização de Sistemas Energéticos, Laboratório Nacional de Energia e Geologia, Lisboa – Portugal, email: vitor.lopes@lneg.pt

[3] Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Porto – Portugal, email: acferreira@esb.ucp.pt

Abstract

The physiological response of plants to external perturbation is complex and occurs at different levels of their metabolism. This is a multivariate and multi-scale phenomena, therefore high-throughput methodologies are required to extract relevant information. The nonexistence of a device with such characteristics constitutes a barrier to the interpretation of the consequences of external inputs.

Spectroscopy is a multivariate methodology with greatest interest for metabolic studies in biological systems. In fact, this technique provides detailed information on the molecular structure and reaction mechanisms. Moreover due to its non-destructive character, this methodology is currently used to characterize proteins, peptides, lipids, membranes, carbohydrates in pharmaceuticals and food products as well as plants and animal tissues.

VinePAT is a vineyard management system based in the Process Analytical Technologies (PAT) methodologies to provide winemakers with state-of-the-art metabolic images of vineyards for precision winemaking by using UV-VIS-SWNIR spectroscopy techniques.

The system hardware is based on miniaturized fiber-optics spectrometer adapted for grape and leaves measurements and suited for outdoor data acquisition. Combining these geo-referenced outdoor measurements collected at the vineyard with state-of-the-art spectroscopy signal processing, the winemaker will be able to observe vine metabolism by using a non-

destructive 'in-vivo' methodology, as well as, the global-picture of the vineyard for implementing precision winemaking technologies based on process analytical technology.
Here we demonstrate the potential of the VinePAT technology for grape-growers by presenting: i) spectroscopy equipment in action; ii) the variance imaging and zone diagnostics; iii) metabolic imaging with especial incidence in key metabolites for grape maturation; iv) how to use multivariate control charts; and v) the full potential of the technology deployed by process analytical technology.

Introduction

1.1. The BioPhotonics Fingerprint in the UV-VIS-SWNIR

Light can carry both physical and chemical information. When light is projected into a grape berry, a distortion its initial spectral information occurs due to the interaction with the grape molecular composition and physical structures. High-end signal processing techniques allows to deconvolute the spectral information to qualify and quantify “in-vivo” metabolic expression (chemical composition) of both grapes, leafs and vine plant.
The ability of the winemaker to monitor “in-vivo” metabolic information allows: a) to monitor all vineyard production tasks and classify grapes before fermentation; and, b) to develop a process analytical technology quality control approaches towards precision wine-making.
The most popular spectroscopy technique in organic chemistry, with widespread applications in biotechnology is FTIR (Fourier transform infrared) spectroscopy. Grapes are no exception, with many available solutions for the wine industry. However, infrared is highly absorbed by water molecules, not being a highly sensitive technology for working with water solutions, and consequently, “in-vivo” biological samples. Recent studies, by our research group, placed UV-VIS-SWNIR as a highly sensitive non-destructive and cheap technology for the measurement of the metabolism of yeasts and a feasible technology for microorganisms identification.

UV spectroscopy records electronic transitions between electron energy levels from molecular levels in the UV-VIS region depend upon the energy involved. For any molecular bound (sharing a pair of electrons), orbitals are a mixture of two contributing orbitals σ and π, with corresponding anti-bounding orbitals σ^* and π^*, respectively. Some chemical bounds present characteristic orbital conditions, ordered by higher to lower order energy transitions: i) alkanes

($\sigma \rightarrow \sigma^*$; 150nm); ii) carbonyls ($\sigma \rightarrow \pi$; 170nm); iii) unsaturated compounds ($\pi \rightarrow \pi^*$; 180nm); iv) molecular bounds to O, N, S and halogens ($n \rightarrow \sigma^*$; 190nm); and v) carbonyls ($n \rightarrow \pi^*$; 300nm). As most UV-VIS spectrometers yield a minimum wavelength of 200nm, this technique has been considered to provide lower information in terms of functional groups when compared to IR, due to spectral differences mostly attributed to conjugated $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions (1,2).

