Quality change in harvested winter squash by enhancement of calcium status and by use of surface coatings

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Abstract

Rots and physiological weight loss cause substantial postharvest losses in harvested winter squash (*Cucurbita maxima* D. hybrid 'Delica').

This research had both preharvest and postharvest components. Preharvest research was aimed at treatments in the field which would decrease storage rots of squash. Postharvest research was focused at reduction of weight loss which is normally caused by storage conditions during shipment.

Calcium concentration affects maintenance of firmness of many fruits and also has a significant effect on fruit resistance to rot development. Enhancement of transpiration of a fruit by an alkaline spray may result in increase of its calcium concentration, since calcium is transported with mass flow of water. Three spray treatments, Na_2CO_3 solution, $CaCl_2$ solution and H_2O (control), and four painting treatments (Na_2CO_3 solution, $CaCl_2$ solution, vapour gard solution and H_2O) were applied to squash growing in the field, and the fruit were stored at ambient temperature after harvest. Squash given Na_2CO_3 spray rotted less in the field and tended to rot later during storage than those sprayed $CaCl_2$ and H_2O, but there was no relationship between the order in which squash rotted and the mineral concentration (calcium, nitrogen and phosphorus) of their tissue. With painting treatments, calcium concentration of both skin and cortex in the squash treated with Na_2CO_3 was higher than those of other three treatments.

Enhancement of transpiration changed fruit calcium status, but it was not confirmed if this changed resistance of the fruit to rot development.

In the postharvest study, three experiments were carried out. In the first, it was shown that 'Primafresh' coating was the most suitable of a number of materials for reducing weight loss without undue modification of the fruit's internal atmosphere.

In the second, squash were given 0, 1, 2, 3, 4, 5, 6 'Primafresh' coatings and stored in a plastic bag at 20°C and 90% RH. Rate of weight loss at 24h intervals, and internal atmosphere composition after 3 days of storage, O_2, CO_2 and C_2H_4, and CO_2 production of each squash were monitored. Coating significantly reduced weight loss of squash, but the order of weight loss did not relate to the number of coatings applied. Coating markedly decreased internal oxygen concentration ($[O_2]_i$, mol mol^{-1}) and increased internal carbon dioxide concentration ($[CO_2]_i$, mol mol^{-1}) and internal ethylene ($[C_2H_4]_i$, mol mol^{-1}) of squash. The decrease of $[O_2]_i$ and increase of $[C_2H_4]_i$ were dependent on the number of coatings applied, but $[CO_2]_i$ was not. On average over all coating treatments, CO_2 production of coated squash was significantly greater than those of non-coated squash. $[O_2]_i$, $[CO_2]_i$ and CO_2 production were highly variable in individual squash within each coating treatment. Off-flavour ratings were highly, positively correlated with $[CO_2]_i$ of individual squash.

Potential benefits of surface coatings to reduce weight loss of squash appear to be quite limited. Increasing the number of coatings resulted in fermentation without achieving worthwhile reduction of weight loss. Use of a fruit system mathematical model with the information gathered from this thesis and a potential alternative method for reduction of weight loss are discussed.

In the third experiment, squash were coated with Primafresh at 2 days intervals to allow physiological equilibration before the following coating was applied. Internal atmosphere composition was measured at 24h intervals. $[O_2]_i$ decreased and $[CO_2]_i$

had increased 24h after each coating. This effect was followed by an increase of $[O_2]_i$ and decrease of $[CO_2]_i$ 48h after each coating, but not to the level present before each coating. This indicated that respiratory response of fruit to the change of internal atmosphere caused by increased skin resistance was not instantaneous. There were a delay between the physical and physiological effects of elevated skin resistance in squash.

Results of the second and third coating experiments showed that the sum of $[O_2]_i$ + $[CO_2]_i$ in coated squash was always more than 0.21 mol mol^{-1} at any level of $[O_2]_i$, which is different from the response of other fruit to surface coatings. Two possibilities for this response were discussed.

This thesis has shown that both preharvest and postharvest surface coatings can have profound effects on the fruit physiology and thereby affect storage behaviour. Further work will be required if these effects are to be optimised to provide treatments suitable for commercial application.

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A	=	surface area (cm^2)
A^{fruit}	=	Fruit surface area (cm^2)
ACP	=	Anaerobic compensation point (mol mol^{-1} O_2)
CA	=	Controlled atmosphere
CO_2	=	Carbon dioxide
$[CO_2]_i$	=	Internal carbon dioxide concentration (mol mol^{-1})
$[CO_2]_e$	=	External carbon dioxide concentration (mol mol^{-1})
$[CO_2]_{jar}$	=	mol fraction of CO_2 in sampled gas (mol mol^{-1})
C_2H_4	=	Ethylene
$[C_2H_4]_i$	=	Internal ethylene concentration (mol mol^{-1})
F	=	Flux (cm^3 s^{-1})
fr	=	air flow through a bag (500 ml min^{-1})
h	=	Hour
$[j]_{external}$	=	external concentration (mol mol^{-1})
$[j]_{internal}$	=	internal concentration (mol mol^{-1})
K	=	Transpiration coefficient (% day^{-1} k Pa^{-1})
K_m	=	Michaelis - Menten constant (mol mol^{-1} O_2)
MA	=	Modified atmosphere
O_2	=	Oxygen
$[O_2]_e$	=	External carbon dioxide concentration (mol mol^{-1})
$[O_2]_i$	=	Internal oxygen concentration (mol mol^{-1})
P^a	=	water vapour pressure at ambient air
P^f	=	water vapour pressure of a fruit

R	=	Resistance to gas diffusion (s cm^{-1})
R^{coat}	=	Resistance of coating to gas diffusion (s cm^{-1})
R_{CO2}	=	Resistance to carbon dioxide diffusion (s cm^{-1})
r_{CO2}	=	Diffusive transmission rate of CO_2 (cm^3 s^{-1})
R_{C2H4}	=	Resistance to ethylene diffusion (s cm^{-1})
R^{cut}	=	Resistance to gas diffusion through cuticle (s cm^{-1})
$R^{cut}{}_{CO2}$	=	Resistance to CO_2 diffusion through cuticle (s cm^{-1})
$R^{cut}{}_{O2}$	=	Resistance to O_2 diffusion through cuticle (s cm^{-1})
$R^{fruit}{}_{CO2}$	=	Total resistance of a fruit to CO_2 (s cm^{-1})
$R^{fruit}{}_{O2}$	=	Total resistance of a fruit to O_2 (s cm^{-1})
r_{H2O}	=	Rate of water loss from a produce (% fresh weight day^{-1})
R_{O2}	=	Resistance to oxygen diffusion (s cm^{-1})
r_{O2}	=	Diffusive transmission rate of O_2 (cm^3 s^{-1})
R^{pores}	=	Resistance to gas diffusion through pores (s cm^{-1})
$R^{pores}{}_{CO2}$	=	Resistance to CO_2 through pores (s cm^{-1})
$R^{pores}{}_{O2}$	=	Resistance to O_2 through pores (s cm^{-1})
R^{c}	=	Resistance of coating to gas diffusion (s cm^{-1})
R^{s}	=	Resistance of skin to gas diffusion (s cm^{-1})
R^{series}	=	Resistance through the series (s cm^{-1})
RQ	=	Respiratory quotient
rr_{CO2}	=	Respiratory CO_2 production (cm^3 s^{-1})
rr_{O2}	=	Fruit respiratory oxygen uptake (cm^3 s^{-1})
rr_{O2}^{max}	=	Fruit inherent maximum rate of O_2 consumption (cm^3 s^{-1})

R^{total}	=	the total of the resistance to gas diffusion between the fruit's internal and external atmosphere
wt	=	Fruit weight (g)
WVPD	=	Water vapour pressure deficit

List of equations

Rate of water loss:

$$r_{H_2O} = K * WVPD \qquad [1.1]$$

Evaporation:

$$r_{H_2O} = K * (P^f - P^a) \qquad [4.1]$$

Respiration:

$$C_6H_{12}O_6 + 6O_2 \text{ --- } 6CO_2 + 6H_2O + 688Kcal \qquad [4.2]$$

Total skin resistance of a fruit:

$$R^{total} = R^{tissue} + \frac{R^{skin} + R^{pores}}{R^{skin} x R^{pores}} + R^{wax} + R^{plastic} + R^{carton} \qquad [4.3]$$

Flux of a gas through a barrier:

$$F = \frac{([J]_{external} - [J]_{internal}) * A}{R} \qquad [4.4]$$

Flux and concentration gradient:

$$\frac{F * R}{A} = [J]_{external} - [J]_{internal} \qquad [4.5]$$

Skin resistance of a coated fruit:

$$R^{series} = R^s + R^c \qquad [5.1]$$

Fruit respiratory oxygen uptake:

$$rr_{O_2} = rr_{O_2}^{max} \frac{[O_2]_i}{(K_m + [O_2]_i)} \qquad [5.2]$$

Respiratory CO_2 production

$$rr_{CO_2} = RQ^{\infty} rr_{O_2}^{max} \left(\frac{[O_2]_i}{K_m + [O_2]_i} + \frac{10^{-10}}{([O_2]_i + a)^b} \right) \qquad [5.3]$$

Diffusive transmission rate of O_2:

$$r_{O_2} = \frac{([O_2]_e - [O_2]_i) * A^{fruit}}{R_{O_2}} \qquad [5.4]$$

$[O_2]_i$ in the steady state:

$$A^{fruit} \frac{([O_2]_e - [O_2]_i)}{R^{total}} = \frac{rr_{O_2}^{max} [O_2]_i}{(K_m + [O_2]_i)} \qquad [5.5]$$

$[O_2]_i$ in the steady state:

$$[O_2]_i = \frac{\left([O_2]_e - K_m - R_{O_2}^{total} \frac{rr_{O_2}^{max}}{A^{fruit}} + \sqrt{\left([O_2]_e - K_m - R_{O_2}^{fruit} \frac{rr_{O_2}^{max}}{A^{fruit}} \right)^2 + 4K_m [O_2]_e} \right)}{2} \qquad [5.6]$$

$[CO_2]_i$ in the steady state: [5.7]

$$[CO_2]_i = \left(\frac{R_{CO_2}^{total}}{A^{fruit}} \right) RQ^{\infty} rr_{O_2}^{max} \left(\frac{[O_2]_i}{(K_m + [O_2]_i)} + \frac{10^{-10}}{([O_2]_i + a)^b} \right)$$

Total resistance of a fruit:

$$R^{total} = \frac{R^{pores} * R^{cut}}{R^{pores} + R^{cut}} \qquad [5.8]$$

Skin resistance through pores:

$$R^{pores} = \frac{\left(R^{pores} + R^{coat}\right) * A^{fruit}}{A^{pores}} \qquad [5.9]$$

Skin resistance through cuticle:

$$R^{cut} = \frac{\left(R^{cut} + R^{coat}\right) * A^{fruit}}{A^{cut}} \qquad [5.10]$$

$[CO_2]_i$, r_{CO_2} and R_{CO_2} :

$$[CO_2]_i = \frac{r_{CO_2} * R_{CO_2}}{A^{fruit}} \qquad [5.11]$$

Total respiratory gas concentration:

$$[O_2]_i + [CO_2]_i = [O_2]_e - \frac{r_{O_2} * R_{O_2} - r_{CO_2} * R_{CO_2}}{A^{fruit}} \qquad [5.12]$$

Total respiratory gas concentration:

$$[O_2]_i + [CO_2]_i = [O_2]_e - \frac{\left(r_{O_2} * \left(R_{O_2} - R_{CO_2}\right)\right)}{A^{fruit}} \qquad [5.13]$$

Rate of CO_2 evolution from a fruit:

$$r_{CO_2} = [CO_2]_{jar} * 60 * fr * \frac{1000}{wt} \qquad [5.14]$$

$[O_2]_i$ from Fick's law:

$$[O_2]_i = [O_2]_e - \frac{r_{O_2} * R_{O_2}^{fruit}}{A^{fruit}} \qquad \textbf{[5.15]}$$

Chapter 1

Introduction

1.1 Importance of squash export for New Zealand

Butter cup squash (*Cucurbita maxima* D. hybrid 'Delica') has become an important crop for New Zealand. The trade has grown rapidly since 1979, when only 400 tonnes were shipped to Japan, to about 60,000 tonnes (worth 40 million $NZ) in 1991. Squash is transported by sea between January and March, which allows its arrival on the Japanese market in a period free both from competing squash produced in Japan and those from other countries. Marketing squash that is ordinarily out of season in Japan can produce large economic benefits to growers, distributors and retailers.

1.2 Problems during transportation

1.2.1 Fungal development

An early problem encountered by the export trade was the variable occurrence of fruit rots during consignments. Studies during that period by Beever et al. (1983, 1984) showed that high (>95%) relative humidity during storage was conducive to development of rots. This resulted in development of the present shipping method in which fruit are transported in "door off" containers (open containers which consist of a solid floor and ends with open sides and top which are covered with tarpaulins)

stowed on deck. Despite provision of improved shipping conditions, apparently random development of high levels of decay continued to be a problem. In some years, 25 percent of the squash has arrived in Japan showing some sort of rot, with some individual consignments being up to 100 percent rotten (Wood, 1983).

1.2.2 Weight loss during transportation

The other problem is weight loss of squash during shipping. Squash can be expected to lose approximately 8 percent of their weight during the six week period from harvest to arrival in Japan. Bins of export squash are expected to contain no less than 500 kg of fruit on arrival in Japan, so are packed approximately 40 kg overweight. The need to pack extra squash to make up this 8 percent of weight loss represents a substantial cost to the industry.

1.3 Potential strategies to reduce rot problem

Harvested fruits are subject to dramatic reductions in disease resistance as they mature and ripen. Respiration continues and the only available nutrients are those stored within the fruit itself so that prolonged storage results in breakdown of the fruit's essential structure and function and a decrease in fruit resistance to postharvest pathogens (Conway, 1989).

Many postharvest storage disease problems arise as quiescent or latent infections that are held in check until the fruit matures. Others occur as a result of injuries during

harvesting and handling operations and subsequent infection from fungal spores infesting the fruit surface. They remain inactive until the fruit becomes susceptible to rots or storage conditions become suitable for fungal development. These fungi, having bypassed the first layer of defence of skin of fruit, may secrete enzymes that macerate or break down the cell walls of the organ (Prusky et al., 1982; Verhoeff, 1974).

1.3.1 Low temperature storage

Rot caused by postharvest pathogens is primarily controlled by manipulating the environment so as to make it less conducive fungal growth. Generally, the development of the pathogen on fresh fruit can be suppressed by storage at temperatures just above freezing point, but this cannot be applied for control of rot in squash, because it would suffer chilling injury if stored below 12°C for more than a few days (Inaba, 1993).

1.3.2 Reduction of damage

Harvested fruit with severe visible wounds which provide pathogens with invasion sites are easily identified and can be discarded outright. Other fruit within the same loads may also be damaged, but the wounds are not readily seen. During subsequent storage prior to sale, these fruit undergo changes (ripen and become less resistant to pathogens) and can be decayed by pathogens (Sommer, 1982; Wood, 1983). Any small injury from rough handling, knife wounds or dashing squash against the sides

of bins are wounds that can become sites of rots that can develop later on during transport (Hawthorne, 1989; Wood, 1983). Pathogens causing rots enter through such breaks in the skin. Healing the cut surface of the peduncle and the mechanically wounded skin tissue in squash by curing involving the process of lignification has been commercially practised in Japan (Hyodo, 1992). Wood, (1983) recommended that squash should have 5 days curing to allow time for the skins to harden, the stalks to dry and encourage rapid wound healing.

1.3.3 Crop management

The heavy use of irrigation and a heavy dense canopy of foliage makes humid conditions underneath; high nitrogen levels tend to give excessive foliage and a very humid atmosphere, which produces ideal conditions for fungi to infect the fruit (Hawthorne, 1989; Wood, 1983) These fungi may not be visible at harvest but develop when given suitable conditions. For this problem, less nitrogen and irrigation, less growth of foliage, produce a lower humidity atmosphere around squash but size of individual squash may be smaller (Wood, 1983).

