



Non-destructive monitoring of flesh softening in the black-skinned Japanese plums 'Angelino' and 'Autumn beaut' on-tree and postharvest

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ABSTRACT

There is a need to develop alternative harvest indexes for black skinned plums. The aim of this research was to analyze and compare the most commonly used indexes for deciding the harvest date for Japanese plum, and evaluate the effectiveness of new approaches for studying maturation. The ripening process was monitored on-tree and during postharvest in a non-destructive way, through the absorbance of chlorophyll (I_{AD}), the compression strength of the intact fruit, and the traditional parameters associated with ripening. Fruit were harvested at commercial ripeness and "tree-ripe", and were stored for 10 d at 22 °C and 75–80% RH. The I_{AD} decreased during the last phase of development of the fruit on-tree, and it was related to the common indexes used for plums. 'Angelino' showed a decrease of the I_{AD} 24% lower than that observed for 'Autumn beaut'. The I_{AD} versus time showed the highest coefficients of determination when compared with the soluble solids concentration (SSC), flesh firmness, hue (H°) and chroma (C^*) of the skin. The compression strength of the intact fruit was associated with flesh firmness, and to a lesser extent with the SSC for 'Angelino', whereas for 'Autumn beaut' higher correlations for both the SSC and flesh firmness were observed. The C^* of the skin on 'Autumn beaut' showed an erratic change during ripening; by contrast, for 'Angelino', this index showed a clear trend. During postharvest it was observed that for 'Angelino' fruit picked at commercial ripeness, the rate of change of the I_{AD} was practically the same as observed on-tree, while for 'Autumn beaut' the rates of change on-tree and at postharvest were $0.075 I_{AD} d^{-1}$ and $0.024 I_{AD} d^{-1}$, respectively. For the "tree-ripe" fruit, the rate of change was practically the same for both cultivars. Similar trends were observed for the compression strength.

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1. Introduction

For plums, the soluble solids concentration (SSC) and flesh firmness are two of the most reliable quality parameters which reflect the stage of ripeness of a fruit. Kader and Mitchell (1989) reported that SSC increases with ripening, but the use of SSC by itself as a ripeness index is limited due to the variation observed among varieties, production area, and season. Furthermore, according to Nunes et al. (2009), for each cultivar, specific maturity parameters need to be defined and adapted according to the destination of the crop.

Flesh firmness is a key quality parameter, since it is directly related to fruit ripeness, and is often a good indicator of shelf-life potential (De Ketelaere et al., 2006; Valero et al., 2007). Japanese plums should be picked before reaching the highest sensory quality potential, because this is reached when the flesh firmness is close to 10–20N, which is too soft for appropriate handling and packaging process. Commercial harvest of stonefruit should start when

the flesh firmness is around 40–50N, since softer fruit are more susceptible to bruising (Crisosto et al., 2004). For the fresh market, measurements of flesh firmness are important for monitoring ripening (Valero et al., 2007), but there is a lack of reference data for different genotypes (Usenik et al., 2008). Fruit softening is a complex process resulting from multiple changes at the morphological and cellular levels (Ponce et al., 2010). These changes are related to the cell wall disassembly and the reduction of turgor pressure because solutes in the cell wall space accumulate and contribute to textural changes during ripening (Brummell, 2006).

In recent years, some research has been focused on measuring fruit ripeness non-destructively based on various principles; for example, impact response (Garcia-Ramos et al., 2003), acoustic properties of the flesh (Zdunek et al., 2009), multispectral images (Leo et al., 2009), near infrared technology (Valente et al., 2009), among others. However, even when these technologies have shown high correlations with physiological parameters related to fruit ripening, they can only be used in the laboratory, so they are not appropriate for monitoring on-tree ripening. In the orchard, the most suitable harvest index is the ground color of the skin. A high correlation between ground color and physiological maturity of stonefruit has been observed (Kader, 1999). However, determining

