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Optical chlorophyll sensing system for banana ripening

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Abstract

An optical chlorophyll sensing system was developed to detect the chlorophyll content of bananas as the fruit ripens. This system shows a high correlation to other peel colour analysis methods (spectral analysis, tristimulus colorimeter analysis, and visual colour matching). Regression equations were developed to predict the chlorophyll content of banana peels, which is primarily responsible for the change in the colour of the peel. This optical chlorophyll sensing system has the following characteristics: rapid response, simple operation, non-destructive measurement, and low cost. The potential application of this system in automatic monitoring of banana ripening is also discussed. © 1997 Elsevier Science B.V.

Keywords: Banana; Chlorophyll; Colour; Spectrum; Reflectance; Sensing; Fruit

1. Introduction

In order to maintain a firm pulp texture, good colour and flavour, and a bruise-free product, bananas are harvested at a mature green stage and shipped to market, where they are ripened under controlled temperature and humidity conditions in an atmosphere with elevated ethylene gas levels. During banana ripening, the peel colour changes, the pulp softens, the flavour develops, and moisture is lost. The first visible sign of ripening is a colour change from green to yellow, caused by a reduced chlorophyll content in the peel. Chlorophyll content of the peel drops from 50 to 100 μg per g fresh peel weight to almost zero (Stover and Simmonds, 1987; Medlicott et al., 1990; Seymour et al., 1993). Results of previous research on banana peel colour change and its relationship to other ripeness indices are summarized in Table 1. The results indicate that peel colour is a good indicator of banana ripeness. However, the results were all based on a visual colour assessment of the peel, which is subjective and can have poor precision. In contrast, the peel chlorophyll content was used as the indicator of peel colour in this research.

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Table 1
Relationships between various changes in bananas during ripening

Relationship	Regression model	Remarks	Reference
Pulp rupture force in kg (y) and peel color score in 1–7 scale (x)	Quadratic $y = 5.91 - 1.08x + 0.31x^2$ $R^2 = 0.884$	Bananas (Musa AAA cv. Valley) under ripening with 1 ml/l (1000 ppm) ethylene at 19–21°C.	Medlicott et al., 1990
Percent soluble solids content (y) and peel color score in 1–7 scale (x)	Linear $y = 3.24 - 0.95x + 7.36x^2$ $R^2 = 0.810$	Bananas (Musa AAA cv. Valley) under ripening with 1 ml/l (1000 ppm) ethylene at 19–21°C.	Medlicott et al., 1990
Peel color score (1–7 scale) (y) and starch score in 1–10 scale (x)	Linear $y = 1.38x + 0.64$ $R^2 = 0.76$	Bananas (Musa AAA Cavendish) under ripening with 150–220 ppm ethylene at 14.4°C	Blankenship et al., 1993
Percent sugar content (y) and peel color score (1–8 scale) (x)	Quadratic $y = -26.2 + 13.8x - x^2$ $R^2 = 0.900$	Bananas (Cavendish) under ripening with 500 ppm ethylene at various temperatures	Seo and Hosokawa, 1982
Percent sugar content (y) and pulp firmness in kg (x)	Linear $y = 25.5 - 34.7x$ $R^2 = 0.867$	Bananas (Cavendish) under ripening with 500 ppm ethylene at various temperatures	Seo and Hosokawa, 1982

During postharvest transportation, handling, and ripening, the maturity status of bananas is checked regularly. There are several methods used to determine banana maturity (Gottreich et al., 1969; Charles and Tung, 1973; Wainwright and Hughes, 1990; Blankenship et al., 1993; Abdullah and Pantastico, 1990; Seymour et al., 1993) (1) fruit respiration rate, (2) pulp firmness, (3) pulp starch content, and (4) pulp reducing sugar content, and (5) peel colour. However, the first four methods are not commonly used because they are complex, time consuming, or destructive.

There are three major methods for peel colour analysis: visual, chemical, and instrumental (Knee, 1980; Wainwright and Hughes, 1989; Medlicott et al., 1990). Visual examination is carried out by a human observer who matches peel colour to a standard colour chart and assigns a colour score (United Fruit Sales Corp., 1964). This method is currently used in commercial practice for maturity determination, although many commercial operators are not able to obtain highly consistent and accurate results.