1.3.4 Reduction of inoculum

In pear (Coyier, 1970) and peach (Luepschchen et al., 1971), preharvest sprays of fungicides are effective in preventing rots in the field, reducing the inoculum level in the field and most importantly, minimising quiescent infections that give rise to rotten fruit in the market. While squash (*Cucurbita maxima)*, Hawthorne (1989)

showed that some fungicide treatments applied in the field did reduce the incidence of storage rots but the effect was inconsistent between years and consequently spraying is unlikely to be a cost effective means of control.

Some fruit are treated with fungicides after harvest, to prevent the development of storage rots, with dips sprays or wax formulations of fungicides in pears (Spotts, 1984), apple (Vir, 1979) and Zucchini (Temkin-Gorodeiski, 1970). However, application of fungicides after harvest are not recommended for any horticultural crops in Japan although the techniques have been shown to considerably decrease decay rates of imported fruit (Inaba, 1993).

1.3.5 Calcium

There are no practical chemical controls for many fungal pathogens which have developed resistance to commonly used chemicals. Conceptually, the strategy of controlling diseases of horticultural produce with treatments that do not contaminate the produce with pesticides has a great appeal to consumers and politicians. Therefore research for natural mechanisms to increase resistance of horticultural produce to pathogens must be beneficial for future trade of horticultural commodities.

There is now considerable evidence that resistance of harvested produce to fungal rots has a positive relationship with calcium concentration of those produce (Conway and Sams, 1987; Hardenburg and Anderson, 1981; Little et al., 1980; McGuire and Kelman, 1984), and this method is utilization of a natural internal mechanism.

Low calcium status has been associated with pathological problems of harvested horticultural produce. Calcium has been used to enhance retention of natural disease resistance in plant tissues (Conway and Sams, 1983; Hardenburg and Anderson, 1981). Calcium plays a special role in maintaining the cell wall structure of horticultural produce by interacting with pectic acid in cell walls to form calcium pectate; those treated with calcium are generally firmer than non-treated ones (Poovaiah, 1986). This might be because calcium strengthens cell walls, making them more resistant to breakdown by rots. Therefore it may be possible to develop postharvest technology to reduce storage loss from fungal rots without causing any concern of pesticides. Whilst various roles of calcium in resistance to pathogens have been studied, controlling mechanisms of enhancing calcium concentration in horticultural produce are just beginning to be unravelled. Here, in chapters 2 and 3, the potential manipulation of calcium concentration of squash and its efficiency against fungal rots of squash are discussed.

1.4 Potential strategies to reduce weight loss of squash

1.4.1 Weight loss

Most fruits and vegetables contain between 80 and 95 % water by weight (Hardenburg et al., 1986), some of which may be lost by transpiration. Loss of water by transpiration from harvested horticultural produce is a major cause of weight loss. Some weight loss is due to loss of carbon in respiration, but this is usually only an important cause of weight loss where produce is in a high relative humidity

environment.

1.4.2 Water loss

There are two ways by which water loss can be minimized - reduction of water vapour pressure deficit (WVPD) between the produce and its environment (the air surrounding produce), and to increase the skin resistance of produce to transfer of water or water vapour.

Rate of water loss from produce can be expressed mathematically as

$$r_{H_2O} = K * WVPD \qquad [1.1]$$

where r_{H2O} = rate of water loss from a produce (% fresh weight day $^{-1}$); K = transpiration coefficient (% day^{-1} k Pa^{-1} Gaffney et al., 1985; Sastry, 1985); *WVPD* = deficit between water vapour pressure inside a fruit and that of external air. K is dependent on the structure and composition of the fruit surface, and therefore is usually treated as constant under all conditions for a given type of produce (Sastry , 1985). Therefore, for a given fruit, variation in r_{H2O} is determined by variation in WVPD. WVPD is affected by both relative humidity (RH) and temperature. The difference in vapour pressure causes water vapour movement from produce to the external air. Capacity of air to hold water increases as the temperature rises; hence, air at 90% RH at 10°C contains more water by weight than air at 90% RH at 0°C. Nevertheless, water would be lost from produce in temperature equilibrium with its environment at about twice the rate at 10°C than at 0°C if RH is 90% in both. Unfortunately, squash need to be stored above 12°C to avoid chilling injury (Inaba,

1993).

On the grounds of water loss alone, it would be possible to suggest desirable storage RH is 100% for squash, but if squash is given such a high RH at temperatures above 12°C, it will probably provide pathogens with suitable conditions for developing rots. Beever et al. (1983, 1984) showed that high (95%) RH during storage was conducive to development of rots.

1.4.3 Coating

Since K in Eq.1 (transpiration coefficient) is inversely related to skin resistance to water diffusion, water loss can be reduced by increasing skin resistance to water or water vapour.

surface coatings can act as an additional resistance to water vapour. The degree to which the rate of water loss is reduced is dependent on the resistance of the coating to water vapour. Much research on coating horticultural products has been done (Ben-Yehoshua et al., 1985; Elson et al., 1985; Erbil and Muftugil, 1986; Kester and Fenema, 1986) which has established that coatings on horticultural produce reduce water loss. Reduction of rate of water loss of each commodity depends on nature of both coating and produce.

Rate of water loss has a significant relationship with the type of protective tissue on the exposed surface and with the area of exposed surface per unit volume. Coating application can also influence gaseous exchange by blocking pores of produce. The consequent physiological effects may or may not be desirable - there can be some

reduction of respiration rate, but there may also be anaerobic respiration and elevated ethanol and acetaldehyde contents (Cohen et al., 1990; Drake et al., 1987; Risse et al., 1987). Even if desirable effects can be obtained, they might not be achieved with certainty because of the difficulty in obtaining a complete and uniformly thick cover (Burton, 1982). The desired effects of reducing water loss substantially whilst having only small effects on the internal atmosphere, may be quite difficult to achieve. Coating treatments which achieved substantial reduction in water loss were sometimes associated with the fruit becoming anaerobic. Inherent variability in skin resistance to gas diffusion and fruit respiration rate, and differing proportion of pores blocked by coating, appear to be responsible for the highly variable response of individual fruit to a given coating treatment (Banks et al., 1993).

In assessing the potential for using surface coating for reducing weight loss of harvested squash, I therefore also investigated their effects on internal atmosphere of fruit.

1.5 Postharvest technology for New Zealand squash export

The reduction of losses in harvested fruit has been a major objective of efforts by New Zealand horticultural industry. It is common for fresh fruit produced in New Zealand to be marketed at great distances from New Zealand due to its situation. Transportation of fresh fruit to distant markets may offer large economic benefits only if the life of fruit is stretched to its limit, near the end of its physiological life. Postharvest loss of fruit by processes such as disease development and weight loss

ordinarily manageable during handling may be excessive when transoceanic marine transport of longer duration is involved.

There is economic necessity for reducing postharvest losses and extending storage life of fresh fruit, because their value increases several times when they are transported from the field to distant markets due to the added cost of sorting, packing, storage and transportation, which may exceed production costs by far.

It is clear that present losses justify a substantial increase in the investment of intellectual and financial resources to improve understanding of their causal factors, methods of control of disease incidence and weight loss and to produce a new technology for preventing losses.

Chapter 2

Literature review: Role of calcium in resistance to diseases

2.1 Introduction

Many postharvest storage diseases occur as quiescent infections that are held in check until the fruit matures (Prusky et al., 1982; Verhoeff, 1974). The cause of these diseases, fungi, secrete enzymes or break down the cell walls of the fruit after they penetrate the skin, which results in decayed fruit. If disease resistance in the host can be enhanced by strengthening or stabilizing the cell walls, making them more resistant to breakdown by macerating enzymes, losses due to decay may be reduced. Calcium helps regulate the metabolism in apple fruit, and adequate concentrations maintain fruit firmness and lower the incidence of such disorders as water core, bitter pit, and internal breakdown (Bangerth et al., 1972; Manson et al., 1975; Reid and Padfield, 1975). Ripening generally is delayed when calcium is increased, and fruit maintain their quality longer.

Enhancement of calcium status could be used to reduce postharvest losses and improve the quality of stored produce by natural mechanisms of resistance to pathogens. In this chapter, a number of possible interactions that can affect calcium uptake and distribution, and the relationship between calcium content of host and its resistance to pathogens are reviewed.

2.2 Physiological functions of calcium

Calcium is a normal constituent of the cell wall and middle lamella. It is essential for structure and function of cell walls and membranes. Poovaiah (1986) showed that calcium maintains cell wall structure in fruit and other storage organs by interacting with the pectic acid in the cell walls to form calcium pectate. Thus, fruit treated with calcium are generally firmer than controls. Membrane stabilization results from the Ca ion bridging phosphate and carboxylate groups of phosphlipids and proteins at membrane surfaces (Legge et al., 1982). In the absence of calcium, the membrane becomes leaky and solutes are lost from the cytoplasm. Typical features of senescence, for example, are similar to those of calcium deficiency and can be retarded by additions of Ca^{2+}. These features include a breakdown in the compartmentation of the cell and an increase in respiration following the leakage of endogenous respiratory substrates from vacuole to the respiratory enzymes in the cytoplasm (Bangerth et al., 1972).

2.3 Relationship of calcium to plant disease resistance

2.3.1 Effect of calcium on plant disease

Certain physiological disorders and diseases of storage organs such as fruit and vegetables are related to the calcium content of their tissues (Shear, 1975). Calcium seems to be closely related to improved disease resistance. Preharvest sprays of $Ca(NO_3)_2$ on apples reduced storage loss due to decay caused by *Gloeosporium*

perennans (Sharples, 1976). Increasing the calcium content of potato tubers also reduced storage losses (Kelman et al., 1989). Calcium content was significantly increased by field fertilization or postharvest vacuum infiltration with $CaSO_4$ or $Ca(NO_3)_2$ and storage losses caused by *Erwinia carotovora* were reduced significantly (McGuire and Kelman, 1984). In a study on the effect of increased flesh calcium content of apples on storage decay caused by *Penicillium expansum*, fruit were treated with solutions of $CaCl_2$ by dipping or vacuum or pressure infiltration. The fruit did not take up enough calcium in the dipping treatment to affect severity of decay, but both vacuum and pressure infiltration increased calcium content of the fruit sufficiently to reduce decay significantly (Conway, 1982). Peaches were sprayed preharvest or pressure infiltrated postharvest with solutions of $CaCl_2$ to increase calcium content (Conway et al., 1987a). Both treatments increased calcium content sufficiently to reduce decay caused by *Monilinia fructicola.*

2.3.2 Relationship of calcium with cell wall

The relationship between calcium and cell wall has been shown to play a key role in disease resistance. Calcium ions are bound to the pectins present in the cell wall (Demarty et al., 1984) There is a high affinity of the carboxylic groups of pectic materials for calcium ions, and the resulting effect on physiological or pathological processes is greater than for other cations present in plant tissues. The middle lamella exists as a gel and calcium very efficiently promotes gel formation in a pectic solution (Tepfer and Taylor, 1981). When ion exchanges are performed in the cell walls, either between calcium and monovalent cations or between calcium and

magnesium, the cell walls have always exhibited a marked preference for calcium (Demarty et al., 1984). Pectins are composed of chains of polygalacturonic acid residues with rhamnose insertions that cause marked kinks in the chain (Preston, 1979). The resulting bunched configuration of the polygalacturonic chain allows spaces for the insertion of a series of cations, all of which may be filled because the binding of one ion causes chain alignment that facilitates the binding of the next (Grant et al., 1973). Cation bridges between pectic acids or between pectic acids and other acidic polysaccharides hinder accessibility to enzymes produced by the fruit that cause softening and to enzymes produced by fungal or bacterial pathogens that cause decay.

2.3.3 Effect of calcium on macerating extract of diseased tissue

In a study by Conway et al. (1987b), calcium applied by pressure infiltration into 'Golden Delicious' apples became bound to cell walls. Cell walls of control fruit contained a calcium concentration of about 0.012 mol kg^{-1} (0.055%) and increased to 0.05 mol kg^{-1} (0.190%) at the highest level of applied calcium. As the amount of calcium in the solution increased, the percent increase of calcium in the cell wall decreased. When 'Golden Delicious' apples inoculated with spore suspensions of Penicillium expansum 6 months after pressure infiltration with 4% $CaCl_2$, the relative effectiveness of the increased calcium in the cell wall increased as the spore numbers in the inoculum decreased. Decay was reduced 52, 37 and 28% with 10^4, 10^5, and 10^6 conidia per millilitre, respectively, in fruit treated compared with controls (Conway et al., 1992).

Conway et al. (1988) purified polygalacturonase from the decayed tissue of untreated apples that had been inoculated with Penicillium expansum, and used cell wall samples from apples which were pressure infiltrated with $CaCl_2$ and with varying calcium content as substrate for P.expansum polygalcturonase to test the effect of cell wall calcium on enzyme activity. Significantly less product, in the form of uronic acid, was released from high-calcium cell walls than from low-calcium cell walls. Since calcium is known to stabilize the cell wall, this indicated that reduction in decay caused by P.expansum was due to a decrease in maceration of cell walls by polygalacturonase, presumably following improved structural integrity proceeding from an increase in calcium content.

Calcium-induced resistance in apples to postharvest fungal pathogens is broad in spectrum. Storage losses caused by Gloeosporium spp. in apple fruit that had been sprayed before harvest with $CaCl_2$ were lower than in untreated fruit (Sharples and Johnson, 1977). Endogenous calcium in apples added by postharvest pressure infiltration with $CaCl_2$ reduced decay caused by Botrytis cinerea and Glomerella cingulata (Conway et al., 1991). Since both B. cinerea and G. cingulata produce polygalacturonase (Wallace et al., 1962), calcium may inhibit the activity of polygalacturonase produced by these fungi, either directly or by stabilizing the cell wall of the host and making them more resistant to breakdown. Calcium however, may differentially inhibit polygalcturonase produced by various pathogens. Differential inhibition of pectolytic enzymes is well known among host protein inhibitors of fungal enzymes (Brown, 1984). Polygalacturonase produced by P. expansum, a highly aggressive pathogen of apple fruit, is the least affected by these

inhibitors, whereas polygalcturonase produced by G. cingulata, a relatively weakly aggressive pathogen, is most inhibited. This seems to be the order in which supplemental calcium inhibits the pathogenicity of these pathogens in apples and similar results have been found for pear fruit sprayed with $CaCl_2$ before harvest (Sugar et al., 1988). Decay caused by P. expansum was not affected by these sprays, while that caused by Phialophora malorum was significantly reduced.

Potato tubers (cv. Superior) were produced with high and low concentrations of medullar calcium (McGuire and Kelman, 1984). They found when tubers were inoculated with serial dilutions of the bacterial pathogen, the relative effectiveness of calcium in reducing decay was increased as inoculum decreased. Injection with a partially purified culture filtrate from Eriwinia carotovora atroceptica with pectate lyase and polygalcturonase activity resulted in decay as with bacteria. Maceration diameters were consistently higher in low-calcium tubers than in those having high medullar calcium. Mixing tuber cell walls and a purified pectate lyase produced significantly slower release of uronides from high-calcium walls than from low-calcium walls (Maher et al., 1986).

The susceptibility of potato tubers to bacterial soft rot has been associated with tissue membrane permeability and electrolyte loss (Kelman et al., 1989). When peel and medullar tissues were immersed in solutions containing polygalacturonase and pectate lyase activity, the rate of electrolyte loss was greater from low-calcium tubers than from high-calcium tubers. Calcium is thought to be effective by binding anionic groups of all membranes to form bridges between structural components, thereby

maintaining selective permeability, structural integrity, and cellular compartmentalization.