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the ground color in black-skinned plums by means of color charts or colorimeters is not very helpful for assessing fruit ripeness. In these kinds of genotypes, in which the ground color is not visible from early stages of fruit development, alternative indexes to decide harvest time are necessary. In this sense, absorption of chlorophyll (I_{AD}) could be a reliable method to determine real ripeness stage. High correlations between absorption of chlorophyll and ripeness level have been reported for peach (Ziosi et al., 2008), and prune (Infante et al., 2011). This parameter could be easily measured by a device such as a DA-meter, which by using absorbance within the chlorophyll active range, allows indirect determination of the chlorophyll content in the fruit skin through non-destructively. The interactance spectrum (I), and fruit absorbance (A) are calculated using Lambert Beer's law ($A = \log_{10} I^{-1}$). Based on fruit absorbance spectrum, the I_{AD} is calculated as the subtraction of absorbance at 670 nm minus the absorbance at 720 nm (Ziosi et al., 2008).

The aim of this research was to analyze and compare the most common indexes used for deciding the harvest time for black-skinned Japanese plum, and for monitoring the changes in fruit ripening on-tree and postharvest.

2. Materials and methods

2.1. Plant material

Ten-year-old Japanese plum trees (*Prunus salicina* Lindell) cvs. 'Angeleno' and 'Autumn beaut', grafted on Marianna 2624 rootstock, and planted 4.0 m apart between rows and 1.0 m apart within the row, were used for monitoring the on-tree fruit ripening. The trees were grown in an experimental station located in the Metropolitan Area, near Santiago, Chile (33°48'14.85"S; 70°40'6.54"W). The postharvest evaluations were conducted in the Fruit Quality laboratory of the University of Chile. The study was carried out between January and March 2009.

2.2. On-tree fruit absorbance of the chlorophyll

Four average trees per cultivar were used on the essay, and 10 fruit from each tree, evenly distributed on the canopy, were selected for monitoring the on-tree fruit ripening. Each fruit was identified through a plastic ribbon tied on the shoot near the corresponding fruit. The I_{AD} was determined every three days, starting 24 d before commercial ripeness, until the fruit was over-ripe. The I_{AD} was measured always in the same area of the skin on both cheeks of each marked fruit, and three measurements per cheek were performed. The average of the six measurements per fruit was used for data analysis. For measuring the I_{AD} on the on-tree ripening fruit, a portable Vis/NIR DA-meter was used (Sinteleia, Bologna, Italy).

2.3. Changes in on-tree fruit quality

On the same days in which the I_{AD} was measured on the marked fruit used for monitoring the on-tree ripening, another 20 fruit at a similar ripeness stage, were harvested and transported to the laboratory in plastic trays. This fruit was submitted to instrumental assessment of fruit quality on the same day of harvest.

The I_{AD} was measured using a DA-meter device on each detached fruit as described above. Further, the skin color was measured with a CR-300 colorimeter (Minolta, Tokyo, Japan). The chroma (C^*) and hue angle (H°) were used to characterize changes in skin color from green to yellow during ripening (McGuire, 1992).

The compression strength was determined non-destructively by measuring the compression of intact fruit through the use of a FTA GS-14 texture analyzer (Guss, Strand, South Africa), equipped with a 21-mm diameter plunger. The haul of the plunger was 1 mm deep

from the time when the plunger made contact with the skin of the fruit.

Flesh firmness was measured with a FTA GS-14 texture analyzer equipped with the plunger traditionally used on stonefruit (7.9 mm), but in this case the skin was removed previously with a sharp knife. The flesh firmness score corresponds to the mean of four measurements performed on both cheeks and on both shoulders of each fruit.

The SSC was measured with a thermo-balanced PAL-1 refractometer (Atago, Tokyo, Japan).

2.4. Postharvest I_{AD} and compression strength

When the fruit used for monitoring on-tree ripening reached commercial ripeness, (day 21 for 'Angeleno' and day 24 for 'Autumn beaut'), 20 fruit at a visually similar ripeness stage were harvested and transferred to the laboratory. The fruit were placed in a temperature-controlled chamber set at 22 °C and 75–80% RH, and then evaluated through non-destructive methods every 2 d, using I_{AD} of the skin and compression strength, following the same methodology described above.

