Technology & Product Reports

Video Imaging for Quantifying Cucumber Fruit Color

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Additional index words. nondestructive, chlorophyll

Summary. A video-imaging technique, using commercial software to process images obtained at 550 nm, was established to estimate chlorophyll content of cucumber fruit disks. The chlorophyll content of excised disks was extracted, determined, and regressed on the video-image grey level. They were linearly related. The change in grey level of the whole visible image accurately indicated the change of green color during fruit development on the vine and the loss of green color after 1 week of storage at 13C. The relationship of the chlorophyll content on grey level was quadratic for three imaging methods: 1)

average grey level of the five disks; 2) average grey level of the whole cucumber image; and 3) average grey level of central one-third of the whole cucumber image. Chlorophyll content was most highly correlated to the grey level of the disks themselves (residual $SD = 6.74 \mu\text{g}\cdot\text{cm}^{-2}$), but this sampling technique was destructive. Both one-third of the fruit image ($SD = 9.25 \mu\text{g}\cdot\text{cm}^{-2}$) and the whole image ($SD = 9.36 \mu\text{g}\cdot\text{cm}^{-2}$) provided satisfactory precision. For simplicity, whole-fruit imaging is suitable for estimating fruit chlorophyll content and for quantifying fruit green color intensity. Potential use of this technique in product sorting and shelf life prediction of long English cucumbers is discussed.

Color change is one of the most important characteristics relating to maturity and senescence of fruits and vegetables. It is desirable that such changes be measured repetitively on the same fruit throughout the entire experiment; i.e., a nondestructive method of measurement (Watada, 1989). At present, tissue samples have to be excised from cucumber fruit and the chlorophyll extracted to quantify the green color (Kanellis et al. 1986; Poenicke et al., 1977). Chlorophyll extraction is laborious and requires the use of solvents. Therefore, this extraction normally is restricted to small areas of fruit surface, which may not be representative of the whole fruit. The procedure is destructive, so each fruit can be measured only once. Alternatively, the surface color can be measured by a reflectance spectrophotometer (Kader and Morris, 1987; Saltveit and McFeeter, 1980). Such a reflectance spectrophotometer normally is equipped with a small aperture. For example, AgTron Colormet

(AgTron, Reno, Nevada) has a 1.9-cm-diameter sampling area. Several measurements are necessary, as indicated by Kader and Morris (1978). The method is somewhat laborious, and the measurements do not include the whole surface. Some modifications have been made to accommodate whole tomato (Kader and Morris, 1978) and field cucumber (Saltveit and McFeeters, 1980). Because greenhouse-grown cucumbers are fairly large in size (up to 43 cm long and 6.2 cm in diameter), neither of these two spectrophotometric methods appear to be practical. There has been no report on nondestructive methods of quantifying surface color of long English cucumber.

Video imaging is nondestructive (Lin and Keng, 1989; Lindsay and Bassuk, 1992), allowing repetitive measurements of the same fruits throughout an experiment. A measurement of average color can be made on a whole fruit surface facing the video camera. This measurement of the "whole image" includes more of the surface than measurements provided by chlorophyll extraction or surface reflectance spectrophotometry. Because the surface of the fruit is curved, the observed color at any point on the surface depends not only on the chlorophyll content, but also on the angle between the line of sight from the video camera lens and the tangent to the surface at the point. This might affect the average green color of a whole image. To reduce this effect, green color can be determined on a central portion of the visible surface. A third alternative is to use the video-imaging system to determine the color of disks excised from the surface of the fruit. Because the disks are small, this ensures that there will be no "edge effect" and provides a standard against which to compare the other two imaging methods. Because the disks are excised, this method is destructive, and therefore would be less useful in practice.

The objective of the first of two experiments was to determine whether the chlorophyll content of cucumbers could be estimated using video imaging. The objectives of the second experiment were to compare three methods of determining chlorophyll using video imaging, and to determine whether the precision of the three imaging methods depended on the size of the fruit.

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Contribution no. 467 of Agriculture Canada, Agassiz Research Station. The study was partially supported by Agri-Food Regional Development Subsidiary Agreement No. 11045 and by B.C. Greenhouse Vegetable Research Council. Technical assistance by G. Block is gratefully acknowledged. Mention of companies or commercial products does not imply recommendation or endorsement by Agriculture Canada over others not mentioned.

