

Towards Improved Maturity Standards for Fuerte Avocado Fruit in the Cool Subtropical Kwazulu-Natal Midlands

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ABSTRACT

For avocado export markets, fruit maturity or time of harvest plays a critical role in determining the quality and firmness of fruit, both during handling and during retailing. Fruit firmness and lipid percentages were monitored at Everdon Estate in the cool, subtropical KwaZulu-Natal midlands, both on a fresh and on a dry-mass basis during the 1994 and 1995 seasons. In addition, the activity of two of the key enzymes involved in the ripening process — pectinmethylesterase (PME) and cellulase — was monitored in the same fruit in an attempt to identify a simple and effective maturity marker. PME activity was too variable between and within replications in 1994 (sed = 0,005) as well as in 1995 (sed = 0,011). Cellulase activity of fruit increased from the day of harvest by about 40 units/g fresh mass to values as high as 210 units/g fresh mass in some cases. Unfortunately, the increase in activity was inconsistent between weeks of harvest in 1994 (sed = 15,06) and 1995 (sed = 37,98). Lipid content proved to be the most accurate marker and the last fruit should have been picked at lipid concentrations of between 16 % (1994) and 18 % (1995) on a fresh-mass basis or 58 % (1994) and 61 % (1995) on a dry-mass basis. In both instances this corresponded with the end of June, which appears to be a target date to complete harvesting of export Fuerte fruit in similar cool environments.

INTRODUCTION

Fruit maturity is a key issue for harvesting and post-harvest handling and avocado fruit are no exception. Physiological maturity may be defined as that stage of development at which the fruit, once detached from the tree, will ripen and result in a product desirable for eating. Immature fruit are known to be bland and likely to shrivel as they ripen and in extreme cases the fruit may not even soften. Although some cultivars, e.g. Hass, may be left to hang on the tree for extended periods of time without subsequent deterioration of fruit quality (Kaiser & Wolstenholme, 1994), others may not be suited to delayed harvesting as fruit senescence while on the tree is probably a limiting factor. The fact that Fuerte, especially late in the season, is more prone to postharvest physiological disorders than are Hass fruit (Witney *et al.*, 1990) is supporting evidence for this. Consequently, it is imperative that maturity standards, both minimum and maximum, be set for Fuerte fruit.

In the past, the only reliable maturity standard for avocado fruit was found to be total lipid content (Eaks & Sinclair, 1978) and this is still the case today (Kaiser, 1994). Since the total lipids and moisture content are reciprocal and sum to a constant for any one cultivar (Swarts, 1976) the moisture content is still used extensively by the South African industry as a maturity standard. However, the moisture content varies according to the prevailing orchard conditions. Consequently, for accurate readings, lipid concentrations should be determined on a dry-mass basis. On a different note, however, Zauberman & Schiffmann-Nadel (1972) examined pectinmethylesterase activity (PME), one of the enzymes responsible for the initiation of the ripening process, in Fuerte fruit at various stages of development and ripening. They found that PME activity on the day of harvest decreased with an increase in the stage of fruit development. In younger fruit, PME activity decreased rapidly, while in mature fruit, PME activity decreased moderately. They suggested from these data that PME activity may be a possible maturity indicator. Apparently, however, no further work has been done in this regard. Consequently, this study was undertaken to determine whether PME activity might act as a suitable maturity marker for Fuerte fruit.

Another hydrolytic enzyme directly associated with fruit ripening (Bennett & Christoffersen, 1986) is cellulase. Cellulase is the collective term given to enzymes capable of hydrolysing cellulose (Sreenath, 1993). According to Awad & Young (1979) cellulase activity increased three days before the increase in polygalacturonase activity. The strong correlation between cellulase activity and softening suggests that in avocado the initial phase of softening may be due to cellulase (Hatfield & Nevins, 1986). This increase in activity is due to the *de novo* synthesis of its protein, correlating with an increase in the steady amount of cellulase mRNA (Kanellis *et al.*, 1989). Maturity indexing based on cellulase activity appears to be well suited to avocado, which is rich in the enzyme. In nearly all reports on cellulase during ripening, the activity identified has been of the carboxymethylcellulase, C_x, type. Hatfield & Nevins (1986) classified the cellulase from avocado fruits as endo-(1-4)- β -D glucanase, and described its role in avocado fruit ripening as one of disrupting and loosening the cell wall matrix rather than one of rapid solubilization. Hence, cellulase activity in its entirety was also monitored over the 1994 and 1995 seasons.