When the fruit reached an the on-tree fully ripe stage ("tree-ripe"), the same procedure was followed for the commercial harvested fruit.

2.5. Data analysis

The I_{AD} on-tree evaluations were performed on 40 individually marked fruit per cultivar for the whole period of evaluation. A linear regression was calculated using the software InfoStat (Infostat, 2008). The means of I_{AD} , compression strength, SSC, flesh firmness, and C^* and H° of the skin, were obtained from 20 fruit every three days from 24 d before commercial ripeness, until the fruit was over-ripe. Linear regression models were calculated for each parameter, with the exception for H° , which was fitted to an exponential model. Postharvest I_{AD} and compression strength were evaluated on 20 fruit as previously described.

3. Results and discussion

3.1. On-tree absorbance of the chlorophyll

A linear reduction of the I_{AD} was observed throughout the last period of fruit development on the tree of both cultivars, which corresponds to physiological and horticultural fruit maturation and ripening (Kader, 1999). The accuracy of the I_{AD} determination, in this case was confirmed by using always the same fruit ($n=40$), and placing the DA-meter sensor always on the same cheek area throughout the whole on-tree evaluation. Hence the natural variability on ripeness of the fruit within a tree at a given time, which is common to stonefruit species, was reduced. The reliability of the data was confirmed by the high scores of the coefficient of determination observed ($R^2 \geq 0.98$; Fig. 1). From the experimental point of view, the use of the same marked fruit, instead of using different samples of fruit for studying ripening of stonefruit on-tree, requires much more time for doing the measurements, but provides much more precise data for studying ripening over time.

A comparison of the curves showed different rates of changes of I_{AD} between the two cultivars; the I_{AD} reduction being nearly two times higher for 'Autumn beaut' than the rate of 'Angeleno' for the same period. This result suggests that it is not possible to consider a single I_{AD} value as a harvest index for different genotypes of black-skinned Japanese plums. In this particular case, 'Angeleno' reaches commercial harvest ripeness when the I_{AD} value is close to 1.3, while 'Autumn beaut' is ripe for commercial harvest when the I_{AD} value is near 0.9. 'Angeleno' fruit never seemed to reach

Table 1

Coefficients of determination among maturity indexes for Japanese plum 'Angeleno' (A) and 'Autumn beaut' (B) measured on a sample of fruit ($n=20$). Commercial harvest was performed on day 21 on 'Angeleno' and on day 24 on 'Autumn beaut' (n.s. stands for non-significant).

	I_{AD}	Flesh firmness	Compression strength	SSC	C^*	H°
A						
I_{AD}	–	0.91	0.85	0.70	0.77	0.44
Flesh firmness	0.91	–	0.92	0.62	0.69	0.61
Compression strength	0.85	0.92	–	0.52	0.77	0.38
SSC	0.70	0.62	0.52	–	0.60	0.52
C^*	0.77	0.69	0.77	0.60	–	n.s.
H°	0.44	0.61	0.38	0.52	n.s.	–
B						
I_{AD}	–	0.89	0.87	0.91	n.s.	0.93
Flesh firmness	0.89	–	0.70	0.89	n.s.	0.92
Compression strength	0.87	0.70	–	0.80	n.s.	0.64
SSC	0.91	0.89	0.80	–	n.s.	0.83
C^*	n.s.	n.s.	n.s.	n.s.	–	n.s.
H°	0.93	0.92	0.64	0.83	n.s.	–

such a low I_{AD} scores as those observed in 'Autumn beaut', even for over-ripe fruit. Thus development of specific I_{AD} curves for different genotypes of Japanese plum is necessary, even when they show similar skin color.

