OPTICAL TECHNIQUES TO ESTIMATE THE RIPENESS OF RED−PIGMENTED FRUITS

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ABSTRACT. *During fruit ripening, chlorophyll degradation is responsible for the degreening of the ground color, which is a well−established ripeness indicator for several species. In completely red−pigmented cultivars of fruits such as apples and peaches, this process is not visible, being masked by anthocyanins in the skin. Two different optical systems were developed to non−destructively assess the chlorophyll content in these fruits, to estimate ripeness, and to optimize harvesting and postharvest management. A fluorescence imaging system equipped with a UV−blue actinic light was used to obtain fluorescence images of fruit in which the gray level of pixels correlated (R^2 = 0.81) with the firmness of fresh apples (*Malus domestica *cv. Red Delicious). With this technique it was possible to estimate changes in the firmness and soluble solids sugar content of stored Red Delicious apples undergoing no detectable hue change in the skin. Using the same system with a red actinic light, fluorescence correlated fairly well with firmness for fresh peaches and nectarines (*Prunus persica *cv. Elegant Lady, Summer Rich, and Morsiani 90), even though the detected fluorescence signal was low in intensity. A laser−diode based, dual−band reflectance probe was developed and tested on fresh peaches (cv. Summer Rich) and stored apples (cv. Royal Gala). The R/IR index, defined as the ratio of the signal measured in red and near−infrared bands, was found to correlate with the chlorophyll content of the fruits (R^2 = 0.66), regardless of fruit species and anthocyanin presence. The R/IR index was used to track the postharvest ripening process for fresh peaches harvested at different maturity stages.*

Keywords. *Chlorophyll content, Fluorescence imaging, Non−destructive, Red−pigmented fruits, Reflectance probe.*

Providing the consumer with high−quality fruit and vegetables is a complex, multidisciplinary task that requires agronomical, biophysiological, technological, and modeling contributions. In this regard, one of the most important aspects in which engineering is directly involved concerns the development of methods and instruments for the non−destructive measurement of the physical−chemical properties of the product, and the definition of appropriate relationships with their evolution and specific threshold values.

During ripening, a fruit undergoes several concurrent and sequential biochemical processes that deeply transform its properties. These changes affect the quality attributes in terms of: texture and firmness (due to solubilization of pectins), flavor (due to starch conversion into sugars, changes in organic acid content, and production of aromatic volatiles), and color (due to chlorophyll degradation in the skin and synthesis and/or unmasking of carotenoids and anthocyanins).

Ground color degreening is an effective ripeness indicator for some fruits (Eccher Zerbini et al., 1991; Merzlyak et al., 1999), and indeed, assessment by means of color charts is a well−established practice for evaluating the optimum harvest date and planning postharvest operations and market distribution so that the fruit is suitably ripe when it reaches the consumer. The color chart method, although inexpensive and non−destructive, can be erratic and provides inconsistent results. Moreover, it cannot be applied on completely red−pigmented cultivars of apples, peaches, and nectarines since, due to anthocyanin synthesis, the blush color is developed in the skin of these fruits at very early stages, and it masks the degreening process induced by the chlorophyll's degradation.

Chlorophyll is a strong light absorber; therefore, optical techniques have been widely applied in order to non−destructively estimate its concentration in a fruit, based both on its reflectance and fluorescence properties. Kempler et al. (1992) were among the first to apply contact chlorophyll fluorescence sensing on a small area of kiwifruit skin, finding a parallel decrease in fluorescence intensity and firmness during storage. Lavrijnsen and Van Kooten (1993) reported that the same technique was unsuccessfully applied to predict the optimum harvest date of apples, due to overly great variations in data. Beaudry et al. (1997) observed a monotone decline of fluorescence intensity in apples during storage at ambient temperature and reported a good correlation between fluorescence intensity and firmness. Mir et al. (1998) used the contact fluorescence technique to determine apple senescence in refrigerated air storage. In addition, Guidetti et al. (1998) showed the potential of applying fluorescence imaging as a contactless, whole−surface investigation technique for monitoring the ripening process in apricots and apples, and found promising correlations between firmness and fluorescence intensity. Similar results for lemons were reported by Nedbal et al. (2000).