Materials and methods

Chlorophyll content can be determined by the reflectivity at 550 nm, when it is normalized by the corresponding reflectivity at 800 nm (Aoki et al., 1986). Our preliminary results showed that there was no need for such normalization. Therefore, all imaging in this study was carried out with a wavelength-specific filter of 550 nm with bandwidth of 40 nm (Andover Corp., 550 FS40-50, Salem, N.H.). A lighting box (70 × 70 × 130 cm, flat-white interior) was built to exclude all external incident light. Inside the light box, there was a stable lighting source of four 60-W incandescent light bulbs placed 50 cm above the platform and 50 cm apart on four corners. These light bulbs were powered at a constant 100 V (DC) using a DC voltage regulator (Xantrex, XKW 150-7, North Vancouver, B.C., Canada). A whole cucumber fruit or a sample disk in a petri dish was placed on the white flat surface of a platform in the box. The effect of the lighting was kept constant throughout the experiment by determining average grey level of a color standard (Royal Horticultural Society, Chart 135B, London) and using this to standardize the sample readings.

The video-imaging apparatus for Expt. 1 consisted of a video camera linked to an IBM-compatible microcomputer (Acer Technologies Corp., Model 1100, San Jose, Calif.) with 1.2-MB internal RAM and a 40-MB hard-disk memory operating at 16 MHz. The image was obtained through a video camera (Kayo International Inc., TVC-6200-2, Hanpange, N.Y.) fitted with a wavelength-specific filter centered at 550 nm (bandwidth 40 nm). The image of a whole cucumber or an excised disk was captured using an imaging board (Imaging Technology Inc., PCVISIONplus Frame Grabber 47-H00010-01, Woburn, Mass.), and stored in the computer memory. The image was displayed on a color monitor (Panasonic, DT-M140, Japan). The cucumber image and its surrounding background constituted a total of 640 × 480 pixels. Each pixel was assigned electronically a grey level ranging from 0 (absolute black) to 255 (absolute white). The image of a fruit surface was isolated from the background on the display by tracing its edge with a mouse device manually. The average grey level of the resulting

cucumber image was calculated with video analysis software (Jandel Scientific, Java version 1.21, Corte Madera, Calif.) and the resulting statistics were displayed on a second computer monitor. After whole-fruit imaging, a disk was isolated from the mid-section of the same cucumber. The green color of 11.5-mm-diameter cucumber disks was quantified from a 19 × 19 pixel square area ($\approx 8 \times 8$ mm of fruit disk) in the center of the disk image.

Flowers of 'Mustang' greenhouse cucumbers were periodically tagged on the date of anthesis so that cucumbers of different ages could be harvested and measured on the same day. Four age groups (12, 16, 20, and 24 days after anthesis) with eight fruit each (except seven fruit in the 12-day group) were harvested on 22 Nov. 1990. Five age groups (17, 19, 21, 23, and 25 days after anthesis) with nine fruit each (except four and seven in the 17- and 19-day group, respectively) were harvested on 10 Dec. 1990 (Table 1). Missing fruit were due to abortion. The average grey level of the whole cucumber was determined on the day of harvest. The fruits were then placed individually in perforated polyethylene tubes (48 × 13 cm) to prevent moisture loss and were stored at 13C with 95% RH. The color of these fruits was measured again after 1 week in storage. At each harvest, 16 additional freshly harvested fruits with four distinct degrees of visual green color intensity were used. These four categories ranged from very yellow to very green. These fresh fruits were used for whole-fruit and disk measurements. After whole fruit imaging, one 11.5-mm-diameter disk was isolated from the mid-section of each fruit with a cork borer and trimmed to a 3-mm thickness for video imaging. The same disk then was homogenized with ice-cold 80% acetone in water (v/v), filtered, and the optical density of the filtrate was determined at 645 and 663 nm with a Bausch and Lomb Spectronic 20 (Rochester, N.Y.). The chlorophyll content ($\text{g}\cdot\text{cm}^{-2}$) was calculated according to Arnon (1949).

For Expt. 2, two modifications appeared to be desirable from the experience in Expt. 1. First, the edge of the image could be defined consistently by selecting a specific threshold grey level rather than tracing it manually. Second, a specific section of an image could be selected for analysis.

In particular, the central one-third of the image was used. A new image system and software allowed this to be done automatically, and it linked all the steps to provide a simplified procedure. The lighting box was the same as in Expt. 1.