The I_{AD} showed a high coefficient of determination ($R^2 \geq 0.7$) regarding flesh firmness, compression strength, and SSC. The first two parameters are both associated with flesh texture and subsequently are associated with determination of the potential postharvest life of the fruit (De Ketelaere et al., 2006). For the European plum cultivar 'D'Ente', the I_{AD} also has been shown to behave as an index capable of sorting fruit into at least three classes with different SSC and levels of flesh elasticity (Infante et al., 2011).

The association of I_{AD} with the components of skin color appeared rather erratic, since the C^* component had an $R^2 = 0.77$ for 'Angeleno', but only 0.09 for 'Autumn beaut'. The same result was found for the H° component, with a R^2 value of 0.44 for 'Angeleno' (Table 1A), and 0.93 for 'Autumn beaut' (Table 1B). With regard to the coefficient of determination between color components and the ripeness indexes, with 'Angeleno' the C^* component had a higher R^2 value with flesh firmness, compression strength and SSC than that observed for the H° component with the same parameters (Table 1A), while the opposite was observed with 'Autumn beaut' (Table 1B). It is paradoxical that even though both genotypes are classified as black-skinned plums, with 'Autumn beaut' the C^* component was related to all the other indexes, but unrelated to any with 'Angeleno'. The components C^* and H° in the ground color of the skin are the harvest indexes most frequently used on stonefruit because they are somehow related to the change from green to yellow color, associated also with the degradation of the chlorophyll during ripening (Abdi et al., 1997; Crisosto et al., 2004).

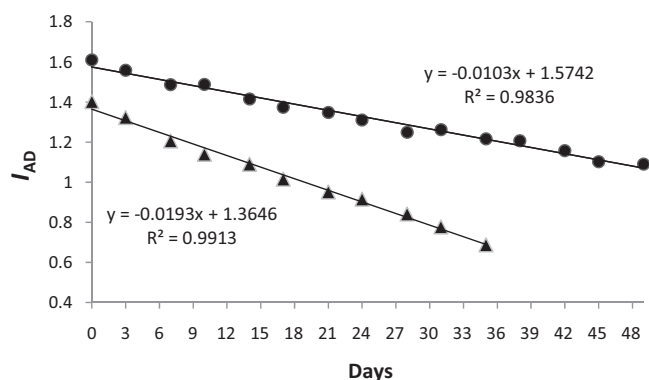


Fig. 1. Chlorophyll absorbance index (I_{AD}) of the Japanese plum cultivars 'Angeleno' (●) and 'Autumn Beaut' (▲), measured on the same fruit ($n=40$). Commercial harvest was performed on day 21 on 'Angeleno' and on day 24 on 'Autumn beaut'.

3.2. Flesh firmness and compression strength

The compression strength of the intact fruit was confirmed as an index associated with flesh firmness determined destructively, and to a lesser extent with the SSC for 'Angeleno', while for 'Autumn beaut' the relationships were high for both the SSC and the flesh firmness (Table 1). The compression strength, which is easily measured in the laboratory, is also a useful index for establishing the softening rate of individually selected fruit throughout a given time span, thereby reducing the inherent variability of plum ripeness within a batch and thus affecting the data reliability.

When the flesh firmness was measured destructively, the softening rate showed a reduction of 0.42 N d^{-1} for 'Angeleno', and 0.55 N d^{-1} for 'Autumn beaut' fruit. 'Angeleno' fruit also had a softening rate 24% lower than that observed for 'Autumn beaut' fruit (Fig. 2B). The common destructive evaluation of flesh firmness using a penetrometer did not follow the same trend observed by the nondestructive evaluation of compression strength of the intact fruit. Thus different physical fruit properties can be measured depending on the method used, as previously observed with destructive (penetrometer) and nondestructive (Sinclair iQTM firmness tester) measurements on Japanese plums performed by Valero et al. (2007), who concluded that direct comparison of both methods for measuring firmness should be avoided. In this experiment, both determinations were based on the maximum strength reached, or just before the collapse of the flesh in the case of the flesh firmness evaluation, or when the plunger reaches 1 mm of haul in the case of the compression strength evaluation. The data interpretation of both methods should take into consideration that in the case of compression strength, an effect due to the resistance of the skin against the plunger is added, while in the flesh firmness the data reflect exclusively the resistance of the flesh.

