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Modified atmosphere storage and transport of avocados what does it mean?

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ABSTRACT

Stored avocado fruit often show an unacceptable incidence of storage disorders. Modification of the storage atmosphere is known to alter disorder incidence, but the manner in which the treatments affect the polyphenol oxidase (PPO) browning mechanism is unknown, making research into improved storage techniques arbitrary. The effect of controlled atmosphere, CO_2 *"shock" and normal atmosphere storage and PPO activity was evaluated. Fuerte fruits were stored for 28 days at 5,5°C, under 2 per cent oxygen, 10 per cent carbon dioxide (controlled atmosphere); 25 per cent carbon dioxide for three days, commencing one day after harvest, followed by normal atmosphere (*CO_2 *"shock") and normal atmosphere (control), in addition, the effect of postharvest fruit moisture loss on storage disorders was evaluated. All treatments showed a lower incidence of physiological disorders than the control. PPO activity was lowest in the* $C0_2$ *"shock" and highest in controlled atmosphere fruit Total phenols tended to be lower in* $C0_2$ *"shock" fruit as compared to other treatments. However, controlled atmosphere fruits were in a physiological state similar to that at harvest, after 28 days of storage. The economic implications of controlled atmosphere storage are discussed.*

UITTREKSEL

Avokadovrugte wat opgeberg word toon dikwels 'n onaanvaarbare hoé voorkoms van afwykings. Wysiging van die bergingsatmosfeer verander die omvang van die afwykings, maar die wyse waarop behandelings die polifenol-oksidase (PFO) verbruiningsensieme affekteer, is nog onbekend. Dit maak navorsing in verbeterde opbergingstegnieke arbitrar. Die effek van beheerde atmosfeer, CO_2*-"skok", en nórmale atmosfeer of PFO-aktiwiteite is bepaal. Fuerte vrugte is opgeberg vir 28 dae teen 5,5°C onder 2 persent suurstof en 10 persent koolstofdioksied (beheerde atmosfeer); 25 persent koolstofdioksied vir drie dae, wat begin het een dag na die oes, gevolg deur nórmale atmosfeer (*CO^{2-}*"skok") en nórmale atmosfeer (kontrole). Verder was die effek van na-oes vrugvogverlies op opbergingskwale ondersoek. Alie behandelings het 'n laer voorkoms van fisiologiese afwykings getoon as die kontrole. PFO-aktiwiteite was die* *laagste in die $C0_2$-"skok" en die hoogste in die beheerde atmosfeer vrugte. In vergelyking met ander behandelings, neig totale fenole om laer te wees in CO_2-"skok" vrugte. Nietemin het vrugte van beheerde atmosfeeropberging na 28 dae 'n fisiologiese toestand getoon soortgelyk aan varsgeplukte vrugte. Die ekonomiese implikasies van beheerde atmosfeer is bespreek.*

INTRODUCTION

The major export markets of South African Avocados are in Europe. The present shipping schedule (nine-day cycle) means that much of the fruit must be stored for approximately 30 days after harvest (Bower & Cutting, 1988). In order to successfully market this fruit it is necessary to ensure that softening is limited to the point where it is undiscernable and external cold damage and internal physiological disorders are minimized.

The avocado is a strongly climacteric fruit, which softens rapidly after harvest (Aharoni, 1984). Low temperature storage can substantially decrease the rate of softening, making it possible to land hard fruit in Europe. However, the avocado, being a fruit with origins in the cool tropics, is also sensitive to chilling (Couey, 1982). Not only can external chilling injury occur, but low temperature storage may also render the fruit susceptible to physiological disorders. Eaks (1976) and Engelbrecht & Koster (1986) found that the incidence of internal disorders is affected by a temperature/time interaction. In order to land hard fruit in Europe, South African exporters are forced to use temperatures of 5,5°C and lower, for the duration of shipping. Eaks found that a period longer than 14 days at such temperatures enhanced the possibility of physiological disorders.