Article was submitted for review in April 2003; approved for publication by Food & Process Engineering Institute Division of ASAE in March 2004. Presented at the 2002 ASAE Annual Meeting as Paper No. 026041.

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Several studies have been conducted using different kinds of VIS (visible, 400−700 nm) and NIR (near−infrared, 700−2500 nm) spectrophotometers in order to quantify pigments and other fruit constituents by means of innovative multiwavelength or whole−spectra statistical analysis methods. Delwiche et al. (1987) investigated ground and flesh color variations in peaches at various stages of ripeness, finding the most distinct spectral differences at 670 nm being induced by chlorophyll degradation. Slaughter (1995), in studying the spectra of peaches of high and low sugar concentrations, reported a consistent difference in chlorophyll content. Ruiz−Altissent et al. (2000) found that including the reflectance at 680 nm among non−destructive estimators of peach firmness significantly increased the prediction accuracy. Zude−Sasse et al. (2002), using the wavelength of the red edge (i.e., the sharp transition in the reflectance spectrum from low VIS reflectance to high NIR reflectance), estimated the chlorophyll content of red−pigmented cultivars of apple with good accuracy, and they found this index well related to the optimal harvest date ($R^2 = 0.64$). Li et al. (1997), using the strong chlorophyll decrease that occurs during banana ripening, developed a LED−based, optical chlorophyll sensor. The system was used to detect the dependence of a combination of reflectances at 610 nm and 660 nm on chlorophyll content in banana peel, which had a correlation of $R^2 = 0.90$.

In this article, we report on the use of two different techniques, one based on fluorescence and the other on reflectance measurements, to assess chlorophyll content in red−pigmented fruits for the purpose of developing an on−line grading approach to classify fruits for their ripeness, even in the case of cultivars characterized by a ground color completely masked by anthocyanins.

Materials and Methods

The two optical systems were used in preliminary experiments on fruits of red cultivars under different conditions: within three days of harvesting on fresh samples of apples (*Malus domestica* cv. Red Delicious) and peaches and nectarines (*Prunus persica* cv. Elegant Lady, Summer Rich, Morsiani 90, Vega, Fayette, and Sweet Lady), and after three months of storage in refrigerated air with ethylene removal on apples (cv. Royal Gala and Red Delicious). All fruits were hand harvested in two commercial orchards in the Milan (Italy) region, and selected to include only healthy and defect−free samples in the experiments.

Chlorophyll fluorescence was detected by measuring the re−emission in the 690−740 nm spectral range of the light absorbed in UV−blue (360−570 nm) and red (630−670 nm) bands. Dual−band chlorophyll reflectance was determined by measuring the intensity of light reflected at 675 nm and at 800 nm by the external layers of the fruit, after multiple scatterings and absorptions in the skin and flesh.

Fluorescence Imaging

The experimental set−up for the acquisition of chlorophyll fluorescence images consisted of a dark chamber (fig. 1) in which a CCD monochrome camera (Sony XC 55, Sony Corp., Japan) equipped with a high−pass filter (Schott RG 690, Schott Italglas, Italy), with edge wavelength at 690 nm, was installed. The camera was operated with fixed gain, and the electronic shutter set at 10 ms. Images in 8−bit format were digitized by a frame grabber (Matrox Meteor−II, Matrox Electronic Systems, Ltd., Dorval, Quebec) connected to the PCI bus of a 128 MB memory Pentium II computer running customized software developed in Labview (Labview 5.1, National Instruments, Austin, Texas).

Figure 1. The experimental fluorescence imaging system. Two actinic sources were tested on red−pigmented cultivars of apple and peach fruit: a filtered UV−blue lamp (360−570 nm) and an array of ultra−bright red LEDs (630−670 nm).

Two sources of actinic illumination to excite the chlorophyll fluorescence were tested: a UV−visible light source consisting of a 600W mercury lamp (Ultramed 600, Osram, Italy) with the emission range limited to 360−570 nm by a low−pass filter (Schott BG 28, Schott Italglas, Italy), and a red light source consisting of an array of ultra−bright LEDs emitting in the range of 630−670 nm (2285073, RS Components Italy, Italy). An adjustable holder kept the fruit sample in the field of view of the camera at a distance of 50 cm from the light source and 40 cm from the camera lens.