MATERIALS AND METHODS

During 1994, 232 Fuerte fruit of count 16 (236-265 g) from wellmanaged orchards on the farm Everdon Estate (30° 16' E and 29° 27' S) near Howick, a cool subtropical area with relatively late fruit maturity, were harvested on a weekly basis between 17 May 1994 and 6 July 1994. Sixteen fruits were sampled on the day of harvest, and fruit firmness, fruit and seed masses were recorded. The moisture content of the fruit flesh was determined gravimetrically, and lipid percentages were determined using a soxhlet apparatus and plotted over time (figure 1). In addition, PME and cellulase activity of radial sections was monitored by modifications of the methods developed by Hagerman & Austin (1986) and Chernaglazov *et al.* (1989) respectively. Meanwhile, 80 fruits were

placed in the laboratory and allowed to ripen at room temperature (21 °C). Eight fruits were sampled on each of the subsequent 10 days of storage, and fruit firmness, fruit and seed masses, and PME activity were determined for all ten days. Cellulase activity was determined only for the first two days after harvest. The change in PME activity (figure 2) and cellulase activity (figure 3) was determined, analysed statistically and plotted over time. The remaining fruits were stored at four different temperature regimes. These storage regimes were identical to treatments 2, 4, 6 and 9 (table 1) of Donkin *et al.*, (1995). during each week of storage, 8 fruits were removed from each treatment and fruit firmness recorded. These values were averaged for each week and the data plotted over time (c.f. Kaiser *et al.*, 1995).

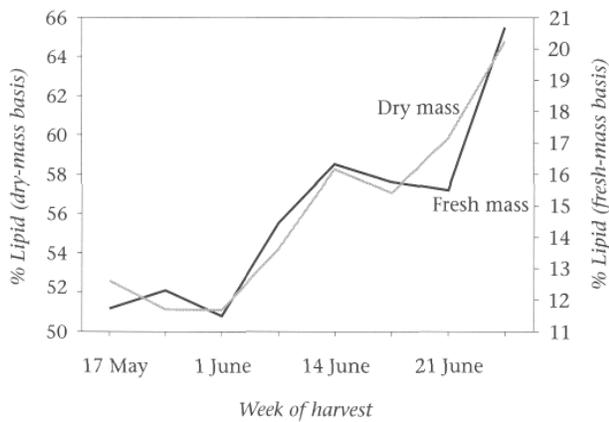


Figure 1

Average lipid content (%) on a dry- and a fresh-mass basis for Fuerte fruit harvested between 17 May and 6 July 1994 at Everdon Estate (Howick)

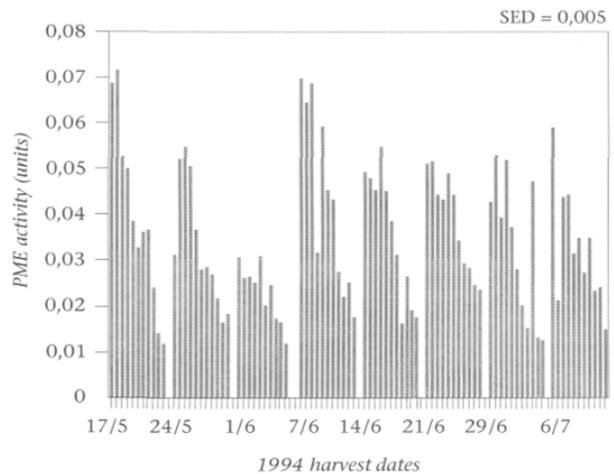


Figure 2

Average values of pectin methylesterase activity (units) for Fuerte fruit harvested at Everdon Estate (Howick) between 17 May and 6 July 1994 and allowed to ripen at room temperature (21 °C)

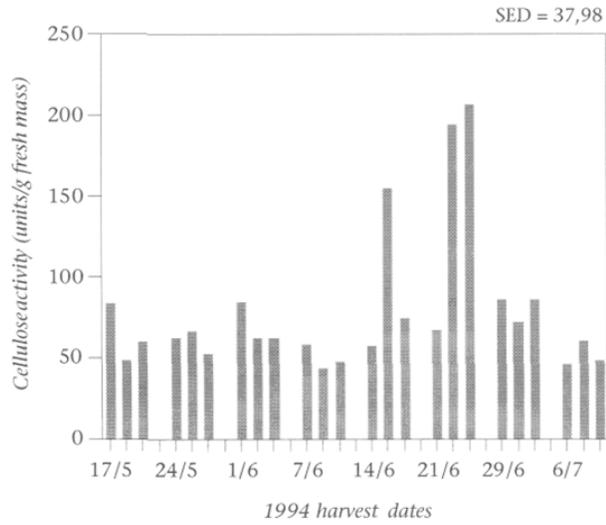


Figure 3

Cellulase activity (units/g fresh mass) over days 0, 1 and 2 for Fuerte avocado fruit harvested on a weekly basis from 17 May to 6 July 1994

