Journal of Food Composition and Analysis 21 (2008) 650–654

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Short Communication

New portable optical sensors for the assessment of winegrape phenolic maturity based on berry fluorescence ☆

Z.G. Cerovic [a,*], N. Moise [b], G. Agati [c], G. Latouche [a], N. Ben Ghozlen [a], S. Meyer [a]

[a] Laboratoire Ecologie Systématique et Evolution, CNRS, UMR 8079 (CNRS, UPS, AgroParisTech), Université Paris-Sud 11, Bât. 362, 91405 Orsay Cedex, France
[b] FORCE-A, Univ. Paris-Sud, Bât 503, Orsay F-91405, France
[c] Istituto di Fisica Applicata 'Nello Carrara', CNR, I-50019 Sesto Fiorentino, Italy

ARTICLE INFO

Article history:
Received 19 December 2007
Received in revised form
27 March 2008
Accepted 28 March 2008

Keywords:
Phenolic compounds
Anthocyanins
Flavonols
Chlorophyll fluorescence
Epidermal absorbance
Vitis vinifera L.
Antioxidants
Food composition

ABSTRACT

Grape phenolic maturity is usually assessed by destructive wet chemistry in the laboratory. Yet, for precision agriculture or continuous monitoring of maturation, more rapid and non-destructive methods are needed. Therefore, in addition to measurements of fruit colour, a new optical method was recently proposed. It is based on the screening of fruit chlorophyll fluorescence that allows both flavonol and anthocyanin contents of intact berry skin to be measured. Here, we present the first results obtained with two commercial devices, Dualex FLAV™ and Dualex ANTH™, and a prototype, Multiplex, all based on this new method. We found that the non-contact optical sensor Multiplex has strong potential for an application in the vineyard for precision viticulture or for crop evaluation at the weighbridge.

1. Introduction

Quality wine can be made only from good quality grapes; therefore, viticultural practice well adapted to the terroir is constantly gaining in importance. Several destructive and non-destructive methods are used in viticulture for crop evaluation and as decision support, both at the plant and at the fruit level (Krstic et al., 2003). Optical methods are particularly appropriate for precision viticulture that tries to answer the problem of spatial and temporal crop heterogeneity, especially in fruit maturity. The optimal berry sugar content and acidity, known as technological maturity, are attained at different times in vineyard blocks. The grape phenolic maturity is even more heterogeneous (Bramley, 2005) and can be attained later than technological maturity (Keller et al., 1998) or simultaneously with it. Selective harvesting has been proposed as a solution to this problem based on zonal vineyard management and on-the-go quality sensing (Bramley, 2005). The assessment of grape quality inside the zones has relied mostly on destructive laboratory analysis, although new optical techniques based on near infrared spectroscopy linked to chemometrics is emerging (Gishen et al., 2005). Estimation of skin anthocyanins (ANTH) through direct berry colour measurement without extraction has also been used (Carreño et al., 1995). More recently, a method was proposed to assess the skin content of phenolics. It is based on their screening of excitation of chlorophyll fluorescence (ChlF). The method is applicable to winegrape leaves (Kolb and Pfündel, 2005) and fruits (Kolb et al., 2003; Agati et al., 2007) using either UV light for flavonols (FLAV) or visible light for ANTH. A visible beam for which the epidermis is transparent is used as a reference (cf. Agati et al., 2007). Assessment of FLAV and ANTH has also been attempted in apple skin by devices originally developed for leaves (Hagen et al., 2006). In summer 2005, we had the opportunity to use the new Dualex ANTH in addition to the leaf-clip Dualex FLAV, and a new prototype hand-held optical sensor Multiplex. The latter stands for multiple excitation fluorescence sensor and was derived from our work on fluorescence lidars (Ounis et al., 2001). The aim of the present work was to validate the use of fluorescence-base sensors on wine grapes known to have large contents of ANTH, and to show their potential usefulness for time surveys of both ANTH and FLAV. Dualex measurements were performed on berry caps in order to calibrate the method on grapes. Multiplex measurements were performed on whole bunches to evaluate the potential use of fluorescence as signature of phenolic maturity. The comparison of

☆ Some of the data were on a poster presented at the Sixth International Congress on Precision Agriculture held in Skiathos Greece in June 2007.