Since the change in peel colour from green to yellow during the ripening of bananas depends mostly on loss of chlorophyll, its content can be used to characterize the change in peel colour. Chemical determination of chlorophyll content involves the extraction and measurement of peel pigment components (Clesceri et al., 1989) which is a destructive method and, therefore, undesirable. Instead, an instrumental assessment of peel colour can be performed by measuring the surface reflectance of the peel (Ramaswamy and Richards, 1980; Chen and Chiu, 1990; Dixon and Hobson, 1984; Wainwright and Hughes, 1989). A spectrophotometer can be used to measure the surface reflectance at specific wavelengths, or a colour difference meter can be used to determine the surface colour as specified by one of the established colour expression systems (e.g. CIE XYZ, or CIE L*a*b*) (Hunter and Harold, 1987). However, all currently available instruments are relatively costly and are not designed to be used in commercial ripening facilities.

Inadequacies in the methods mentioned above resulted in the need to develop a rapid, accurate, and inexpensive device for automatically monitoring peel colour during commercial handling. Ideally, a sensor could be placed on the banana surface during transportation, handling, and ripening processes for ripening control. Such a device would reduce the labor involved in inspecting fruit and would be useful in developing an automatic ripening room.

The objectives of this research were to:

- Develop an inexpensive optical chlorophyll sensing system.

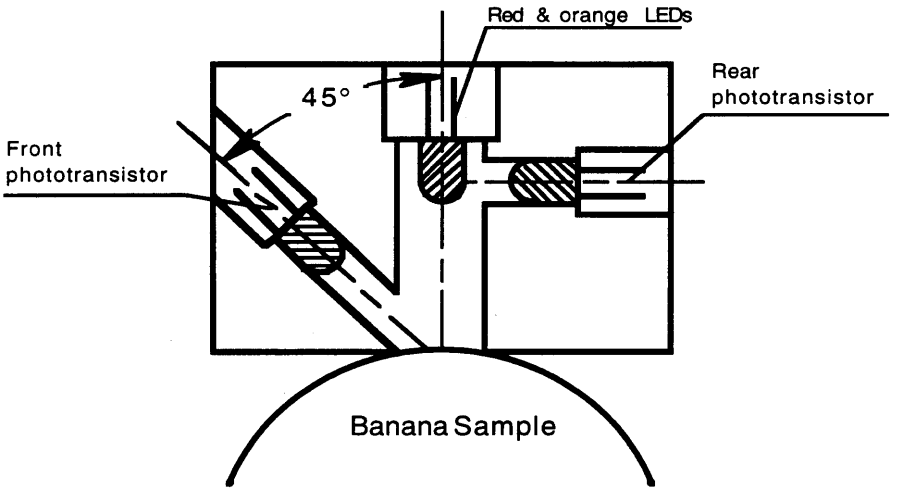

Fig. 1. Schematic drawing showing the cross-section of the sensing head.

- Determine the correlation between the optical chlorophyll sensing system measurement and chlorophyll content of banana peel measured by chemical analysis.
- Determine the correlation between chlorophyll content and colour difference meter measurement and visual colour assessment.

2. Materials and methods

2.1. Development of the optical chlorophyll sensing system

Recent advances in electro-optical technology make low cost colour measurement possible. In this study, an optical chlorophyll sensing system was developed which included a sensing head, an amplifying circuit, and a datalogger. The sensing head was composed of a support structure with two monochromatic illuminating sources and two detectors. Light emitting diodes (LED) and phototransistors were chosen to be illuminating sources and detectors because of their long life (e.g. LEDs have a mean time between failure of 1 million continuous hours, Hewlett Packard, 1996a,b), availability and low cost (in 1995 each LED cost US$0.32 and each phototransistor cost US$0.62).

It is generally believed that a single wavelength measurement is not sufficient to set up a quality index because it may be influenced by other product parameters and measurement variables (Powers et al., 1953; Mohsenin, 1984). Li (1995) conducted a preliminary spectrophotometric study of banana peel chlorophyll content and used an exhaustive search procedure, PROC REG (SAS Institute, 1990) to determine which visible wavelengths, corresponding to commercially available LEDs, best predicted chlorophyll content. Li determined that a model based upon the reflectance at two wavelengths best predicted chlorophyll content. The SAS program selected a red LED (Gilway, model E169 with peak wavelength at 660 nm and spectral line halfwidth of 25 nm), and an orange LED (Gilway, model E102 with peak wavelength at 610 nm and spectral line halfwidth of 40 nm). A sensor system was designed that used both LEDs and two phototransistors (Gilway, model D40). One phototransistor was positioned to detect the luminous intensity of either LED and another was positioned to detect the reflectance from the peel (see Fig. 1). The current applied to each LED was optimized to provide a stable light intensity that allowed the phototransistor to operate within its linear sensitivity region.