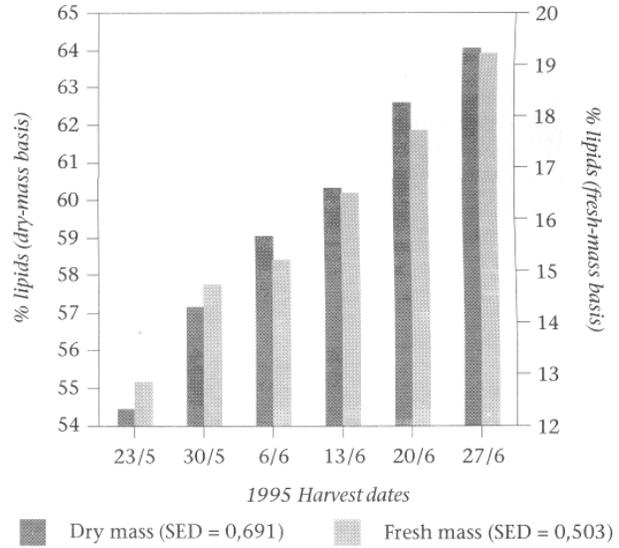


Figure 4

Average lipid content (%) on a dry- and a fresh-mass basis for Fuerte fruit harvested between 23 May and 26 June 1995 at Everdon Estate (Howick)

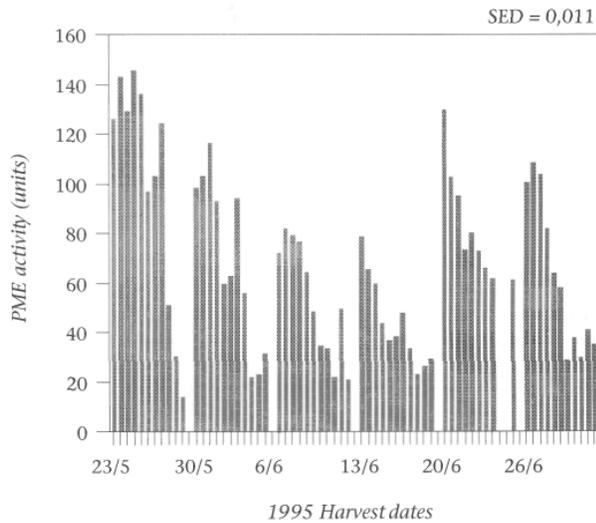


Figure 5

Average values of pectin methylesterase activity for Fuerte fruit harvested during 1995 at Everdon Estate (Howick) between 23 May and 26 June 1995 and allowed to ripen at room temperature (21 °C)

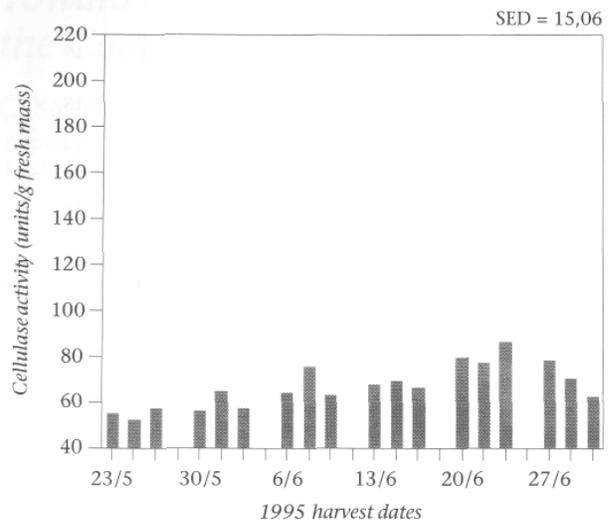


Figure 6

Cellulase activity (units/g fresh mass) over days 0, 1 and 2 for Fuerte avocado fruit harvested on a weekly basis from 23 May to 27 June 1995