Many organic molecules present conjugated unsaturated and carbonyls bounds, such as aminoacids, phospholipids, free fatty acids, phenols and flavonoids, peroxides, peptides and proteins, sugars and their polymers absorb in these bands. UV-VIS not only records the effect of electron excitation, but also the effect of return to lower orbitals, which result in vibrational and rotational modes, increasing the characteristic spectra of biological materials. This effect enhances photochemical reactions and fluorescence which are important features for microbiological identification (3,4) and help to identify metabolic states of yeast. Many biological molecules also present chromophore groups, which increase the absorption in the UV-VIS region, such as: nitro, nitroso, azo, azo-amino, azoxy, carbonyl and thiocarbonyl (3,4). Moreover the sensitivity of today's spectrometers has highly increased, being possible to obtain low noise to signal ratios which expands the detection limits (5). UV-VIS-SWNIR has among other advantages, the minimization of both liquid water absorbance and temperature effect. As state of the art spectrometers include high frequency vibrational infrared (SWNIR), it is also possible to obtain important information on water, fats and proteins (6).
The main difficulties in spectral processing of “in-vivo” biological samples are: i) establishing the hardware set-up; ii) eliminating systematic errors from environmental variables (e.g. sun-light, dust, plant morphology); and iii) processing the signal for spectral variance interpretation and metabolism quantification. After dealing with the hardware and methodology optimization, the key factor in spectroscopy is signal processing. In this reasoning, a good part of this research note is devoted to spectroscopy signal processing needed for implementing the VinePAT system.

1.2. The hyper spectral spectroscopy principle

With the advent of bioinformatics, there are different strategies for signal processing to obtain

a "holistic" vision of the phenomena putting in evidence the multiple players in the biochemical pathways.

In this study, a “non-target approach” was applied i.e. specify metabolites were highlighted by comparison of different fingerprints collect from grape berries, harvested from different water regimes at different maturation levels.

Two mathematical procedures were used to extract the relevant fingerprints: (i) non-supervised, aiming to isolate the global metabolic differences, and, (ii) supervised, aiming to explain a given parameter, i.e. metabolite using the overall spectra.

Hence, this work aims to present a methodology which includes both: device and software. This application uses “hyperspectral” spectroscopy technique, which acquires the spectra at each point of an image; as a non-destructive, “in-situ”, “in-vivo” and in real-time to obtain information of the metabolic state of ripeness of the grapes, allowing a detailed view of the composition of the grape fields over time. This new tool will provide more information that can be used to assist the development of new approaches to wine production.

1.3. The VinePAT System

The VinePAT is a data-driven system based the following key components: i) Geo-referenced point-to-point acquisition mesh; ii) control chart models; iii) metabolite calibration models; iv) hyper-spectral “in-vivo” acquisition equipment and signal processing modules; v) “in-vivo” variance and metabolic imaging; and the system cerebrum, vi) VinePAT computational system. The following information is provided on how the system components work: i) Geo-referenced point-to-point acquisition mesh: user defined geo-referenced mesh depending on precision necessity (variance and representativity); ii) Control chart models: high-end pattern recognition multivariate control charts based on latent variables modeling for monitoring and diagnose production deviations; iii) Metabolite calibration models: high-end signal spectral modeling by pattern recognition, allow to identify and quantify a vast variety of metabolites; iv) Hyperspectral “in-vivo” acquisition equipment and signal processing modules; v) “In-vivo” variance and metabolic imaging: direct spectral variance maximization imaging allow to rapidly verify space-time differences in the vineyard.