Fluorescence images with the UV−blue source were acquired for 20 fresh Red Delicious apples within three days of harvesting at three dates: 13, 20 (commercial harvest), and 27 September 2000; for 45 Red Delicious apples harvested on the commercial harvest date and stored for three months; and for four groups of 16 fresh peaches of red−pigmented cultivars (Vega, Fayette, Sweet lady, and Elegant lady) within three days of harvesting on the commercial harvest date (between 27 June and 1 August 2000, depending on the cultivar).

Fluorescence images with the red actinic light were acquired for groups of 30 fresh red−pigmented peaches (cv. Elegant lady and Summer Rich) and nectarines (cv. Morsiani 90) within three days of harvesting on the commercial harvest date (between 24 July and 12 August 2000, depending on the cultivar).

After placing a fruit sample in the holder, a monochromatic image ($\lambda > 690$ nm) of the side facing the camera was

acquired after an actinic illumination period of 1 s, when the chlorophyll fluorescence emission reaches its maximum intensity. The fluorescence images were analyzed by measuring the mean gray value in three regions of interest (ROIs) of 10 × 10 pixels (corresponding to 7 × 7 mm approx.), two located in the upper equatorial part of the fruit's imaged face and one in the lower. The ROIs were selected at a distance of 1 cm from the center of the fruit's face to minimize the effect of surface curvature. The mean gray value of the three ROIs was assumed to represent the fluorescence intensity of fruit skin as detected by the imaging system.

Dual−Band Reflectance Measurements

Two wavebands were considered for reflectance−based chlorophyll estimation. One is its red absorption band, centered at 675 nm, at which the intensity of the reflected light is minimally influenced by anthocyanins and, at the same time, strongly dependent on the chlorophyll content. The second is the near−infrared band centered at 800 nm, where neither chlorophyll nor anthocyanins absorb light (Merzlyak et al., 2003). Therefore, the R/IR index, obtained as the ratio between the intensity of reflected light in the red (R) and infrared (IR) bands, is expected to be negatively correlated with the chlorophyll content, regardless of anthocyanin concentration.

With these initial assumptions, a hand−held, dual−band probe was developed (fig. 2). The probe contained one 10 mW laser diode (RLT6710MG, Roithner Lasertechnik, Austria) emitting red light at 675 ±2 nm, two LEDs emitting near−infrared light at 800 ±20 nm (2678380, RS Components Italy, Italy), and two photodiode sensors (OPT301M, Burr−Brown, Tucson, Ariz.) measuring the sampled area from symmetrical directions.

The probe was connected to a PC through an input−output, 12−bit card (PC−LPM−16, National Instruments, Austin, Texas), for light source control purposes and for digitizing and storing the output of the sensors. During measurements, the probe was manually placed in contact with the intact surface, which in turn was sequentially illuminated by the laser diode and the LEDs. The intensity of the signal sensed by the photodiodes due to the reflected red laser light was acquired first, followed by the signal from the LED light. A measurement typically involved a region of the fruit's surface of 7 to 8 mm in diameter, and consisted of ten illumination/sensing repetitions. Measurements for a fruit required a total time of 200 ms.

Detected values in reflection measurements are highly sensitive to sample/sensor geometry. Indeed, natural morphological non−homogeneities, such as surface orientation, concavity, or convexity, vary the actual distance between the reflecting area and the sensor, consequently influencing the measured data. To compensate for these effects, the signals detected by the two opposite photodiodes were averaged, and the ratio (R/IR) between the intensity of reflected light under red laser illumination (R) and under LEDs infrared illumination (IR) was calculated.

The dual−band reflectance probe was tested on three sets of 16 fresh peaches (cv. Summer Reach) within three days of harvesting on three dates (14, 19, and 24 July 2000, commercial harvest) to obtain different maturity stages, and on 50 apples (cv. Royal Gala) harvested on the commercial harvest date (4 September 2000) and after stored for three months in refrigerated air.