The electronic circuit in this sensing system included an LED driver and a signal amplifier. LED on/off controls were connected to the analog output channels on a datalogger (Campbell Scientific, model 21X) which was capable of data collection, storage and control. The amplified signals from the circuit were also connected to the input channels of the datalogger.

Phototransistor output was influenced not only by the optical characteristics of the sample, but also by variation in the intensity of the illuminating source, sensor sensitivity, and temperature. The basic design of the sensor is similar to that of a double beam spectrophotometer, where the rear phototransistor observes the illumination source at the same time that the front phototransistor observes the sample reflectance. One advantage of this design is that the rear sensor can be used to automatically correct the front sensor readings for interfering influences such as temperature. To develop the necessary equations for sensor correction, the stability of the sensing system was extensively studied prior to testing bananas using a standard green ceramic tile (Hunter Associates Laboratory, CIE $L^* = 71.6$, $a^* = -18.0$, $b^* = 9.9$) as a calibration surface. During stability testing, the temperature of the sensing system was controlled by placing both the ceramic tile and the sensing head into a modified cooler (Igloo, Koolrider) controlled by a temperature controller (Omega, Model CN9000A). The cooler's temperature was varied from 10 to 30°C (which encompasses the commercial banana handling temperatures of 13–18°C) to observe temperature influences on the sensing system. The output signals from the front and rear phototransistors were measured as each of the LEDs were operated sequentially. The signal output from the phototransistors was also measured while both LEDs were off to check the dark signal variation of the phototransistors at different temperatures. Detector readings were collected every 20 s over a 12 h period. The stability tests were replicated four times over a period of several weeks. Since the colour of the ceramic tile was constant, formulas (Eqs. (1) and (2)) were developed using multivariate regression analysis which correlated the changes in the front phototransistor signals with temperature, sensor sensitivity and illumination intensity to those of the rear phototransistor.

$$\text{Frt\_org} = 1279 - 0.583*\text{R\_org} - 0.542*\text{Frt\_dark} + 0.767*\text{R\_dark} \quad r^2 = 0.967 \tag{1}$$

$$\text{Frt\_red} = 944 - 0.781*\text{R\_red} - 0.546*\text{Frt\_dark} + 0.675*\text{R\_dark} \quad r^2 = 0.991 \tag{2}$$

where, Frt_org and R_org are the unadjusted readings from the front and rear phototransistors, respectively, when the orange LED is turned on; Frt_red and R_red are the unadjusted readings from the front and rear phototransistors when the red LED is turned on; while Frt_dark and R_dark are the unadjusted readings from the front and rear phototransistors when both LEDs are off. The intercept values (1279 and 944) were then assigned to be the adjusted readings for the Frt_org and Frt_red of the standard green tile.

To automatically correct the readings for interfering influences such as temperature the following equations were defined.

$$\text{Frt\_org}_{adj} = \text{Frt\_org} - 0.583*\text{R\_org} - 0.542*\text{Frt\_dark} + 0.767*\text{R\_dark} \tag{3}$$

$$\text{Frt\_red}_{adj} = \text{Frt\_red} - 0.781*\text{R\_red} - 0.546*\text{Frt\_dark} + 0.675*\text{R\_dark} \tag{4}$$

where, Frt_org_{adj}, is the adjusted reflectance intensity from the front phototransistor when the orange LED is on and Frt_red_{adj} is the adjusted reflectance intensity from the front phototransistor when the red LED is on.

A control program was written in the datalogger to turn the red LED on for 0.5 s, then turn the red LED off for 0.5 s, then turn the orange LED on for 0.5 s, and then turn the orange LED off in a cyclical pattern. The reflectance intensity from the sample and the light intensity of the illuminating source were measured and saved when either of the LEDs were on and when both were off. According to a preliminary study, the phototransistors had a response time of about 0.2 s. The sensor readings were measured after the LEDs had been on (or off) for nearly 0.5 s. For each

test, the datalogger recorded ten replicate measurements and saved the average value. Fig. 2 shows the flow diagram of the peel colour measurement program.

The output from the prototype sensor was determined by placing a banana sample and the detector head in a light-tight chamber in order to avoid interference from other light sources. Because of slight colour variations on the fruit surface, the reflectance intensity of each banana was measured twice on both sides of the main axis near the center region of the banana and the average was taken.

The correlation between sensor output and banana peel chlorophyll content was developed using several brands of Cavendish bananas (Chiquita, Dole, Del Monte, and Nutriclean), randomly selected from a local wholesaler or from a local retail store. Only undamaged fruit, free from surface defects were chosen for this study.