The compression strength required to deform an intact fruit is a useful parameter which could be easily measured on the same fruit repeatedly, allowing build up of a real softening rate throughout a given time span. The practical meaning of what the compression strength could represent is related to the strength applied to a fruit between the thumb and the index finger, which is one of the most frequent actions used by consumers when buying stonefruit, as this gives a clear idea of the consistency of the flesh, ranging from ripeness to over-ripeness.

3.3. Sugar accumulation

The SSC accumulation for both cultivars showed similar trends, although 'Autumn beaut' fruit were harvested 10 d before 'Angeleno' fruit. Throughout the evaluation period, the SSC of 'Angeleno' increased from 12.5 to 16.0%, while 'Autumn beaut' increased from

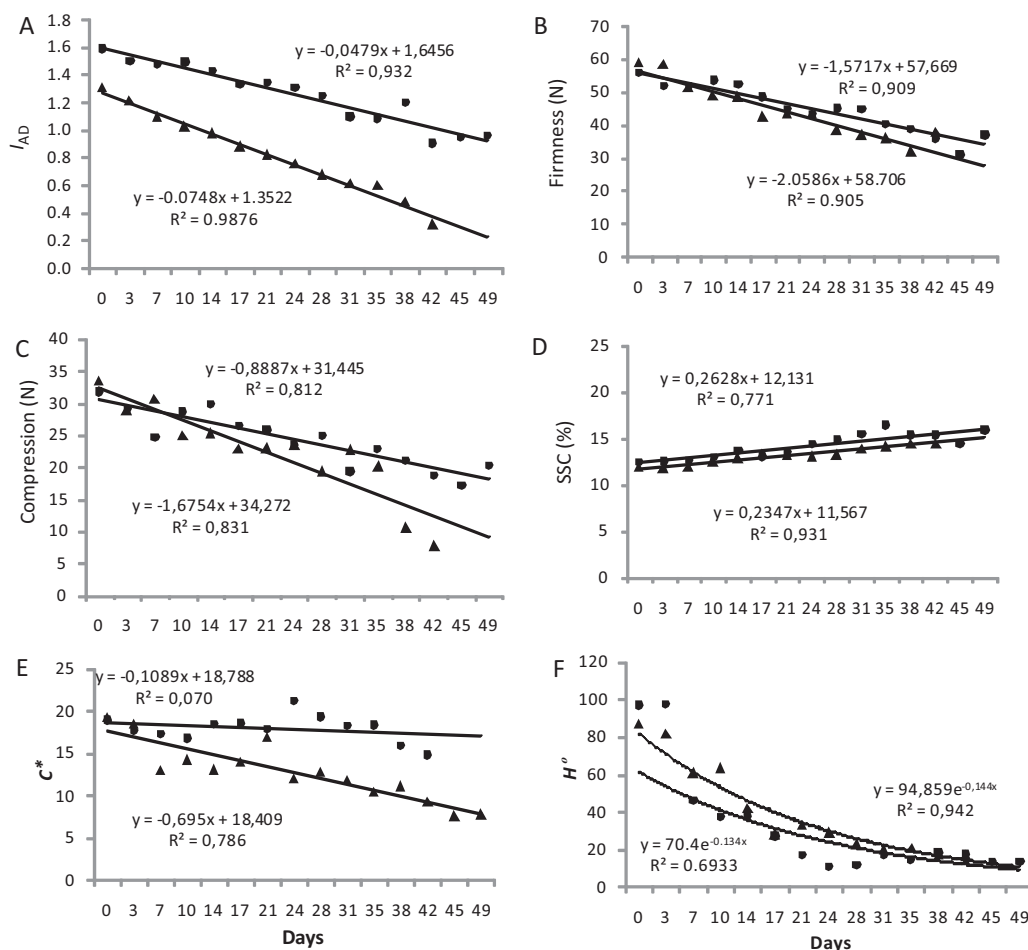


Fig. 2. Chlorophyll absorbance index (I_{AD}) (A), flesh firmness (B), compression strength of the fruit (C), soluble solids concentration (D), C^* of the skin (E), and H° of the skin (F) of the Japanese plum cultivars 'Angeleno' (●) and 'Autumn Beaut' (▲) measured in a sample of fruit ($n = 20$) throughout time. Commercial harvest was performed on day 21 on 'Angeleno' and on day 24 on 'Autumn beaut'.

