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INHIBITION OF RIPENING OF AVOCADOS WITH CALCIUM

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ABSTRACT

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Ripening of intact avocado fruit was inhibited by infiltration under reduced pressure with solutions containing calcium chloride. The increase in storage life was about 50% greater than control fruit. When the fruit did ripen, they were found by organoleptic evaluation to be highly acceptable and of similar quality to fruit that ripened normally. The fruit, however, had ripened without exhibiting any rise in respiration and with a greatly reduced climacteric pattern of ethylene evolution.

INTRODUCTION

Calcium (Ca) has been shown to affect a range of physiological processes in plants (Jones and Lunt, 1967) and to inhibit aspects of abnormal senescence in many fruit and vegetable tissues (Faust, 1975). Studies on apples have shown that fruit with a higher level of Ca have a lower rate of respiration regardless of whether the differences in Ca were endogenous (Bramlage et al., 1974; Faust and Shear, 1972) or due to applied Ca (Bangerth et al., 1972), although no difference in the time of occurrence of the climacteric peak was observed (Bramlage et al., 1974). A limitation on the use of Ca to reduce respiration and other metabolic activity of whole fruits is the relatively low rate of uptake of Ca from a dipping-solution by the fruit. Scott and Wills (1977) obtained much greater uptakes of solution by dipping apples under vacuum, and this resulted in a greater retardation of senescence generally. The use of vacuum infiltration of Ca solution into tomatoes, whereby the Ca content of the fruit was raised from about 10 mg/100 g to about 40 mg/100 g, was found to prevent tomatoes from ripening although the fruit never subsequently ripened (Wills and Tirmazi, 1979; Wills et al., 1977). Tirmazi and Wills (1981) were able to delay the ripening of mangoes by about 7 days at 25°C after vacuum infiltration had increased the Ca content of the flesh tissue from about 11 mg/100 g to 14 mg/100 g, and the fruit then ripened satisfactorily.

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Avocados are climacteric fruit with a relatively short life at ambient temperatures but they cannot be stored at low temperature due to the onset of chilling-injury (Pantastico et al., 1975). Tingwa and Young (1974) found that avocados with a higher endogenous Ca level took longer to ripen and had a lower peak production of ethylene (C_2H_4). They also found that vacuum infiltration of whole fruits with Ca solutions resulted in inhibition of peak carbon dioxide (CO_2) and C_2H_4 production, but the time to reach the climacteric was not markedly affected. In the present paper we have examined in more detail the effect of infiltration with Ca on the ripening-pattern of avocados.

MATERIALS AND METHODS

Avocados (*Persea americana* Mill.), cultivars 'Fuerte' and 'Hass', were obtained from commercial orchards in Northern New South Wales, Australia. The fruit in each replicate of an experiment were of uniform size and picked from a single tree. The study with each replicate was conducted at different times with fruit from different orchards. Ca was applied by vacuum infiltration with calcium chloride ($CaCl_2$) solution, as described by Wills and Tirmazi (1979). Control fruit were either infiltrated with water under the same conditions or untreated. There were 5 fruit/treatment in each replicate. The fruit after treatment were placed singly in jars at 20°C and ventilated with C_2H_4-free air at about 1.5 l/h. Respiration, as expressed by oxygen consumed by the fruit, was determined with an Oxygen Analyser (Servomex Controls, England), and C_2H_4 production was determined by gas chromatography (McGlasson, 1969). Ascorbic acid of flesh tissue was measured by the dye titration method of Kefford (1957), alcohol-insoluble solids by AOAC Method No. 32.012 (AOAC, 1980) and total lipids by the method of Bligh and Dyer (1959). The Ca content of flesh and peel tissue was determined by atomic absorption spectrophotometry (Tirmazi and Wills, 1981). Peel thickness was measured under a can-seam microscope on sections of peel that had been scraped free of flesh.