Reference Measurements

In order to investigate how the results obtained with the two optical techniques were related with quality attributes, different conventional reference indices were determined destructively for the same fruit regions that were measured optically: firmness, expressed as force applied at rupture point, by means of a penetrometer (Samson, Andilog, France); soluble solids content, by means of a digital refractometer (Brixstix, Sati, Italy); and chlorophyll content of a disk of fruit skin and associated tissue (approximately 20 mm in diameter and 1 mm thick) by means of extraction for 24 h in 5 mL of dimethylformamide and subsequent quantification by absorbance spectrophotometry (Lambda 6, Perkin Elmer, Wellesley, Mass.) according to Porra et al. (1989). Moreover, skin color was measured with a colorimeter (CR200, Minolta, Japan) and expressed in terms of hue.

Results and Discussion

Fluorescence Imaging Measurements

Chlorophyll fluorescence intensity as detected in UV−blue excited images of fresh Red Delicious apples correlated (R^2 = 0.81) with firmness measurements, as shown in figure 3.

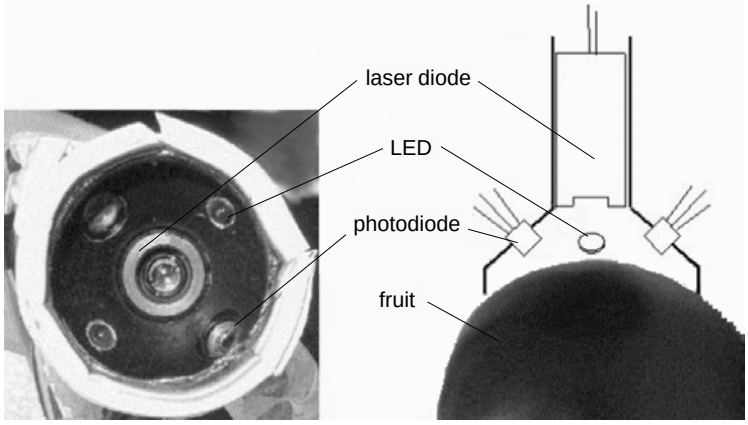

Figure 2. The hand−held probe for dual−band reflectance measurements on apple and peach fruit.

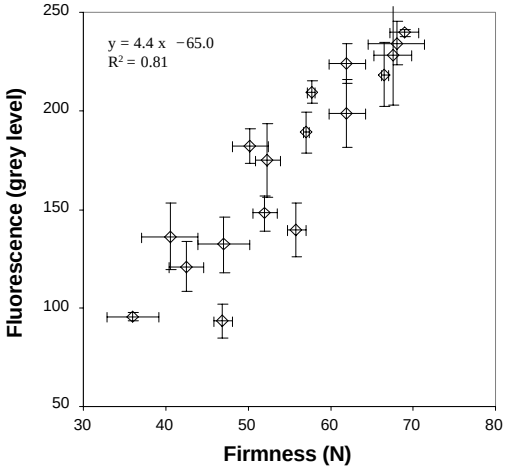

Figure 3. Relationship between firmness and fluorescence intensity in images of fresh Red Delicious apples illuminated with UV−blue actinic light.

Subsets of the stored apples of the same cultivar were optically and destructively measured immediately after storage removal and after 8 and 16 days of ripening at 20˚C. In these fruits, fluorescence intensity was found to decrease during postharvest ripening, as was firmness (fig. 4), even though limited variations of the two parameters were observed (approximately a 35% decrease from the initial value, for both fluorescence and firmness), probably induced by postharvest storage. Also in this case, fluorescence was linked to both firmness and sugar content, even though with poorer correlation (R^2 = 0.44 and R^2 = 0.51, respectively). Firmness and sugars in these samples were found to be unrelated to skin hue (R^2 = 0.14 and R^2 = 0.19, respectively).

The gray levels in UV−blue induced fluorescence images of fresh peaches were found to estimate fairly the chlorophyll content only for cv. Vega (R^2 = 0.48) among the four red−pigmented cultivars considered. Reasons for this were their low chlorophyll content (typically <5 μg/g), together with the non−homogenous distribution of anthocyanins in the superficial layers of the fruits, causing an uneven absorption of the UV−blue actinic light before it could reach the chlorophyll layer and excite its fluorescence. This is why fluorescence imaging of fresh peaches and nectarines was