Previous work (Spalding & Reeder, 1974; Ahmed & Barmore, 1980; Eksteen & Truter, 1982; 1985) indicated the possibility of a decrease in the incidence of physiological disorders at a modified storage atmosphere. Both traditional controlled atmospheres for the duration of the shipping period, during which gas concentrations must be carefully controlled (Smock, 1979), as well as a more economical CO_2 "shock" method (Collin, 1984; Eksteen & Truter, 1985), have shown promise. However, neither technique has been evaluated for its effect on the biochemistry of the fruit, especially the flesh browning mechanism, which causes the most important physiological disorder: pulp spot and mesocarp discolouration (Swarts, 1984). The potential for browning is said to be primarily a function of the activity of the browning enzyme, polyphenol oxidase (PPO) (Kahn, 1975). However, phenolic substrates may also be of importance (Kahn, 1976; 1977). Therefore both PPO activity and an indication of the phenolic content of the fruit should be investigated (Golan, Kahn & Sadovski, 1977).

While modified atmosphere normally concerns only oxygen and carbon dioxide, Bower & van Lelyveld (1985) showed that the fruit/water relation could be an important factor in fruit quality. The role of postharvest container humidity as a means of decreasing fruit moisture loss (and thereby stress) during transport, should also be included as an atmospheric variable. Before technical recommendations can be made about shipping under modified atmosphere conditions, the physiology of the process, particularly in relation to the browning reaction involved in physiological disorders, must be investigated. This is particularly important in view of literature showing modified atmospheres to be deleterious to fruit quality in some cases (Spalding & Marousky, 1981), while advantageous in other cases.

A further aspect which requires at least some comment is that of the economics of modified atmosphere.

MATERIALS AND METHODS

Fruit used in the modified atmosphere experiment (humidity trial excluded), were picked from healthy, irrigated Fuerte trees during the second week in May, at Westfalia Estate, Duivelskloof, Northern Transvaal. After the normal packhouse treatment, the following conditions were applied, in each case to 100 cartons of fruit:

- Control fruits, stored at 5,5°C in normal atmosphere for 28 days.

- Controlled atmosphere storage, using 2 per cent oxygen and 10 per cent carbon dioxide commencing four days after harvest (arrival in Cape Town) with a storage temperature of 5,5°C for the remainder of the 28-day storage period.

- Carbon dioxide "shock", using an atmosphere of 25 per cent CO_2 in a normal oxygen atmosphere for three days, commencing one day after harvest. Thereafter the container was ventilated and normal atmosphere used at a temperature of 5,5°C for the remainder of the 28-day storage period.

The fruit was transported by refrigerated road transport to Cape Town, where it was held for the duration of the experiment. At the end of the 28-day period, the fruit was allowed to soften at 20°C before visual evaluation of internal physiological disorders. Fruit from 10 cartons (count 12) was scored for both incidence of severity of the disorders, mesocarp discolouration and vascular abnormalities (pulp spot and vascular blackening). For biochemical analysis, five fruits from each treatment were randomly selected on the day of transfer from the 5,5°C cold store to the 20°C ripening room (designated hard fruit); when fruits reached 50 per cent soft (firmometer readings as according to Swarts (1981)) and when fruits had reached eating ripeness (100 per cent soft according to firmometer readings).

After selection for biochemical analysis, fruit exocarp was removed as was the seed, and the flesh (meso- and endocarp) cut into 10mm cubes. These were immediately frozen in liquid nitrogen and freeze-dried for later analysis.

The specific activity of soluble PRO was analysed by a modified form of the method described by Bower & van Lelyveld (1985). An ultraturrax was used to grind 1 g of freeze-dried fruit material in 10ml cold (4°C) sodium acetate buffer pH 5, with insoluble PVP (Polyclar AT) added to remove phenolic compounds. The pulp was centrifuged at 15000g for 10 minutes. The clear supernatant was used as the source of crude enzyme extract, which was assayed with 4-methyl catechol as substrate, as previously described by Bower & van Lelyveld (1985). The Lowry method of protein determination was used.