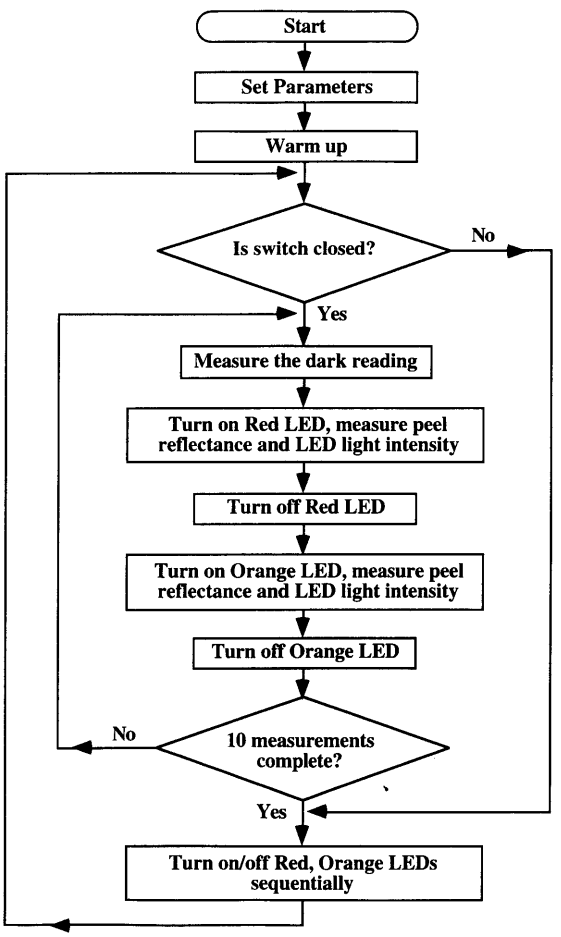

Fig. 2. Flow chart of banana peel color testing program.

2.2. Testing the sensor

Two sets of banana samples were collected approximately 2 weeks apart and the regression model was developed using 22 samples from the first set and tested against 21 samples from the second set. The banana samples were selected with different stages of ripeness as determined by visual colour assessment. All banana samples were equilibrated to room temperature (25°C) before testing.

The colour of each banana was evaluated subjectively by comparing the fruit with a standard colour chart (Del Monte fresh produce N.A.) to obtain banana maturity ratings for each fruit. The visual colour score of each banana peel was assessed on a seven point scale, where 1 = green, 2 = light green with a very light tinge of yellow, 3 = half green half yellow, 4 = three quarters yellow with green, 5 = yellow with green tips, 6 = full yellow, and 7 = yellow with brown spots. All fruit had a rating of 5 or less because bananas are not commercially marketed at a 6 or 7 ripeness level.

A colorimeter (model D25-PC2, Hunter Associates Laboratory) with a 45° illumination, 0° viewing angle, and 0.64 cm diameter opening was used to determine the CIE L*, a*, b* and X, Y, Z coordinates of each peel. These quantities were measured twice on both sides of each banana near the mid-section and the average determined.

A scanning spectrophotometer (Model 6500, NIRSystems) was used to obtain the reflectance spectrum of each banana. Samples were cut along one side and the pulp was removed. A circular portion of the peel (50.8 mm diameter, 20.27 cm^2 surface area) was cut near the mid-section of the banana sample and placed in the spectrophotometer. NSAS software (NIRS systems, 1990) was used to control the spectrophotometer with a selected scanning range of 400–1100 nm and a 1.8 scans/s scan rate. Each peel sample was scanned 250 times and the average was recorded.

The peel chlorophyll was extracted and quantified using a spectrophotometric method (Clesceri et al., 1989). This was carried out immediately after the spectral reflectance analysis to avoid any breakdown of the chlorophyll components in the peel. To extract the chlorophyll, the peel sample was cut into small pieces with a knife, and ground and mixed for about 1 min with a solution of 90% acetone and 10% distilled water using an electric homogenizer (ESGE, Model M122). The chlorophyll solvent solution was transferred to a screw-cap brown bottle, shaken for 5 min using a wrist-action shaker (Burrell scientific, Model 75), then stored in a refrigerator at 4°C. After about 24 h, the solution was clarified by passing it through filter paper (Whatman, No. 54). The filtrate was then collected in a syringe (Monoject) and injected into a 1 cm cuvette through a 0.2 μm nylon acrodisc filter. Chlorophyll content was measured using a diode array spectrophotometer (Beckman Instruments, model DU7500). The chlorophyll extract was scanned and optical densities (OD) at 647, 664, and 750 nm (750 nm was used to correct for background interference) were obtained. A 90% acetone solution was used as the optical reference in the test. The following equations were used to determine the concentration of chlorophyll in the extract (Clesceri et al., 1989):

$$C_a = 11.85*(\mathrm{OD664} - \mathrm{OD750}) - 1.54*(\mathrm{OD647} - \mathrm{OD750}) \quad (5)$$

$$C_b = 21.03*(\mathrm{OD647} - \mathrm{OD750}) - 5.43*(\mathrm{OD664} - \mathrm{OD750}) \quad (6)$$

where C_a and C_b are the concentrations of chlorophyll a and b in mg/l, respectively; and OD647, OD664, OD750 are the optical densities at the 647, 664, and 750 nm wavelengths, respectively.

