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IFAC-PapersOnLine 49-16 (2016) 315–319

Fluorescence spectral study in grapes (Vitis Vinifera L.) Benitaka variety to different solid soluble values

Rodolpho C. R. Tinini,* Caio Wetterich, Luís G. Marcassa**, Danilo G. Figueiredo***, Barbara J. Teruel*****

** Institute of Agricultural Sciences, Federal University of Minas Gerais,BRA (Tel: +5538-2101-7923; e-mail: rtinini@yahoo.com.br*

*** Physics Institute of São Carlo/São Paulo State Universitys,São Carlos, SP BRA*

**** Facyulty of Agricultural Engineering , Campinas States University, Campinas, Brazil*

Abstract: The spectral images used as an alternative to the quality nondestructive evaluation, can contribute to improve monitoring and control of variables involved in partial dehydrated grapes for production of juice and / or wine, with the product quality as goal. This study aimed to differentiate Benitaka variety grapes with different concentrations of soluble solids using spectral image of fluorescence. The grape samples come from to Vale do São Francisco - State of Bahia, and were forwarded to the Laboratory of Atomic Interactions - Institute of Physics of São Carlos, University of São Paulo. The treatments consisted by two (T1 and T2) being T1 - fresh grapes (12.54 +/- 0.09 °Brix), and T2 - partial dehydrated grapes (in forced air oven at T=65°C and RH=50%) (13.32 +/- 0.08°Brix). The fluorescence image system used consists in a scientific CCD camera, lens system, a variable optical filter, and illumination system with LEDs (UV-405 nm). The wavelengths spectrum used in this study were between 480 to 750 nm. The spectral calibration occurred through a principal component analysis (PCA) in Matlab, wherein principal components which represent most the data were selected, separating T1 e T2. To select the best wavelength was applied a linear regression forward a PCA model to image data. After the PCA implementation was possible to distinguish the different soluble solids values in grapes to T1 and T2, setting the best wavelengths were to 480; 493; 552; 568; 605; 645; 666; 690 and 715 nm. The applied technique can contribute to technological advancement in the non-destructive evaluation field and real-time in high added value products such as derivatives Viticulture.

Keywords: Temperature; Spectral images; nondestructive analysis;

1. INTRODUCTION

Dehydration of grapes at low temperatures and air humidity (temperatures below 10 ° C and relative humidity below 40%) began to be studied with more emphasis recently, for some varieties basically aimed at obtaining sweet wines, seeking the concentration of phenolic compounds and the decreasing the amount of water in the wort (BARBOSA-CANOVAS & VEGA MARKET, 2000; BELLINCONTRO et al., 2004; CONSTANTINI et al., 2006).

This scenario then points to the need for research to leveraging the development and technological improvement of the viticulture sector, seeking the Supply Chain, supported by the concept of Precision Agriculture, or even more comprehensive, Precision Agro Industry.

In order to play sugar, dehydration postharvest changes consistent results in post-harvest, thus obeying a positive development of the flavour of the wine and grape. Besides facilitating the transport, storage and microbiological stability, the dehydration process of agricultural products causes physical, chemical and organoleptic changes; therefore, it must be performed in a controlled manner and meet the limits established to not affect the quality (SAMPAIO & QUEIROZ, 2006). The reduction of the moisture content in fruits for processing, whether for juice, pulp and/or concentrates, causes an increase in the concentration of soluble solids (DIONELLO et al., 2009). When the grapes are used for winemaking, it is desirable the highest possible concentration of soluble solids and phenolic compounds. Phenolic compounds, as well as anthocyanins and flavonoids, provide the sensory characteristics of the wine, and they are indicated as beneficial in the prevention of cardiovascular diseases (BRADAMENTE et al.,2004; FREITAS et al., 2010; LASA et al., 2011).

The concentration of soluble solids in the must is the key factor so that, during winemaking, a significant amount of alcohol is produced. When the concentration of soluble solids is low, it becomes difficult, or even impossible, to obtain table wines with alcohol levels according to the required by the Brazilian law, which must be between 8.6 at 14%, according to note published in the Official Gazette.