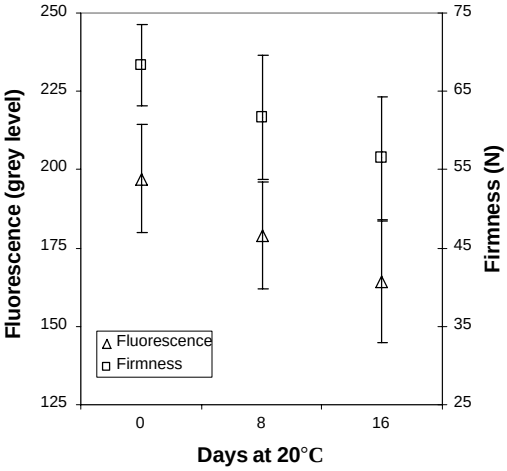

Figure 4. Decrease of firmness (squares) and fluorescence intensity (triangles) in Red Delicious apples stored for three months in refrigerated air and subsequently for 0, 8, and 16 d at 20˚C.

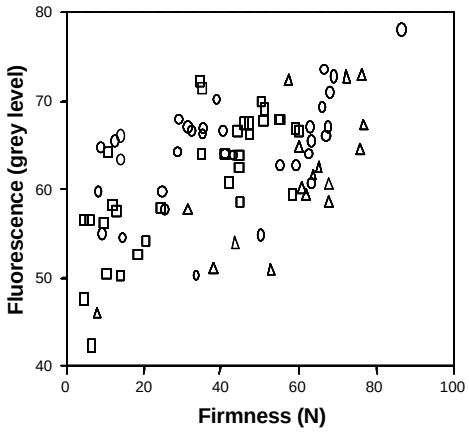

Figure 5. Relationship between firmness and fluorescence intensity in images of fresh Summer Rich (squares), Elegant Lady (circles) peaches, and Morsiani 90 (triangles) nectarines illuminated with red actinic light.

carried out using the red actinic source. Gray levels in images were found to be fair estimators of fruit firmness (R^2 = 0.59), regardless of cultivar (fig. 5). However, the detected fluorescence intensity was quite low (gray levels between 40 and 80, on a 255 scale), confirming the low chlorophyll content in peaches when they are close to optimal ripeness, and emphasizing that the sensitivity of such a system must be carefully considered for further implementations.

Dual−Band Reflectance Measurements

A robust logarithmic relationship was found between the destructively determined chlorophyll content and the R/IR index measured by the dual−band probe on red−pigmented Royal Gala apples and Summer Rich peaches (fig. 6). The overlapping of the data referring to the two fruit species and the total correlation coefficient (R^2 = 0.66) illustrate that in apples and peaches the probe accuracy was not influenced by differences in species or anthocyanin content.

The stored Royal Gala apples were found to be characterized by rather uniform organoleptic properties, with a low variability in terms of firmness (avg. = 49 N, SD = 7 N) and soluble solids (avg. = 11.9 ˚Brix, SD = 1.0 ˚Brix). Despite the limited range in variability, the R/IR index correlated with the firmness of the samples with R^2 = 0.48 and with their

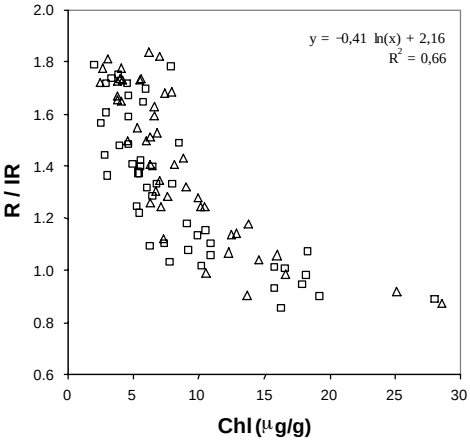

Figure 6. Relationship between the R/IR index measured by the dual−band probe and the chlorophyll content analytically determined in fresh Summer Rich peaches (squares) and Royal Gala apples (triangles) stored for three months in refrigerated air.

