In situ assessment of quality-related compounds in fruits by using fluorescence sensors

Elisa Fierini, Lisa Banelli, Patrizia Pinelli, Annalisa Romani
Dipartimento di Statistica, Informatica, Applicazioni-DiSIA,
Università di Firenze
Firenze, Italy

Damiano Remorini
Dipartimento di Scienze Agrarie, Alimentari e Agro-ambientali (DiSAAA-a)
Università di Pisa
Pisa, Italy

Giovanni Agati
Istituto di Fisica Applicata "Nello Carrara"
Consiglio Nazionale delle Ricerche
Sesto Fiorentino (Firenze), Italy
g.agati@ifac.cnr.it

***Abstract*—Fruit quality compounds, such as antioxidant phenolics and chlorophyll, were assessed in situ by using a fluorescence method applied by a portable sensor. Indices of anthocyanins (ANTH) and flavonols (FLAV) localized on the fruit surface were obtained based on their screening of chlorophyll fluorescence excitation. The chlorophyll content was estimated by the far red to red chlorophyll fluorescence ratio (CHL index), due to the partial reabsorption of red fluorescence by chlorophyll itself. In kiwifruits, the CHL index was found to be well linearly correlated to the chlorophyll content determined by wet chemistry on the same fruit samples. Full sunlight exposed kiwifruits possessed a higher content of chlorophyll than shaded kiwifruits. This is an important parameter to know for assessing fruit quality and storability. Based on the estimation of the red-pigmented anthocyanins, we defined a new rapid method to determine the maturity level of olives after harvest, giving the proportion of red and green olives, important for the quality of the olive oil produced. In plums, ANTH and FLAV were found to be linearly correlated to the actual content of compounds measured by HPLC analysis of skin extracts. These indices can be, therefore, used to predict the phenolic antioxidant potential of plums and to define their maturity stage.**

Keywords— Actinidia deliciosa; chlorophyll fluorescence; non-destructive methods; Olea europaea L.; optical sensors; Prunus domestica L..

I. INTRODUCTION

Fruit compounds, such as antioxidant phenolics and chlorophyll, are important parameters to be assessed for the evaluation of fruit maturity, quality and storability. They can also be used to predict the quality of derived products such as wine and olive oil. For this, optical tools have been developed, with the advantage to be non-destructive, fast and repeatable on the same samples during the different stages of ripening as well as post-harvest treatments and storage.

Optical methods for measuring fruit quality were usually based on reflectance and interactance spectroscopy in the visible and near infrared (NIR), colorimetry and imaging [1-3].

Fluorescence spectroscopy techniques were early developed to assess phenolic compounds in the fruit exocarp in wine grape [4] and olives [5]. They are based on the screening by superficial molecules of chlorophyll fluorescence excitation, allowing the absorbance of intact fruit skin to be measured [6]. Yet, the far red to red chlorophyll fluorescence ratio (CHL index) was used as a proxy of the chlorophyll content, due to the partial reabsorption of red fluorescence by chlorophyll itself [7]. The fluorescence screening method is particularly suitable to investigate in situ absorbance of skin in fruits that retain chlorophyll in the flesh. By using a portable fluorescence sensor, this technique was recently applied to estimate pigments and colorless compounds in the apple peel and kiwifruits skin.

Here, we present some other applications of a fluorescence sensor on kiwifruits (*Actinidia deliciosa*), olives (*Olea europaea* L.) and plums (*Prunus domestica* L.). We provided accurate calibration curves for the CHL index in kiwifruits and for flavonoids indices in plums. Furthermore, optical determination of anthocyanins in olives allowed defining a new maturity index of olive drupes.