Since peel thickness varies with the maturity of the banana, the chlorophyll content per unit surface area was used, rather than content per unit fresh weight or per unit volume. The chlorophyll content (μg/cm^2) was calculated as follows:

$$C_a(\mu\mathrm{g/cm^2}) = 1000\ \mu\mathrm{g/mg}*C_a^**\mathrm{TSV/PSA} \quad (7)$$

$$C_b(\mu\mathrm{g/cm^2}) = 1000\ \mu\mathrm{g/mg}*C_b^**\mathrm{TSV/PSA} \quad (8)$$

where C_a is the chlorophyll a concentration (mg/l) of the extract solution determined in Eq. (5), C_b is the chlorophyll b concentration (mg/l) of the extract solution determined in Eq. (6), TSV is the total volume (l) of the chlorophyll extract solution and PSA is the peel surface area (20.27 cm^2) of the sample.

3. Results

3.1. Calibration of the optical chlorophyll sensing system

The spectral reflectance curves of some banana peel samples are shown in Fig. 3. Reflectance in the 550–700 nm wavelength region shows a strong correlation with peel chlorophyll level. A peel with a high chlorophyll content has a low reflectance in this region.

The highest correlation between the peel chlorophyll level and the change in reflectance occurs at 678 nm (chlorophyll absorption band). It was also found that the sample peel chlorophyll contents (a and b) were linearly correlated ($r^2 =$ 0.88 and 0.85, respectively) with visual colour score.

Since a red LED with a peak wavelength at 660 nm and an orange LED with a peak wavelength at 610 nm were used as illuminating sources in the sensor, reflectance values at 610 nm and 660 nm were used to predict the chlorophyll values. The regression models using spectrophotometric data to predict chlorophyll a and b levels were developed by using several different statistical regression approaches. The performance of these models is shown in Table 2.

The multiple linear regression model, Eq. (9) and Eq. (10), had the best performance.

$$C_a = 22.4 - 0.675*\mathrm{Refl}_{610} + 0.287*\mathrm{Refl}_{660} \quad r^2 = 0.927 \quad (9)$$

$$C_b = 6.02 - 0.216*\mathrm{Refl}_{610} + 0.112*\mathrm{Refl}_{660} \quad r^2 = 0.904 \quad (10)$$

where C_a and C_b are the concentrations of chlorophyll a and b in μg/cm^2; and Refl_{610}, Refl_{660} are