2.4 Calcium transport into fruit

2.4.1 Calcium transport with transpiration

The level of control of disease resistance in stored fruits and vegetables by calcium is mostly related to the amount of calcium taken up by fruit when on the plant (Clarkson, 1984; Lang, 1990). Calcium is moved largely in the xylem and only to a very limited extent in the phloem. Movement of calcium in the xylem is preferentially toward meristematic and transpiring tissues. Plants have limited ability to regulate calcium distribution between fast growing tissues such as leaves and the low transpiring organs such as fruits (Clarkson, 1984). Translocation of calcium to any organ depends on the existence of a transpiration stream to that organ, and therefore on the existence and behaviour of stomata on that organ (Allaway, 1976). A number of physiological disorders of fruits correlated with calcium deficiency or imbalance in the whole plant are thought to be more strongly expressed in fruit since transpiration is low in maturing fruit (e,g. blossom end rot in tomatoes; Wiersum, 1966). There is a close correlation of calcium transport with transpiration rate. The long distance movement of calcium in the xylem is to a considerable extent one of mass flow, calcium movement following the upward direction of the transpiration stream. Older leaves, and tissues, therefore, have a much higher calcium content than younger tissues (Allaway, 1976). Apple leaves may contain 0.2-4.0% of calcium per

unit dry weight, whereas the internal concentration of calcium in fruit may range from 0.015-0.030% dry weight (Conway and Sams, 1983).

2.4.2 Calcium transport with xylem

In contrast to the xylem sap the concentration of calcium in phloem sap is extremely low (Mengel and Haeder, 1977). The reason for the minute concentration of calcium in phloem sap is not clear. Epstein (1973) is of the view that the exclusion of Ca^{2+} from sieve tubes is part of a development process whereby the sieve tubes obtain a relatively structureless condition and this allows the conduction of a flowing solution. Marschner (1974) suggests the presence of calcium specific efflux pumps in the membranes of the sieve elements. This is in agreement with the relatively high concentrations of calcium in the area of the phloem strands (Wieneke, 1979). The relatively low concentration of calcium in the phloem means that organs which are mainly supplied with phloem (leaf tissue, fleshy fruits, storage roots or tubers) are likewise low in calcium. Most of calcium has to be translocated via the xylem to meet the demand of these growing tissues. The calcium supply is correlated with the amount of water entering fruit by means of xylem.

2.4.3 Variation of calcium transport

There can be large variability of calcium concentration between individual fruit of the same cultivar. Davenport and Peryea (1990) reported the variability of calcium concentration (fresh weight basis) in the different fruit of the same cultivar analyzed

in the whole fruit of Delicious apple were 0.008±0.003 mol kg in control, 0.009±0.004 mol kg^{-1} with $CaCl_2$ sprayed 5.9 kg ha^{-1} year. Calcium concentration (dry weight basis) of 'Kent' strawberries without treatment at harvest was 0.07±0.001 mol kg^{-1} (Cheour et al., 1991).

In kiwifruit calcium concentration (dry weight basis) at maturity (Brix 6.2%) were 0.117±0.008 in skin and 0.05±0.014 mol kg^{-1} (Clark and Smith, 1988). Usually variability of individual fruit in the same cultivar developed in response to a number of factors. Variation in the speed of growth of each fruit governs balance of proportion between phloem flow and xylem flow into fruit. Position of a fruit in a tree can influence its transpiration. For example, a fruit growing in more humid condition (ie. in the shade of many leaves) has less transpiration than a fruit in dry condition (a fruit relatively exposed to the sun light). This increases calcium flow into a fruit in the exposed condition. Location of a fruit also affects relation between tree water potential (governed by foliar transpiration) and fruit water potential (governed by fruit skin transpiration) which influence Ca uptake in apple fruit (Lang, 1990) and has even been reported to cause withdrawal of Ca from fruit (Landsberg and Jones, 1981).

2.5 Methods to increase calcium content in a fruit

2.5.1 Calcium application by fertilization

Fertilization and liming cannot guarantee increasing the calcium content of fruit by the required amount (Bain and Robertson, 1951). This is due to an inefficient

distribution of calcium within the plant rather than poor calcium uptake by the plant, since leaves usually have a much higher calcium concentration than fruit on the same plant.

2.5.2 Direct application of calcium

The direct application of calcium to fruit seems to be a more efficient method for increasing calcium in fruit. The calcium content of apples has been increased by foliar sprays (Drake et al., 1987). In studies comparing treatment of 'Delicious' and 'Golden Delicious' apples with solutions of $CaCl_2$ by dipping, vacuum infiltration, or pressure infiltration, the calcium concentration of fruit flesh was increased the most using pressure infiltration, followed by vacuum infiltration, with dipping being a distant third (Conway, 1982).

2.5.3 Calcium application by enhancement of natural mechanism

Martin and Stott (1957) showed that the drying of the sultana grape by dipping in alkaline solution is controlled by the rate at which water diffuses through the fruit's waxy cuticle. Alkaline solution was thought to cause the wax platelet of grapes to become reoriented and become adpressed to the berry surface. Radler (1965) reported that dipping solution (2.5% potassium carbonate) had very little effect on increasing drying rate of grapes after removal of the soft wax fraction (soft wax consist of long chain alcohols, esters, free acids and hydrocarbons, which is readily removed by petroleum, and a hard wax consist of mainly of oleanolic acid which is removed by

chloroform) of the cuticles with petrol or the total removal of cuticular wax. He suggested the soft wax fraction prevents water loss from the fruit probably acting simply as a hydrophobic layer that repels water both from within and without, and the addition of lipids (fatty acids) with hydrophillic groups to the cuticular wax establishes a hydrophillic link between the hydrophobic surface of the grape and its watery contents, thus facilitating the flow of moisture through the cuticle. Possingham (1972) reported that the reorientation decreases the distance between adjacent platelets and almost certainly reduces the effective thickness of the wax layer. It has the effect of bringing the hydrophillic groups of overlapping platelet into more intimate contact with each other, and this change may facilitate the movement of water from the grape to the atmosphere.

During and Oggionni (1986) reported that grape berries treated with $Na_2(CO_3)$-olive-emulsion promoted transpiration of the berries, and the calcium content increased faster than untreated berries. In their study, calcium accumulation in berries and transpiration rate of these berries were closely correlated. Increased rate of transpiration (drying of fruit) could increase the amount of calcium which flows into fruit by the treatment with sodium carbonate. Tullberg and Minson (1978) studied that field drying rate of alfalfa treated with a potassium carbonate solution approximately doubled over the untreated control. Other studies reported an increased field drying rate for alfalfa hay with application of potassium carbonate or potassium carbonate-sodium carbonate solutions, proposing that application of drying agent acts to solubilize or breakdown the cutin layer (Nocek et al., 1986). From these studies above, it can be recognised that alkaline solution emulsion could increase evaporation through skin of both fruit and leaves.

Hartley et al., (1982) showed a different result of their study of Lucerne (alfalfa) from other studies, which indicates that cell-level action of potassium ions are unlikely to affect superficial waxes but interferes with the tight closing of stomata which is their normal reaction to wilting. Many stomata in leaflets treated with potassium carbonate remained slightly open even when 14% of their initial water content had been lost. This suggested that a small interference with stomatal closure could increase the rate of drying in treated leaflets of Lucerne. This effect is likely to be less important in fruits than in leaves, in which the stomatal density is orders of magnitude higher.

2.6 pH effect on pathogens

There were some observations of an inhibitory effect of elevated pH on disease control. Punja and Grogan (1982) found that ammonium salts, carbonate and bicarbonate salts were fungicidal to *Sclerotium rolfsii* apparently due to the prevalence of free NH_3, CO_3^{2-} and HCO_3^- respectively, at the high pH. Arimoto et al. (1977) reported that $NaHCO_3$ had an inhibitory effect on rice blast, citrus melanose and cucumber powdery mildew. Homma et al. (1981a) found $NaHCO_3$ had a control effect against citrus penicillium decay and cucumber powdery mildew. Arimoto et al. (1976) described the inhibitory effect of $NaHCO_3$ on citrus common green mold due to the alkaline condition. Therefore, it might be expected that Na_2CO_3 has potential to control diseases on squash fruit such as powdery mildew.

2.7 Prospect of use of calcium in resistance of squash to a postharvest rot problem

Postharvest losses due to infections by microorganisms can be prevented or reduced by several means. Commonly used practices are proper harvesting and handling of materials to minimize infection of susceptible plant parts and treatment with chemicals (Helgeson, 1989). Low temperature management is central to modern postharvest handling systems, which slows fungus development (Sommer, 1982). However, squash should be stored at above 12°C to avoid chilling injury (Inaba, 1993), and postharvest use of fungicide for horticultural produce is not recommended in Japan which is the main market of squash (Inaba, 1993).

The incorporation of resistance in the host to the potential pathogens provides a valuable alternative approach. This way may possibly eliminate the need for fungicide treatment or low temperature treatment. Increasing the resistance of storage organs to rot by elevating the tissue concentration of calcium enables use of a natural, internal mechanism of resistance of fruit (Conway, 1989). Loss due to rot may be reduced if calcium concentration in the fruit at harvest is above a certain threshold to prevent fruit rot from developing (Monselise and Goren, 1987).

There are a number of possible interactions that can affect calcium uptake and distribution as addressed in this review, there is no established practice that can manipulate calcium distribution within a plant, without a direct application of calcium to the susceptible organ (Sharples, 1976). Therefore, development of a practical technique to manipulate calcium distribution in a plant which may enhance resistance

of a fruit to pathogens is in much need. A new approach to this problem forms the basis for the study in Chapter 3.

Chapter 3

Relationship between calcium contents and fungus development in squash

3.1 Seasonal changes of mineral contents of squash fruit

3.1.1 Introduction

Mineral nutrient composition has been shown to be important in the subsequent storage behaviour of a wide range of fruits (Conway, 1989; Demarty et al., 1984; Sharples and Johnson, 1977) and their resistance to fungal development. (Bateman, 1964; Conway et al., 1988; McGuire and Kelman, 1984; Tepfer and Taylor, 1981). Clark and Smith (1988) suggested that an understanding of how the nutrient content of kiwifruit varied throughout the season would be central to defining optimum conditions for both crop yield and fruit storage quality, and in determining the timing and quantities of nutrient required by the fruit. Thus, the pattern of accumulation of minerals during fruit growth on the plant has important implications for development of strategies to overcome any deficiencies which occur in normal production systems. Since squash (*Cucurbita maxima* D. hybrid 'Delica') is a relatively new crop, there is little basic data on the mineral requirements of the fruit and how individual mineral concentrations change during fruit growth. In this study, I examined the change of mineral concentrations of squash fruit from 3 weeks after pollination until harvest time.

3.1.2 Materials and methods

Fruit were taken from vines of *Cucurbita maxima* ('Butter cup') squash growing at Grocorp orchard in Onga Onga in Hawkes Bay. Thirty fruit at the same growth stage were marked on 21 January 1992. Five fruit were sampled at weekly intervals, beginning 21 January 1992, three weeks after pollination. The final sample was taken at mid-commercial harvest on 24 February. In the laboratory, cortex and skin (peel) samples were separated and freeze dried. The entire fruit skin was removed to a depth of up to 2 mm using a peeler. Cortex tissue samples comprised half of a 2 cm equatorial slice from which the outermost 10 mm of tissue underlying the skin had been removed.

For mineral analysis, samples of 0.1 g dried tissue were individually digested (in HNO_3 for calcium or in Kjeldahl digestion solution for nitrogen) and phosphorus, and analyzed.

To 0.1 g sample (which had been at 60°C for 24 hours, and then cooled in a desiccator prior to weighing), 4ml concentrated nitric acid was added for calcium analysis. Digestion tubes were set in a heating block with small funnels in the top to cause refluxing and heated to 150°C for 4 hours or until solution cleared. Then temperature was turned up to 250°C and funnels were removed. Acid was boiled off to dryness. And then 5 ml 2M HCl was added whilst warm; deionised water was added to make up to 50 ml.

In the analysis of nitrogen and phosphorus, 4ml Kjeldahl digestion solution was added to a sample, then heated to 350°C for 4-5 hours, or until solution cleared.

Distilled water was added to make up to 50 ml. Resultant solutions were analyzed by atomic absorption spectrophoresis Data were subjected to analysis of variance using the linear models procedure of SAS (Littell et al., 1991). Trends with time within tissues were examined on the raw data using orthogonal polynomial contrasts (Ridgman, 1975). For comparing mineral levels between tissues, data were transformed to natural logarithms to overcome heterogeneity in variance between samples.

3.1.3 Results

Calcium concentration in the cortex declined continuously from week 3 to week 8 ($P < 0.001$ for the linear effect of time; Fig. 3.1a) but the rate of decline was greater over the initial period than later on ($P < 0.01$ for quadratic effect of time). Calcium concentrations in skin tissue were about 4 times as high as in the cortex averaged over the entire experimental period ($P < 0.001$) but values for individual samples of fruit tissue were highly variable and showed little consistent change with time (Fig. 3.1b). In this case the polynomial model was inappropriate as there was a significant quartic effect of time, to which it is difficult to ascribe any little biological meaning.

Nitrogen concentration in the cortex declined from week 3 to week 6 and rose from week 6 to week 8 ($P < 0.0001$ for the quadratic effect of time; Fig. 3.2a). Nitrogen concentration in the skin decreased from week 3 to week 6 and slightly increased from week 7 to week 8 ($P < 0.001$ for the quadratic effect of time; Fig. 3.2b).

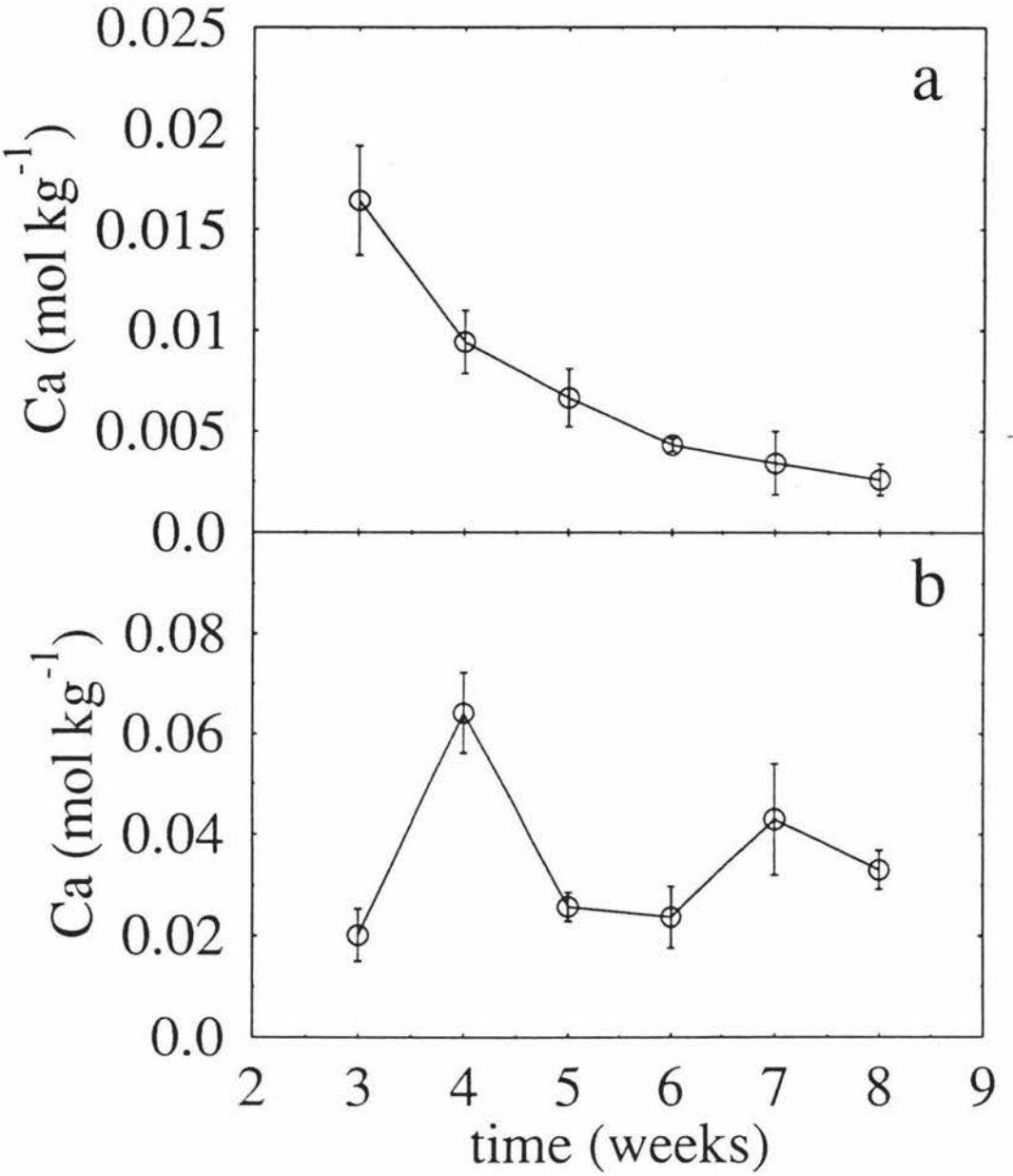


Figure 3.1 Change of total calcium concentration of squash during development from start of flowering in a) cortex, b) skin. Circles represent mean (n = 5 observations each). Vertical bars indicate SE_{mean}.