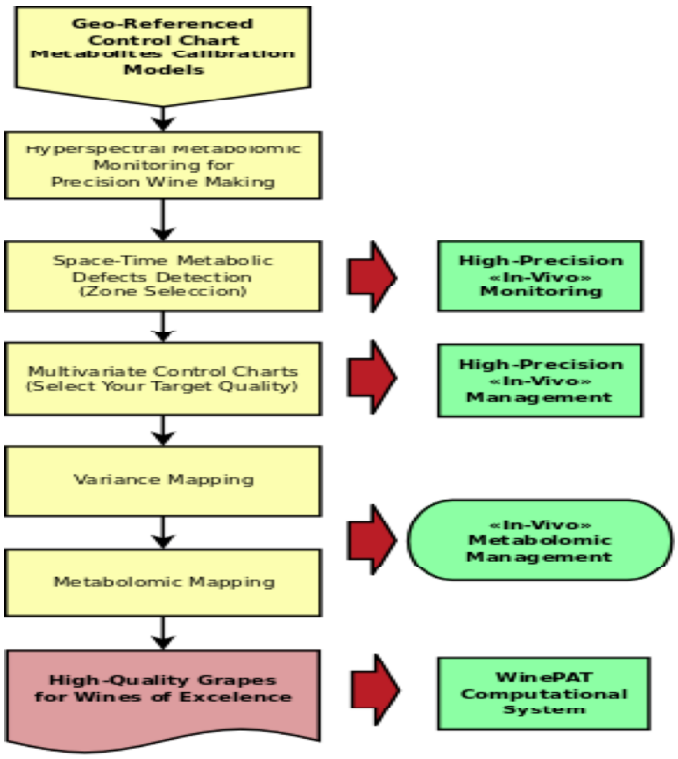

Figure 1. VinePAT system philosophy: I) "in-vivo" monitoring, ii) "in-vivo" management; iii) "in-vivo" computational process analytical technology.

vi) WinePAT computational system: a) manipulate hyperspectral data; b) metabolic and variance imaging; c) usage of multivariate control charts for defects detection; d) pattern recognition for metabolic identification/interpretation/classification; e) grapes quality classification; and f) vineyard and harvest management.

2. Materials and Methods

2.1. Spectroscopy

The WinePAT systems comprises in its simpler version of an: i) UV-VIS-SWNIR spectrometer of Ocean Optics (model HR 4000, 200 to 1100 nm; 3648 pixel) (Ocean Optics, 2010); ii) reflection VIS-SWNIR probe (Ocean-optics) modified to meet our specifications; iii) tungsten light source (HL-2000 Tungsten Halogen Light Sources, 360 nm-2000 nm) and; iv) Spectrasuite software which can be used for data acquisition.

Spectra were obtained at the environmental temperature, with an adapted probe curved tip to maintain the signal under 100 to 500 ms of integration time, in all types of grapes and different degrees of maturation. It is recommended that the tungsten lamp is let to stabilize during 15 min before measurements at the grape surface are performed.