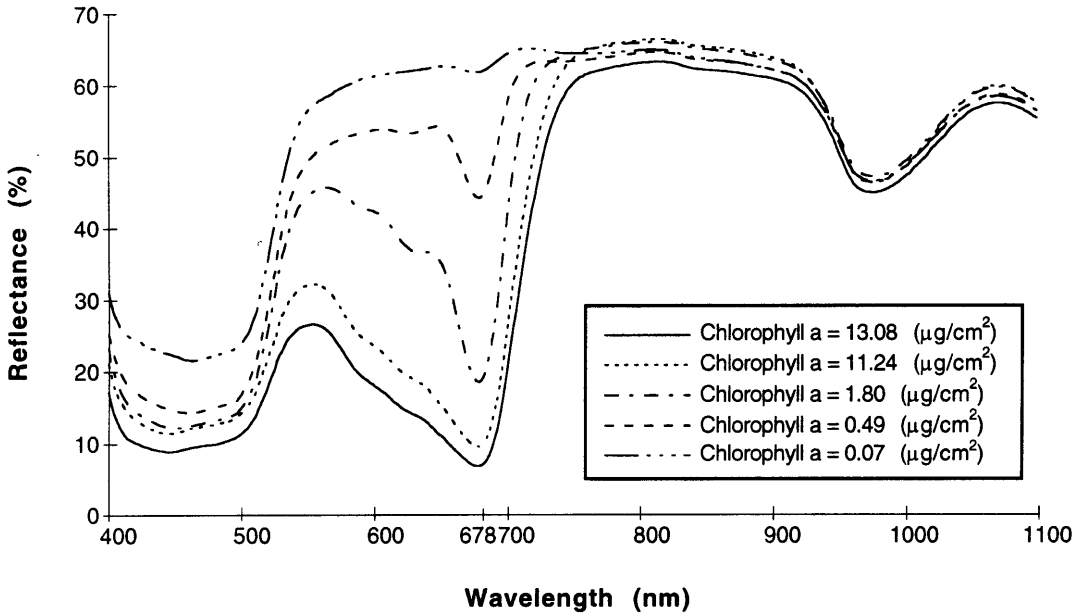

Fig. 3. Spectral reflectance of banana peels with different chlorophyll *a* levels

percent reflectance at 610 nm and 660 nm, respectively (Fig. 4).

We next used multiple linear regression to correlate chlorophyll content with the adjusted sensor output.

$$C_a = -17.9 - 0.0187*\text{Frt\_red}_{adj} + 0.0329*\text{Frt\_org}_{adj} \quad r^2 = 0.904 \qquad (11)$$

$$C_b = -5.40 - 0.00678*\text{Frt\_red}_{adj} + 0.0106*\text{Frt\_org}_{adj} \quad r^2 = 0.880 \qquad (12)$$

where Frt_red_{adj} is the adjusted reading from the optical chlorophyll sensing system when the red LED is on and Frt_org_{adj} is the adjusted reading from the optical chlorophyll sensing system when the orange LED is on (Fig. 5).

3.2. Model validation

Chlorophyll levels for the 21 fruit test set were predicted using Eqs. (9)–(12), and then the predicted values were compared with the measured values. The summary of calibration and validation results are shown in Table 3.

The root mean squared prediction error (S.E.P.) of these regression models indicates the actual predictive capability of the regression models. The S.E.P. for each model is fairly close to the root mean squared calibration error (S.E.C.), indicating that the S.E.C. for each model is not seriously biased and gives a good indication of the model's predictive ability. The relatively low bias values and slope values approaching one also indicate the good predictive capability of the models.

3.3. Colour testing results

Figs. 6 and 7 and Table 4 show that the visual colour assessment and the colorimeter data correlate well with chlorophyll content. The visual colour score of the 43 banana samples ranged from 1 to 5. Colour is a good predictor of chlorophyll content for scores of 1–4. The hue angle is also a good predictor of chlorophyll content except when chlorophyll content approaches zero. Visual colour evaluation inherently has more error because colour is only measured to the closest

Table 2
Performance of regression models for predicting peel chlorophyll content

Regression Model	Variables	Chlorophyll *a*			Chlorophyll *b*		
		r^2	S.E.C.	S.E.P.	r^2	S.E.C.	S.E.P.
Simple linear	$Refl_{610} - Refl_{660}$	0.211	4.664	4.731	0.121	1.259	1.116
	$Refl_{610} + Refl_{660}$	0.829	2.170	1.751	0.731	0.696	0.514
	$Refl_{610}/Refl_{660}$	0.817	2.246	1.904	0.713	0.719	0.551
	$Refl_{660}/Refl_{610}$	0.715	2.804	2.333	0.598	0.852	0.636
Multiple linear	$Refl_{610}$ and $Refl_{660}$	0.927	1.458	0.949	0.904	0.427	0.317

S.E.C. = Root mean square error of calibration.
S.E.P. = Root mean square error of prediction.

unit value. However, both these relationships show a strong correlation between peel colour and chlorophyll content. Our chlorophyll sensor should correlate well with the commercially used visual colour method of determining banana ripeness.

Higher chlorophyll contents cause lower colour scores, L*, a*, b*, h_{ab}, and C_{ab} values. Since the hue angle ($h_{ab} = \arctan(b^*_{CIE}/a^*_{CIE})$) represents the dominant wavelength of a colour, it can predict the chlorophyll *a* and *b* contents with r^2 values of 0.949 and 0.933, respectively.