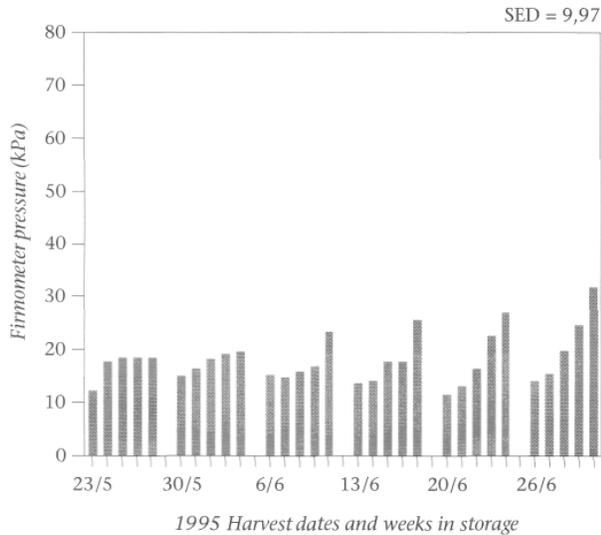


Figure 7

Firmometer readings (kPa) for fruit harvested weekly during 1995 at Everdon Estate and kept in cold storage for 4 weeks at temperatures of 8,5 °C, 8,5 °C, 7,5 °C, 6,5 °C respectively (treatment 2)

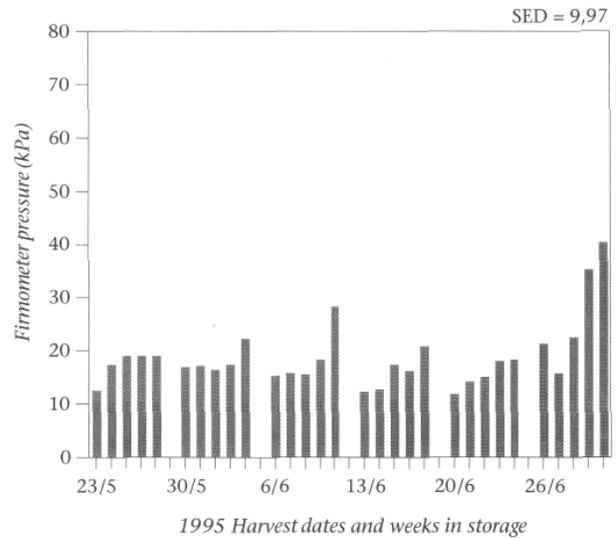


Figure 8

Firmometer readings (kPa) for fruit harvested weekly during 1995 at Everdon Estate and kept in cold storage for 4 weeks at temperatures of 7,5 °C, 7,5 °C, 6,5 °C, 5,5 °C respectively (treatment 4)

During 1995, 144 Fuerte fruit of count 16 were harvested at the same site on a weekly basis between 23 May 1995 and 26 June 1995. Sixteen fruits were sampled on the day of harvest, and fruit firmness and fruit and seed mass were recorded. The moisture content and lipid percentages were determined as above, and the results plotted over time (figure 4). In addition, 80 fruits were placed in the laboratory and allowed to ripen at room temperature (21 °C), and 8 fruits sampled on each of the subsequent 10 days of storage. Again the changes in PME activity (figure 5) and cellulase activity (figure 6) were determined as before, analysed statistically and plotted over time. The remaining fruits were again stored at the same temperature regimes (treatments 2, 4, 6 and 9) and the firmness of 16 fruits was recorded each week. These weekly values were averaged and the data plotted over time (figures 7-10).

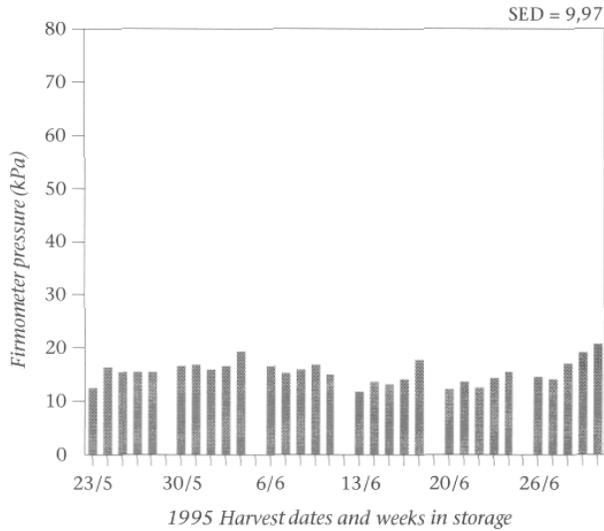


Figure 9

Firmometer readings (kPa) for fruit harvested weekly at Everdon Estate in 1995 and kept in cold storage for 4 weeks at temperatures of 5,5 °C, 5,5 °C, 5,5 °C, 5,5 °C respectively (treatment 6)