II. MATERIALS AND METHODS

A. Plant Material

Kiwifruits (*A. deliciosa* (A. Chev.) Liang & Ferguson, cv 'Hayward') were collected from a commercial orchard in Rigoli (Pisa, Italy) at the end of the 2010 growing season. Soluble solid content of kiwifruits ranged between 7.8 and 9.8 °Bx. Fruits were harvested from positions in the plants exposed to different light conditions (full sun, partial shade and shade). The full sun midday photosynthetic photon flux density measured at harvest was 1100 µmol/m^2/s. Partial shade and shade light regimes were 15% and 2% of full sun, respectively. During fruit collection, the most exposed side of each fruit was marked. Under shade condition, the most exposed side was defined as that facing the external part of the canopy. Samples (25 fruits per light condition) were measured the same day of harvest by the fluorimetric sensor.

Olive (*Olea europaea* L.) samples of the Itrana cultivar were collected at different ripening stages from the Casale del Giglio farm (Le Ferriere (Latina), Italy) during October 2011.

Plum (*Prunus domestica* L.) samples were obtained from the Banfi farm (Montalcino (Siena), Italy). Fruits were collected at three different ripening stages during August-September 2012

and transported under cool conditions to the laboratory. Each fruit was measured individually by the Mx, within 24 h from harvest, and then processed by removing the skin for the extraction and HPLC determination of phenolic compounds as described before [8].

B. Kiwifruit Chlorophyll Extraction and Determination

The flat wider side of each fruit was measured by the Mx through a 2×3 cm mask with the longer length parallel to the longitudinal axis of the fruit. From the measured fruit area, a 2x2 cm sample, 5 mm thick, including both skin and flesh was cut off using a razor blade. Each fresh sample was weighted, homogenized and extracted with 5 ml of 80% acetone. The acidic pH of the solution was rapidly neutralized by adding small volumes of NaOH 1N to avoid degradation of Chl. After 30 min, the sample was centrifuged and the supernatant measured spectrophotometrically without any dilution. The Chl concentration of extracts was calculated according to Porra [9] and expressed as μg g^{-1} of fresh weight.

C. The Fluorescence Sensor

The Multiplex (Mx) fluorescence sensor (FORCE-A, Orsay, France) [10], is a portable battery-powered fluorimeter that measures fluorescence emitted by chlorophyll, in the red (RF), at 680–690 nm, and far-red (FRF), at 730–780 nm, bands, under excitation with light-emitting diodes in the UV-A (375 nm), blue (450 nm), green (520 nm) and red (630 nm) spectral regions. The combination of the RF and FRF fluorescence signals under different excitations (indicated by subscripts) provided indices of anthocyanins,

$$\text{FER(fluorescence excitation ratio)}_{RG} = FRF_{red}/FRF_{green} \quad (1)$$

$$ANTH_{RG} = \log(FRF_{red}/FRF_{green}), \quad (2)$$

flavonols,

$$FLAV = \log(FRF_{red}/FRF_{UV}), \quad (3)$$

and chlorophyll,

$$CHL = FRF_{red}/RF_{red}. \quad (4)$$

III. Results and Discussion

A. Kiwifruits Chlorophyll Index

In kiwifruits, red excitation light, used to measure the signals used for the CHL index, was able to cross the external fruit skin and to reach chlorophyll in the pericarp. The CHL index was found to be well (coefficient of determination, R^2=0.85) linearly correlated to the chlorophyll content determined by wet chemistry on the same kiwifruits (Fig. 1).

For full sun and partial shade kiwifruits, the CHL values of the more exposed sides were significantly higher than those of the less exposed sides (Fig. 2A). No difference between sides was observed for shade fruits.