The Faradyne iP/600 Imaging System (Faradyne System Group Inc., Richmond, B.C., Canada) consisted of a black/white solid-state CCD video camera (Cohu 48151-5100/AL25, San Diego, Calif.) linked to an IBM-compatible microcomputer (Comtex, Richmond, B.C., Canada). The microcomputer had an 80386 CPU main processor, 2.0 RAM, and a 80.0-MB hard-disk memory. The cucumber or disk was viewed through the video camera fitted with a 550-nm filter (bandwidth, 40 nm). The image was captured by an imaging board with RS170 input and 512 × 512 × 8 buffer capacity, and then stored on the hard disk. The image was displayed on an RGB color monitor (CTX model CVG-5432, CTX International Inc., Walnut, Calif.). The image was isolated from background automatically by the edge function of image analysis software VISILOG (NOESIS Vision Inc., St. Laurent, Que., Canada). The average grey level of the whole image then was determined. The central one-third of the image surface then was identified by "eroding" the edge of the whole image. The depth eroded on each side was 80% of one-third of the maximum diameter of the image. The maximum diameter was determined automatically by the software, and the 80% factor was found empirically to leave one-third of the visible image. Finally, the average grey level of the one-third image was determined. Five 9-mm-diameter disks were excised from the area of each fruit corresponding to the center one-third of the fruit image, and their average grey level was determined. These five disks were combined and the chlorophyll content was extracted and determined as before.

In Expt. 2, 100 long English 'Mustang' cucumbers were harvested on 12 Apr. 1992 and divided visually into four groups of 25 cucumbers each and stored at 13C. The size groups were composed of the largest 25 fruit, the next largest 25, and so on. The weight, length, and maximum diameter of each fruit was recorded. After 0, 1, 2, 3, and 4 weeks, green color measurements were made on five cucumbers of

each size. Thus, a full range of fruit sizes was studied each week. Because the chlorophyll deteriorated with time in storage, this procedure provided a range of surface colors for study.

Statistical methods. The SAS statistical system (SAS Institute Inc., Cary, N.C.) was used for statistical computations. In Expt. 1, the chlorophyll content of disks was regressed on video grey level. The effects of age at harvest and after storage on the video grey level of whole fruit was determined by analysis of variance. In Expt. 2, the log of weight was regressed on log of length and diameter to determine whether they could be used to characterize fruit size. The chlorophyll content was regressed separately on the grey readings from the whole image, one-third image, and disks. The residual standard deviations were calculated to indicate the precision of the relationships. The residuals from the regression lines were regressed on log (length) and log (diameter) to determine whether they were related to fruit size.

Results

Experiment 1. The green color of cucumber fruits varied significantly with age at harvest (Table 1). The most-intense green, indicated by the lowest grey value, occurred 20 to 23 days after anthesis. Subsequent 1-week storage resulted in significant loss of green, which was evident from increasing grey levels (Table 1). Disk chlorophyll was related linearly to average disk grey level, but the intercepts and slopes of the lines differed significantly between harvests and between fresh and stored cucumbers (Table 2). The grey levels ranged from 99 to 250.

Experiment 2. Chlorophyll concentrations ranged from 33.7 to 116.4 $\mu\text{g}\cdot\text{cm}^{-2}$. The range of the grey levels

Table 2. Relationship between chlorophyll content ($\mu\text{g}\cdot\text{cm}^{-2}$) and video image grey level^a of cucumber disks (11.5 mm in diameter).

Sample type	Chlorophyll/grey level relationship
<i>Harvested 22 Nov.</i>	
Fresh (n = 16)	Chlorophyll = 124 - 0.47 (grey), $r^2 = 0.74$
Stored (n = 31)	Chlorophyll = 92 - 0.28 (grey), $r^2 = 0.66$
<i>Harvested 10 Dec.</i>	
Fresh (n = 16)	Chlorophyll = 111 - 0.39 (grey), $r^2 = 0.92$
Stored (n = 38)	Chlorophyll = 92 - 0.31 (grey), $r^2 = 0.77$

^aGrey level was unitless (0 = absolute black; 255 = absolute white).

^bFresh fruit were assayed on the date of harvest, and stored fruit after 1 week at 13C.

was similar for the two whole-fruit determinations (84-131 for one-third surface and 87-127 for whole image), but the range was somewhat wider for the disks (73-185). The relationship between chlorophyll content and grey level was quadratic for all three video-imaging determinations (Fig. 1). The amounts of variation explained by the relationships were very similar for the two whole-fruit determinations. The standard deviations for the whole-fruit readings (9.25 $\mu\text{g}\cdot\text{cm}^{-2}$ for one-third fruit surface and 9.36 $\mu\text{g}\cdot\text{cm}^{-2}$ for whole fruit) were $\approx 40\%$ larger than that for the disks (6.74 $\mu\text{g}\cdot\text{cm}^{-2}$).