* Corresponding author. Tel.: +33 1 69 15 72 24; fax: +33 1 69 15 72 38.
E-mail address: zoran.cerovic@u-psud.fr (Z.G. Cerovic).

0889-1575/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.jfca.2008.03.012

red grape to white grape varieties during the whole season revealed two fluorescence ratios as potential signatures of grape phenolic maturity.

2. Materials and methods

Each week from mid July to the end of September 2005 two bunches of grapes (*Vitis vinifera* L.) of two red varieties, Pinot Noir, Pinot Meunier, and one white variety, Chardonnay, were sampled from the experimental vineyard Fort Chabrol in Epernay, France (Long. 03°57′ E, Lat. 49°02′N). They were chosen stochastically within a size range of 10–15 cm. Bunches were shipped to the laboratory in Orsay where all spectroscopic measurements were performed on the next day after harvesting.

2.1. Dualex measurements and extractions of flavonoids

The Dualex™ (FORCE-A, Orsay, France) technology, involving the use of ChlF excitation screening by UV-absorbing compounds of the epidermis, was described by Goulas et al. (2004). In the new Dualex ANTH, the original UV-A LED is replaced by a green sampling LED emitting at 528 nm, and is therefore dedicated to the measurement of ANTH (Fig. 1). At véraison, which occurred on August 16, day of the year (DOY) 228, 30 berries were chosen to cover six different grades of colour from green to dark violet. There was a single dark-blue almost black berry (cf. uppermost point in Fig. 2A). Berry caps of 7 mm diameter were cut with a cork borer and razorblade and placed on a cover slip for microscopy, the flesh side on the glass. This preparation was measured with Dualex ANTH and then with Dualex FLAV. The juice of the remaining berry part was used for total soluble solids (TSS) measurements expressed in °Brix (Pocket PAL-1, Atago, Japan). The caps were frozen in liquid nitrogen and stored at −80 °C until freeze-dried and ground. Dried berry caps were ground (ball mill MM301, Retsch, Haan, Germany) and extracted by the method of Pirie and Mullins (1977). The powder was transferred in 1 ml of acidified extraction solvent (50% MeOH, 0.1% HCl) vortexed 30 s, and, after 3 min standing, centrifuged for 3 min at 4100*g*. The pellet was re-extracted twice by the same procedure and the final pooled three supernatants adjusted precisely to 3 ml, then centrifuged again for 5 min at 4100*g* for final clarification. The whole procedure was performed at room temperature (20–25 °C). Absorbance spectra were immediately measured upon extraction from 200 to 1100 nm on the spectrophotometer HP 8453 (Agilent, Courtabeouf, France). Anthocyanin and flavonol contents were calculated on skin surface basis. ANTH were expressed in equivalents of malvidin 3-*O*-glucoside (oenin), the major Pinot Noir anthocyanin (Hebrero et al., 1988), using the molar absorptivity at 530 nm of 28,500 $M^{-1}\,cm^{-1}$ (28.5 $\mu mol^{-1}\,cm^2$). Flavonol glycosides were expressed in equivalents of quercetin 3-*O*-glucoside (quercitrin) (Extrasynthèse, Lyon, France), using the molar absorptivity of 16,700 $M^{-1}\,cm^{-1}$ (16.7 $\mu mol^{-1}\,cm^2$) at its absorption maximum (351 nm), and of 9700 $M^{-1}\,cm^{-1}$ (9.7 $\mu mol^{-1}\,cm^2$) at 375 nm, the wavelength used in Dualex FLAV (Fig. 1).

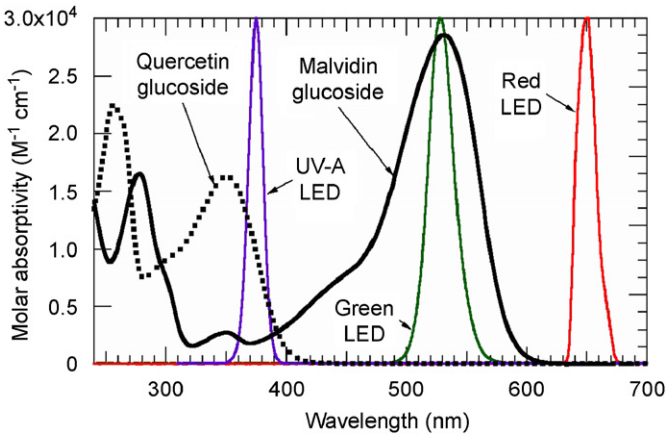

Fig. 1. Absorption spectra of the main berry skin absorbers compared to the light emission spectra of the LED source (thin full lines) used in Dualex FLAV and Dualex ANTH optical sensors (normalised to their maximum). The absorbers are presented in units of molar absorptivity: the thick full line for malvidin derivatives (ANTH) and dotted line for quercetin derivatives (FLAV).