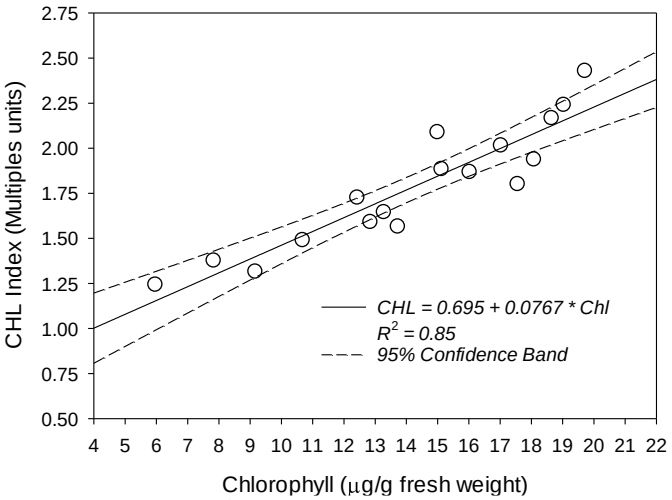

Fig.1 Calibration curve for the CHL Mx index obtained through the actual chlorophyll kiwifruit content quantified spectrophotometrically.

As per fruit average was considered, the sensor showed that full sunlight exposed and partial shade kiwifruits possessed a higher content of chlorophyll than shaded kiwifruits (Fig. 2B). This is an important parameter to know for assessing fruit quality and storability.

Our observation agreed with previous studies on the effect of canopy sunlight shading on the Chl content of kiwifruits determined destructively [11, 12].

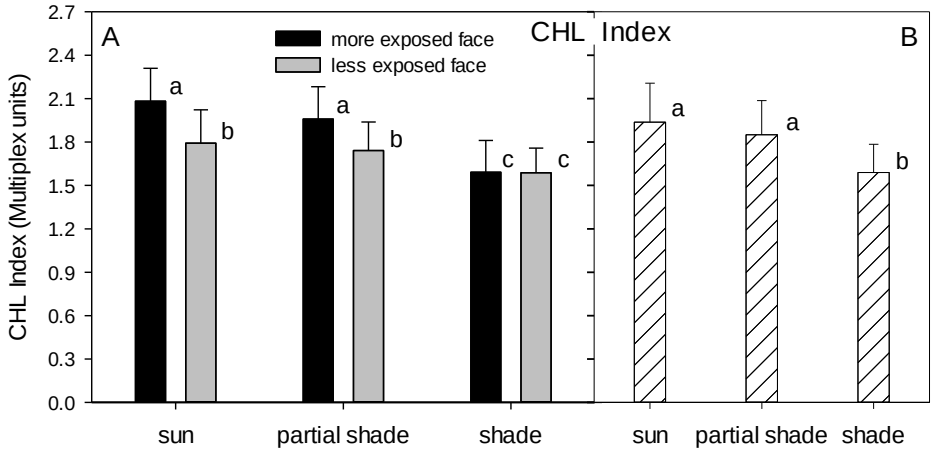

Fig.2 Values (±SD) of the non-destructive CHL Mx index measured at harvest (05/11/2010) on kiwifruits under different light regimes, averaged (n=25) over fruit sides (A) or per fruit (B). Within each panel, bars with different letters are significantly different at P<0.05.

B. *Estimation of the Red to Green Olives Proportion*

According to a previously published model applied to grape berries [10], defining *p* as the proportion of red olives, the FRF signals should be proportional to the sum of the fluorescence of green olives having unscreened chlorophyll, $1 - p$, and red olives, *p*, in which chlorophyll is screened, as described by the following equations for green and red excitations:

$$FRF_G = k_G [(1 - p) + (p\ T_G)] \quad (5)$$

$$FRF_R = k_R [(1 - p) + (p\ T_R)] \quad (6)$$

Re-arranging (5) and (6), the fluorescence excitation ratio, FER_{RG}, as function of p results to be

$$FER_{RG} = k_R [(1 + p\ (T_R - 1)]/\ k_G [(1 + p\ (T_G - 1)], \quad (7)$$

that is the equation of an holographic function.

To check this model, a sample of 14 green olives was measured by the Mx sensor, then red olives were progressively introduced in steps of about 7% and the Mx measurements were repeated. As showed in Fig. 3, the FER_{RG} index as function of the red olive proportion, *p*, was indeed well fitted by an holographic function, in accordance with (7).