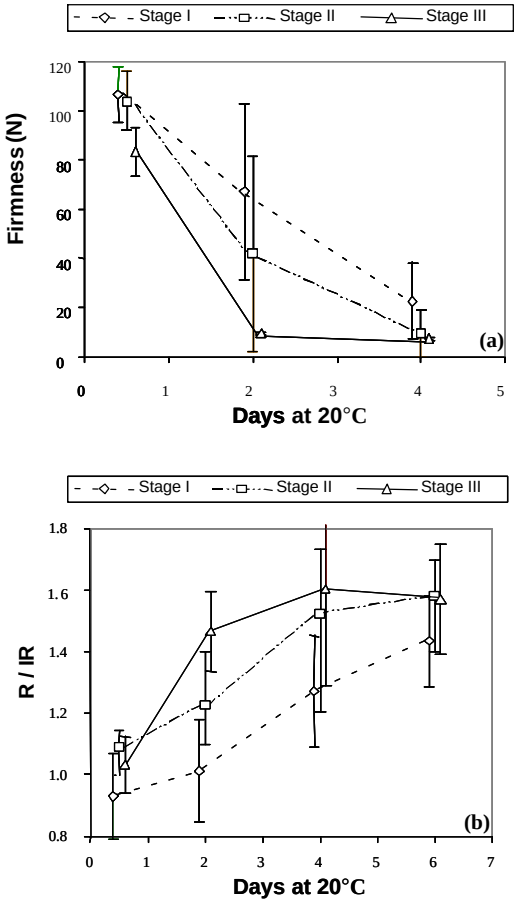

Figure 7. Evolution of (a) R/IR index and (b) firmness during postharvest ripening of fresh Summer Rich peaches harvested at three different maturity stages: diamonds = stage I (10 d before commercial harvest), squares = stage II (5 d before commercial harvest), and triangles = stage III (commercial harvest).

soluble solids content with R^2 = 0.40. The R/IR index for groups of fresh Summer Rich peaches harvested at three different maturity stages increased with storage duration at 20˚C (fig. 7a). Fruit firmness of subsets extracted from each group declined during the same period (fig. 7b).

The comparison of the two processes highlighted the strong relationship between firmness and R/IR index changes (correlation of R^2 = 0.70), as well as the possibility of monitoring the ripening of the fruits even without an observable hue change in the skin (e.g., correlation coefficient between hue and firmness was found to be R^2 = 0.09).

Slight differences in the ripeness stage (10 d advanced harvest, 5 d advanced harvest, and commercial harvest) were detected as differences in increase of R/IR. The most unripe group (stage I) exhibited a slower increase of R/IR and did not reach a steady−state level, even after 6 d at ambient temperature; on the other hand, the ripest group (stage III) underwent a faster increase of R/IR, followed, after 2 or 3 d at ambient temperature, by a steady−state level corresponding to complete ripeness.

Conclusions

Two different systems were developed and used in preliminary experiments for the non−destructive evaluation of the chlorophyll content in red−pigmented fruits in order to assess their ripeness. The results, although obtained only for a limited number of samples, allow us to draw the following conclusions.

A fluorescence imaging system was tested on fresh and stored apples and on fresh peaches and nectarines, using two different actinic sources. With the UV−blue light, it was possible to estimate the firmness of fresh apples and track changes in the firmness and sugar content of stored apples, even in the absence of observable hue change in the skin. Results obtained with fresh peaches were more variable, probably due to the low chlorophyll content, together with the uneven distribution of anthocyanins in the skin which absorb the UV−blue actinic light. A red light−based version of the system provided good correlations between fluorescence and firmness of fresh peaches and nectarines. In general, chlorophyll fluorescence imaging was confirmed as an interesting non−destructive, contactless technique that can provide useful information on the ripeness of red−pigmented fruits. Nevertheless, the experiments stressed that the sensitivity of the system must be carefully considered for further implementations.

A hand−held, dual−band reflectance probe was tested on fresh peaches and stored apples. The R/IR index, defined as the ratio of the signals measured at 675 nm and 800 nm, was found to be accurate in estimating the chlorophyll content of apple and peach fruit, regardless of anthocyanins content and differences due to species. Moreover, the R/IR index monitored the postharvest ripening of fresh peaches, showing different evolution patterns for fruits harvested at different dates.

The results obtained with the dual−band reflectance probe, which was a simple, low−cost, hand−held system, encourage further research devoted to characterizing the ripening process and defining appropriate threshold values for different fruits and cultivars in order to consider its possible applications in field and postharvest practice.

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