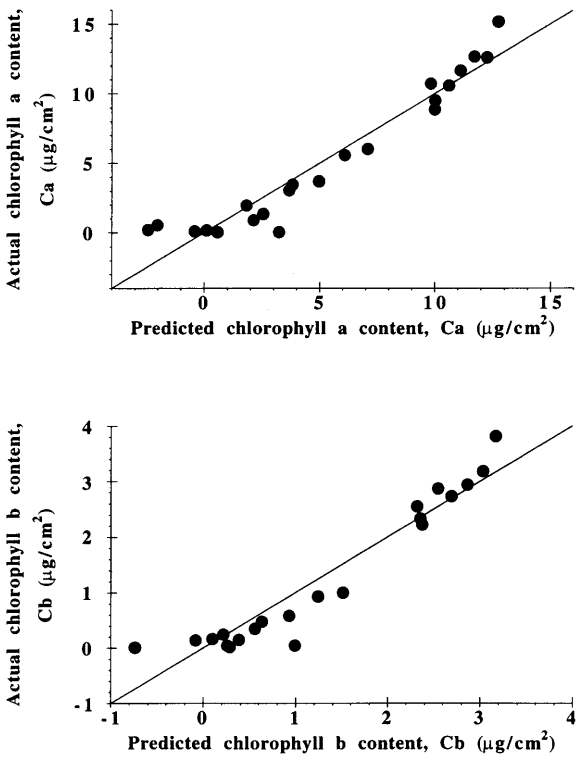

Fig. 4. Plot of actual chlorophyll content versus chlorophyll content predicted by banana peel reflectance at 610 nm and 660 nm for the calibration data set.

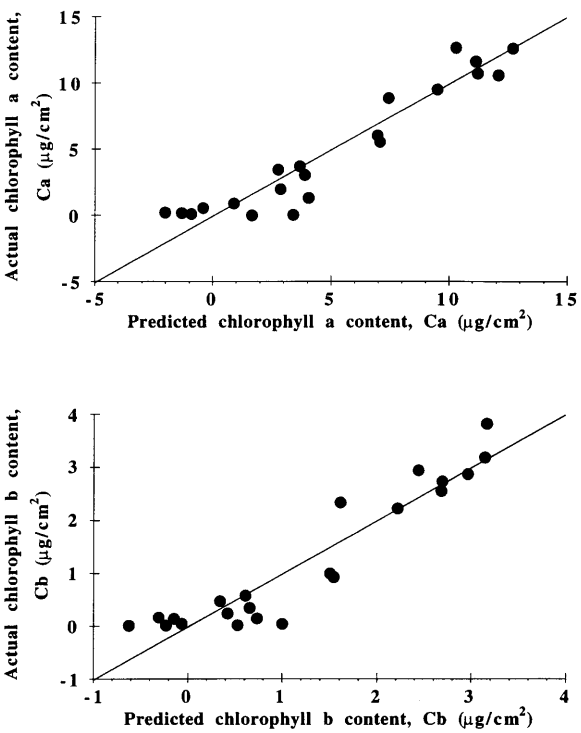

Fig. 5. Plot of actual chlorophyll content versus chlorophyll content predicted by the LED phototransistor for the banana peels in the calibration set.

Table 3
Summary of calibration and validation results.

Predicted variable	Sensed variable(s)	Calibration results (22 samples)		Validation results (21 samples)		
		r^2	S.E.C.	S.E.P.	Bias	Slope
C_a (Eq. (7))	$Refl_{610}$, $Refl_{660}$	0.927	1.458	0.949	0.358	0.950
C_b (Eq. (8))	$Refl_{610}$, $Refl_{660}$	0.904	0.427	0.317	0.205	0.830
C_a (Eq. (9))	Frt_red_{adj}, Frt_org_{adj}	0.904	1.670	1.605	0.388	0.941
C_b (Eq. (10))	Frt_red_{adj}, Frt_org_{adj}	0.880	0.477	0.454	0.274	0.804

4. Discussion

This optical chlorophyll sensing system rapidly and accurately measures the chlorophyll content of banana peels. It is also simple to operate, non-destructive, and inexpensive. It is envisioned that this device could be used to monitor banana peel colour during ripening, replacing the standard manual colour determination. This would eliminate inspection during ripening, thereby improving the quality of ripening and preventing the release of ethylene gas.

Peel chlorophyll content (C_a and C_b) did not change much between colour score 4 and 5 (Fig. 6). This might result in difficulty predicting visual colour scores above 4 using the chlorophyll sens-