Was shown to undergo dehydration grapes suffer significant changes in the concentration of sugars, which involve volatile compounds, phenolic compounds and enzyme activity, but is controlled way, these changes bring the final product quality (LERMA et al., 2012; ROLLE et al., 2012). However, there is the need to control the variables affecting the process of heat and mass transfer (temperature, flow, humidity) so that benefits are achieved both from a physiological point of view as food safety by eliminating the risk of fungal growth and thus pollution, for example, ocratoxin (BELLINCONTRO et al., 2004).

10.1016/j.ifacol.2016.10.058

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This scenario shown the need for research that leverage the development and technological improvement of the viticulture sector, with a look of Production Chain and support the Precision Agroindustry.

The power of the spectral images lies in its ability to handle spectral and spatial information (ELMASRAY, 2007). So, the spectral images have been applied at different stages of production, harvest, post-harvest and / or processing of agricultural products and food, and it showing a potential tool and that can be used as an alternative of nondestructive technique and for fast evaluation.

In this sense, the use of image analysis techniques can be an alternative to improve the grapes dehydration, reduce costs and make the process more secure, ensuring a quality food to consumers.

Specifically, the spectral images show its use potential as a basis for the detection system development, evaluation of chemical components and physical characteristics and other properties (BARANOWSKI et al, 2012; BAIANO et al, 2012; QIANG & MINGJIE, 2012; RAJKUMAR et al, 2012).

This article had as goal to differentiate grapes of Benitaka variety under partial dehydration, by using fluorescence spectral images.

2. MATERIALS AND METHODS

Seeking to get subsidies for understanding the spectral response of grapes used for processing, the experiments using a fluorescence imaging system were performed.

The experiment was conducted at Atomic Interactions Laboratory of Physics Institute of Universidade de São Paulo (São Carlos city). The fluorescence imaging system (SIF) used consists of a scientific CCD camera, a system of lenses, an optical filter of variable liquid crystal, an illumination system with LEDs, a laptop and a metal frame for fixing components and samples. The optical filter of tunable liquid crystal (Liquid Cristal TunableFilter, Meadowlark Optics, USA) responds to applied voltage, allowing to select the wavelength from 420 to 750 nm (±10nm).

The lightning system is composed of high power LEDs with about 200mW per diode (totalizing 10W). The system is composed of LEDs 365, 405, 470 and 530 nm, and also white LEDs for reflectance images acquisition. In these preliminary experiments were captured fluorescence images for excitations in 405nm. The diodes are fixed to a metallic support with adequate slope to the even distribution of light on the surfaces of the leaves, and powered by a voltage source. Coupled to the variable filter there is a lens system and a CCD camera to images obtaining. The CCD camera used was mvBlueFox 120G (Matrix Vision, Germany), with resolution of 640 x 480 pixels on the active area of the sensor.

It was made a metal structure with shape a dark black box to align and lock the components.

The Benitaka (Vitis vinifera L.) variety grape samples, came from Vale do São Francisco - BA, and after harvest and transportation, were sent to the Atomic Interactions Laboratory of Physics Institute of Universidade de São Paulo (São Carlos site - Brazil). Two types of samples were studied in this experiment (30 samples – 15 for each treatment), fresh grapes (without any treatment) and partially dehydrate grapes (dehydrated in a forced air stove for one hour at 60°C).

The fluorescence test was consisted of exposing the fresh berries and dehydrated berries over a period 2.0 minutes at each excitation wavelength of each fluorescent wavelength. The fluorescence wavelength was changed in the variable filter.