11.0 to 15.0% (Fig. 2D). The relative increase of SSC through the period of observation was rather low, indicating that this parameter, although it is associated with flavor and fruit quality, is not a clear parameter that could aid decisions on the best date of harvest (Crisosto et al., 2004). Furthermore, the SSC in stonefruit is one of the quality parameters most influenced by environment (i.e. radiation and heat accumulation) and rootstock; therefore, it is a weak quality index for black-skinned plums (Ruiz-Altisent et al., 2006). In peaches, a yearly effect has been reported for SSC, sucrose, glucose and sorbitol contents. Cantin et al. (2009) suggest that the year-to-year variation in sugar profile is explained by the differences in climate and crop load. By contrast, the softening rate in stonefruit is a parameter that shows low variability on a year-to-year analysis, behaving very similar in different years of evaluation, as has been observed in 'Spring bright' peach, even when the climatic conditions were quite different (Eccher Zerbini et al., 2006). The SSC in stonefruit is certainly an essential quality index, which is highly associated with sensory quality, and consumer acceptability (Di Miceli et al., 2010). Nevertheless SSC is not very informative and reliable during the last phase of fruit development, so it is an unsuitable index to decide the harvest time for plum.

3.4. Skin color

When the C^* component of the skin of 'Autumn beaut' fruit was monitored on-tree during ripening, it showed an erratic trend with an $R^2 = 0.07$ suggesting that C^* in this particular cultivar is

not a suitable index of ripeness (Fig. 2E). By contrast, with 'Angeleno' fruit, a clear trend was observed, and this parameter proved to be related to the changes in SSC, flesh firmness, and compression strength measured on the same fruit during the ripening time (Table 1; Fig. 2A). The H° component of the ground color is not easily detectable, because it is masked by the blush when the fruit is near commercial harvest. An H° of 180° represents pure green and a H° of 0°, pure red (Shewfelt et al., 1988), and on tomatoes a dramatic decrease of the H° was observed when the change occurs from the green to the orange stage, but after this stage it remains constant (Arias et al., 2000). A similar trend has been observed in this case for the last ripening phase of 'Angeleno' and 'Autumn beaut' fruit. It seems that the H° component is a quite useful parameter in black-skinned plums until the commercial harvest date, although during the period between commercial harvest ripeness and the "tree-ripe" stage, the H° component showed imperceptible changes, inadequate for sorting fruit on different ripeness classes. In fact, around this phase of fruit development, H° acquires an asymptotic trend, being difficult to discriminate ripeness classes based on this parameter (Fig. 2F). The H° component has been chosen in other plum cultivars to show changes in color during on-tree ripening, but the decrease of H° in yellow-pigmented cultivars is even slower than in purple/black-pigmented cultivars (Díaz-Mula et al., 2008). For genotypes where it is possible to distinguish the ground color in the fruit even in the final phase of ripening, the color-related parameters remain a reliable tool because they are easily measured and synchronized with the softening process, as