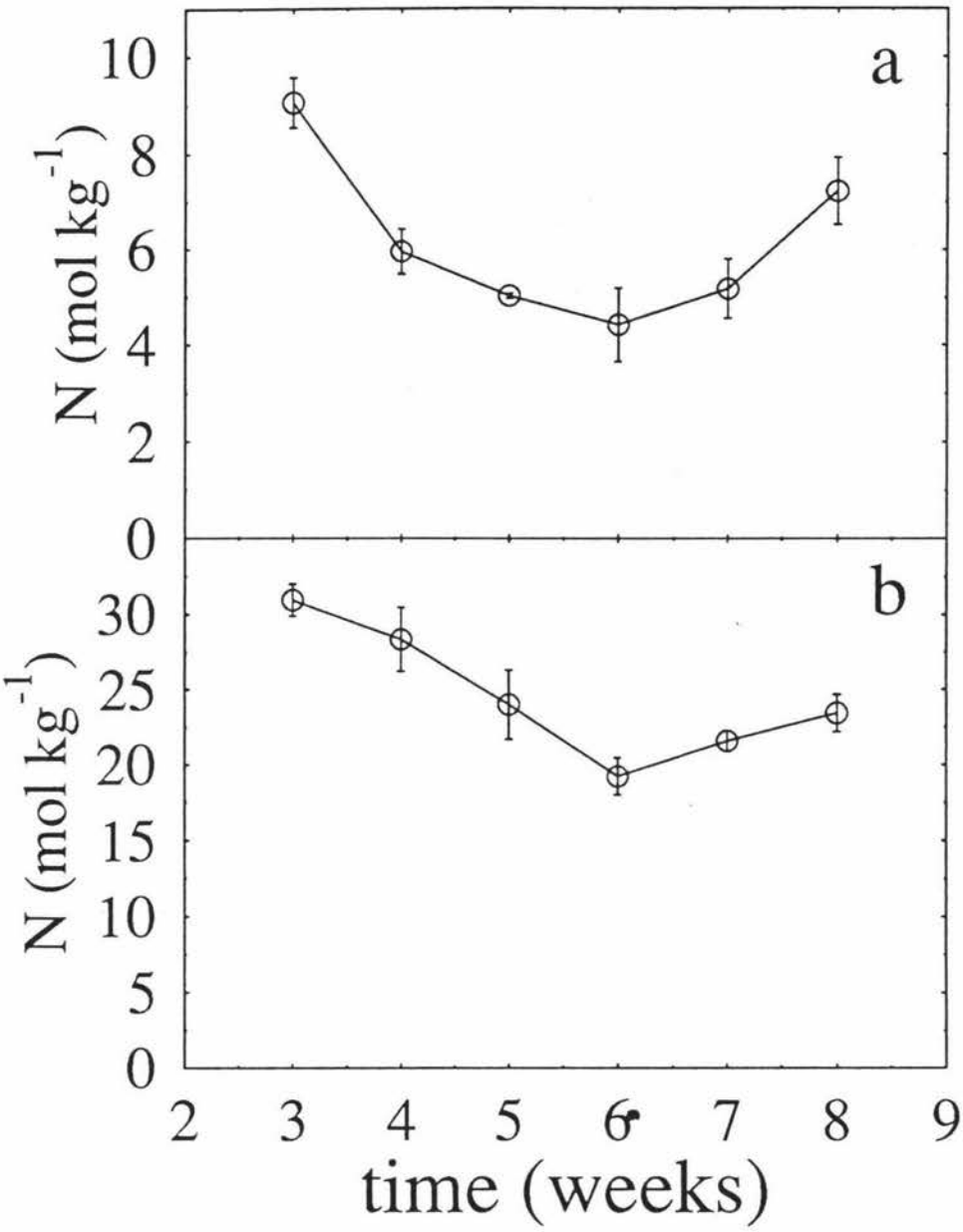


Figure 3.2 Seasonal change of total nitrogen concentration of squash during development from start of flowering in a) cortex, b) skin. Circles represent the mean data (n=5 observations each). Vertical bars indicate SE_{mean}.

Phosphorus concentration in the cortex declined from week 3 to week 6 and increased from week 6 to week 8 ($P < 0.0001$). Phosphorus concentration in skin declined from week 3 to week 5, and it rose from week 5 to week 6, then remained at the same level ($P < 0.01$ for quadratic effect of time; Fig. 3.3), but change of phosphorus concentration from week 4 to week 5 was much greater than the rest of the period.

Both nitrogen and phosphorus concentration in the skin were also 4 times as high as in the cortex ($P < 0.001$).

Fresh weight of the sampled fruit increased from week 3 to week 6 and then remained unchanged from week 6 to week 8 (Fig. 3.4).

3.1.4 Discussion

Calcium concentration in cortex declined from week 3 to week 8. This, as well as decline of nitrogen and phosphorus concentration over weeks 3 to 6, can be attributed to the dilution effect by volume increase of fruit during growth. However, the decline in calcium concentration as time passed indicated that calcium intake to the fruit might cease earlier than those of nitrogen and phosphorus. The same patterns have been found in apple (Wilkinson and Perring, 1964), kiwifruit (Ferguson, 1980) and pickling cucumber (Engelkes et al., 1990). A major proportion of calcium in plants is supplied by way of the xylem (Pate, 1976) which means that its rate of uptake depends heavily on the rate of transpiration. This decline of calcium concentration could largely be a consequence of a reduction of transpiration associated with decrease in the surface area to volume ratio with fruit growth. A similar effect would

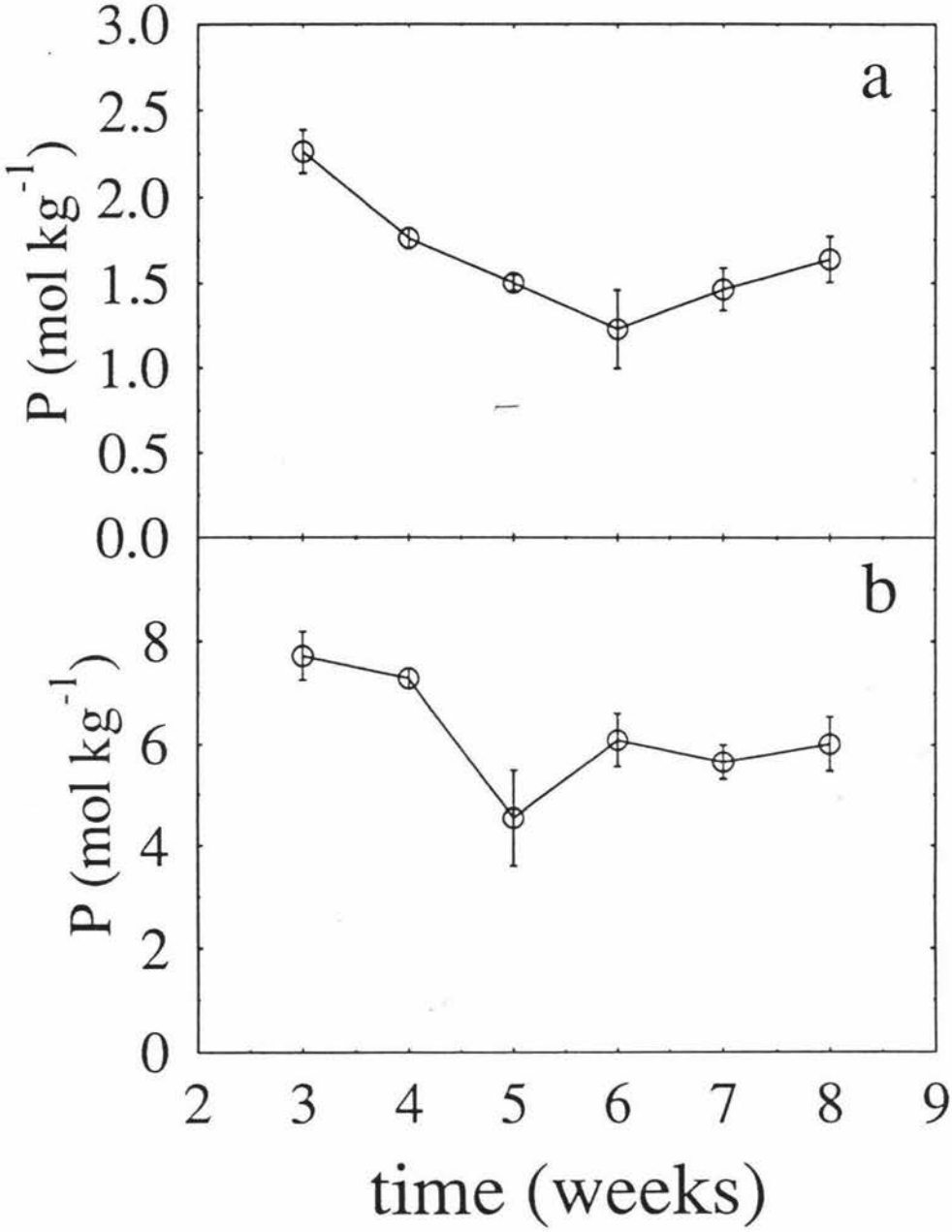


Figure 3.3 Seasonal change of phosphorus concentration of squash during development from start of flowering in a) cortex, b) skin. Circles represent the mean data. Vertical line indicates SE_{mean}.

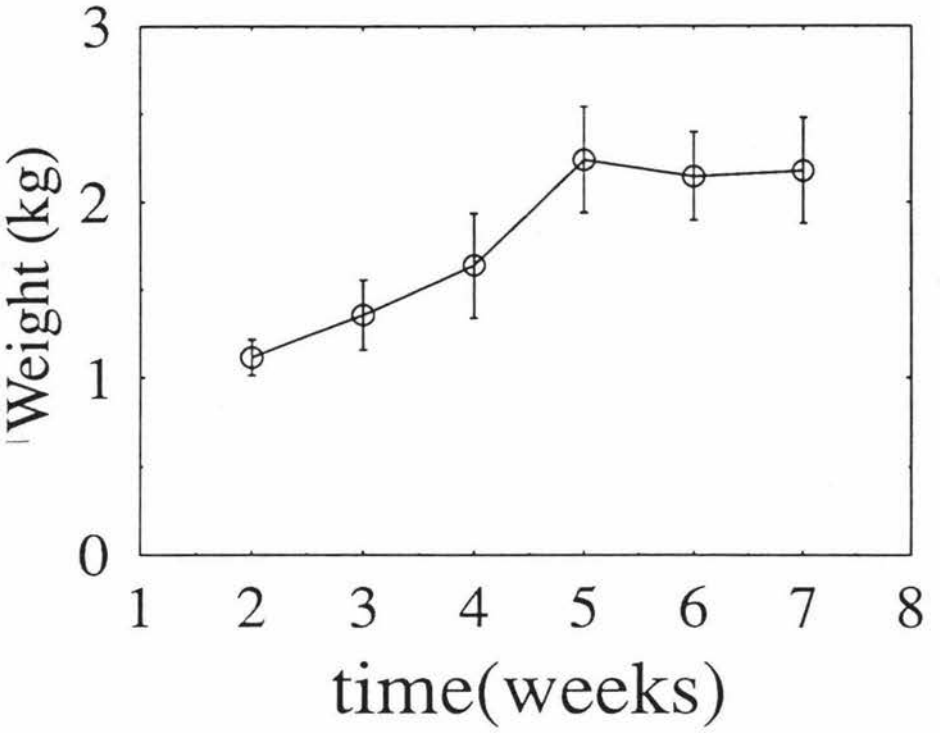


Figure 3.4 Change of average fresh weight of squash during development from start of flowering. Circles represent mean (n = 5 observations each). Vertical bars indicate SE_{mean}.

result from increased resistance of the fruit surface to transpiration loss. An alternative possibility is that there was a shift from xylem to phloem as the major supply route for materials moving into the fruit. A reduction of transpiration of fruit as they mature was suggested in kiwifruit by Clark and Smith (1988). Conversely, nitrogen and phosphorus concentration in both skin and cortex rose from week 6 after fruit ceased their volume development (Figs. 3.2 and 3.3). The major supply route of nitrogen and phosphorus is the phloem, and they are more able to move in phloem than calcium (Pate, 1976). Therefore nitrogen and phosphorus continued to move into fruit and accumulate at a rate above that of volume (dry matter) in the late stage of fruit development. This has also been shown in kiwifruit by Ferguson (1980).

Calcium concentration in skin showed little consistent change with time (Fig. 3.1), but it continued to be significantly higher than that in the cortex from week 4 to week 8. This may be that the water transporting calcium through the tissue evaporates from the surface of a fruit, leaving its calcium behind, while almost calcium in water merely pass as through cortex. It can also be attributed to the difference of water potential gradients as postulated by Engelkes et al. (1990) in cucumber. They suggested that volume flow of water via the xylem into the cortex close to seed cavity is driven by osmotically generated water potential gradients; here, the rate of calcium transport within xylem would be expected to be slow. On the other hand, volume flow of water via xylem into the skin is driven by much higher water potential gradients than that to cortex due to transpiration from skin. Therefore more calcium may accumulate in skin than in cortex.

Nitrogen concentration and phosphorus concentration in both cortex and skin had a

similar trend that they declined from week 3 to week 6 and slightly increased from week 6 to week 8 (Figs. 3.2 and 3.3). These changes in both nitrogen and phosphorus were correlated with changes of fruit fresh weight (Fig. 3.4). Fruit fresh weight increased from week 3 to week 6, which made a dilution effect from volume increase of fruit with steady rates of intake of phosphorus and nitrogen to fruit. The steady rates of intake through the season for nitrogen in kiwifruit (Clark and Smith, 1988), and the same trend for phosphorus in apple (Quinlan, 1969: Haynes and Goh, 1980) were shown. Fruit ceased its volume development (dry matter increase) after week 6, but intake of phosphorus and nitrogen continued. Continuous intake of nitrogen and phosphorus to fruit without further increases in fruit volume would have reduced the dilution effect and increased nitrogen and phosphorus concentration in fruit from week 6 to week 8 (Figs. 3.2 and 3.3). Concentration of both nitrogen and phosphorus in skin was significantly higher than that in the cortex. This might be explained by suggesting that the solution of phloem which transports most nitrogen and phosphorus into a fruit terminated at the surface area (skin) of a fruit where it evaporated. Therefore it would probably leave much of its contents in the fruit surface (skin). Another explanation might be if the imported nitrogen and phosphorus passes first through the pericarp part of the skin (which is just below skin). Cells along the vascular pathway would have first opportunity to absorb these minerals and thus deplete them from the pathway going into the cortex. This may make a significant gradient of concentration of nitrogen and phosphorus between skin and cortex.