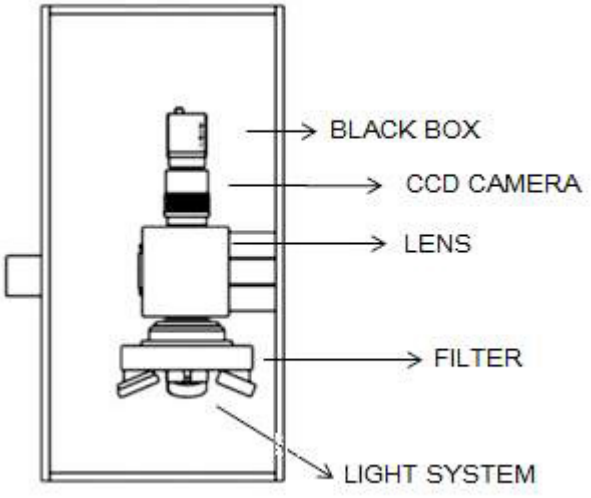

Fig.1. SIF Mount Scheme, highlighting main optical components: camera, lens, tunable optical filter, lighting system and the support for setting the polarizers and filters to the LEDs.

The images were then preprocessed and normalized so that they could be compared, was made an image normalization according to the CCD response curve and optical filter transmission curve. This normalization consists in multiply this function at wavelengths which they were acquired. Then applied in to images to eliminate the spectral and exposure time information, and eliminating the need for a white reference.

The wavelength of interest ranged from 480 at 750 nm. The spectral calibration occurred by a principal component analysis (PCA), in which the main components that referred for the majority of data and also separating the grapes with different soluble solids concentrations were selected.

Statistical analysis was done in Matlab® environment, it was used multivariate analysis of main components for separation

of treatments, then it was performed a mean comparison test nonparametric Wilcoxon to check the difference between each wavelength.

2. RESULTS AND DISCUSSION

The physical-chemical analysis results for grapes characterization before and after treatment to observe the soluble solids content (°Brix) are shown in Table 1 (below). We observed an increase in the concentration of soluble solids.

Table 1. Soluble solids values of grapes (cv. Benitaka) before and after the drying treatment in air circulating oven at 60 ° C for 1 hour.

Treatment	Fresh	Dry partial
Average	12,54	13,32
Standard Deviation	±0,09	±0,08

As observed on Figure 2, there are significant differences in fluorescence emission between partially dehydrated and fresh grapes, indicating the feasibility of applying the technique to differentiate due to loss of water and soluble solids concentration, allowing non-destructive evaluation and obtaining answers to support control of variables of the grapes dehydration process.

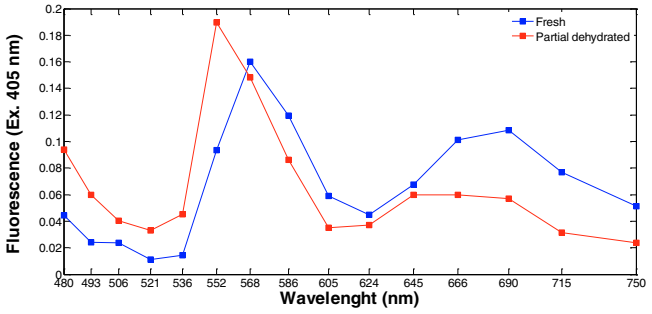

Fig.2. Average spectral response of grapes samples to the fluorescence excitation of 405 nm.

It can be observed that the excitation values show a peak spectral region of wavelengths 536-605 nm. This difference between the fluorescence emission may be explained by loss of water in the product, this region corresponds to the region of chlorophyll, and the water loss promotes the concentration of these pigments in vegetal material, thereby generating an increase in a percentage of emission, as discussed by BAIANO et al. (2012) and DIEZMA et al. (2013). In order to verify the differentiation of spectral data, the fluorescence values at light excitation in 405 nm for all spectra were subjected to Principal Component Analysis (PCA) wherein the results are presented in Figure 3. The results showed the differentiation between fresh and partially dehydrated grapes for principal component 1 compared to the principal component 2, the others components are excluded because don't show difference between fresh and partially dehydrated grapes (each component represent less thasn 10% of the data).