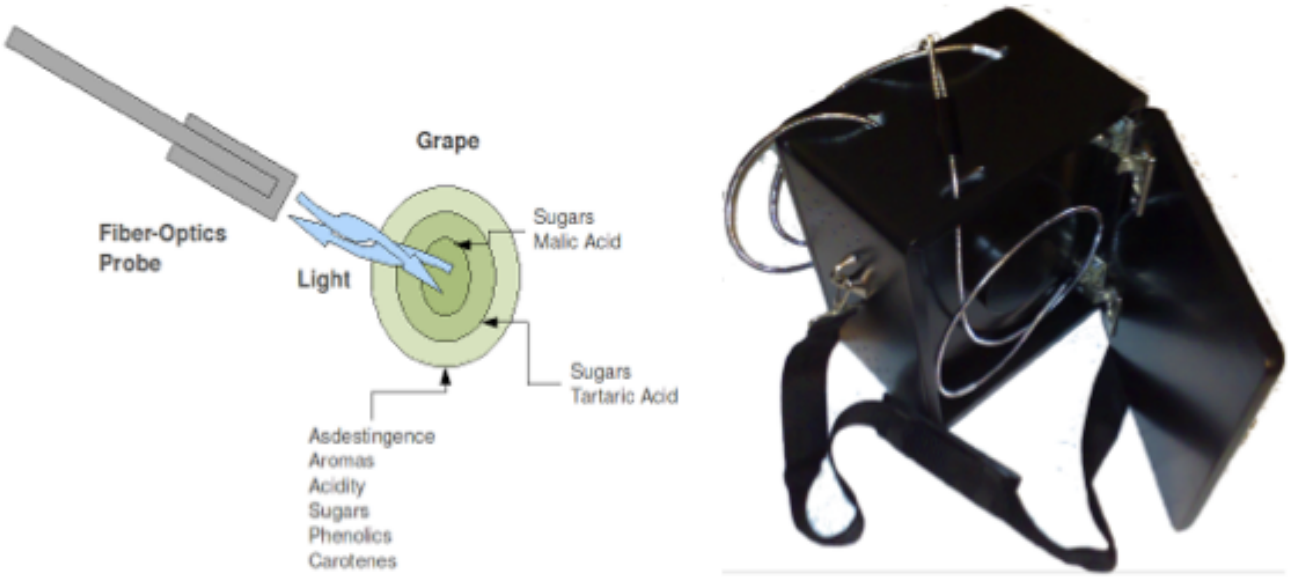

Figure 2. VinePAT spectroscopy prototype: a) schematic of fiber-optics probe and corresponding radiation penetration areas/compounds: b) first portable prototype used to develop the system.

The dark spectra were recorded and measurements were taken with linear and electric dark correction. Both light spectra were monitored by statistically assessing the reproducibility of the light source with measurements of light during the several days of the experiment. Twenty spectra replicates were recorded.

2.2. Variance and Metabolic Imaging

In order to develop a hyperspectral image of the vineyard, the system records the spectra in a pre-established mesh with defined nodal points. The mesh is obtained by Voronoi tessellation, and produces triangular elements were vertices's are the nodal points, corresponding to a vine sample. This is thereafter used for signal processing to obtain the variance imaging and metabolic imaging.

Variance imaging is a technique that plots the variance decomposition (singular value decomposition) of the spectral data in each coordinate, similar to a heat map. Such maps allow one to use spectroscopy as a high-throughput system to identify and classify grapes quality by spectral features similarity. As the decomposition uses only the relevant singular values, the user will be able to identify the causes of relevant spectral differences among the different points of the mesh.

Metabolic imaging is technique that plots the expected composition of the grapes at each point of the mesh. Such is performed by the supervised mathematical modeling of the spectra vs chemical composition obtained by laboratory methodologies, such as, HPLC-DAD, LC-MS and GC-MS. Under these circumstances, the system calculates using the spectra the metabolic composition.

