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Skin color and chlorophyll absorbance: Indices for establishing a harvest date on non-melting peach



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ABSTRACT

The aim of this research was to develop an objective method to determine the best harvest date for non-melting peach cultivars. For this purpose the absorbance of chlorophyll ($I_{\rm AD}$) on the skin of 'Andes Du-1', 'Loadel' and 'Bowen' peach cultivars was assessed. The on-tree fruit development was monitored for two consecutive seasons, one month before harvest. The correlation between $I_{\rm AD}$ of the skin and the flesh firmness was positive and highly significant (r=0.9). Also, $I_{\rm AD}$ and h° correlation was found to be positively and significantly associated (r=0.75 to 0.91). The greatest observed correlations were between $I_{\rm AD}$ and flesh firmness, and the lowest correlations were between soluble solids concentration (SSC) and the other indices. The relationship between the h° of the skin and the h° of the flesh was high and significant, reaching r=0.89, 0.84, and 0.88, for 'Andes Du-1', 'Loadel, and 'Bowen', respectively. However, the relationship between the two variables was not linear, so the data was adjusted to segmented regressions. The relationship between $I_{\rm AD}$ and the *Munsell Book of Color* defined the five categories of $I_{\rm AD}$, ranging from less than 0.180 $I_{\rm AD}$ units for the category holding the ripest fruit (2.5Y 8/10) to the category of $I_{\rm AD}$ greater than 1.545 units (10Y 8/10) for the less ripe fruit. The $I_{\rm AD}$ has shown being an informative ripeness index for evaluating non-destructively the evolution of ripeness during the last phase on-tree, and particularly this index is associated with the flesh firmness.

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1. Introduction

One of the main challenges of a peach orchard is how to determine the optimal harvest time (Tijskens et al., 2007), as the proper peach harvest maturity level is the key factor to assure a high-quality product (Infante, 2012). The maturity level of peach at harvest is naturally heterogeneous: fruit are at different stages of development and maturity because the bloom time can span two or more weeks (Shinya et al., 2013). Further, the peach on the tree canopy are exposed to uneven light and temperature conditions, which is dependent on spacing and position, so each fruit will ripen at different times and rates (Marini et al., 1991).

The ground color of the skin has been used on peach as an indicator of maturity, comparing it with standardized color charts (Eccher Zerbini et al., 1994). Other parameters to determine the beginning

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of the harvest time for the industry have been the fruit size and soluble solids concentration (SSC) (Kader, 1997). However, these parameters are greatly affected by environmental conditions, thus they are not always reliable indices (Infante et al., 2011a). Additionally, the color of peach skin it is not always a reliable index because in some cases, it has not been shown to relate to the real maturity of the fruit in terms of physiological ripeness (Ferrer et al., 2005). In this regard, Lewallen and Marini (2003) note that the ground color is not always satisfactory to define fruit maturity, as fruit with the same color have wide variability in flesh firmness, acidity, and SSC.

For the above reasons, there is a need to define indices that can be measured easily in the orchard in a non-destructive way and that can be tightly associated with the parameters that reflect the maturity stages of peach, such as the flesh firmness. The index related to the absorbance of chlorophyll ($I_{\rm AD}$), calculated as $I_{\rm AD}$ = A670–A720, which is near the peak of absorbance of chlorophyll-a (Ziosi et al., 2008), allows an indirect determination of the concentration of chlorophyll in the skin (Lurie et al., 2013). This is also related to ethylene biosynthesis (Ziosi et al., 2008). Together, these are the parameters that, to a large extent, can determine the maturity of the peach.

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The $I_{\rm AD}$ has been proven to predict the state of maturity on melting fleshed peach cultivars (Ziosi et al., 2008; Bonora et al., 2013; Lurie et al., 2013), but there have been no studies that validate this index on non-melting peach genotypes. Non-melting peach cultivars are harvested according to the ground color of the skin, which is then compared with color charts. The equivalence of an $I_{\rm AD}$ value to a standardized color may allow getting a more objective physiological parameter of ripeness instead of only the visual comparison of the skin of the fruit with a chart of colors.