3.2 Relationship between fungus development and calcium contents in squash fruit

3.2.1 Introduction

Recently people have become aware that the calcium status of horticultural products is important, particularly for storage of fruit. Status of calcium in fruit strongly influences its postharvest life as explained in literature review (chapter 2). It has been reported (chapter 2) that calcium-related problems in fruit can be regarded as localized deficiencies or imbalances of calcium which result from peculiarities of calcium transport in a plant, and are not necessarily a result of low soil calcium. This means that it may be possible to manipulate the calcium level of fruit by modification of physiological function of plants, especially transport of calcium by water.

Many fungal pathogens have developed resistance to commonly used fungicidal chemicals. Enhancing a plant's natural mechanisms of resistance to pathogens by changing its mineral concentration might reduce dependency of production regimes on fungicidal chemical treatments. As calcium strengthens a plant rather than directly affecting the pathogen, there may be little selection pressure for the development of more aggressive strain of pathogen. Therefore, increase of plant's resistance to pathogens by calcium are of significant importance.

Fruit tissue calcium levels influence growth and quality of a large number of fruits, including cucurbitaceous crops. Frost and Kretchman (1989) showed that fruit with calcium deficiency developed water soaked and necrotic lesions on the epidermis and

pericarp of the distal end of fruit in pickling cucumbers. Adhikari (1980) reported a negative correlation between incidence of misshapen cucumbers and fruit calcium concentration when chlorflurenol was applied to induce parthenocarpic fruit set. Staub et al. (1988) produced the pillowy disorder (styrofoam-like, porous-textured tissue) by culturing cucumbers in a calcium - deficient nutrient solution.

Much more information is available on the role of calcium in the quality of apples, in which it has been shown to affect maintenance of fruit firmness and the incidence of such disorders as water core and bitter pit (Bangerth et al., 1972; Reid and Padfield, 1975). Decay in potatoes (McGuire and Kelman, 1984) and decay by fungal pathogens in apples (Conway, 1982) can also be effectively reduced with applications of calcium. Several researchers have studied the influence of $CaCl_2$ additions on cucumber fruit quality (Buescher et al., 1987; McFeeters, 1986). Unfortunately, increasing the calcium content in fruit tissue to a level that reduces postharvest losses is difficult (Conway et al., 1992). Direct application of calcium to the fruit seems to be a more efficient method for increasing calcium of fruit than fertilizer regimes such as liming practice due to uneven distribution of calcium from roots to fruit and leaves. Since calcium absorbed into plants via their roots is distributed throughout the plant principally with water, it moves upwards in the xylem in the plant and is deposited in plant organs with a large evaporative surface for transpiration, principally the leaves. Ferguson et al., (1987) indicated that early in apple fruit development, the surface to volume ratio of the fruit is high, and thus favourable for the maintenance of a transpiration stream under tension, which is required for xylem water flow. With further fruit development, the surface to volume

ratio of the fruit declines as the fruit expands. The result is a decrease in transpirational pull by the fruit, and water movement and flow of calcium into the fruit by the xylem is reduced. Staub et al, (1988) reported that processing cucumbers grown under high relative humidity produced significantly more fruit with pillowy disorder than those grown in ambient relative humidity. This was thought to be due to reduced calcium translocation into fruit under high RH, which reduced transpiration rate. Gerard and Hipp (1968) reported that the incidence of blossom-end rot in tomato was negatively correlated with the number of hours above a certain temperature or high evaporative conditions which promote input of calcium to the fruit. Similarly, incidence of blossom-end rot in watermelon could be reduced by foliar sprays with calcium chloride or irrigation during highly evaporative weather conditions (Cirulli and Ciccarese, 1981). Thus, the more transpiration a specific plant organ has, the more calcium is transported into it.

Vacuum or pressure infiltration of calcium reduces bacterial decay in potato tubers and fungal decay in apples (Conway et al., 1992). Hewett and Watkins (1991) reported that calcium applications ($CaCl_2$ sprays) over the growing season are superior to postharvest vacuum-infiltration with calcium in the prevention of bitter pit of 'Cox' Orange Pippin' apples, especially internal bitter pit. The reduced effectiveness of vacuum infiltration on internal, compared to external bitter pit is presumably related to the time required for calcium to move from the skin into the fruit and through cortical tissue to potential lesion sites (Ferguson and Watkins, 1989). In commercial practice, control of bitter pit is often less successful than can be shown experimentally, possibly due to incomplete coverage by calcium sprays

(Ferguson and Watkins, 1989).

Lidster and Webster (1983) investigated the possibility of enhancing apple fruit calcium contents by selectively increasing fruit water loss with a surfactant (8% Tween 20) and suppressing leaf water loss with an antitranspirant (8% Vapour Gard) during fruit growth on the tree. They found that Vapour Gard spray to leaves decreased calcium concentration of leaves and also decreased the amount of calcium per apple fruit. Several researchers have found a positive effect of potassium carbonate and sodium carbonate on water loss from grape berries (Grncarevic et al., 1968; During and Oggionni, 1986) and alfalfa (Ziemer et al., 1990). Sodium carbonate application increased transpiration of berry fruit, resulting in increasing calcium concentration of fruit (During and Oggionni 1986). If it was possible to enhance transpiration of squash fruit during growth then fruit calcium contents might be enhanced far more uniformly within the fruit tissue and perhaps also with greater efficiency than with calcium sprays. However, at this point it was unknown whether or not increased calcium contents would be beneficial in reducing susceptibility of squash fruit to fungal infection. The objectives of this experiment in this study were therefore to evaluate the effect of sprays promoting transpiration and transpiration-inhibiting sprays on fruit calcium concentrations and storage life.

3.2.2 Methods

3.2.2.1 Experiment 1

Three spray treatments were applied, one to each of three groups of 200 squash at one week intervals from 21 January to 16 February. All solutions contained 2 ml l^{-1}

Tween 20 to increase their ability to wet the fruit surface. Fruit were harvested on 24 February and stored in a bulk bin covered by a tarpaulin in a shed at ambient temperature (at 12°C to 18°C). Weights of 50 squash of each treatment were recorded at one week intervals from 25 February to 26 April. Rotten fruit were removed each week and sampled for mineral contents. In the laboratory, cortex and skin (peel) samples were separated and freeze dried. The entire fruit skin was removed to a depth of up to 2 mm using a peeler. Cortex tissue samples comprised half of a 2 cm equatorial slice from which the outermost 10 mm of tissue underlying the skin had been removed. Individual samples from each treatment were combined into groups of 24 on the basis of the time at which they showed signs of rotting.

3.2.2.2. Experiment 2

Eighty squash fruit at the same growth stage were randomly selected and divided into 4 groups of twenty at Grocorp's orchard in Onga onga in Hawkes Bay. Each fruit was painted (using a paintbrush) with a treatment solution on one side whilst the other half was left as a control for each of 4 solutions at weekly intervals from 21 January to 16 February. All solutions contained 0.2% Tween 20 to increase their ability to wet the fruit surface. The solutions were as follows: Water (control), 20 g (0.2 mol) l^{-1} sodium carbonate (Na_2CO_3) plus 20 ml l^{-1} olive oil, 20 g (0.19 mol) l^{-1} of calcium chloride ($CaCl_2$), or an antitranspirant (2 % Vapour gard). All fruit were harvested 24 February and each half of fruit was analysed for calcium, nitrogen and phosphorus concentration as described in section 3.1.2.

3.2.3 Results

3.2.3.1 Experiment 1

Mineral contents of squash

There was no relationship between the order in which squash succumbed to rots (time at which the fruit rotted) and their mineral concentrations in cortical tissue of squash fruit form any of the three treatments except for phosphorus concentration in the calcium treatment, which was higher in fruit that rotted late than in those which rotted early ($P < 0.01$). Given that nine comparisons were made and that only one was significant, it seems that a conclusion that minerals were not significantly associated with time to develop rots might be justified.

Two significant relationships in skin tissue were found between time to rot and mineral concentrations: a negative one with calcium concentration in the water treatment and a positive one with nitrogen in the $CaCl_2$ treatment. However, there seemed to be little consistency in these relationships which could be used in developing a model of the role of minerals in rot development in harvested squash. Number of rotten squash at harvest was significantly less in the Na_2CO_3 treatment (37) than in either the $CaCl_2$ and H_2O treatments (which contained 57 and 56 respectively; $P < 0.01$, $X^2 = 6.76$ with 1 df Fig. 3.5). After harvest, there was a sigmoidal pattern of rot development over time which was reasonably well described by a logistic model for fruit in each treatment (Fig. 3.5).

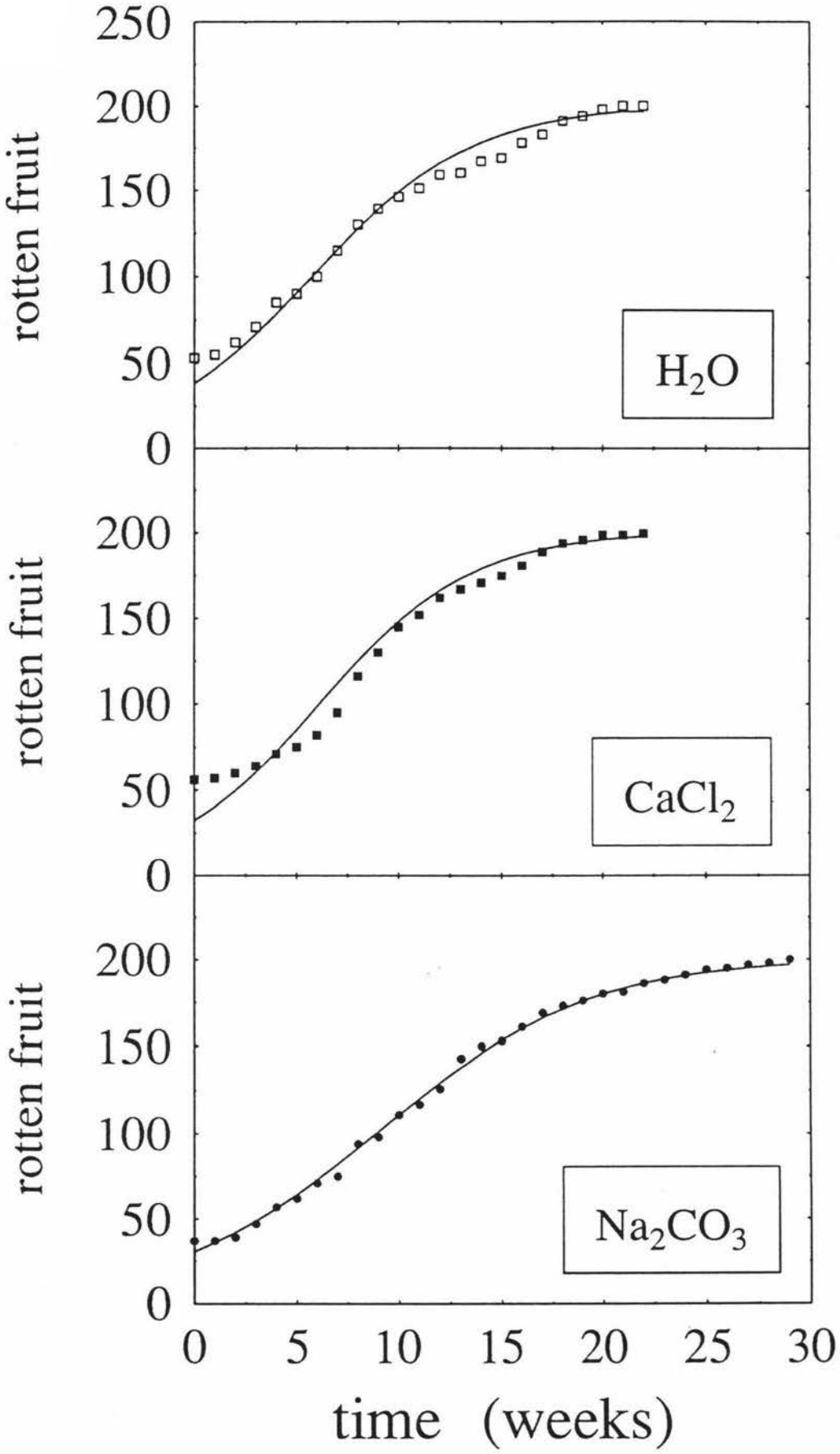


Figure 3.5 Accumulated number of rotten squash in 3 treatments after harvest.

logistic equation used to fit data

LGTH20 = $\log(H_2O/(201-H_2O))$

LGTCa = $\log(Ca/(201-Ca))$

LGTNa = $\log(Na/(201-Na))$

where P is parameter estimate, SE is standard error, Ca is calcium and H_2O is water. Comparison of slopes of the log-transformed data against time showed that squash in the Na_2CO_3 treatment succumbed to rots less quickly than either the $CaCl_2$ or H_2O treatments ($P < 0.01$) but no significant difference between $CaCl_2$ and H_2O ($T = (P_{ca} - P_{H2O}) / \{(SE_{ca})^2 + (SE_{H2O})^2\}^{1/2}$.

Weight loss

There was a highly significant decline of weight loss rate with time in all treatments (Fig. 3.6). Fruit with Na_2CO_3 had significantly lower weight loss rate in weeks 4, 5 and 6 than those with H_2O and $CaCl_2$ treatments ($P < 0.01$, 0.001 and 0.0001 in week 4, 5 and 6 respectively).

3.2.3.2 Experiment 2

There was no significant difference in calcium concentrations between painted and control halves of each fruit in either cortical or skin tissue. Calcium concentration in fruit skin was significantly higher than that in the cortex in all treatments ($P < 0.001$; Table 3.1). On average over both halves of each fruit, cortical calcium concentration was higher in the fruit treated with Na_2CO_3 than those of other three treatments

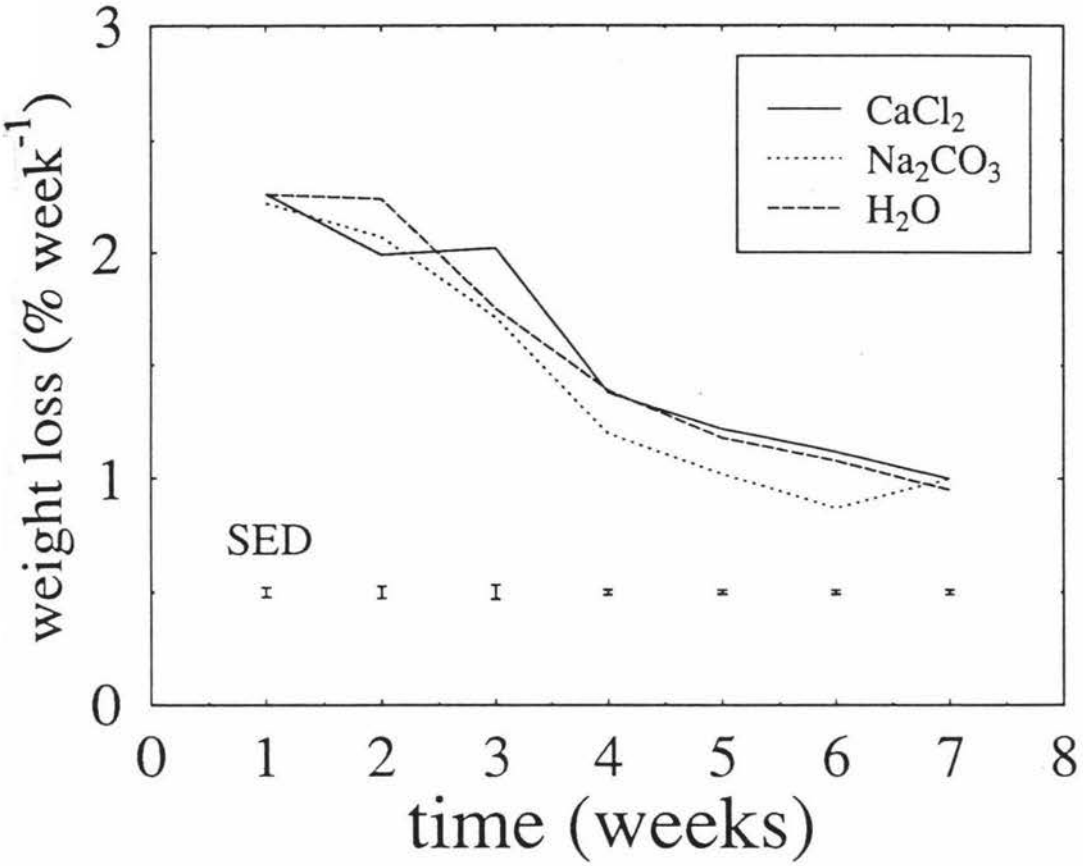


Figure 3.6 Change of weight loss rate of squash in 3 preharvest treatments. during storage time. Vertical bar indicates SED.