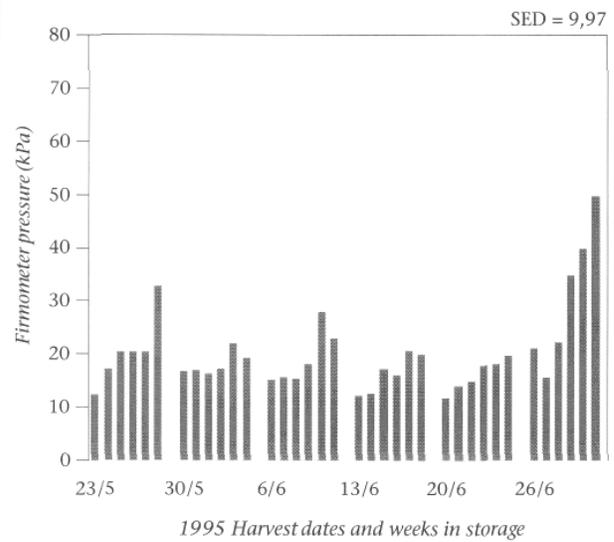


Figure 10

Firmometer readings (kPa) for fruit harvested weekly at Everdon Estate and kept in cold storage for 4 weeks at temperatures of 7,5 °C, 7,5 °C, 6,5 °C, 5,5 °C, 5,5 °C respectively (treatment 9)

RESULTS AND DISCUSSION

During 1994, lipid percentages increased over the season from about 11 % on a fresh-mass basis (or 53 % on a dry-mass basis) on 17 May 1994 to slightly more than 20 % on a fresh-mass basis (or about 65 % on a dry-mass basis) on 6 July 1994, with some minor fluctuations between these times (figure 1). During 1995, a similar trend was seen where lipid percentages increased over the season from about 12 % on a fresh-mass basis (or 54,5 % on a dry-mass basis) on 23 May 1995 to slightly more than 19 % on a fresh-mass basis (or about 64 % on a dry-mass basis) on 27 June 1995 (figure 4).

In most instances, both 1994 and in 1995, PME activity usually increased the day after harvest but then declined steadily while the fruit was ripening. No definite trends in initial activity nor the rate of change of activity could be observed during either season. Consequently, PME activity was not a suitable maturity marker. Two definite decreases in initial activity were, however, observed in 1994: first from 17 May to 1 June, and then from 7 June to 6 July (figure 2). Two similar trends were observed in 1995: first from 23 May to 13 June, and then from 20 to 26 June (figure 5). PME activity was thus modelled against fruit firmness using a simple linear regression model. The relationship between fruit firmness and PME activity was highly significant ($P < 0,0001$). Consequently, firmness definitely increased with decreasing PME activity, however, the r^2 value was only 22,8 %, which means that PME activity describes only 23 % of the variability in fruit firmness. This implies that PME activity is also not a very good marker for fruit softening.

Table 1
Storage temperatures (°C) for treatments 2, 4, 6 and 9 over 4 or 5 weeks
of regular atmosphere storage

Treatment	Week 1	Week 2	Week 3	Week 4	Week 5
2	8,5	8,5	7,5	6,5	—
4	7,5	7,5	6,5	5,5	—
6	5,5	5,5	5,5	5,5	—
9	7,5	7,5	6,5	5,5	5,5

For days 0, 1 and 2 of ripening after the day of harvest, cellulase activity between the weeks was greater in 1994 (up to 210 units/g fresh mass) than in 1995 (up to 90 units/g fresh mass) (figures 3 and 6 respectively). During 1994 the peak activity of 210 units/g fresh mass was observed in fruit harvested on 21 June, and in 1995 peak cellulase activity showed a less definitive peak of 90 units/g fresh mass in fruit harvested on 20 June. Unfortunately, the differences in cellulase activity between and within the weeks of harvest can largely be explained by variability within the individual days, since the standard error of the difference of means was 37,98 in 1994 and 15,06 in 1995.