In terms of both sensory quality and consumer acceptance, the SSC is an informative parameter for establishing quality levels, or even thresholds, of peach (Crisosto and Crisosto, 2005); however, this parameter is solely valid for the fresh peach industry. When dealing with non-melting fleshed cultivars destined for canning, the SSC is rarely useful because the final product is enriched with extra sugar, in the form of syrup, where are cooked. With canning peach, the flesh texture and the color of the flesh are quite valuable parameters to define quality (Infante, 2012). For this reason, a non-destructive parameter that could be associated with flesh color would be quite valuable for the industry.

In this research, the $I_{\rm AD}$ of the skin of three non-melting peach cultivars was monitored on-tree for approximately one month before harvest. This index was then associated with maturity parameters that are important as either harvest or quality parameters for peach destined for canning. In consequence, the objectives of this research were to evaluate the evolution of some non-destructive indices that are most tightly related with fruit ripeness during the last phase on-tree, and particularly with flesh firmness; and also to define the relationships between different ripeness parameters that change during this phase on peach.

2. Materials and methods

2.1. Plant material

The trial was conducted during two consecutive seasons (November 2010 and December 2011) in two orchards located in the VI Region (34°19′49.68″S; 70°50′1.88″W) and the Metropolitan Region (33°48′12.57″S; 70°45′6.17″W), both of which are located in the Central Valley of Chile. The plant material corresponds to the non-melting peach cultivars 'Andes Du-1', 'Loadel', and 'Bowen', grafted onto 'Nemaguard', which were planted in a north-south orientation and irrigated by drip lines.

2.2. Monitoring peach ripening

Trees were randomly selected in a uniform field area of the orchards. To assess fruit maturity, in the first season, 30 fruit were collected weekly, and in the second season, 20 fruit were collected twice weekly. Each time, the sampling occurred about four weeks before the time of harvest, picking the fruits from three neighbor trees. The fruit were transferred to the lab, and the I_{AD} was assessed in the equatorial zone of each cheek with a DA-meter (Sinteleia, Bologna, Italy). The ground color of the skin was assessed with a Minolta portable colorimeter model CR- 400 (Minolta, Tokyo, Japan), with a source illuminant D65 and an observer angle of 0° using the CIELab system, calibrated with a white standard. The SSC was determined by a thermo-compensated portable refractometer (Atago, Tokyo, Japan), and the flesh firmness was analyzed with a FTA GS -14 texture-meter (Guss, Strand, South Africa) on each cheek before the skin removal, using a 7.9-mm diameter probe with a penetration distance of $10 \, \text{mm}$ at $5 \, \text{mm} \, \text{s}^{-1}$ speed. Harvest was performed when the ground color changed from yellow-green to yellow (Munsell Book of Color, 1958), which was associated with flesh firmness between 35 and 44N. During the second season, the color and the $I_{\rm AD}$ of the flesh were also assessed, measured after removing the skin on each cheek.

On 'Andes Du-1', a descriptive evaluation of the evolution of the ground color was performed using a Munsell color chart. For this purpose, five weeks before harvest, 20 fruit per tree were scored twice a week. The ground color determination was always performed by the same assessor, and under regular daylight conditions.

2.3. Data analysis

We performed correlations and linear and nonlinear regressions. The linear regression models described the relationship between the I_{AD} and the other variables, under the framework of General Linear and Mixed Models, during the period in which the I_{AD} decreased linearly, approximately 10 to 15 days before harvest. The I_{AD} , the cultivar, and their interaction were considered as fixed effects, and the seasons were the random effect. Segmented model nonlinear regressions were used to describe the relationship between the hue (h°) of the skin and the h° of the flesh. The parameters of the regressions (i.e., inflection point and slope) were statistically significant ($\alpha = 0.05$). Akaike Information Criterion (AIC) (Akaike, 1974) and Bayesian Information Criterion (BIC) (Schwarz, 1978) were used. An analysis of the classification and regression tree (CART) was performed to identify the rank of I_{AD} for each color category. Lastly, the InfoStat statistical program (National University of Córdoba, Argentina) version 2013 was used in all the statistical analysis.