Ninety-six percent of the variation in weight log of the cucumbers was explained by multiple regression on length log and diameter log. Therefore, fruit size was characterized by length log and diameter log. The residuals from each of the three relationships of chlorophyll content on grey level were regressed on length log and diameter log. In no case was a significant relationship found.

Discussion

The relationship between disk chlorophyll content and grey level was linear in Expt. 1, but quadratic in Expt. 2. In Expt. 1, chlorophyll concentrations ranged from 10 to 65

$\mu\text{g}\cdot\text{cm}^{-2}$. These low chlorophyll levels were the results of low light intensity during November and December. In Expt. 2, the fruit were produced in April, when light intensities were higher, and the chlorophyll concentrations ranged from 34 to 116 $\mu\text{g}\cdot\text{cm}^{-2}$. The quadratic component of the relationship likely was observed because of the wider range of values in Expt. 2. The significant differences in slopes and intercepts observed in the first experiment may be accounted for by the curvature of the overall relationship. The observed values depended on the harvest and storage time, and thus lay on different segments of the curve.

Cucumber chlorophyll content can be determined from the grey level of the whole-fruit image (i.e., half of whole fruit surface) or the center one-third of visible image. The relationships were both quadratic, but the residual standard deviations were not significantly different. Because the standard deviations were not different, there was no advantage in reducing the image to the center one-third. The standard deviations for these two methods were $\approx 40\%$ more than that for the excised disks. This disadvantage is offset by two advantages. The whole-fruit image is more representative of the entire fruit surface and the procedure is nondestructive. The latter advantage is of considerable importance when fruits are to be measured repetitively. The usefulness of whole-fruit imaging was exemplified by the grey readings obtained from cucumber harvested at different ages, and by an increase in grey level (i.e., their loss in green) after 1 week in storage. The range of grey levels of whole-fruit imaging and center one-third were narrow (78 to 127 and 84 to 131, respectively) as compared to that of excised disks (grey level of 73 to 185) (Fig. 1). The measurement of grey

Table 1. Effects of fruit age (days from anthesis to harvest) and postharvest storage at 13C for 1 week on video image grey level^a of cucumber fruit surface.

Fruit age (days)	Harvested 22 Nov.		Fruit age (days)	Harvested 10 Dec.	
	Fresh	Stored		Fresh	Stored
12	136	156	17	136	140
16	123	134	19	134	142
20	109	118	21	129	138
24	110	116	23	115	121
			25	119	125
SE = 3.0 ^b			SE = 4.2		

^aGrey level measured by video imaging (0 = absolute black; and 255 = absolute white).

^bStandard error for a harvest date.

Fig. 1. The quadratic relationship between chlorophyll content of cucumber disks and image grey levels obtained by three imaging methods. (A) Chlorophyll content and disk grey level: $\text{chlorophyll} = 220.8 - 1.92(\text{grey}) + 0.0050(\text{grey})^2$; $r^2 = 0.89$. (B) Chlorophyll content and grey level of whole image: $\text{chlorophyll} = 923.8 - 14.73(\text{grey}) + 0.0611(\text{grey})^2$; $r^2 = 0.78$. (C) Chlorophyll content and grey level of center one-third fruit image: $\text{chlorophyll} = 629.2 - 9.45(\text{grey}) + 0.0377(\text{grey})^2$; $r^2 = 0.77$. Symbols on the figure denote the number of weeks in storage at 13C (solid square = 0 week; open circle = 1 week; open square = 2 weeks; solid triangle = 3 weeks; solid circle = 4 weeks).

level for a specific fruit is accurate and reproducible (Lin and Keng, 1989). A small difference in grey level is sufficient to indicate the difference in cucumber green color. For cucumber, a 550-nm wavelength-specific filter was used because of its high sensitivity to chlorophyll (Aoki et al., 1986). Other wavelengths might be necessary for

other commodities (Searcy and Reid, 1989). A color camera was not tested in this study because color imaging produces a much larger data set and its processing is too time-consuming.