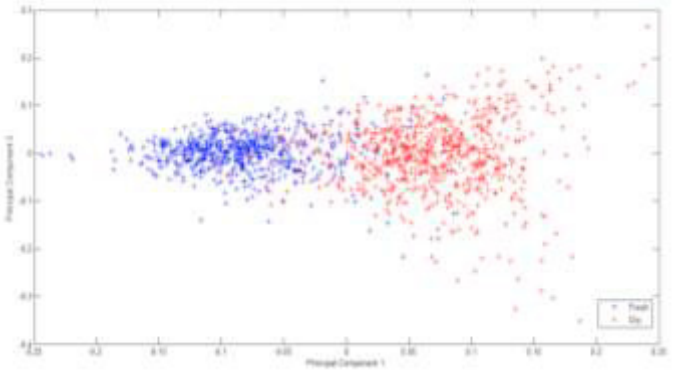

Fig. 3. "Score plot" generated by principal component analysis - CP1 (30.70%) and CP2 (15.00%) for differentiation of spectra for partially dehydrated and fresh grapes.

In order to statistically prove this differentiation and compare fresh and partially dehydrated grapes by CP1 and CP2, it was performed the Wilcoxon test that is signifier in comparison between CP1 (pvalor 1,0413x10^-19), represented by Figures 4 and 5.

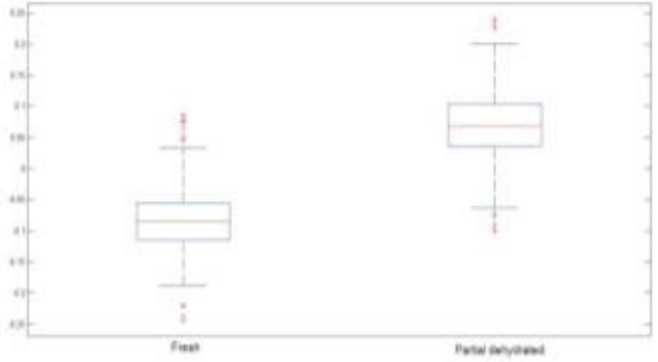

Fig. 4. Box Plot Wilcoxon test for CP1.

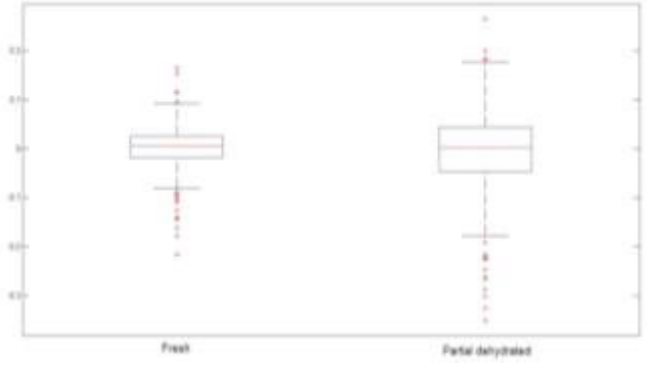

Fig. 5. Box Plot Wilcoxon test for CP2.

The model generated by principal component 1 is the best to represent the data, and to select the best wavelength it was generated predicted data by the model obtained by the principal components and correlating measured values and

predicted values, and selecting the models with high correlation (R^2):

Table 2. Correlation values of multivariate analysis model of principal components and the data obtained from the fluorescence spectral images for each wavelength.

Wavelenght (nm)	R^2
480	0,985
493	0,928
506	0,193
521	0,446
536	0,525
552	0,99
568	0,995
586	0,994
605	0,975
624	0,171
645	0,991
666	0,996
690	0,997
715	0,993
750	0,861

The principal component analysis shows potential for differentiation between fresh and partially dehydrated grapes. Practically we can correlate the model at any wavelength studied, except for: 506, 521, 536, 624 nm.

4. CONCLUSIONS

It was possible to differentiate treatments by using spectral fluorescence images. It can be observed that the product has the reflection peak of chlorophyll (~ 500nm), indicating that the concentration of the compounds due to loss of water can be used for evaluations for the process and quality of grapes. Thus, fluorescence spectral images show the potential to evaluate the properties of grapes, and it may be applied for determining the concentration of phenolic compounds and degradation of chlorophyll in grapes and thus this technique can be applied in viticulture.

6. CONCLUSIONS

A conclusion section is not required. Although a conclusion may review the main points of the paper, do not replicate the abstract as the conclusion. A conclusion might elaborate on the importance of the work or suggest applications and extensions.

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