Fruit softening is commonly used as the primary indicator of ripeness. In this study, the degree of ripeness was determined subjectively by softening of the flesh as judged by hand pressure and translated to numerical values as: hard unripe (pressure $<$ 12 kg if measured with penetrometer) = 1; starting to soften = 2; medium-hard = 3; medium-soft = 4; soft = 5. A fruit with a score of 5 was considered as consumer-ripe. A small panel of 10 tasters evaluated some fruit when ripe (as determined by flesh softening) for acceptability using a 6-point scale (0—5) where 0 = very poor, 1 = poor, 2 = fair, 3 = good, 4 = very good, 5 = excellent.

RESULTS

After preliminary studies to determine the strength of vacuum that

needed to be applied to produce a measurable uptake of solution by the fruit, 'Fuerte' avocados were dipped in $CaCl_2$ solutions under 375 mm Hg pressure and the time to ripen as expressed by fruit softening was found to be delayed. Untreated fruit and water-infiltrated fruit were fully ripe after 9 days, while fruit dipped in 4% w/v $CaCl_2$ ripened after 15 days ($P<0.001$). Fruit dipped in 8 or 12% $CaCl_2$ were just starting to soften after 13 days but they developed skin injury and microbial growth started to appear on injured areas. Therefore, observations on softening were terminated and it was not possible to determine whether these fruit would have ripened fully on longer storage.

Studies with dips of lower $CaCl_2$ concentration showed that infiltration with even 1% $CaCl_2$ solution retarded ripening, while infiltration with 2 and 4% $CaCl_2$ solution had a greater effect. The results of one study are given in Fig. 1, which shows the development of ripening and that control fruit had softened fully after 10 days whereas fruit treated with $CaCl_2$ took 15 days or longer to soften. The taste panel evaluated the water-infiltrated fruit and 2 and 4% $CaCl_2$-treated fruit when they became ripe but did not find any significant difference in the level of acceptability. The mean scores for control, 2% $CaCl_2$ and 4% $CaCl_2$ were 3.75±0.59, 3.8±0.67 and 3.5±0.58, respectively. No taster reported the presence of any off-flavour or off-odour.

The production of C_2H_4 was significantly affected by the application of $CaCl_2$ (Table I); the peak value of C_2H_4 evolution at the climacteric was reduced ($P<0.001$) and the time of occurrence of the climacteric was delayed ($P<0.05$).

The pattern of C_2H_4 production in 1 typical fruit from each treatment is shown in Fig. 2. With many fruits infiltrated with 4% $CaCl_2$, it was difficult to determine when the climacteric in C_2H_4 production was attained. Figure 2 also shows that the climacteric precedes by some days the attainment of full ripeness as determined by fruit softening.

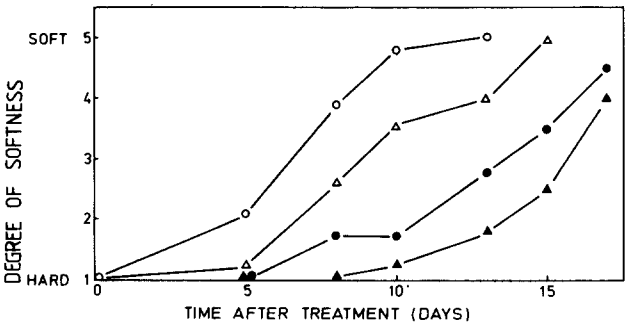

Fig. 1. Effect of dipping in $CaCl_2$ solution under 375 mm Hg pressure on ripening of 'Fuerte' avocados. Each point is the mean of 5 fruit. Treatments were: ○; water; △, 1% $CaCl_2$; ●, 2% $CaCl_2$; ▲, 4% $CaCl_2$.

TABLE I

Effect of dipping in $CaCl_2$ solution under 375 mm Hg pressure on production of C_2H_4 by 'Fuerte' avocados. Each value is the mean of 10 fruit (2 replicates × 5 fruit)

Treatment	C_2H_4-peak value (μl/kg/h)	Time to reach C_2H_4-peak (days)
Untreated	38.8	3.9
2% $CaCl_2$	15.5	6.4
4% $CaCl_2$	7.2	8.0