Therefore, based on the estimation of the red-pigmented anthocyanins, we can propose a new rapid method to determine the maturity level of olives after harvest, giving the proportion of red and green olives. This method is important to predict the quality of the olive oil produced.

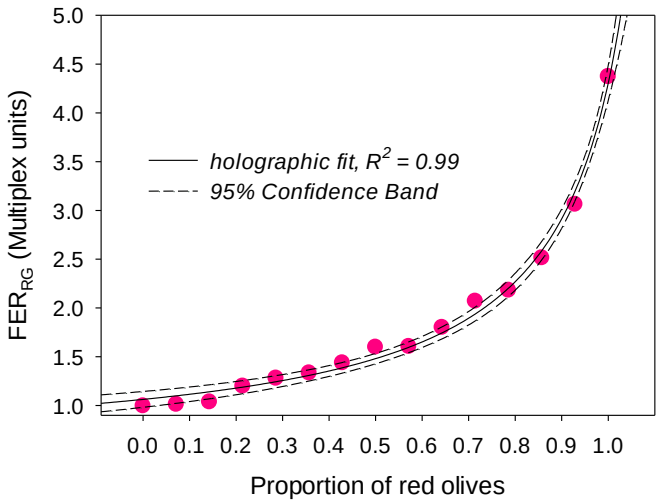

Fig. 3. The FER_{RG} anthocyanins Mx index as a function of the proportion of red olives (p).

C. *Compound Indices in Plums*

The anthocyanins and flavonols content of plum skins was indirectly determined by the fluorescence sensor. The anthocyanins ($ANTH_{RG}$) (Fig. 4A) and flavonols (FLAV) (Fig. 4B) indices were found to be linearly correlated to the actual content of compounds measured by HPLC analysis of skin extracts. These indices can be therefore used to predict the phenolic antioxidant potential of plums and to define their maturity stage.

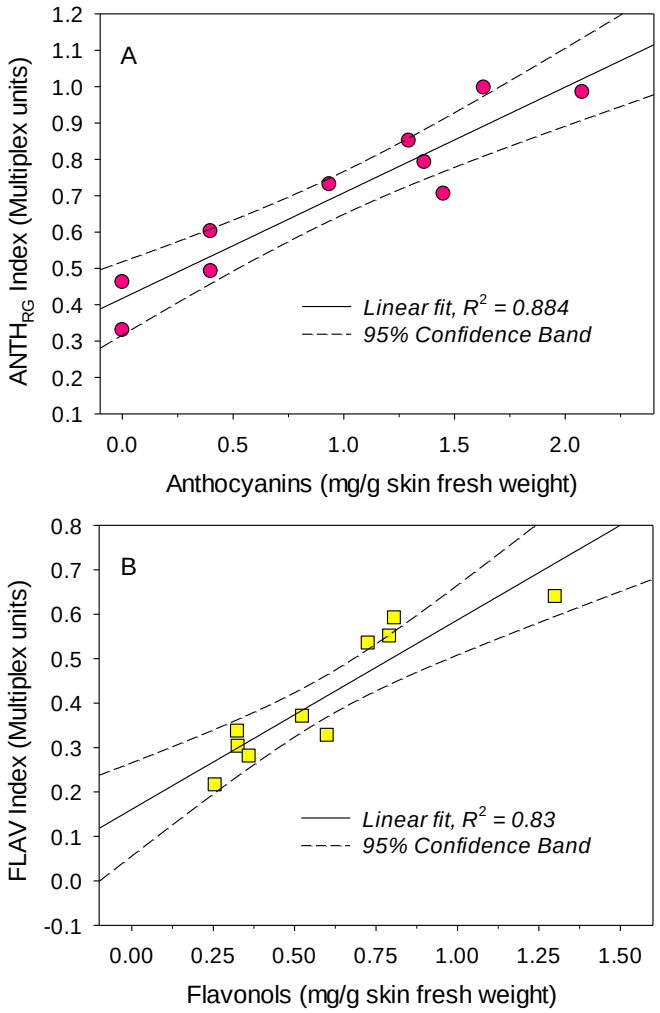

Fig. 4. Calibration curve for the $ANTH_{RG}$ (A) and FLAV (B) Mx indices obtained through the actual anthocyanins and flavonols contents, respectively, of plum skins quantified by HPLC analysis of extracts.