2.2. Multiplex fluorescence measurements

The details of the Multiplex prototype will be presented elsewhere (patent pending). It is a non-contact hand-held optical sensor controlled by a computer. It has three LED-matrix light sources (Shark series, Opto Technologies, Wheeling, IL, USA): 375 nm UV-A (UV), 530 nm green (G) and 630 nm red (R), pulsed at 3.3 kHz (20 μs per flash). There were three synchronised detectors for fluorescence recording: blue-green (BGF), red (RF) and far-red (FRF), based on three $20 \times 20\,mm^2$ silicone photodiodes (PDB-C618, Photonic Detectors, Simi Valley, CA, USA) protected by a 447WB60 (Semrock, Rochester NY, USA), 678WB22 and 750WB65 (Intor, Socorro, NM, USA) interference filters, respectively. Here we concentrated on the signal ratios most important for phenolic maturity, based on FRF_{UV}, FRF_G, FRF_R (FRF excited by UV, G and R light, respectively), and UV-excited BGF signals. The advantage of ratios over individual signals is their lower dependence on orientation and distance for future measurements in the vineyard. Although it can be used in the field under daylight (Fig. 2D), Multiplex was used indoors in this study (cf. Fig. 2E). Each one of the two bunches per date was measured twice on the two sides; so each point in Fig. 2C and Fig. 3B–D is a mean of eight measurements. Pinot Noir and Pinot Meunier bunches were available from véraison onwards (DOY 228).

The Multiplex index for FLAV was defined here as log (FRF_R/FRF_{UV}) like in Dualex FLAV (Goulas et al., 2004). The Multiplex index for the ANTH was also based on a ChlF excitation ratio, but with excitation wavelengths specific for ANTH screening, as defined by Agati et al. (2007). In addition, in order to eliminate the difference in light source intensities, the ANTH index can be calibrated to yield a value of 1 for an average pre-véraison green bunch devoid of ANTH. Therefore, it was calculated as

$$\log(FRF_G/FRF_R) - \text{constant} + 1$$

the constant being equal to the log (FRF_G/FRF_R) of a green pre-véraison bunch. Here, we used the average value for green bunches of Chardonnay just before véraison. Note that the fluorescence ratio used in the equation is the reciprocal of the one used in Agati et al. (2007) (FRF_R/FRF_G), in order to have increasing values of the Multiplex index with ANTH accumulation. Indeed, due to very large skin ANTH content, both the FRF_G and the FRF_R are decreased with increasing ANTH skin content during maturation, and because of the relatively low power of the green light source the FRF_G signal saturated earlier than the FRF_R signal. In other words, in the proposed index the FRF_R signal does not have the role of the reference like in Agati et al. (2007), but is a second signal influenced by ANTH screening.

After Multiplex measurements, six berries were sampled individually for their sugar content (TSS in °Brix) and then the pH of the pooled juice was measured. Additional sets of 20 berries