Table 3.1 Calcium contents of squash fruit with or without four different chemical treatments expected to modify calcium levels ($\pm$ SD averaged value over samples taken from treated and control halves of each fruit).

Treatment	Tissue	mol kg^{-1}
H_2O (Control)	Cortex	0.013 $\pm$ 0.005
	Skin	0.061 $\pm$ 0.024
Vapour gard	Cortex	0.014 $\pm$ 0.005
	Skin	0.059 $\pm$ 0.028
$CaCl_2$	Cortex	0.014 $\pm$ 0.008
	Skin	0.066 $\pm$ 0.043
Na_2CO_3	Cortex	0.026 $\pm$ 0.026
	Skin	0.085 $\pm$ 0.065

Table 3.2 Nitrogen contents of squash fruit with or without four different chemical treatments expected to modify calcium levels (± SD values here averaged over samples taken from treated and control halves of each fruit).

Treatment	Tissue	mol kg^{-1}
H_2O (Control)	Cortex	0.864 ± 0.204
	Skin	3.012 ± 0.484
Vapour gard	Cortex	0.840 ± 0.231
	Skin	3.094 ± 0.537
$CaCl_2$	Cortex	0.964 ± 0.216
	Skin	3.104 ± 0.634
Na_2CO_3	Cortex	0.836 ± 0.226
	Skin	3.115 ± 0.527

Table 3.3 Phosphorus contents of squash fruit with or without four different chemical treatments expected to modify calcium levels (± SD values here are averaged over samples taken from treated and control halves of each fruit).

Treatment	Tissue	mol kg^{-1}
H_2O (Control)	Cortex	0.091 ± 0.022
	Skin	0.371 ± 0.081
Vapour gard	Cortex	0.086 ± 0.020
	Skin	0.358 ± 0.102
$CaCl_2$	Cortex	0.102 ± 0.024
	Skin	0.367 ± 0.113
Na_2CO_3	Cortex	0.079 ± 0.019
	Skin	0.344 ± 0.081

($p < 0.05$; Table 3.1). On average over both halves of each fruit, calcium concentration in the skin of fruit given the H_2O and vapour gard treatment were significantly lower than those of other two treatments ($P < 0.05$). Nitrogen concentration (Table 3.2) was similar in the painted and control halves of each fruit in both skin and cortex tissues in all treatments. Nitrogen concentration in the skin was not significantly different from that in the cortex.

There were no significant differences between phosphorus concentrations (Table 3.3) in painted and control halves of either skin or cortex tissue in any of the treatments. Phosphorus concentration of cortex in Na_2CO_3 treatment was marginally significantly higher than other treatments ($P < 0.05$). There were no significant differences in phosphorus concentration in the skin between the four treatments.

3.2.4 Discussion

3.2.4.1 Experiment 1

In contrast to expectations, there were no consistent differences in mineral concentrations in either skin or cortex between fruit which became rotten soon after harvest and those which were the last to develop rots. However, the number of fruit rotting in the field was less in those fruit given sprays with Na_2CO_3 than in the other treatments; similarly, fruit from this treatment tended to rot later than fruit from the other two treatments. Given that the calcium contents of the fruit in all three treatments were similar, this may indicate that the effect of Na_2CO_3 treatment was through some other mechanism. This might be because of the effect of pH on disease development. This was observed by Punja and Grogan (1982) for the fungicidal effects of NH_3, CO_3^{2-} and -HCO_3-, and by Homma et al. (1981a) for the effect of $NaHCO_3$ on suppression of citrus green mold and cucumber powdery mildew. They considered that alkaline conditions suppressed rot development.

When $NaHCO_3$ and Na_2CO_3 dissolve in water, they are separated as:

$NaHCO_3 + H_2O$ ----- $Na^+ + HCO_3^- + OH^-$

in case of Na_2CO_3,

$Na_2CO_3 + H_2O$ ----- $2Na^+ + HCO_3^- + OH^-$

Solutions of either Na_2CO_3 or $NaHCO_3$ in water are therefore alkaline (pH > 7). The lower number of rotten fruit with Na_2CO_3 treatment in the field may therefore have been due to the alkaline condition of its solution.

Homma et al. (1981b) also reported that sodium bicarbonate solution combined with emulsifier of some food additives, such as soybean lecithin, glycerol fatty acid ester, sucrose fatty acid ester had more inhibitory effect on citrus common mold and cucumber powdery mildew than sodium bicarbonate alone. This may have been because the emulsifiers strengthened the adhesiveness of sodium bicarbonate and avoid crystallization so that sodium bicarbonate was distributed uniformly on the surface of leaves. Perhaps olive oil (2%) mixed with Na_2CO_3 solution acted in a similar manner.

Rates of weight loss (Fig. 3.6) were quite similar between 3 treatments except those with Na_2CO_3 treatment in 3rd week and 4th week. This indicates that none of the 3 treatments had a stimulative effect on fruit transpiration of fruit in the postharvest phase. Fruit in all treatments had less weight loss rate with the passage of time. This might be due to change of physical characteristics of fruit affected by water loss during storage. After fruit had lost a certain amount of the water because of water vapour pressure deficit, shrivelling of the skin might have resulted in a reduced transpiration coefficient which in turn would reduce the rate of water loss. From 5th to 7th week, fruit treated with Na_2CO_3 had more declined weight loss rate than those with $CaCl_2$ and H_2O treatments. This might be due to Olive oil (2%) mixed with

Na_2CO_3 solution. Olive oil could have functioned like a wax on the fruit skin when fruit skin shrivelled, which reduced transpiration from the skin (increased resistance to transpiration), and resulted in less weight loss rate of fruit than those with $CaCl_2$ and H_2O treatments.

3.2.4.2 Experiment 2

In this experiment, half of the surface of all fruit was treated with solutions and the other half was left as a control in order to eliminate variability of individual fruit. However, there was no significant difference of mineral concentrations(Ca, N, P) found between painted and control halves for any of the treatments. This may indicate that nitrogen and phosphorus were relatively mobile through vascular system or other tissues in squash fruit. Overall, fruit which were half painted with Na_2CO_3 had significantly higher calcium concentrations in both skin and cortical tissues. This conforms to the expectation that this treatment would stimulate transpiration and therefore carry more calcium into fruit treated in this way. However, it is difficult to reconcile this mechanism with the lack of difference in calcium concentration between the painted and control halves of the fruit, since it might be expected that the painted half should have transpired more quickly and therefore have accumulated more calcium. Given the low mobility of calcium ions within fruit tissues (Clarkson, 1984: Ferguson, 1984: Pate, 1976), it would be expected that the painted half would always remain at a higher calcium status than the control half. There was large variability around the average values in these treatments and, given the marginal levels of significance of these effects, it may be inappropriate to try to read too much

into these data.

Higher calcium concentration of squash treated with Na_2CO_3 than in fruit with other treatments which might be from the raised calcium intake enhanced by increased transpiration could be explained by the mechanisms as investigated by several researchers. Postharvest transpiration rate of a fruit such as apple (Horrocks, 1964) and grape (Grncarevic, 1968) depends on the resistance to water vapour movement of its cuticle when stomata are closed. It has been postulated by Chambers and Possingham, (1963) that water diffuses as a liquid from the cell through the cuticular layer until it reaches the wax-platelet region, then it moves as a vapour. Water film or water droplets cannot form in wax layer because of the hydrophobic nature of the wax. Dipping grapes in alkaline emulsion (potassium carbonate) forms a continuous aqueous zone permeating the capillary spaces of wax, which convert the wax layer from hydrophobic to hydrophillic. Therefore, water moves through the treated wax layer much more easily, and a given driving force created by water vapour pressure deficit between external atmosphere and saturated internal condition draws more water from the fruit. Schonherr (1976 a) observed that water diffusion through isolated cuticle (= polymer matrix) of citrus (*Citrus aurantium L.*) leaves increased five fold with increasing pH between 3 and 11. This was thought to be due to the dissociation of carboxyl groups (-COOH) which are fixed to the membrane matrix. Clusters of carboxyl groups were thought to become continuous from one side of the membrane to the other, developing into continuous pores when the carboxyl groups dissociated with increasing pH, thereby increasing water diffusion. Alternatively, he suggested that pores exist in the membrane even at relatively low pH (pH 6 and below) but most of them were discontinuous because of a "bottle neck" lined with

weakly acid groups. These acid groups would have become dissociated and the pores become continuous when pH was raised.

Schonherr (1976 b) reported that removal of soluble lipids (cuticular waxes) from cuticle of citrus, pear leaves and onion bulb scales decreased resistance to water vapour by a factor of 300 to 500, indicating the resistance of waxes to water would be 2 orders magnitude larger than that of the polymer matrix. This means that water transport across the cuticles is determined by water permeability of these waxes. Chambers and Possingham (1963) also suggested that potassium carbonate saponified the fatty acid constituents of grape wax. Substances produced by saponified fatty acids would also have been alkaline and therefore presumably Na_2CO_3 treatment in this experiment acted to increase water diffusion through the cuticle. In experiment 2, the cortex of fruit treated with Na_2CO_3 by paintbrush had significantly higher calcium concentration. Perhaps the more effective wetting of the cuticle achieved by painting rather than spraying Na_2CO_3 solution increased transpiration from skin. Conversely, Na_2CO_3 solution sprayed onto fruit in experiment 1 did not adhere to the skin as much as those which were rubbed with the paintbrush and rolled off the fruit. This might be cause of the difference in calcium concentrations of fruit treated with Na_2CO_3 in experiments 1 and 2.

Calcium concentration of fruit painted with Vapour gard was not significantly different from those of other treatments in both skin and cortex. This result contrast with my expectation that vapour gard would reduce transpiration and therefore suppress calcium movement into the fruit. Two potential reasons for this finding

might be identified. Fruit size increased rapidly between week 3 and week 6 (Fig. 3.4). As the fruit surface expanded with volume increase, the vapour gard film applied on the skin of fruit would probably have become stretched and cracked, and decreased any effects it might have on resistance of the fruit surface to transfer of water vapour. Alternatively, it may be that the increment in resistance to water vapour transfer achieved by vapour gard was low because its resistance was low compared to the inherent resistance of the fruit skin. Vapour gard has been widely reported to have an effect on transpiration rates of vegetative plant materials (Lidster, 1981; Lidster and Webster, 1983). However, in a fruit which already has a high resistance to water vapour transfer (ie. a low water vapour permeance), surface coatings may not necessarily be expected to make a large difference to their rate of transpiration (Banks et al., 1993). Either way, with no effect on transpiration, vapour gard treatment would not be expected to have affected calcium movement into fruit.

3.2.5 Conclusion

There was no indication that Na_2CO_3 treatment had raised calcium concentration sufficiently to give the fruit strong resistance against postharvest rot development. As discussed before, the effect of Na_2CO_3 might be due to a pH effect, suggesting Na_2CO_3 has potential for direct control of rot development, and therefore may not cause surface injury like that caused by calcium solution (Conway and Sams, 1985). Results of experiment 2 indicated that manipulation of transpiration by Na_2CO_3 may be superior to direct calcium application in increasing calcium concentration of squash. It would be useful to know the optimum concentration of Na_2CO_3 required

to manipulate transpiration and increase calcium concentration to a level which could give squash sufficient resistance against rot development. It would also be useful to know if the effects of Na_2CO_3 are affected by time and frequency of application during growth or the form of emulsion which is applied. The absence of a significant difference in weight loss between the three treatments during storage indicated that there may be little concern that Na_2CO_3 preharvest application would increase weight loss of squash during storage period.

Probably we can expect two beneficial effects from Na_2CO_3, one is to increase resistance against rot by increase a calcium concentration of squash; the other is to suppress rot development directly by a pH effect. By increasing fruit calcium concentration to strengthen natural resistance of fruit to pathogens, there is perhaps less possibility of development of more aggressive strains of pathogen which is often caused by fungicide application. In addition, there need be no concern about chemical residues as there would be with postharvest fungicide application.

Therefore, further study of this approach could benefit both industry and consumer, as well as expand our knowledge of factors affecting rot development in harvested squash.

Chapter 4

Literature review: Factors which affect weight loss of squash

4.1 Introduction

The loss of weight of horticultural commodities through water loss results in changes in structure, texture and appearance. A loss in weight will cause many perishable commodities to appear wilted or shrivelled. Even in the absence of visible wilting, water loss can cause a loss of crispness, and undesirable changes in colour and palatability (Wills et al., 1989). Water loss also causes loss of saleable weight and thus is direct loss in marketing (Woods, 1990). In addition, respiratory carbon loss may account for a significant fraction of the weight loss of some crops, especially at high relative humidity (Gaffney et al., 1985).

In this chapter, the literature on factors affecting weight loss of horticultural commodities is reviewed.

4.2 Transpiration

4.2.1 The process of transpiration

Transpiration is the process responsible for water loss from horticultural commodities after harvest (Kader, 1983). Transpiration of fresh fruits and vegetables is a mass transfer process in which water vapour moves from the surface of the plant organ to the surrounding air. While crop is attached to the plant, loss of water due to

transpiration is replaced from the flow of sap which is mostly water. Transpiration of horticultural produce continues after harvest, and since the produce is now removed from its normal source of water, it becomes dependent entirely on its own water content (Grierson and Wardowski, 1978).

4.2.2 Transpiration coefficient

A crop's transpiration coefficient, expressed in terms of quantity of water evaporated per unit surface or weight of product per unit water vapour deficit per unit time, is a measure of the ease with which a surface of fruit or vegetable gives off moisture. Vegetables such as carrots lose water at a rate close to that of a free water surface when first exposed to drying conditions (Van den Berg, 1987). The transpiration coefficient of carrots is 15.4% day^{-1} kPa^{-1} when rate of air flow is 0.05 m/s at 80% relative humidity (Van den Berg, 1987). Vegetables with a thick, tough skin, such as potatoes, have a very low transpiration coefficient ; 0.52 - 1.47% day^{-1} kPa^{-1} in similar conditions (Van den Berg, 1987). A free water surface generally has the highest coefficient of transpiration (Lentz, 1966).

The transpiration coefficient value expressed on a mass basis (% day^{-1} kPa^{-1}) is only be valid for a given size of product, since the surface area to mass ratio of a given product depends on the product dimensions (Sastry, 1985). The area-based transpiration coefficient, on the other hand, is not dependent on product size, but requires knowledge of surface area of individual products (Sastry and Buffington, 1982)

4.2.3 Water vapour pressure deficit

Evaporation is proportional to the difference between the water vapour pressure in the air and the equilibrium water vapour pressure of the produce. This difference is termed the water vapour pressure deficit (WVPD) and is the driving force in the process of evaporation from the produce (Van den Berg, 1987).

Evaporation (r_{H2O}) is expressed mathematically with Fick's law:

$$r_{H_2O} = K * (P^f - P^a) \qquad [4.1]$$

where: K = transpiration coefficient (% day^{-1} kPa^{-1})

P^a = water vapour pressure at ambient air

P^f = water vapour pressure of fruit

In Eq. 4.1 driving force is represented by WVPD (P^f - P^a).

In isothermal conditions the deficit is sometimes expressed in terms of relative humidity (RH) e.g. the difference between the equilibrium RH of the produce and the actual RH of the air (Wills et al. 1989). Assuming the equilibrium water vapour pressure to be equivalent to relative humidity of 100%, evaporation is then proportional to the difference between RH in the air and 100%.