3. Results

Significant linear correlations between the $I_{\rm AD}$ of the skin and the maturity variables were observed during the on-tree development phase, except for SSC (Table 1). The correlation between $I_{\rm AD}$ of the skin and flesh firmness was positive and highly significant ($r\!\approx\!0.9$). Also, the $I_{\rm AD}$ and the h° of the skin were positively and significantly associated, with r values between 0.75 and 0.91. The strongest observed correlation was between the $I_{\rm AD}$ of the skin and flesh firmness, reaching a higher score than the relationship between the h° of the skin and the flesh firmness. In contrast, the association between SSC and the other indices were found to be not significant, which is in agreement with previous results with peach (Cantín et al., 2009). Chroma, as h° and $I_{\rm AD}$, is correlated with firmness in all three cultivars, but it is weakly associated with SSC. On non-melting peach cultivars, which are commonly destined

Table 1 Pearson's correlation coefficients (r) among maturity parameters assessed non-destructively on the skin $(h^{\circ} \text{ and } I_{AD})$ and the soluble solids content (SSC) and the flesh firmness of three non-melting peach cultivars. Data was registered during the last phase of fruit development on-tree on two consecutive growing seasons.

Cultivar		I_{AD}	Chroma	h°	SSC
Andes Du-1	Chroma h° SSC Firmness	-0.27* 0.88* -0.04 ^{NS} 0.94*	- 0.13 ^{NS} 0.19* -0.30*	- -0.02 ^{NS} 0.80*	- - - 0.04 ^{NS}
Loadel	Chroma h° SSC Firmness	-0.67* 0.75* -0.15 ^{NS} 0.92*	- -0.17 ^{NS} 0.05 ^{NS} -0.67*	- -0.27* 0.64*	- - - 0.05 ^{NS}
Bowen	Chroma h° SSC Firmness	-0.88* 0.91* -0.02 ^{NS} 0.90*	-0.77^{*} -0.00^{NS} -0.84^{*}	- -0.07 ^{NS} 0.83*	- - - 0.09 ^{NS}

ns Not significant.

^{*} Level of significance $p \le 0.05$.

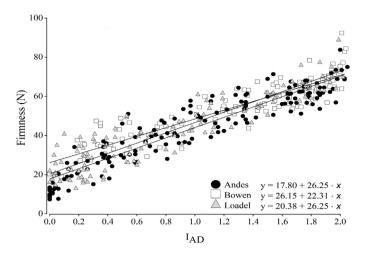


Fig. 1. The flesh firmness (N) as a function of I_{AD} of the fruit skin in 'Andes Du-1', 'Loadel' and 'Bowen' during the last phase of fruit development on-tree on two consecutive growing seasons. The solid lines represent models adjusted to linear regression functions.

for canning, the standard cultivars lack blush, whereas the melting fleshed cultivars, destined mainly for fresh consumption, have a much more extended blush that masks the ground color (Brovelli et al., 1999). For this reason, on non-melting cultivars, the skin color is practically the same as that of the ground color, so these terms could be used interchangeably. The study observed that flesh firmness decreases simultaneously with a decrease in $I_{\rm AD}$ (Fig. 1). This relationship is linear, with $I_{\rm AD}$ scores less than 2, which occurs about two weeks before harvest. However, if the change in $I_{\rm AD}$ and flesh firmness are considered during stage III of fruit growth (Chalmers and Ende, 1975), the relationship between the two variables are better represented by an exponential curve (data not shown).

The relationships between the h° angle, the C*, and the I_{AD} are direct and linear (Figs. 2 and 3 and); the change of skin color during ripening occurs in peach because both chlorophyll content decreases and carotenoid content increases during this time (Lancaster et al., 1997). In this relationship 'Andes Du-1' showed a more pronounced slope (Fig. 2); it reached an h° score nearer to an orange h° color, with angles of 55–60°. This was unlike 'Loadel' and 'Bowen', which showed an h° maximum of yellow-orange h° with values close to 75° (McGuire, 1992). In the case of C* for both 'Bowen' and 'Loadel', the correlation with the I_{AD} was negative, showing an increase in the C* while the I_{AD} decreased (Figure 3).

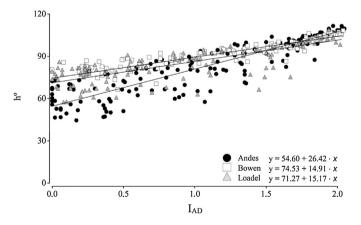


Fig. 2. The skin hue (h°) as a function of I_{AD} of the fruit skin on 'Andes Du-1', 'Loadel' and 'Bowen' during the last phase of fruit development on-tree on two consecutive growing seasons. The solid lines represent models adjusted to linear regression functions.