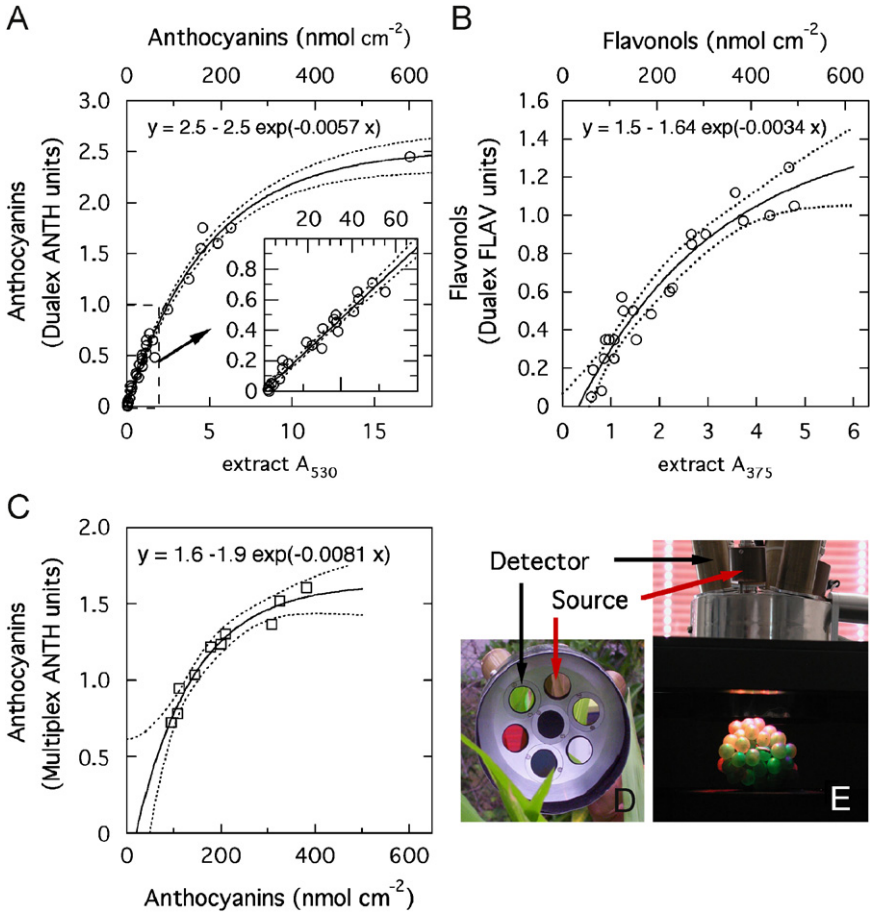

Fig. 2. Validation of the optical sensors. ANTH measured by Dualex ANTH (A) and by Multiplex (C) and FLAV measured by Dualex FLAV (B) at their characteristic wavelengths are compared to the absorbance of methanolic extracts of both Pinot Noir and Pinot Meunier berry skins. In (A) and (B), each data point represents a single berry cap. In (C), each data point is for skins obtained from 20 representative berries of the bunch measured with the Multiplex. Dotted lines indicate the 95% confidence interval. In (A) and (B), in addition to the flavonoid molar contents in nmol cm^{-2} shown on the upper axis, the equivalent skin absorbance, calculated as the absorbance of the extract of 1 cm^{-2} of skin in 1 cm^{-3} of solution measured in 1-cm-pathlength cell, is indicated on the lower axis. A front view of the Multiplex sensor is presented in (D). The picture (E) illustrates a Chardonnay grape bunch illuminated by the Multiplex during optical measurements.

per bunch were frozen and stored at −20 °C. Subsequently, skins were peeled from still frozen berries and ground under liquid nitrogen. Aliquots of skin powder (0.1–0.5 g) were extracted in 50 ml of acidified methanol and the flavonoids quantified like for berry caps. Curve fitting and statistical analysis were performed using Igor Pro 4.0 software (WaveMetrics, USA).

3. Results and discussion

3.1. Dualex

Dualex ANTH gave a very good estimation ($r^2 = 0.98$, fit standard error = 0.083, root mean square error of estimation: RMSE = 11 nmol cm^{-2}) of the skin ANTH content, albeit for an exponential relationship as expected from our recent spectroscopic studies (Agati et al., 2007) (Fig. 2A). This non-linearity above 100 nmol cm^{-2} is due to incomplete ANTH and chlorophyll spatial separation in the skin, and due to inherent limits of absorption spectroscopy at high concentrations. FLAV were also measured reliably by Dualex FLAV ($r^2 = 0.91$, fit standard error = 0.112, RMSE = 48 nmol cm^{-2}), showing tendencies towards saturation at higher skin contents (above 200 nmol cm^{-2}) (Fig. 2B). This is due to the three-fold smaller molar absorptivity for FLAV at their measuring wavelength compared to ANTH (cf. Fig. 1). One of the solutions to extend the working span of Dualex ANTH would be to use a less absorbed sampling wavelength. Thanks to their low absorptivity in UV-A (Fig. 1), ANTH will interfere little with the measurements of FLAV. The 375/530 nm absorptivity ratio for the former is only 0.064 (Fig. 1). The FLAV do not contribute to absorbance at 530 nm at all (Fig. 1). So, Dualex devices can be used to follow optically the two flavonoid families to about halfway up the maturation curve (DOY 240) (cf. Fig. 3A). Thereafter, individual berries can show saturation, especially for ANTH. This is to be expected since extracted ANTH then give skin absorbances of over 15 (Fig. 2A). Actually, these values might be much smaller in vivo because at skin pH above 3 less than a half of ANTH would be in the red-coloured flavylium cation form (Moskowitz and Hrazdina, 1981; Agati et al., 2007). For other fruits like apples that have much smaller skin ANTH contents than grapes, 2.5–50 nmol cm^{-2} according to Merzlyak et al. (2003), Dualex ANTH response would be in the linear part of the curve (insert Fig. 2A).