IV. Conclusions

Our results suggest that fluorescence spectroscopy can represent a rapid non-destructive tool for the evaluation of quality characteristics in fruits. Use of available portable fluorescence sensors allows application of the technique in the field directly on fruit attached to the plant. This can be advantageous in having an index of Chl content as a complementary decision tool of determine optimum harvest maturity and to rapidly assess variability of Chl content within and between fruit depending on exposure to light. The indices of Flav and Anth antioxidants can also represent a further valuable characterization of fruits.

Acknowledgments

The authors are grateful to 'Camillo Pacini e Figli' (Rigoli, Pisa, Italy), Casale del Giglio (Le Ferriere, Latiìna, Italy) and Banfi (Montalcino, Siena, Italy) for supplying the fruit samples used in this study.

REFERENCES

[1] M.N. Merzlyak, A.E. Solovchenko, and A.A. Gitelson, "Reflectance spectral features and non-destructive estimation of chlorophyll, carotenoid and anthocyanin content in apple fruit," Postharvest Biol. Technol., vol. 27, pp. 197-211, 2003

[2] P. Butz, C. Hofmann, and B. Tauscher, "Recent developments in noninvasive techniques for fresh fruit and vegetable internal quality analysis," J. Food Sci., vol. 70, pp. R131-R141, 2005

[3] H. Cen and H. Yong, "Theory and application of near infrared reflectance spectroscopy in determination of food quality," Trends Food Sci. Tech., vol. 18, pp. 72-83, 2007

[4] G. Agati, S. Meyer, P. Matteini, and Z.G. Cerovic, "Assessment of anthocyanins in grape (Vitis vinifera L.) berries using a non-invasive chlorophyll fluorescence method," J. Agric. Food Chem., vol. 55, pp. 1053-1061, 2007

[5] G. Agati, et al., "Nondestructive evaluation of anthocyanins in olive (Olea europaea) fruits by in situ chlorophyll fluorescence spectroscopy," J. Agric. Food Chem., vol. 53, pp. 1354-1363, 2005

[6] G. Agati, Z.G. Cerovic, P. Pinelli, and M. Tattini, "Light-induced accumulation of ortho-dihydroxylated flavonoids as non-destructively monitored by chlorophyll fluorescence excitation techniques," vol. 73, pp. 3-9, 2011

[7] C. Buschmann, "Variability and application of the chlorophyll fluorescence emission ratio red/far-red of leaves," Photosynth. Res., vol. 92, pp. 261-271, 2007

[8] L. Tuccio, et al., "A rapid and non destructive method to assess in the vineyard grape berry anthocyanins under different seasonal and water conditions," Aust. J. Grape Wine R., vol. 17, pp. 181-189, 2011

[9] R. Porra, "The chequered history of the development and use of simultaneous equations for the accurate determination of chlorophylls a and b- Minireview," Photosynth. Res., vol. 73, pp. 149-156, 2002

[10] N. Ben Ghozlen, Z.G. Cerovic, C. Germain, S. Toutain, and G. Latouche, "Non-destructive optical monitoring of grape maturation by proximal sensing," Sensors, vol. 10, pp. 10040-10068, 2010

[11] A. Tombesi, E. Antognozzi, and A. Palliotti, "Influence of light exposure on characteristics and storage life of kiwifruit," New Zeal. J. Crop Hort., vol. 21, pp. 85-90, 1993

[12] D. Remorini, et al., "Influence of canopy position on kiwifruit quality," Acta Hort., vol. 753, pp. 341-346, 2007