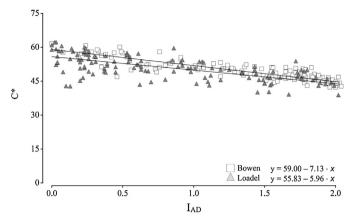


Fig. 3. The skin chroma (C^*) as a function of I_{AD} of the fruit skin on 'Loadel' and 'Bowen' during the last phase of fruit development on-tree on two consecutive growing seasons. The solid lines represent models adjusted to linear regression functions.

Thus, by decreasing the concentration of chlorophyll during ripening, the ground color of the skin becomes more intense. 'Andes Du-1' was not included in the analysis because of the low correlation observed (r = 0.27).

The h° angle shows that the flesh color of fruit development evolves from a green color (near 120°) to an orange color ($h^\circ > 80^\circ$) (Fig. 4). In contrast, the ground color of the skin changed differently for each cultivar, moving from a yellow color to an orange color ($85-60^\circ$). 'Andes Du-1' reached an h° under 60° in advanced maturity stages. 'Bowen' (Fig. 4) reached values close to 85° , but no notable differences between the color of the skin and flesh were observed. The relationship between the h° of the skin and the h° of the flesh was both high and significant, reaching r values of 0.89, 0.84, and 0.88, for 'Andes Du-1', 'Loadel', and 'Bowen', respectively. However, the relationship between the two variables was not linear, so the data was adjusted to segmented regressions (Fig. 5). 'Loadel' and 'Bowen' showed similar behavior, whereas 'Andes Du-1' showed a greater change in skin color, greater variability, and more intense coloring.

It was also noted that 'Loadel' and 'Bowen' showed a skin's h° below 88° and 'Andes' around 69°; the change in skin color, in terms of h° , was not associated with significant changes in the flesh color (Fig. 5). The I_{AD} decreased progressively over time, always reaching higher values on the skin than on the flesh (Fig. 6). The drop in I_{AD} values had an equivalent trend on the skin and on the flesh, reaching similar values for both 'Loadel' and 'Andes Du-1'. This is

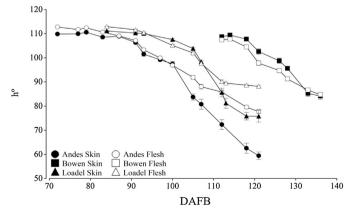


Fig. 4. Evolution of the hue of skin and the hue of the flesh in terms of days after full bloom (DAFB) during the last phase of fruit development on-tree on two consecutive growing seasons on 'Andes Du-1', 'Bowen' and 'Loadel' peach cultivars.

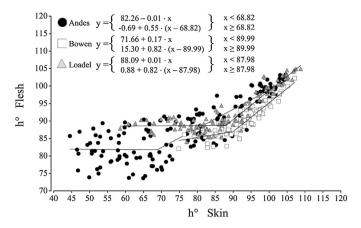


Fig. 5. The hue of the flesh (h°) as a function of the h° of the skin in 'Andes Du-1', 'Bowen' and 'Loadel' peach cultivars. The solid lines represent models adjusted segmented non-linear regression function.

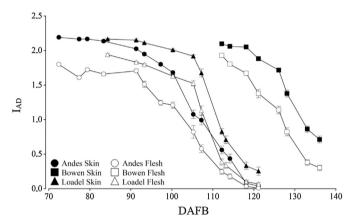


Fig. 6. Evolution of the $I_{\rm AD}$ of skin and the $I_{\rm AD}$ of the flesh in terms of days after full bloom (DAFB) during the last phase of fruit development on-tree on two consecutive growing seasons on 'Andes Du-1', 'Bowen' and 'Loadel' peach cultivars.

unlike what happened with the values of h° , where slightly different values between the I_{AD} skin and flesh were observed on the 'Bowen' fruit. The relationship between the skin I_{AD} and the flesh I_{AD} showed significant r values, of 0.98, 0.97, and 0.96 for 'Andes Du-1', 'Loadel', and 'Bowen', respectively, thus demonstrating a curvilinear relationship (Fig. 7) that was very similar in the three analyzed cultivars. In addition, it was observed that the I_{AD} evolution was