4.2.4 Air velocity

Woods (1990) reported that air velocity influences both transpiration coefficient and the temperature difference between the produce and the surrounding air. At low

velocities, the boundary layer resistance has its greatest effect in reducing transpiration. It also restricts heat transfer to the produce necessitating a greater temperature difference in order to transfer the heat for evaporation. Conversely, at a large velocity, the effect of boundary layer on transpiration coefficient is small and the produce to air temperature difference is minimized through the large heat transfer rate. Van den Berg (1987) also noted that the resistance to evaporation decreases with increasing rate of airflow. As a result, the effective transpiration coefficient increases.

4.2.5 Surface area / Volume effects

A major factor in the rate of water loss from produce is the surface area to volume ratio of commodities. Large commodities possess a smaller surface -to- volume ratio than small ones and hence lose less moisture on a per unit weight basis (Wills et al., 1989). The shape of vegetables also affects the ratio of surface area to volume. Long, thin, cone-shaped carrots lose more weight than thick cylindrical-shaped ones in a given environment (Sastry et al., 1978). Hass (1936) found that small (and young) avocado fruit of a given cultivar lost water more rapidly than large (and old) fruit of the same cultivar. He attributed this difference to one of two factors : the larger avocados have fewer stomata per unit area of skin than smaller fruit, or the smaller fruit have more surface exposed per unit volume. Pieniazek (1944) also found that the transpiration rate of apples was proportional to surface area, that is, large apples lose more weight than small ones.

4.2.6 Temperature

RH interaction with temperature is critical with regard to VPD. When RH is kept constant, the effect on weight loss of apples of a 1°C increase in temperature from 15 to 16°C is double that of a 1°C increase from 2 to 3°C (Smith, 1933).

4.3 Respiration

4.3.1 Respiration on storage life of commodity

Respiration is the overall process by which stored organic materials (carbohydrates, proteins) are broken into simple products with a release of energy. Oxygen is used in this process and carbon dioxide is produced by the commodity. Kader (1985) explained that the loss of stored food reserves in the commodity during respiration causes : (1) onset of senescence as the reserves which provide energy for maintaining the living status of the commodity are exhausted, (2) loss of food value (energy value) for the consumer, (3) reduced flavour quality, especially sweetness, and (4) loss of saleable dry weight. Therefore, the rate of deterioration of harvested commodities is generally proportional to their respiration rate.

4.3.2 Respiration effects on weight loss

Respiration has two effects on weight loss one of which is direct effect of carbon loss whilst the other is an indirect effect of heat production on transpiration.

Respiration can be summarised by the following equation:

[4.2]

$$C_6H_{12}O_6 + 6O_2 \text{ --- } 6CO_2 + 6H_2O + 688Kcal$$

The water produced remains within the tissue, however CO_2 escapes and accounts for part of the weight loss of harvested fruit (Pantastico, 1975; Burton, 1982). O_2 diffuses into fruit and the net mass loss due to respiration is that of the carbon in the CO_2 evolved. This carbon loss rate is directly proportional to the respiration rate (Gaffney et al., 1985). It is usually an insignificant proportion of the total weight loss, but since it directly related to respiration, it is a function of temperature. Carbon loss rate is unaffected by relative humidity. Therefore, carbon loss by respiration can become significant in total weight loss at high relative humidity at which transpiration loss is lowered.

The generation of heat within fruit by respiration may lead to additional loss of weight (Burg and Kosson, 1983). This heat is dissipated through direct heat transfer to the environment and through evaporation of water. The heat of respiration raises the tissue temperature and therefore increases transpiration. Lentz and Rooke (1964) showed that apples lost weight even in water-saturated air at presumably the same temperature as the fruit. They presumed that heat of respiration tends to raise the temperature of the fruit, which in turns raises the vapour pressure deficit and thus increases transpiration even in water-saturated environments.

4.3.3 Factors affecting respiration

4.3.3.1 Maturity at harvest

The respiration rate is usually very high during the early stages of development and

decrease as plant organs mature (Wills et al., 1989). Thus, vegetables harvested during active growth phase, such as leafy, floral, and immature-fruit vegetables, have high respiration rates. Generally the respiration rate declines steadily after harvest, and the decrease is slow in mature fruit vegetables and rapid in vegetative tissues and immature fruit vegetables. This rapid fall reflects depletion of respirable substrate, which are typically low in such tissues (Kader, 1987). Exceptions to the declining pattern of postharvest respiration are those of climacteric fruit.

4.3.3.2 Temperature

Rate of respiration is governed by temperature. In plant material the immediate effect of a rise of 10°C in temperature, over the range where no harmful effect of temperature are experienced, could well be an approximate doubling of the rate of O_2 uptake, or CO_2 evolution, or carbohydrate breakdown (Burton, 1982). The rate of most chemical and biochemical reactions increase two or three times with every 10°C in temperature. Hardenburg et al. (1986) showed that an apple held at 10°C ripens and respires about three times as fast as one held at 0°C, and one held at 20°C respires about three times as fast as one held at 10°C. Head lettuce respires about three times as fast at 10°C as at 0°C and two or three times as fast as at 20°C as at 10°C.

4.3.3.3 Modified atmospheres

Modified atmosphere (MA) and controlled atmosphere (CA) storage involves removal

or addition of gases resulting in an atmosphere composition surrounding the commodity that is different from that of air. Usually this involves reduction of oxygen and/or elevation of carbon dioxide concentrations.

From Eq. 4.2, it can be suggested that fruit respiration could be slowed by limiting the oxygen, or by raising the carbon dioxide concentration in storage. Kader (1987) showed a general schematic representation of the effects O_2 concentration on respiration rate of fresh vegetables, suggesting that a significant reduction in respiration rate as O_2 concentration was reduced below that in air (20.9%), especially below 10%, and when O_2 concentration dropped to less than about 2% (exact concentration depended on commodity, temperature, and duration), anaerobic respiration rate and total CO_2 production increased. In the same study, elevated CO_2 levels reduced aerobic respiration (O_2 consumption). However, at concentrations above 20%, a significant increase in anaerobic respiration (ethanol and acetaldehyde accumulation) caused irreversible tissue damage.

The beneficial and detrimental effects of CA and MA on fresh fruits and vegetables have been investigated (Blankenship, 1985; Brecht, 1980; Dilley, 1983; Isenberg, 1979; Kader, 1980; Lipton, 1975; Wolfe, 1980). They suggested that CA/MA conditions reduce respiration rates as long as the levels of O_2 and CO_2 are within those tolerated by the commodity. These reduction of respiration rates combined with the decreased C_2H_4 production and reduced sensitivity to C_2H_4 action, results in delayed senescence as indicated by retention of chlorophyll (green colour) and textural quality. In their study, it was indicated that exposure of fresh fruits and vegetables to O_2 levels below their tolerance limits or to CO_2 levels alone their

tolerance limits may increase anaerobic respiration and the consequent accumulation of ethanol and acetaldehyde causing off-flavours.

4.3.3.4 Influence of coating on respiration

The effects of coating are directly related to gas exchange between the fruit and its environment. The diffusion barrier formed by the coating increase skin resistance to both O_2 and CO_2 diffusion, resulting in elevated CO_2, reduced O_2, and concomitantly reduced respiration rate (Ben-Yehoshua, 1967; Banks, 1984a; Erbil and Muftugil, 1986; Smith et al. 1987; Nisperos-Carriedo et al., 1990; Hagenmeier and Shaw, 1992). Benefits of low respiration caused by coating were that slowing the rate of chlorophyll degradation in coated unripe banana and retardation of the rate of passage from the optimum stage of ripeness into senescence of banana (Banks, 1984a). Loss of flesh firmness was markedly reduced in coated apples (Smith and Stow, 1984).

4.4 Routes of different gases through fruit skin

4.4.1 Differential resistance of fruit skin to different gases

The resistance of various fruits to water vapour is less than 100-fold that to CO_2, O_2, or C_2H_4 (Cameron and Reid 1982; Ben-Yehoshua et al., 1985). Burg and Kosson (1983) and Ben-Yehoshua et al. 1985) have proposed that, unlike water, C_2H_4 and CO_2 pass through apple peel in the air phase of a limited area of that peel. They based this proposal on the fact that the rate of movement of water out of the fruit is

inversely proportional to the atmospheric pressure, and if passage were limited by a liquid phase, resistance to CO_2 and ethylene would be inversely proportional to their solubility in that phase. Thus, resistance to C_2H_4 would be much higher than it actually is, if the phase were water and much higher to CO_2 if it were lipid. The skin resistance to the passage of ethylene, CO_2 and O_2 of untreated Valencia oranges is similar and very high (6000 s cm^{-1}), although its resistance to water is only one-sixtieth as great (Ben-Yehoshua et al., 1985). These result show there are separate pathways for various gases. Therefore, resistance of fruit to various gases differ between different species of fruit.

4.4.2 Stomata

Stomata occur in most fruits, such as squash (Sutherland and Hallett, 1993) except for a few, such as the grape berry and blueberry (Martin and Stott, 1957). Stomatal density varies among fruit species and with age. Contribution of stomatal transpiration to water loss is probably of little significance in harvested fruits, because stomata often close after fruit are detached from a plant. Moreshet and Green (1980) showed that stomata stopped functioning after harvest. However, Ben-Yehoshua et al. (1985) showed with scanning electron microscope photomicrographs that the stomata of harvested oranges are partially open and allow some gas exchange. The slightly opened stomata on harvested citrus fruit could account for 0.4 % of the initial area of fully open stomata. Johnson and Brun (1966) reported that stomata of fully mature harvested banana fruit open in high relative humidity (RH) and light and close in low RH and in darkness.

4.4.3 Cuticle

The cell wall surface of fruits exposed to the ambient air are completely covered by a waxy, water resistant excretion which forms the cuticle. This cuticle, the layer which lines all interfaces between the plant and atmosphere, comprises a matrix of cellulose, proteins, and phenolic compounds, and these are combined with varying amounts of waxes and deposited superficially over its surface (Kolattukudy 1980). Evaporation from these surfaces is described as cuticular transpiration. When stomata of fruit are closed little flow of water takes place through them and the only pathway is through the cuticle. No correlation was found between the thickness of the cuticle and the cuticle transpiration rates in *Prunus* or *Agave* (Sitte and Rennier, 1963) or in grapes (Radler 1965), which might indicate rate of water loss is not principally limited by cuticle thickness. The structure and chemical composition of the cuticle is thought to play a more important role (Martin and Juniper 1970). The cuticular waxes constitute the major barrier to water movement through the cuticle of apples (Horrocks 1964). Disruption of the wax on the surface of the grape berry greatly increased the rate of water loss (Possingham et al., 1967). Martin and Juniper (1970) reported that all cuticles contain various amounts of soluble lipids (so - called cuticular waxes) varying greatly in amount and composition. Removal of soluble lipids from cuticular of Citrus leaves, pear leaves, or onion bulb scales decreased their resistance to water diffusion (Schonherr, 1976b). Resistance of cuticle to each gas would vary inversely with its solubility to cuticle, which is, on its solubilities in water and waxy materials, and there is potential for differential cuticular resistance as H_2O is infinitely self-soluble and CO_2 is very much more soluble in water and

lipids than O_2 (Mitz, 1979).

4.4.4 Stem scar

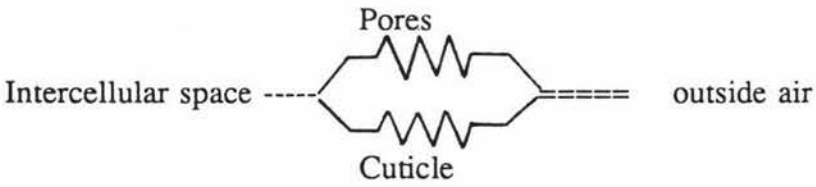
The contribution of the stem scar as an avenue for gas diffusion was investigated by comparing fruit with plugged and open scars. Burg and Burg (1965) showed that sealing the stem scars of peppers reduced CO_2 emanation by 60%. Cameron (1982) showed that the contribution of the calyx to the passage of different gases varied in different fruits. In 'Golden Delicious' apples, the calyx provided for the diffusion of 42% of the ethylene, 24% of the CO_2, and only 2% of the water. In tomatoes, the respective percentages were 94, 81 and 67. Paull and Chen (1989) showed that water loss in papaya had the highest rate per unit area through the stem scar, but the bulk of weight loss occurred through the skin stomata and cuticle.

4.4.5 Resistance network

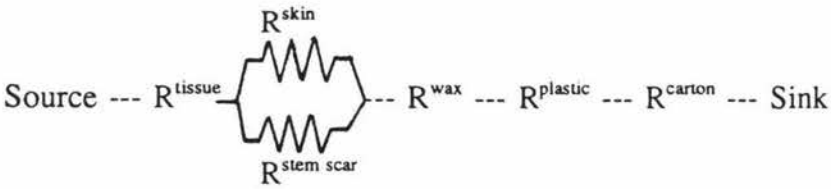
Resistance to gas exchange in fruit consists of the relative contribution of each of the following components:stomata, stem end and cuticle. Contribution of each of these components to total fruit resistance differs between species. Burton (1982) found that lenticels and stomata account for only 3% of transpiration in water loss of potato tuber because of their sparse distribution on the tuber surface. The other 97% of transpiration was through surface of the periderm on the surface. Within the fruit, gases move throughout the intercellular spaces from walls of cells to the stomata and then into the outside air. These spaces act as a continuum, extending throughout the

fruit (Ben-Yehoshua, 1987). This gas phase is responsible for the adequate gas exchange of bulky organs (Burg and Burg, 1965; Burton, 1982), and any action that results in clogging of this space by a liquid leads to inadequate gas exchange and fermentation (Sacher, 1973).

Nobel (1983) recognized and applied the analogy of resistance in electrical circuits to gas movement of a harvested fruit with its stomata closed. This can be represented diagrammatically as follow:



Cameron and Reid (1982) have utilized this approach for the study of various barriers to diffusion encountered by ethylene. As it leaves a nonstomatal fruit which is waxed, wrapped in plastic, and placed in a carton, the resistance network is described as follows:



Total resistance can be calculated as for the analogous electrical circuit as follows:

[4.3]

$$R^{total} = R^{tissue} + \frac{R^{skin} + R^{pores}}{R^{skin} \, x \, R^{pores}} + R^{wax} + R^{plastic} + R^{carton}$$

The advantage of the resistance network approach is that it permits the segregation

of the total resistance into its many components, thereby enabling investigation of how a change of any one component might affect the total resistance. An important consequence of the resistance network approach is an understanding of the concentration profile of a certain gas species from the atmosphere to the tissue at steady state. The steady state condition for any gas species is met when the mass flux of that species is constant throughout the entire system under study (Ben-Yehoshua and Cameron, 1988). For example, when diffusion of oxygen is at steady state, it is utilized at a constant rate of x mol s^{-1}, precisely x moles moves across the plastic film every second, x moles per second crosses the wax barrier, and x moles per second crosses the cuticle and skin. However, whilst the flux of oxygen is constant, the concentration of oxygen drops from the atmosphere to the site of respiration. The concentration difference across any barrier for a given steady state flux will depend only on the resistance of the barrier and its surface area.

4.5 Surface coating

4.5.1 Resistance of coating materials

The presence of a surface coating, which is an artificial barrier to gas diffusion, on a fruit surface may result in reduced O_2 and increased CO_2 concentrations inside fruit, and altered water and ethylene concentrations as well. The degree to which these factors are altered for a given commodity will depend on species, cultivar, mass: surface ratio, and respiration rate. The rate of respiration itself is dependent on maturity at harvest, source of produce, season, and temperature. The nature and

thickness of the coating material and how it interacts with temperature and the presence of water will determine its resistance and hence the degree in modification of internal atmosphere.