3.2. Multiplex

The Multiplex ANTH index gave also a good estimation ($r^2 = 0.96$, fit standard error = 0.065, RMSE = 49 nmol cm^{-2}) of the skin ANTH content of whole bunches (Fig. 2C). More

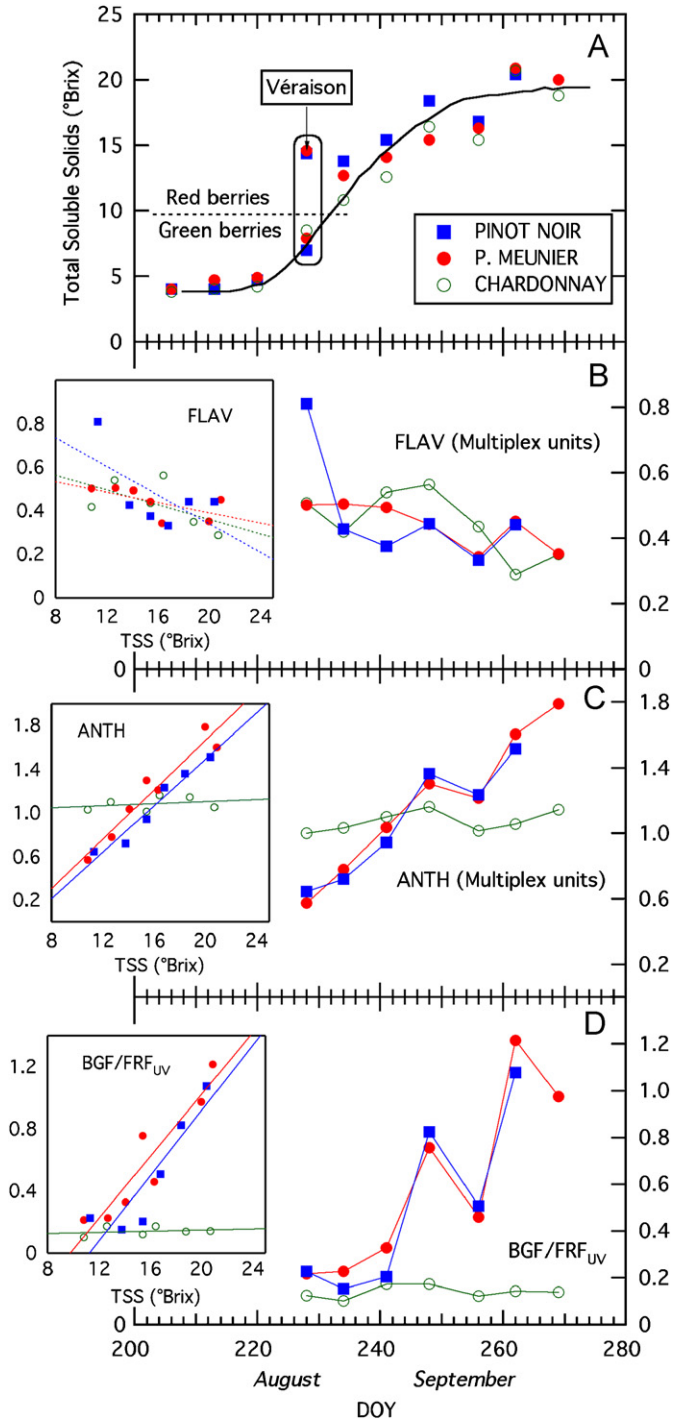

Fig. 3. Changes in indicators of technological (A) and phenolic (B, C and D) maturity during the summer 2005. Multiplex indices related to the skin content of FLAV (B) or ANTH (C and D) measured on whole bunches are plotted against the DOY or compared to the sugar content of the berry juice (inserts) obtained from six representative berries of the bunch. Pinot Noir and Pinot Meunier bunches were available for Multiplex measurements only starting from DOY 228 (véraison). Multiplex indices are based on fluorescence ratios and therefore have no dimensions.

importantly, the Multiplex ANTH index was capable of estimating ANTH throughout the maturation period, as opposed to the Dualex that was tested only at véraison. This is possible thanks to the new role of the FRF_R signal in the Multiplex ANTH index (cf. Materials and Methods).