Total phenols in soft fruits were extracted by the method described by Torres, Mau-Lastovica & Rezaaiyan (1987). Assay was done by the Folin Ciocalteau method of

Amerine & Ough (1974) as modified by Torres *et al* (1987). Results are expressed as 4-methyl catechol equivalents.

To ascertain the effect of postharvest moisture stress on fruit quality, fruit from a commercial packer (ex-non-stressed, irrigated orchards) was waxed and packed in mid-May, as for export. Two adjacent similar cold rooms were each packed with a standard shipping pallet of cartons. Ten cartons were selected from each pallet, and fruit weighed before and after storage at 5,5°C for 30 days. A humidifier was placed in one cold room to raise the relative humidity in the atmosphere, therefore decreasing moisture loss in fruit. After the storage period, fruit was ripened at 21°C. The 20 cartons of 14 fruits each were evaluated for pathological and internal physiological disorders.

RESULTS

On cutting soft fruit, the incidence of pulp spot and blackening of cut vascular bundles was significantly higher ($P<0,05$) in the control as compared to the CO_2 "shock" and controlled atmosphere treated fruits. The disorder mesocarp discolouration, while not showing significant differences, did indicate an increasing trend from controlled atmosphere through $C0_2$ "shock" to the control treatment. The Kruskal Wallis analysis for non-parametric data was used. Table 1 shows the results.

TABLE 1 Index of incidence and severity of physiological disorders in soft Fuerte fruit after storage under normal atmosphere (control), modified atmosphere (CO_2 "shock") and controlled atmosphere (CA). Means are of 120 fruits per treatment

Treatment	Disorder incidence and severity index			
	Mesocarp discolouration		Vascular abnormalities	
	%	Rank average	%	Rank average
Control	5,06	15,90	14,13	24,50
CO_2 "shock"	3,67	15,20	1,93	11,75
CA	3,48	15,40	1,19	10,25
LSD (P=0,05)		NS		5,22

The changes found in specific PPO activity due to the influence of container atmosphere during fruit softening are shown in Table 2.

TABLE 2 Specific PPO activity ($\triangle OD^{420}$ $min^{-1}mg$ $protein^{-1}$) for fruit stored under normal atmosphere (control), modified atmosphere (CO_2 "shock") and controlled atmosphere (CA) immediately after removal from 28 days storage at 5,5°C, when half soft and soft. SE of means represent five fruits with the analysis replicated three times

Treatment	PPO activity			
	Control	CO_2 "shock"	CA	LSD (P=0,05)
Hard	1,94 (±0,14)	1,82 (±0,14)	5,14 (±0,83)	1,54
50 % soft	1,31 (±0,18)	1,03 (±0,04)	1,98 (±0,23)	0,67
Soft	0,96 (±0,15)	0,51 (±0,05)	1,26 (±0,13)	0,34
LSD (P=0,05)	0,52	0,30	1,63	

All treatments showed a decrease in specific PPO activity during softening, as has previously been shown (Sharon & Kahn, 1979) with the greatest change occurring in the controlled atmosphere stored fruits. Throughout the softening period, the CO_2 "shock" treated fruit tended to have a lower PPO activity than that from other treatments, (($P<0,05$) for soft fruits). The controlled atmosphere fruit (on the other hand), showed the highest PPO activity.

The analysis of total phenols in soft fruit is shown in Table 3.

TABLE 3 Total phenolic content (μg g fruit dry $mass^{-1}$ in 4-methyl catechol equivalents) for fruits stored under normal atmosphere (control), modified atmosphere (CO_2 "shock") and controlled atmosphere (CA) when soft. SE of means represent five fruits

Treatment	Total phenol concentration
Control	68,1 (±10,1)
CO_2 "shock"	49,4 (±2,6)
CA	61,3 (±5,0)
LSD (P=0,05)	23,6

The total phenol content in soft, controlled atmosphere fruit was not different to that of control fruit, but there was a tendency for CO_2 "shock" fruit to exhibit a decreased total phenol content, which is consistent with the work of Siriphanich & Kader (1985) on lettuce.