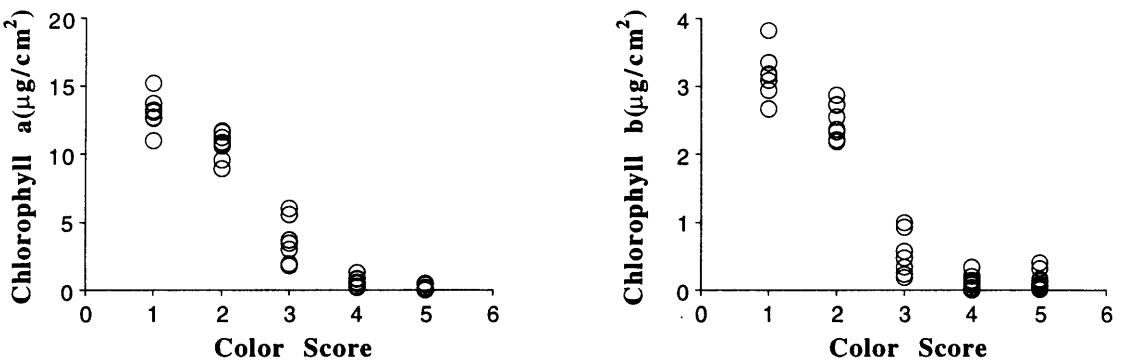

Fig. 6. Relationship between chlorophyll content and visual color score in banana peels.

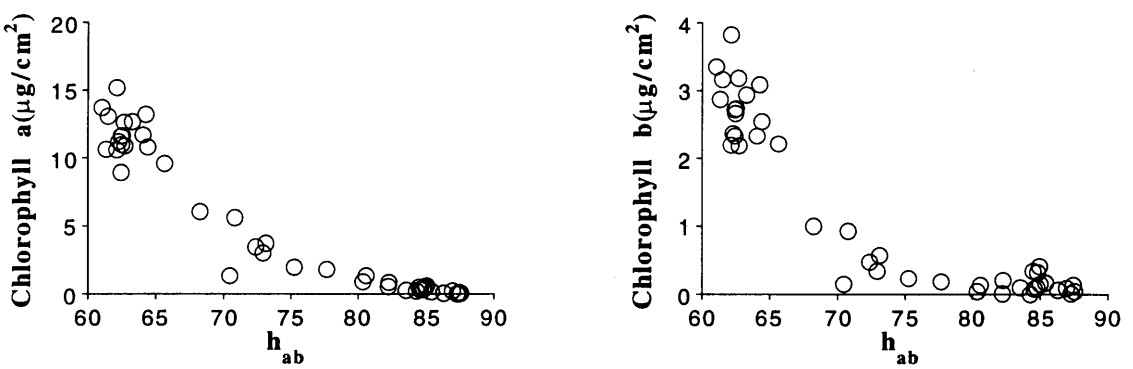

Fig. 7. Relationship between chlorophyll content and hue angle (h_{ab}) in banana peels.

Table 4
Correlation table of different measurements

	Chlorophyll		Spectral reflectance		Optical chlorophyll sensing system reflectance		Visual color	CIE color values				
	a	b	$Refl_{610}$	$Refl_{660}$	Frt_org_{adj}	Frt_red_{adj}	Score	L^*_{CIE}	a^*_{CIE}	b^*_{CIE}	h_{ab}	C_{ab}
Chlorophyll a	1.00											
Chlorophyll b	0.99	1.00										
$Refl_{610}$	−0.96	−0.91	1.00									
$Refl_{660}$	−0.91	−0.85	0.99	1.00								
Frt_org_{adj}	0.94	0.91	−0.97	−0.96	1.00							
Frt_red_{adj}	0.92	0.87	−0.97	−0.97	0.99	1.00						
Visual color score	−0.94	−0.92	0.94	0.93	−0.92	−0.92	1.00					
L^*_{CIE}	−0.95	−0.93	0.95	0.92	−0.97	−0.95	0.91	1.00				
a^*_{CIE}	−0.93	−0.89	0.97	0.97	−0.97	−0.97	0.93	0.95	1.00			
b^*_{CIE}	−0.86	−0.82	0.85	0.83	−0.88	−0.87	0.84	0.87	0.86	1.00		
h_{ab}	−0.95	−0.91	0.97	0.97	−0.97	−0.97	0.94	0.96	1.00	0.89	1.00	
C_{ab}	−0.72	−0.69	0.71	0.69	−0.76	−0.75	0.71	0.74	0.72	0.97	0.76	1.00

$h_{ab} = \arctan\,(b^*_{CIE}/a^*_{CIE})$ and $C_{ab} = (a^{*2}_{CIE} + b^{*2}_{CIE})^{1/2}$

ing system. However, bananas are removed from ripening before they reach a score of 4.

As variability may exist between banana cultivars and between the reproducibility of components in the optical chlorophyll sensing system, the coefficients in the regression models developed might be different for each variety of banana. If the optical chlorophyll sensing system is placed into practical use, a calibration may have to be done first for each cultivar and sensor combination. In order to increase the accuracy of determining banana ripeness, multiple sensing heads would be required to account for variability in ripening status among fruit within a carton or ripening room.

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