Both the pH (not shown) and sugar concentration increased along a typical maturation curve despite the fact that only two bunches per cultivar were sampled each week (Fig. 3). At véraison the intra-bunch variability was very large, with coefficients of variations for TSS of the order of 25% (not shown) even among the berries of the same colour, green or red. Individual red berries had characteristics that will be attained by the whole bunch only 2 weeks later. As can be seen from Fig. 3B, the Multiplex index for FLAV changed little with time, with a small tendency to decrease. It was weakly and negatively correlated to the sugar content, with an r^2 of only 0.35, 0.40 and 0.39 for Chardonnay, Pinot Meunier and Pinot Noir, respectively (Fig. 3B insert). On the other hand, the Multiplex index for ANTH and the simple emission ratio BGF/FRF_{UV} were far more responsive to ANTH accumulation with grape maturation. Soon after véraison, FRF_R and FRF_{UV} were also decreased by ANTH accumulation in addition to FRF_G that was already very low. So, it was the changes in FRF_R and FRF_{UV} that influenced the Multiplex indices, the FRF_G and BGF signals being one order of magnitude smaller and almost constant (not shown).

The behaviour of the two ANTH indices was very similar for Pinot Noir and Pinot Meunier. They showed the same increase with time (Fig. 3C and D) and a good correlation to the grape sugar content (insert Fig. 3C and D). The r^2 for Pinot Noir was 0.95 and 0.80, and for Pinot Meunier 0.93 and 0.88, for Multiplex ANTH and the BGF/FRF_{UV} ratio, respectively. This good correlation confirms that the transient decrease at DOY 256 was just a problem of sampling of less mature grapes with less sugar and less ANTH than expected for that date. As expected, ANTH-related Multiplex indices changed little in white grapes (Chardonnay) and were therefore totally disconnected from technological maturity (r^2 of 0.08 and 0.05) (inserts Fig. 3C and D).

3.3. ANTH, FLAV and phenolic maturity

In a skin-disc study on Pinot Noir grapes, Price et al. (1995) have shown that sun-exposed bunches have 10 times more quercetin glycosides in their berry skins compared to their shaded counterparts, and that sun exposure did not affect ANTH. So, since FLAV and ANTH skin contents are differently controlled by environmental constraints they may not be correlated, as seen here. Still, FLAV, which are sun-exposure markers of grapes (Lenk et al., 2007), can also be important for wines because of their co-pigmentation with ANTH, and their contribution to the formation of precipitates and bottle deposits (Krstic et al., 2003). It is therefore important to have a means to assess them directly in each berry. Dualex can fulfil that need for research on light intensity, temperature, nitrogen and other nutrient effects on accumulation of phenolics. Preliminary tests have shown that, taking certain precautions, Dualex measurements can be performed even on whole berries. In the vineyard, the largest problem for maturity assessment in general is the representativity of the sample. A large variability exists among vines, grape bunches and even among berries inside a single bunch. So, the faster and the friendlier the method, the larger and the more representative will be the analysis. For the Multiplex type sensor, the number of samples to be measured is not limited, and in theory, even total harvest can be sensed through on-line measurement. In addition, for maturation surveys, thanks to the non-destructive nature of the measurements, marked bunches representative of the block can be followed during the whole season. More generally, these optical sensors can be useful for the agro-food industry for an easy non-destructive assessment of ANTH and FLAV as antioxidants present in table grape and other fruits.

In conclusion, both types of optical sensors tested can be useful for viticulture. The "leaf-clip" type Dualex is inherently more precise and can yield quantitative data on ANTH and FLAV in individual berries. These would be valuable for studies on environmental effects on grape maturation. The non-contact sensor Multiplex, on the other hand, has real potential for precision viticulture. Still, for very mature grapes an increase in green source power would be welcome.