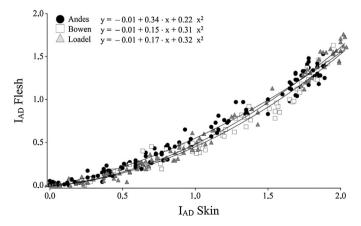


Fig. 7. Evolution of the $I_{\rm AD}$ pulp as a function of the $I_{\rm AD}$ skin on during the last phase of fruit development on-tree on two consecutive growing seasons on 'Andes Du-1', 'Bowen' and 'Loadel' peach cultivars.

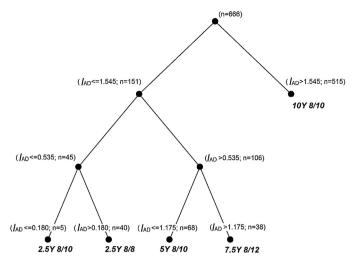


Fig. 8. Classification and regression tree (CART) of the categories of the *Munsell book of color*, depending on the I_{AD} of the skin in the peach cultivar 'Andes Du-1'.

higher in the skin than in the flesh, where the skin values were 1.0 and the I_{AD} flesh values were near to 0.5.

The relationship between $I_{\rm AD}$ and the Munsell Book of Color by the CART procedure, using 666 'Andes Du-1Munsell Book of Color fruit samples, defined five categories of $I_{\rm AD}$, ranging from less than 0.180 $I_{\rm AD}$ units for the category holding the ripest fruit to an $I_{\rm AD}$ higher than 1.545 units for the category holding the less ripe fruit (Fig. 8). In terms of Munsell colors for this batch of fruit, 77% of them were classified in the 10Y 8/10 category (less ripe), whereas the ripest categories were represented by only 6% of the sample (2.5Y 8/8) and 0.8% of the sample (2.5Y 8/10).

4. Discussion

From this investigation, we obtained significant associations between I_{AD} and maturity variables, as similarly reported by Costa et al. (2005) on melting fleshed peach, Costa et al. (2010) on apricot, and Infante et al. (2011a) on Japanese plum. Together, these results confirm the value of I_{AD} as a reliable, non-destructive, and objective index to determine the state of maturity in non-melting peach cultivars. Several authors have already shown that the reduction of the skin's chlorophyll content is a reliable indicator of fruit development and maturity (Solovchenko et al., 2005; Herold et al., 2005; Zerbini et al., 2006). The high correlations between I_{AD} with flesh firmness and the h° of the ground color are promising. These correlations can be explained by the absorbance measurements that are affected by both light absorption and light scattering (Zerbini et al., 2006; Ziosi et al., 2008), thus providing information on the chemical (pigments) and physical (texture) properties of the biological tissue, respectively (Cubeddu et al., 2001; Muhua et al., 2007). With regard to the SSC, there was no relationship between the I_{AD} and the other parameters related to ripeness, which agrees with the results of Lewallen and Marini (2003), Infante et al. (2011a), and Cantín et al. (2009). It also confirms that SSC on melting fleshed peach is only a quality index, and not a harvest index (Crisosto, 1994).

It is interesting to underline that linear regressions of $I_{\rm AD}$ with flesh firmness and ground color were observed after the occurrence of the "break" of the skin color, from green to yellow, which is registered approximately three weeks before harvest. The remarkable association of flesh firmness with $I_{\rm AD}$ confirms the existence of the synchronization of the degradation of chlorophyll with flesh softening, as reported earlier by Lurie et al. (2013). Most specifically, the changes in texture occur via the solubilization and degradation

of pectins due to the action of different enzymes affecting in cell walls; this process is related to biosynthesis and the effect of ethylene (Trainotti et al., 2003). Meanwhile, the ethylene also serves to promote the degradation of the chlorophyll (Giovannoni, 2004).

It is important to note that the softening rates were very similar between the two seasons for the same cultivar, even when the weather conditions were different (Zerbini et al., 2006). Thus, through proper validation of the models, flesh softening on peach can be a reliable predictive parameter, and also can be considered an important trait not affected by the environment, hence with high genetic determinants.