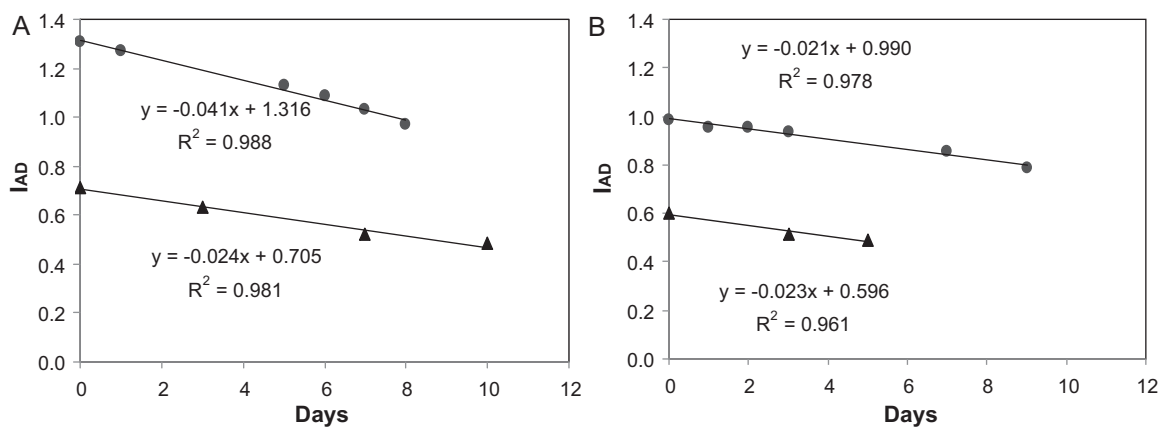


Fig. 3. Postharvest chlorophyll absorbance index (I_{AD}) of the Japanese plum cultivars 'Angeleno' (\bullet) and 'Autumn Beaut' (\blacktriangle) measured on fruit maintained in a temperature-controlled chamber set at 22 °C and 75–80% relative humidity of fruit harvested at commercial flesh firmness (A) and on "tree ripe" fruit (B).

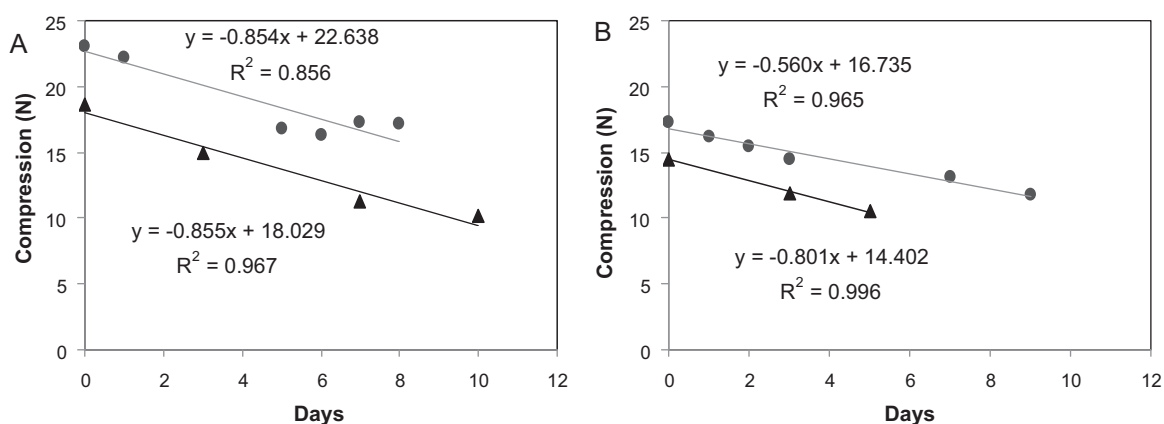


Fig. 4. Postharvest compression strength of the intact fruit of the Japanese plum cultivars 'Angeleno' (\bullet) and 'Autumn Beaut' (\blacktriangle) measured on fruit maintained in a temperature-controlled chamber set at 22 °C and 75–80% relative humidity of fruit harvested at commercial ripeness (A) and on "tree ripe" fruit (B).

observed in a four-year experiment performed by Tijssens et al. (2007).