Of the four temperature trials on fruit softness, treatment 6 (5,5 °C; 5,5 °C; 5,5 °C; 5,5 °C) the industry norm, proved the best in both seasons where fruit softness was concerned. None of the fruits receiving this treatment exceeded the maximum 35 kPa firmometer readings after 4 weeks of storage (figure 9). During the 1994 season, however, fruits from treatment 6, harvested after 21 June 1994, were beginning to soften after 4 weeks in storage, but still had an acceptable firmness (c.f. Kaiser *et al.*, 1995). Indeed, some of the fruit harvested in KwaZulu-Natal left Durban harbour on Vessel 906 on 25 June 1994, and arrived in Europe soft. It appears that the best harvesting period for Fuerte fruit in the KwaZulu-Natal midlands was up to 21 June in 1994. Indeed, this was confirmed by Donkin *et al.*, (1995) who found that there was a definite increase in physiological and pathological disorders after this date. Fruit harvested at that time had a lipid content of about 58 % on a dry-mass basis (or 16 % on a fresh-mass basis). A similar trend was seen in 1995 when post-harvest physiological problems were seen in several of the fruit cut after 20 June 1995. Here, the lipid content was about 61 % on a dry-mass basis (or 18 % on a fresh-mass basis).

Fruit from treatments 2, 4 and 9 (figures 7, 8 and 10 respectively) were only firm after four weeks of cold storage for the first two weeks of harvest in 1994. In contrast during 1995, fruit which underwent these same treatments had an acceptable firmness after and until the last week of harvest but was significantly higher (i.e. softer) than treatment 6 (sed = 0,997). Consequently, if a single temperature regime is to be recommended then 4 weeks of 5,5 °C was more than adequate.

CONCLUSIONS

In respect of fruit softness, Fuerte fruit harvested in the KwaZuluNatal midlands at Everdon Estate, Howick during the 1994 and 1995 seasons stored best in regular atmospheric cold storage at a constant 5,5 °C over 4 weeks when compared to other steppedup or stepped-down temperature regimes. The experimental fruits stored under

this regime in South Africa were still relatively firm even when harvested after 21 June 1994. However, some of the commercial consignments of fruit (on Vessels 906 and 907) which underwent similar storage conditions arrived in Europe 4 weeks after that date and were soft. During 1995, however, all fruit was firm after 4 weeks at a constant 5,5 °C. For the first three weeks of storage during 1994 (17 May to 1 June) and the first 5 weeks of storage during 1995 (23 May to 20 June), however, there were no marked differences in fruit firmness between treatments 2, 4, 6 and 9. Consequently, it is recommended that fruit is stored at a constant 5,5 °C for 4 weeks to reach the European markets firm. If a stepped down temperature regime is to be used, it is advisable to do so only during the first two weeks of harvest.

Lipid content, to date still the most reliable maturity standard for avocados, plateaued on 21 June 1994 at approximately 58 % on a dry-mass basis (or 16 % on a fresh-mass basis). After that time it declined to about 57 % on a dry-mass basis (or 15 % on a fresh-mass basis) by 29 June 1994. This, along with increased physiological browning disorders of the mesocarp (Donkin *et al.*, 1995) and distal-end browning (Kaiser *et al.*, 1995) after 21 June 1994, indicates a maximum fruit maturity date. In 1995, a similar trend was seen where post-harvest physiological disorders appeared in fruit harvested after 20 June 1995. Here, fruit lipid content was 61 % on a dry-mass basis (or 18 % on a fresh-mass basis). Based on these results, it is recommended that Fuerte fruit from Everdon Estate, Howick should be harvested before lipid concentrations reach a maximum of 58-61 % on a dry-mass basis (or 16-18 % on a fresh-mass basis). During 1994 and 1995, this coincided with the last week of June.

Analysis of PME activity showed two definite decreases in initial activity, both in 1994 and in 1995 (figures 2 and 4). However, they were not coincidental. Consequently, PME activity was not a good fruit maturity marker. In addition, modelling of PME activity showed that PME activity described only 23 % of the variability in fruit firmness. Similarly, no appreciable trends in cellulase activity were observed for Fuerte avocado fruit harvested between 17 May and 6 June 1994, and between 23 May and 27 June 1995. Besides a peak in cellulase activity of 190 and 210 units/g fresh mass on days 1 and 2 of ripening respectively, for fruit harvested on 21 June 1994, no other significant differences were observed between and within the weeks of harvest in both 1994 and 1995. Consequently, cellulase should not be used as a marker for predicting maturity of Fuerte avocados.

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