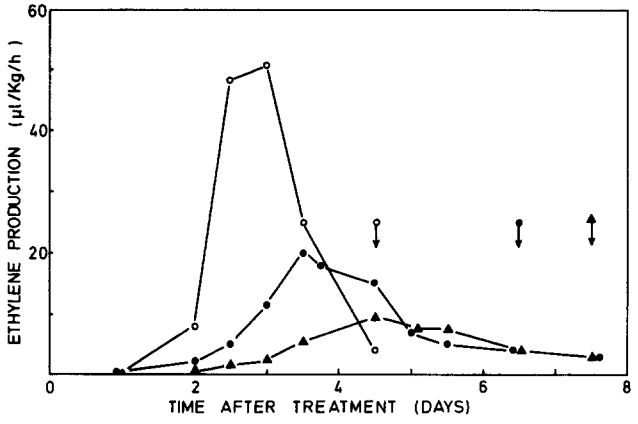

Fig. 2. Evolution of C_2H_4 by 'Fuerte' avocados dipped in $CaCl_2$ solution under 375 mm Hg pressure. Arrows above curves indicate the time when fruit were ripe as judged by softening. Treatments were: ○, water; ●, 2% $CaCl_2$; ▲, 4% $CaCl_2$.

The effect of Ca on respiration was to completely eliminate any climacteric rise prior to the fruit softening. Figure 3 shows that water-infiltrated fruit attained a respiratory climacteric about 2 days before being fully ripe, whereas the Ca infiltrated fruit had an initial fall in respiration immediately after infiltration, an effect common to all infiltrated fruit, and then continued to respire at a relatively steady rate. The studies on C_2H_4 evolution and respiration were carried out on separate fruit and hence there were differences in the times to soften of fruit in the 2 studies.

The levels of ascorbic acid, total lipid, and alcohol-insoluble solids were determined in unripe fruit and at the ripe stage in control as well as Ca-treated fruit (Table II). Although there was a significant ($P<0.001$) decrease in ascorbic acid content of fruit during the transition from the unripe to the ripe stage, the fruit infiltrated with 2 and 4% $CaCl_2$ had significantly ($P<0.05$) higher retention of ascorbic acid than control fruit. There was no significant change in alcohol-insoluble solids and total lipid content during ripening, and Ca-treated fruit had similar levels of these constituents as control fruit.

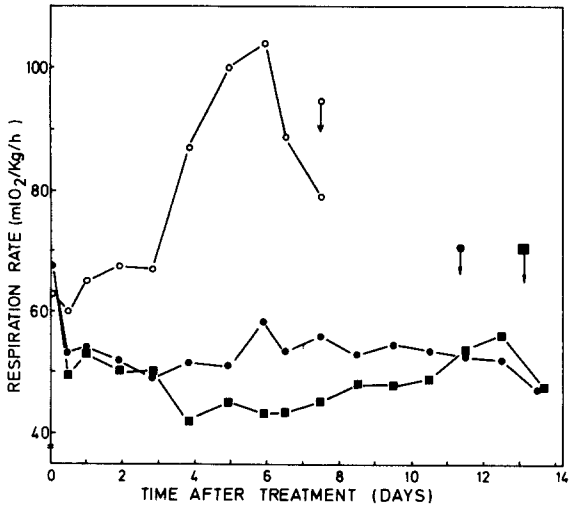

Fig. 3. Respiration of 'Fuerte' avocados dipped in $CaCl_2$ solution under 375 mm Hg pressure. Arrows above curves indicate the time when fruit were ripe. Treatments were: ○, water; ●, 2% $CaCl_2$; ■, 4% $CaCl_2$.

TABLE II

Effect of dipping in $CaCl_2$ solution under 375 mm Hg pressure on chemical composition of 'Fuerte' avocados when ripe. Each value is the mean of 20 fruit (4 replicates × 5 fruit)

	Unripe fruit	Ripe fruit (score 5)		
		0% $CaCl_2$	2% $CaCl_2$	4% $CaCl_2$
Ascorbic acid (mg/100 g fresh wt.)	11.4	3.2	4.7	5.7
Alcohol insoluble solids (%)	21.8	22.4	21.9	22.9
Total lipid (g/100 g fresh wt.)	18.1	19.0	17.9	19.5

TABLE III

Uptake of Ca by 'Fuerte' avocados on dipping in $CaCl_2$ solution. Each value is the mean of duplicate estimations on 3 composite samples, each of 5 fruit