Software and Examples

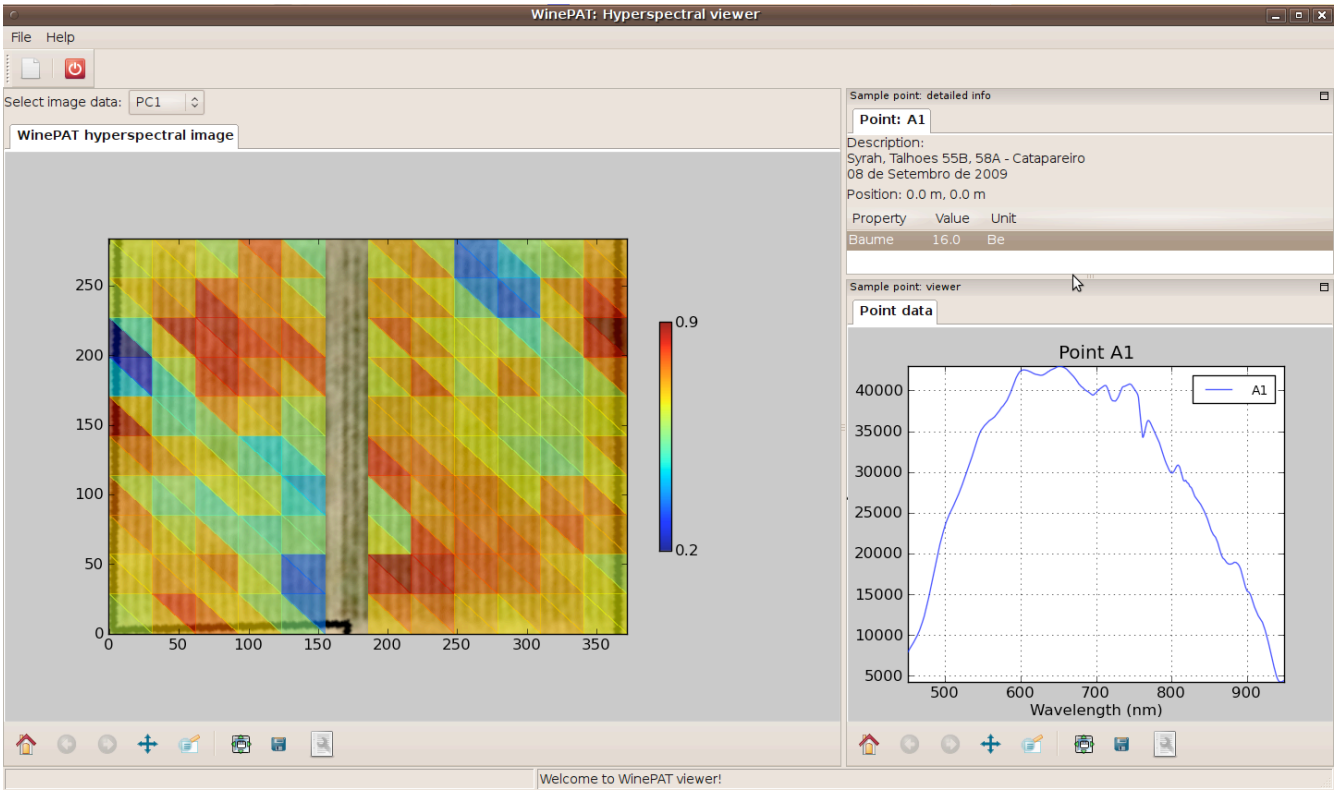

Figure 3. VinePAT viewer interface

Hyperspectral Imaging

Figure 3 presents the VinePAT viewer interface. The software demonstrates in this screen-

shoot a variance imaging with the corresponding spectra at each point of the mesh. The user can navigate through the image and obtain the spectra collected at each point.

Variance Imaging

Variance imaging is provided for each relevant component in the software. The user chooses the principal component that wishes to observe, and the system provides the super-imposed image over the mesh and vineyard. Similar colors in these images provide the information on grapes clusters similarity in each component. By using this information, it is possible to identify possible causes of differences of non-supervised metabolic differences.

Metabolic Imaging

Metabolic imaging is provided to the user by selecting the metabolite to view in the user interface. The user can navigate the metabolic composition by clicking at each node. The composition is provided in a list in the right corner of the software GUI.

Figure 4. Time course metabolomic imaging for b-carotene from Verasion (19th July) to harvest (30th of August).

Time-Course Metabolomics

Figure 4 presents the b-carotene fraction throughout 19 July (before verasion) to 30 -August (harvest), presenting from non-detected to maximum concentration (1mg.kg-1). The effect of irrigation on changes in carotenoid contents is shown in figure 4. Results showed that carotenoid content decreased during ripening, which is in agreement with previous works, where the presence of carotenoids in grape berries showed that b-carotene and several xanthophylls are abundant before veraison, and tend to decrease during ripening. Nevertheless, the image shows a high variability of b-carotene along the field during

maturation. For example, it is possible to observe that in the 1st sampling point (veraison) the highest content of b-carotene is present in high-left corner. This dislocates to the center in the next frames and attains lower levels at the harvest. At the harvest, is also observable that lower concentrations exist in the right side.

Conclusions

The examples presented demonstrate the usefulness, applications and advantages of having the VinePAT system for the collection of grape metabolic information. The system allows the vineyard manager to monitor the grape maturation process and to collect information on the variance of “grape quality”. The winemakers can also compare the database records containing the information from different vintages as way to adjust and plan corrective actions at the vineyard.

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