3.5. I_{AD} and compression strength in postharvest

The postharvest period at 22 °C and 75–80% RH for the fruit of both cultivars lasted for 10 d, which is unusually long for Japanese plums (Figs. 3 and 4). However, even after this period, no dehydration symptoms were found. A continuing decrease in I_{AD} was observed when fruit were detached from the tree. When the slope of the function I_{AD} versus time was compared with the same fruit still on the tree, the rate of change was practically the same in both conditions with 'Angeleno' fruit picked at commercial ripeness, showing a reduction of $0.048 I_{AD} d^{-1}$ for the on-tree fruit (Fig. 2A), and $0.041 I_{AD} d^{-1}$ during the postharvest period (Fig. 3). For 'Autumn beaut' fruit the rate of change on the tree was $0.075 I_{AD} d^{-1}$ (Fig. 2A), and $0.024 I_{AD} d^{-1}$ postharvest (Fig. 3). For "tree-ripe" fruit, the slope of the curves was the same for both cultivars, reaching a very low I_{AD} rate of change of (Fig. 3).

The fruit softening determined by the change of the compression strength measured on the intact fruit showed a decreasing trend at the same rates ($0.85 N d^{-1}$) for both cultivars when fruit were harvested at commercial ripeness (Fig. 4A), which was practically the same rate of change observed for on-tree fruit for 'Angeleno' ($0.89 N d^{-1}$), but half of the observed rate for on-tree fruit of 'Autumn beaut' ($1.68 N d^{-1}$) (Fig. 2C).

Although there are no previous reports describing different flesh types for Japanese plum as for peach, (i.e. melting, non-melting, and

recently the 'stony hard' flesh type), in this case 'Angeleno' behaves like a non-melting peach, whereas 'Autumn beaut' behaves like a classic melting genotype. Furthermore, 'Angeleno' could be cold-stored for eight weeks, or even more, maintaining a high quality. Other plum cultivars such as 'Oullins Gage' have shown adaptation to cold storage due to their slow softening rate, so harvest date could be delayed in order to achieve a higher quality of fruit after shelf-life (Casquero and Guerra, 2009). In another study, Singh and Singh (2008) showed that 'Angeleno' showed an increase in acid concentration even 8 d after commercial harvest, in contrast to a sudden decrease in 'Blackamber' and 'Amber Jewel' plums during the same period. The explanation for such behavior was attributed to a suppressed climacteric change that maintained a high concentration of malic acid (Khan and Singh, 2007). This is yet further evidence of the suitability of 'Angeleno' for longer shelf-life in comparison to the other cultivars (Singh and Singh, 2008). Other studies in plum have demonstrated that if the climacteric peak is suppressed or delayed, flesh firmness is retained for longer periods (Dong et al., 2002; Argenta et al., 2003; Luo et al., 2010). On the other hand, treating 'Angeleno' fruit with exogenous ethylene, reduces the time required to reach maximum ethylene production at 20 °C (Candan et al., 2008).

3.6. Conclusions

The I_{AD} and compression strength measured on-tree and postharvest of 'Angeleno' and 'Autumn beaut' plums are reliable parameters for a nondestructive monitoring of ripening either for

deciding the optimum date for harvest or for helping retailers in sorting fruit batches with similar ripeness levels.

In this experiment, the variability of the samples was reduced by evaluating the same fruit each time. This experimental approach does not consider the real situation of a plum orchard that is characterized by the heterogeneity of the ripeness of the fruit, nevertheless it is useful for learning whether these new methods for studying fruit maturation are associated or not with other known physiological parameters.

Regarding I_{AD} , a decreasing trend during the last phase of fruit development was confirmed, showing the highest R^2 among all the indexes evaluated (Fig. 2). Thus, I_{AD} could reflect better than the other indexes the real on-tree fruit age, which is associated with ripeness.

The practical suitability of using I_{AD} for monitoring ripeness of black skinned plums should be confirmed by measuring it under different climatic conditions over different years. Furthermore, the effect of orchard conditions or local climate should be considered for a proper use of the DA-meter data, for its industrial implementation.

The evaluation of the daily change in the compression strength of plum flesh would also help to define characteristic curves of the softening rate for different cultivars, useful for supplying the market with homogeneous, high quality plums.

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