Treatment	Ca content (mg/100 g fresh wt.)	
	Peel	Flesh
Untreated	15.5	11.0
2% $CaCl_2$	21.6	15.5
4% $CaCl_2$	26.0	18.6
8% $CaCl_2$	31.8	21.5

TABLE IV

Uptake of Ca by 'Hass' avocados on dipping in $CaCl_2$ solution. Each value is the mean of duplicate estimations on 3 samples

Treatment	Ca content (mg/100 g fresh wt.)			
	375 mm Hg pressure		250 mm Hg pressure	
	Peel	Flesh	Peel	Flesh
Untreated	15.2	10.0	16.0	12.9
4% $CaCl_2$	23.0	12.8	26.2	19.8
8% $CaCl_2$	—	—	30.0	23.7

The levels of Ca in peel and flesh tissue following infiltration are shown in Table III. The increase in Ca concentration was slightly higher in the peel than in the flesh.

A smaller number of studies were carried out on 'Hass' fruit. An initial study showed that infiltration with 4% $CaCl_2$ under 375 mm Hg pressure did not significantly affect the time of ripening. The peel of 'Hass' fruit was found to be about 1.6 mm thick, compared to about 0.7 mm for 'Fuerte' fruit. To overcome the increased resistance to Ca penetration due to the thicker peel, 'Hass' fruit were infiltrated under even lower pressures. Infiltration with 4% $CaCl_2$ under 250 mm Hg pressure was found to result in a retardation of ripening from about 11 days in control fruit to about 16 days in Ca-treated fruit ($P<0.01$). Fruit infiltrated with 8% $CaCl_2$ had not ripened by 16 days, but the development of rots prevented further observations. As with 'Fuerte' fruit, the climacteric peak of C_2H_4 was markedly reduced in Ca-infiltrated fruit and it was often difficult to determine when the climacteric peak was attained. The average peak value was 155 μl/kg/h in control fruit and 40 μl/kg/h in 4% Ca-infiltrated fruit. The respiration rate was not determined on 'Hass' fruit. Measurement of Ca in the peel and flesh tissue (Table IV) showed that there was a higher uptake of Ca in fruit infiltrated at the lower pressure, and that the use of 250 mm Hg pressure on 'Hass' fruit gave a similar uptake as 375 mm Hg on 'Fuerte' fruit.

DISCUSSION

Infiltration with $CaCl_2$ has been shown to be effective in delaying the ripening of avocados at 20°C. The use of 4% $CaCl_2$ at 375 mm Hg with 'Fuerte' and at 250 mm Hg with 'Hass' fruit appeared to give an optimal Ca uptake that resulted in an extension of about 50% in the time before ripening. When the infiltrated fruit did ripen, it was of an eating-quality comparable to control fruit and chemical parameters such as alcohol-

insoluble solids and lipid content were also similar in control and Ca-treated fruit, which, however, had significantly higher ascorbic acid content than control fruit.

The ability of Ca-infiltrated fruit to soften and attain full ripeness, as determined organoleptically, without passing through a well-defined respiratory or C_2H_4 climacteric supports the view (Biale et al., 1954) that in avocados endogenous C_2H_4 is a product and not the primary endogenous initiator of ripening, even though exogenous C_2H_4 will initiate normal ripening. Softening is due in large part to breakdown of the cell wall and middle lamellae induced by pectinases (Pilnik and Voragen, 1970) and cellulases (Awad and Young, 1979), and the consequent loss of cell-wall integrity has been proposed as leading to the production of C_2H_4 (Awad and Young, 1979; Solomos and Laties, 1973; Strand et al., 1976). It would seem that, in avocados, respiration is more susceptible to inhibition by Ca than C_2H_4 production, which in turn is more susceptible to inhibition than the cell-wall degrading systems.

The greater effects observed in this study compared to those reported by Tingwa and Young (1974) are no doubt due to a greater uptake of Ca by the fruit, brought about by using infiltration solutions at higher concentrations. Although 'Hass' fruit required the use of a lower vacuum to attain a physiological response, the Ca content was similar in both cultivars when the response occurred.

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