The relationship between h° and I_{AD} with 'Andes Du-1' is the one that presented the highest variability of all the observations. This is because 'Andes Du-1' is suitable for both canning and fresh consumption, as the skin is covered with a blush that reaches more than 50% of the fruit surface (Infante et al., 2011b), with skin h° near to 60° in advanced stages of maturity, which is similar in appearance to melting fleshed peach cultivars. In contrast, 'Bowen' and 'Loadel' are classic cultivars for industrial use, as they exhibit a yellow skin (h° near to 80°) and almost no blush.

Ferrer et al. (2005) showed that with 'Calanda' peach, which does not develop a blush, the h° of the skin may be a good predictor of maturity, so that under similar conditions, the ratio of h° with I_{AD} becomes important. Generally speaking, canning peach cultivars show little reddish color (Brovelli et al., 1999), thus they do not completely mask the background color. Therefore, the change of color tones also reflects the degradation of chlorophyll.

The association between the h° evaluated on the skin and on the flesh was high, with r values over 0.84, which is contrary to the observations of Crisosto et al. (2007) and Slaughter et al. (2013), who determined r values of 0.35 and 0.50, respectively. The segmented regressions described for the h° of the flesh dependent on the skin h° confirm the nonlinear relationship reported by Slaughter et al. (2013). The models for 'Bowen' and 'Loadel' fit properly, but this was not the case for 'Andes Du-1', in which the same skin color is highly variable in its flesh color. Moreover, Slaughter et al. (2013), who studied similar canning peach cultivars in terms of ground color, determined that h° scores less than 70° do not reflect changes in the color of flesh. In our study, this response was observed only with 'Andes Du-1'; with 'Bowen' and 'Loadel' this response occurred with h° angles near 90°. The discrepancies in these results make it doubtful that the ground color, measured as h°, could be used to predict the flesh color of all peach varieties used for industrial purposes. Specifically, this is because color development can be affected by genetic factors, growing conditions, the position of the fruit on the tree (Bible and Singha, 1993), the microclimate in which it grows (Génard and Baret, 1994), and the fruit load (Alcobendas et al., 2012). This is why studies are needed in different seasons to determine whether the relationship holds true, as was observed on varieties without blush, such as 'Loadel' and 'Bowen'.

The association between the $I_{\rm AD}$ of the skin and the flesh reached a value of r=0.97. This relationship can be expressed through quadratic regressions for all three evaluated cultivars, which would simplify a future prediction of flesh color using $I_{\rm AD}$ evaluations, compared to the segmented models of h°. Considering that there are peach cultivars with remarkable visual differences between the color of the skin and the color of the pulp, it is feasible to think that the $I_{\rm AD}$ ranges should be cultivar-specific, as is true with apple varieties (Cubeddu et al., 2001). In this regard, it is important to validate these results with different cultivars and in different seasons. However, it seems that $I_{\rm AD}$ could be a promising tool for growers and the industry as an objective method for estimating the moment to begin the harvest, examining the change in the $I_{\rm AD}$ flesh as a function of the $I_{\rm AD}$ skin.

5. Conclusions

For many years, as a criterion for a peach harvest, the industry used the ground color, as measured by comparing it with color charts (Slaughter et al., 2006). The close association between $I_{\rm AD}$ and physiologic parameters and its relationship with a color chart allows establishing the beginning of harvest objectively on canning peach cultivars. Although the methodology was successful in 'Andes Du-1', this first approach aimed to objectively relate the visual color assessment of the skin with the real level of ripeness of a non-melting peach, should be validated for the other genotypes and in other environments, given the high variability observed in the color development of peach flesh and skin.

The present study confirms that $I_{\rm AD}$ can be employed to establish the optimal harvest time for non-melting peach cultivars, as it strongly correlates with the main parameters normally assessed for harvest: flesh firmness and ground color. Linear models developed to predict $I_{\rm AD}$ of the flesh as a function of $I_{\rm AD}$ of the skin have not yet been validated, but these models could be a promising tool to improve the harvest management on those cultivars in which the color of the skin is not easily associated with ripeness. Moreover, it is feasible to establish $I_{\rm AD}$ ranges for the categories of a color chart on non-melting peach cultivars.

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