The incidences of pathological and physiological disorders, as influenced by fruit moisture loss during storage, are shown in Table 4. Fruit from the non-humidified cold room lost significantly ($P<0,01$) more moisture than fruit from the humidified room, and fruit from the room with added humidity was of better internal quality (P<0,01).

TABLE 4 Percentage incidences of Fuerte avocado fruit disorders as influenced by fruit moisture loss during storage

Fruit moisture loss	% Fruit with disorder			Internal physiological disorders +
	External cold damage	Anthracnose	Stem-end rot	
High	0,69	22,00	3,81	31,20
Low	1,04	13,90	0,35	17,8
Significance NS		★	★★	★★

Significance at P = 0,05 by ★ and P=0,01 by ★★, calculated from frequency of disorder by x^2 test

+ Indicates pulp spot, vascular and mesocarp discolouration.

DISCUSSION

The results, particularly those pertaining to PPO activity, indicate that controlled atmosphere storage results in physiologically different fruits to those from the other treatments at the end of the storage period.

The final browning potential of controlled atmosphere fruit, bearing in mind both the PPO activity and total phenolic content, should have been the highest of all treatments (Golan, Kahn & Sadovski, 1977). Visual observations of the experimental fruit did not, however, show as high in incidence of physiological disorders as the control fruit. There are a number of possible explanations. Subsequent work (Cutting & Bower, unpublished results) has shown that an avocado picked in early May, and in a non-stressed state, has a specific soluble PPO activity of approximately 5.This rapidly decreases during the initial stages of softening, followed by a more gradual decrease until finally complete (eating) softness is attained. The results therefore imply that controlled atmosphere storage decreased the postharvest physiological changes to the extent that after 28 days storage, fruits had maintained a state similar to that at harvest. The other treatments were unable to achieve this, with partial ripening taking place. Such ripening at low temperatures can result in membrane damage (Bower & Cutting, 1988). The reaction which results in physiological disorders is reliant on polyphenol oxidase together with phenolic substrate in the presence of oxygen. This does not normally occur in the cell and implies some form of cellular damage (Vaughn & Duke, 1984).

An aspect presently receiving attention, relates to the individual phenolics within the totals measured. This may be of considerable importance (Martinez-Cayuela, Plata, Faus & Gil, 1988). A better perspective may be gained of the actual PPO substrates present, and the possible role of postharvest container atmosphere in modifying substrates as found in lettuce by Siriphanich & Kader (1985). Whether the effect on PPO substrates is direct or indirect is unknown. Pruskey, Kobiler, Jacoby, Simms & Midland (1985) showed that epicatechin (which inhibits lipoxygenase and thereby membrane destruction) did not decrease under high CO_2 conditions, but decreased in normal atmosphere.

Increased postharvest moisture loss with greater moisture stress during storage, caused enhanced visual symptoms of physiological disorder and increased the prevalence of pathological disorders. The mechanism is unknown, but the consequences for the avocado industry are clear.

Unfortunately, however, humidification of containers is not a practical proposition at present. Humidification of packhouse cold stores is an area worth investigating in view of the fact that the greatest fruit moisture loss occurs during the initial cooling phase to shipping temperatures.

Overall results indicated that $C0_2$ "shock" held promise as a storage technique. Controlled atmosphere storage, however, "held" fruit in a physiological condition similar to that of freshly picked fruit. This provides a considerable marketing advantage over fruit from other forms of storage.

Although controlled atmosphere is clearly technologically superior to other forms of transport, the economic and logistical realities must be taken into account. At present, controlled atmosphere shipping costs (Cape Town to Europe) are approximately 90 cents per kg, as opposed to 40 cents per kg for conventional containers. However, this 50 cents per kg premium must be seen in the light of increased quality and the lesser likelihood of having to apply "price dumping" tactics (of up to R1,50 per kg) to move fruit through the market when buyer confidence is poor, due to quality uncertainty.

Logistically, lack of controlled atmosphere container availability makes a full controlled atmosphere service for avocados impossible. However, future developments in shipping and a competitive attitude make it imperative to develop the commercial aspects of controlled atmosphere transport of avocados.

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