Video imaging quantifies the green color of the image of the half of the surface facing the video camera on the basis of peel chlorophyll content. Video imaging measures reflectance of the fruit surface in the same way as spectrophotometric methods. The former includes the whole visible surface facing the video camera, while the latter covers a specific area (with a probe) or a strip of surface (with a rotating device in a chamber) (Kader and Morris, 1978). A trained person can use our video-imaging system to measure one cucumber fruit in ≈ 3 min. Much time can be saved with simplifications in software.

The video grey level of the whole cucumber fruit is related to its chlorophyll content (Fig. 1B). The precise relationship between grey level and whole-fruit chlorophyll content was not determined because of the volume of organic solvent needed for the extraction. However, the grey level of the whole image correlated well with the chlorophyll of five disks, which were sampled from the same fruit (Fig. 1B). The effectiveness of video imaging in quantifying cucumber green color was evaluated in two separate experiments: Cucumbers were grown under two different growing conditions and two different imaging systems were used. The results were consistent. In Expt. 1, average chlorophyll content of fresh cucumbers was $45.9 \mu\text{g}\cdot\text{cm}^{-2}$, which was associated with grey level of 126 to 250. In Expt. 2, the average chlorophyll content was $88.5 \mu\text{g}\cdot\text{cm}^{-2}$, with grey levels of 89 to 98.

Video imaging for quantifying cucumber green color is nondestructive. The resulting average grey level is not affected by fruit size. It has potential in product sorting, which is commonly carried out by visual inspection. In commercial practice, product colors can be standardized by taking color photographs of product standards, and establishing their grey levels. Individual products then can be inspected on line according to these color standards by means of video imaging. In cucumber, fruit green color at harvest is related to the shelf life (incipient yellowing) (Lin and Ehret, 1991). Therefore, it might be possible to predict cucumber shelf life with its grey level obtained at

harvest. Such prediction in cucumber shelf life is under further investigation. Our preliminary data obtained from various experiments involving training methods (Klieber et al. 1993) and canopy light levels (Lin and Jolliffe, unpublished data) indicate such a possibility. When such predictability is established, the marketing strategy can be devised. For example, cucumbers with predicted long shelflives could be marketed in a distant market. Conversely, the cucumbers with predicted short shelf lives should be sold very quickly.

Literature Cited

- Aoki, M., F. Yabuki, T. Totsuka, and M. Nishida. 1986. Remote sensing of chlorophyll content of leaf (I) effective spectral reflection characteristics of leaf for the evaluation of chlorophyll content in leaf of dicotyledons. *Environ. Control in Biol.* 24:21-26.
- Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.* 24:1-15.
- Kader, A.A. and L.L. Morris. 1978. Tomato fruit color measured with AgTron E5-W reflectance spectrophotometer. *HortScience* 13:577-578.
- Kanellis, A.K., L.L. Morris, and M.E. Saltveit. 1986. Effect of stage of development on postharvest behavior of cucumber fruit. *HortScience* 21:1165-1167.
- Klieber, A., W.C. Lin, P.A. Jolliffe, and J.W. Hall. 1993. Training methods affect canopy light level and shelf life of long English cucumber. *J. Amer. Soc. Hort. Sci.* 118:Nov. Get Pages.
- Lin, W.C. and D.L. Ehret. 1991. The effects of nutrient concentration and fruit thinning on shelflife of long English cucumber. *HortScience* 26:1299-1300.
- Lin, W.C. and J.C.W. Keng. 1989. Use of digital image processing for nondestructive measurement of cucumber fruit color. ASAE paper No. PNR89-304. Penticton, B.C., Canada.
- Lindsay, P.A. and N.L. Bassuk. 1992. A nondestructive image analysis technique for estimating whole-tree leaf area. *HortTechnology* 2:66-72.
- Poenicke, E.F., S.J. Kays, D.A. Smittle, and R.E. Williamson. 1977. Ethylene in relation to postharvest quality deterioration in processing cucumber. *J. Amer. Soc. Hort. Science* 102:303-306.
- Saltveit, M.E. and R.F. McFeeters. 1980. Polygalacturonase activity and ethylene synthesis during cucumber fruit development and maturation. *Plant Physiol.* 66:1019-1023.
- Searcy, S.W. and J.F. Reid. 1989. Machines see red...and so much more. *Agr. Eng.* 70(7):10-15.
- Watada, A.E. 1989. Nondestructive methods of evaluating quality of fresh fruits and vegetables. *Acta Hort.* 258:321-329.