Acknowledgements

This project received financial support from the CNR/CNRS bilateral cooperation project no. 11409. We would like to thank Alexandre Tonnellier & Laurent Panigai of CIVC (Comité Interprofessionnel des Vins de Champagne) for the supply of grapes and useful advice, and Dr. Spencer Brown for a critical reading of the manuscript.

References

Agati, G., Meyer, S., Matteini, P., Cerovic, Z.G., 2007. Assessment of anthocyanins in grape (*Vitis vinifera* L.) berries using a non-invasive chlorophyll fluorescence method. Journal of Agricultural and Food Chemistry 55, 1053–1061.

Bramley, R., 2005. Understanding variability in winegrape production systems. 2. Within vineyard variation in quality over several vintages. Australian Journal of Grape and Wine Research 11, 33–42.

Carreño, J., Martínez, A., Almela, L., Fernández-López, J.A., 1995. Proposal of an index for the objective evaluation of the color of red table grapes. Food Research International 28, 373–377.

Gishen, M., Dambergs, R.G., Cozzolino, D., 2005. Grape and wine analysis-enhancing the power of spectroscopy with chemometrics. A review of some applications in the Australian wine industry. Australian Journal of Grape and Wine Research 11, 296–305.

Goulas, Y., Cerovic, Z.G., Cartelat, A., Moya, I., 2004. Dualex: a new instrument for field measurements of epidermal UV-absorbance by chlorophyll fluorescence. Applied Optics 43, 4488–4496.

Hagen, S.F., Solhaug, K.A., Bengtsson, G.B., Borge, G.I.A., Bilger, W., 2006. Chlorophyll fluorescence as a tool for non-destructive estimation of anthocyanins and total flavonoids in apples. Postharvest Biology and Technology 41, 156–163.

Hebrero, E., Santos-Buelga, C., Rivas-Gonzalo, J.C., 1988. High performance liquid chromatography-diode array spectroscopy identification of anthocyanins of *Vitis vinifera* variety. Tempranillo 39, 227–233.

Keller, M., Arnink, K.J., Hrazdina, G., 1998. Interaction of nitrogen availability during bloom and light intensity during veraison. II. Effects on anthocyanin and phenolic development during grape ripening. American Journal of Enology and Viticulture 49, 341–349.

Kolb, C.A., Pfündel, E.E., 2005. Origins of non-linear and dissimilar relationships between epidermal UV absorbance and UV absorbance of extracted phenolics in leaves of grapevine and barley. Plant, Cell and Environment 25, 580–590.

Kolb, C.A., Kopecky, J., Riederer, M., Pfündel, E.E., 2003. UV screening by phenolics in berries of grapevine (*Vitis vinifera*). Functional Plant Biology 30, 1177–1186.

Krstic, M., Moulds, G., Panagiotopoulos, B., West, S., 2003. Growing Quality Grapes to Winery Specifications: Quality Measurement and Management Options for Grapegrowers. Winetitles, Adelaide.

Lenk, S., Buschmann, C., Pfündel, E.E., 2007. In vivo assessing flavonols in white grape berries (*Vitis vinifera* L. cv. Pinot Blanc) of different degrees of ripeness using chlorophyll fluorescence imaging. Functional Plant Biology 34, 1092–1104.

Merzlyak, M.N., Solovchenko, A.E., Gitelson, A.A., 2003. Postharvest Biology and Technology 27, 197–211.

Moskowitz, A.H., Hrazdina, G., 1981. Vacuolar contents of fruit subepidermal cells from *Vitis* species. Plant Physiology 68, 686–692.

Ounis, A., Cerovic, Z.G., Briantais, J.-M., Moya, I., 2001. Dual excitation FLIDAR for the estimation of epidermal UV absorption in leaves and canopies. Remote Sensing of Environment 76, 33–48.

Pirie, A.J.G., Mullins, M.G., 1977. Interrelationships of sugars, anthocyanins, total phenols and dry weight in the skin of grape berries during ripening. American Journal of Enology and Viticuture 28, 204–209.

Price, S.F., Breen, P.J., Valladao, M., Watson, B.T., 1995. Cluster sun exposure and quercetin in pinot noir grapes and wine. American Journal of Enology and Viticulture 46, 187–194.