The application of surface coatings has been practised by the fruit and vegetable industries of the world for the past five decades for the purpose of rendering the fruit more glossy and attractive in appearance and of reducing shrinkage and water loss (Ben-Yehoshua, 1967; Hulme, 1949; Pieniazek, 1944; Trout et al., 1953). Experiments with artificial waxing of apples were first reported by Magness and Diehl (1924), who found that oil and paraffin coatings reduced the permeability of the epidermis and the rates of respiration and softening. Platenius (1939) applied wax to several vegetables, notably to topped carrots and cucumbers, and found a significant reduction in water loss. However, Trout et al. (1942) reported that such coatings on 'Granny Smith' apples interfered with normal ripening and, if applied too thickly, also caused off-flavours and breakdown of the flesh. They recommended careful application of coatings and temperature control to reduce injury. Their measurements of the internal atmosphere of coated apples revealed reduced internal oxygen and increased internal carbon dioxide concentrations.

With the advent of new emulsifiable synthetic polymers, new formulations for surface coatings became available. Ben-Yehoshua (1996b) suggested the use of plastic coatings made of these emulsions in order to extend the storage life of fruits and vegetables. The introduction of polyethylene into wax formulations resulted in greater resistance to diffusion of water vapour without much effect on the diffusion of other gases (Ben-Yehoshua, 1969). Davis and Harding (1960) reported the

reduction of rind breakdown of grapefruit by an orchard spray of polyethylene emulsion. Gassner et al. (1964) developed a polyethylene wax emulsion called 'Tag' for use as a skin coating for fruits. Its major effects were the reduction of weight loss by up to 60% (during the sixth day after treatment at 21-23°C, 60-75% RH) and inhibition of shrinkage. Tag also significantly extended storage life of coated fruit by delaying other physiological aspects of deterioration (Ben-Yehoshua, 1967). Tag-coated oranges and bananas had slightly higher CO_2 and markedly lower O_2 concentrations in their internal atmosphere and lower respiration rates (Ben-Yehoshua 1966a). Interestingly, Tag-coated oranges showed about 1.8 times as much resistance to O_2 diffusion as to CO_2 diffusion, whereas the resistance to O_2 and CO_2 diffusion in uncoated fruit are equal (Ben-Yehoshua, 1967).

Ben-Yehoshua (1966b) showed that the better result on the keeping quality of oranges achieved by dipping the fruit in Tag in the laboratory, as compared with packing-plant application, were largely due to the higher amount of coating left on the fruit in the laboratory. Alternatively, too thick a coating can be detected by measuring the oxygen and ethanol concentration inside the fruit (Ben-Yehoshua, 1967). A high ethanol level was well correlated with the presence of off-flavours (Horrocks, 1964).

4.5.2 Effect of surface coating on gas exchange

4.5.2.1 Control of water loss

Water transpires continuously from fruit and vegetables during handling and storage

whenever the water vapour pressure of the surrounding air is lower than that beneath the skin of commodity.

Rate of water vapour flow is expressed by Eq. 4.1. The inverse of K (transpiration coefficient) represents the skin resistance to water vapour of commodity. A increase in the skin resistance by surface coating may reduce water loss.

Some coatings can result in marked reduction in weight loss (Ben-Yehoshua, 1967; Smith, 1982), but only slight reductions of weight loss in banana (Banks, 1984a) and apples (Smith and Stow, 1984) have been found with some coatings. This might be thought that coatings plugged stomata and created a barrier to CO_2 and O_2 exchange (Ben-Yehoshua et al., 1985), and water loss occurred via the cuticle, through the liquid water phase (Burton, 1982). It may be also be related to the resistance properties of the coating materials themselves.

4.5.2.2 Modification of internal atmosphere by surface coating

Surface coating increase the resistance of fruit skin to gas diffusion, and modifies internal atmosphere composition. This can be described by Fick's Law (Nobel, 1983). Flux of a gas through a barrier varies with resistance (R) and concentration gradient as follows:

$$F = \frac{([j]_{external} - [j]_{internal}) * A}{R} \qquad [4.4]$$

where:

F - Flux of a gas through a barrier ($cm^3\ s^{-1}$)

$[j]_{external}$ - external concentration (mol mol^{-1})

$[j]_{internal}$ - internal concentration (mol mol^{-1})

R - Resistance through a barrier (s cm^{-1})

A - Surface area (cm^2)

Eq. 4.4 can be rewritten as:

$$\frac{F * R}{A} = [j]_{external} - [j]_{internal} \qquad [4.5]$$

As external concentration of a gas and flux are constant at a given temperature, change in the skin resistance results in modification of internal atmosphere composition. At any given temperature, increased resistance to diffusion of CO_2 and O_2 lead to elevated CO_2, reduced O_2 and concomitantly reduced respiration rate, which were reported for apples (Elson et al., 1985; Smith et al., 1987) and banana (Banks, 1984a). Reduced respiration rate leads to establishment of new steady rate concentrations of internal atmosphere. This equilibrated atmosphere achieved by coating primarily depends on resistance of the coating and the respiration rate of the fruit and holding temperature (Banks, 1985a; Smith and Stow, 1984; Hagenmaier and Shaw, 1992). Smith et al. (1987) reported that modified internal concentration of O_2 and CO_2 in apples depended on coating type and cultivar. Cultivar differences in respiration to coating application may reflect difference in their respiration rate, efficiency of the coating - fruit surface interface, or natural differences in the resistance of individual fruit skin (Banks et al., 1993; Dadzie, 1992).

The beneficial effects of modification of the internal atmosphere of fruit with

coatings has been reported for slowed degreening associating with delayed ripening of banana (Banks, 1984a; Al-Zaemey et al., 1989), suppression of respiration rate and ethylene evolution in apple (Drake et al., 1987; Elson et al., 1985; Smith et al., 1987), strawberry (Ghaouth et al., 1991) and papaya (Paull and Chen, 1989) and mango (Dhalla and Hanson, 1988). On the other hand, modifications of internal atmosphere composition by the use of coatings may increase disorders associated with high CO_2 and low O_2 concentrations, such as flesh browning and breakdown, accumulation of ethanol and alcoholic off- flavours (Ben-Yehoshua, 1967; Cohen et al., 1990; Erbil and Muftugil, 1986; Paull and Chen, 1989). Adverse flavour changes have been attributed to the inhibition of O_2 and CO_2 exchange, thus resulting in anaerobic respiration and elevated ethanol and acetaldehyde content (Banks, 1984a; Drake et al., 1984; Cohen et al., 1990).

Coating, specially fruit coating, should be prescribed according to the physiological requirements of the fruit. The formulation selected should permit desirable gas exchange through the coating. The thickness of the coating and its uniformity is important. Too thick a coating may cause deterioration of fruit by creating partly anaerobic conditions within it; too thin a coating may not be effective enough to achieve the desired results such as reduction of water loss (Ben-Yehoshua, 1967).

Data on resistance of coatings which control gas exchange between the fruit and the surrounding atmosphere may give a general improvement in the technology of fruit coatings. First, data are needed on the performance of coatings of known resistance and thickness. Second, information is needed on what coating resistance is optimum for storage of various commodities. 'Tag' coating is relatively less resistant to CO_2

diffusion than O_2, which makes it suitable for citrus fruit, which is sensitive to CO_2 injury when the fruit has too high an internal CO_2 concentration. However, an emulsion which would form a coating with more resistance to CO_2 diffusion may be advantageous for kinds of fruit that are relatively resistant to CO_2 injury (Ben-Yehoshua, 1987). Hagenmaier and Shaw (1992) reported values for permeability (1 / resistance * thickness) to O_2, CO_2, C_2H_4, and water vapour determined for 19 commercial fruit wax coatings. O_2 permeability at 50% RH and 30 °C ranged from 470 to 22.000 ml * mil (m^2 * day * atm) (1 mil = 0.0254 mm) whilst CO_2 permeability was two to eight times as high. With this range in resistance (1 / permeance) characteristics, it should be possible to devise optimum fruit wax coatings for different commodities.

4.6 Prospect for reducing weight loss in harvested squash

One of the major requirements for extending the postharvest life of horticultural commodities is to slow their transpiration. The water loss resulting from transpiration causes not only shrinkage, drying and softening of commodities (Ben-Yehoshua, 1987; Sastry, 1985; Woods, 1990), but triggers the change from juvenile hormonal balance to a senescent one, leading to accelerated deterioration of the fruit (Ben-Yehoshua, 1985). Prevention of water loss (reduction of transpiration) enables commodities to retain high quality.

Recently, export of squash from New Zealand to Japan encountered weight loss problem during shipping. This weight loss was thought to be caused by mostly water

loss (Buys, D. personal communication, 1991). Low temperature and high relative humidity are the principal techniques to lower transpiration. However, there are some difficulties in the application of these techniques to storage of squash, since there were frequent occurrences of rotten squash during shipping, which might be due to high (95%) relative humidity during storage conducive to rot development (Beever et al., 1983, 1984). Although low-temperature storage with high relative humidity is effective in reduction of water loss of many commodities, there are limitations to its use for the storage of chilling-sensitive produce. The lowest safe temperature for storage of squash is about 12°C (Inaba, 1993). Practically, refrigeration to keep squash at low temperature may expensive, it is not known if these costs during transportation could be recovered by the marketing system. Because of these limitations, a different approach was sought that would achieve reduction of weight loss of squash. This approach involved investigating the potential for reducing weight loss by surface coating on squash.

Based on the above review, there is clearly a need to learn about the basic as well as applied aspects of coating technology.

Chapter 5

Relationship between surface coating, weight loss and gas exchange in squash fruit

5.1 Introduction

Recently it has been reported that squash fruit lose approximately 8 % of their fresh weight during the six week period from harvest to arrival in Japan (D, Buys. personal communication, 1991). The need to pack extra fruit to compensate for this weight loss represents a substantial cost in terms of potential sales. Weight losses occur through the processes of respiration and transpiration of fruit after harvest. Surface coatings have been suggested as a potential means of overcoming this problem (Mancktelow, personal communication, 1991).

The use of coatings as an artificial barrier to water loss (water vapour) has been studied for many horticultural commodities (Cohen et al., 1990; Lidster, 1981; Hardenburg, 1967; Long and Leggo, 1959; Pieniazek, 1944). The most studied property of waxed fruit is its weight loss during storage (Cohen et al., 1990; Paull and Chen, 1989; Erbil and Muftugil, 1986). Coating reduced markedly the rate of weight and volume loss of oranges (Ben-Yehoshua, 1967). These effects are achieved because coatings produce greater resistance to diffusion of water vapour. Weight loss of fruits is mostly due to transpiration, a process which is driven by the water vapour pressure deficit (WVPD, Pa) between the internal and external atmospheres of the fruit. Transpiration is retarded by the fruit's resistance to water vapour transfer.

Rate of water loss (r_{H2O}, % fresh weight day^{-1}) from a fruit can be predicted from Fick's law (section 4.1.2):

$$r_{H_2O} = K * WVPD \qquad \textbf{[1.1]}$$

where: K = transpiration coefficient (% day^{-1} kPa^{-1})

Whilst there are some anomalies in convenient units for expressing transpiration coefficients in terms of weight loss and skin resistance to the exchange of gases, the transpiration coefficient defined above is essentially the inverse of skin resistance to water vapour diffusion. For barriers in series, such as a surface coating on a fruit skin, the resistance through the series (R^{series}) is related to the resistance of the skin of a fruit (R^s) and coating (R^c) as follows (Crank, 1956):

$$R^{series} = R^s + R^c \qquad \textbf{[5.1]}$$

To achieve a substantial reduction in transpiration, a surface coating should have a high resistance to water vapour transfer.

Another factor which affects weight loss is loss of carbon from CO_2 evolution in respiration, which is directly proportional to the respiration rate. Though respiration rate is not directly affected by WVPD at a single temperature, its proportional contribution to total weight loss increases with reduced WVPD at high relative humidities (RH) as weight loss through transpiration is reduced (Eq. 4.1). Therefore, the contribution to total weight loss by respiration rate could become considerable at high values of RH.

Any surface coating applied to reduce weight loss must achieve a compromise between preventing water loss from the fruit and maintaining an adequate level of exchange of O_2 and CO_2. However, sometimes modifications of internal atmosphere composition by the use of coatings produce disorders associated with high CO_2 and low O_2 concentrations, such as flesh browning and breakdown, accumulation of ethanol and alcoholic off-flavours (Ben-Yehoshua, 1967; Cohen et al., 1990; Erbil and Muftugil, 1986; Paull and Chen, 1989). Adverse flavour changes have been attributed to the inhibition of O_2 and CO_2 exchange, where results in anaerobic respiration and elevated ethanol and acetaldehyde contents (Banks, 1984a; Cohen et al., 1990; Drake et al., 1987). The optimal coating should maximally reduce transpiration loss without creating an injurious atmosphere inside the fruit, so the internal atmosphere must be within the range in which there is neither a deficiency of O_2 nor an excess of CO_2 during storage life at the range of temperatures that the fruit would be exposed to (Banks et al., 1993).

In addition to reduction of transpiration loss, coating can affect respiration rate by reducing O_2 and CO_2 diffusion which causes depression of internal oxygen ($[O_2]_i$, mol mol^{-1}) and elevation of internal CO_2 ($[CO_2]_i$, mol mol^{-1}). Lowering respiration presumably retards breakdown of carbohydrates and should therefore result in longer shelf life of fruits. It also contributes to reduction of weight loss by decreasing output of heat (which causes evaporation of water) and carbon loss. Little benefit is achieved when $[O_2]_i$ remains high because there is very little effect on respiration rate (Banks et al., 1993). Progressively greater benefits are obtained as $[O_2]_i$, and consequently respiration, are depressed more and more. However, if $[O_2]_i$ is lowered

below a certain point (anaerobic compensation point), fruit begins anaerobic respiration and development of off flavours (Kader et al., 1989), and hence very low $[O_2]_i$ must be avoided. There is therefore an optimum $[O_2]_i$ at which respiration is minimised without development of anaerobic respiration.

When fruit is coated to reduce water loss, the increase in resistance to CO_2 and O_2 by coating can cause adverse effects such as anaerobic respiration. Therefore, the ideal coating should have low resistance to O_2 and CO_2 but high resistance to water vapour. The main objectives of the work described in this chapter were to investigate the effects of coating on weight loss of squash and how this affected the internal condition of the fruit.

5.2 Description of the model system

A steady state model for the gas exchange of coated fruit presented by Banks et al. (1993) was used to predict steady state (equilibrated) $[O_2]_i$ and $[CO_2]_i$ values in coated fruit. The following information is summarised from that paper.

Fruit respiratory oxygen uptake (rr_{O_2}, $cm^3\ s^{-1}$) was modelled as a Michaelis-Menten function of $[O_2]_i$ (Dadzie, 1992):

$$rr_{O_2} = rr_{O_2}^{max} \frac{[O_2]_i}{(K_m + [O_2]_i)} \qquad [5.2]$$

The term $rr_{O_2}^{max}$ ($cm^3\ s^{-1}$) represents an inherent maximum rate of oxygen consumption for that fruit under prevailing conditions of organic substrate levels,

ethylene concentration, developmental stage and temperature. A value of 0.02 mol mol^{-1} O_2 was used for the Michaelis-Menten constant (K^m; Dadzie, 1992).

Respiratory CO_2 production comprises two components : aerobic and anaerobic (Boersig et al., 1988). A combined equation was used to describe these two components (Dadzie, 1992):

$$rr_{CO_2} = RQ^{\infty} rr_{O_2}^{max} \left(\frac{[O_2]_i}{K_m + [O_2]_i} + \frac{10^{-10}}{([O_2]_i + a)^b} \right) \qquad [5.3]$$

where: RQ^{∞} is the respiratory quotient (ie. rr_{CO_2} / rr_{O_2}) when $[O_2]_i$ is unlimiting (assumed in this analysis to have a value of unity). The preliminary estimates of constants a and b were calculated by Dadzie, (1992).

Fick's Law was used to quantify diffusive transmission rate of O_2 (cm^3 s^{-1}) between the internal and external atmospheres of the model fruit through the skin (Cameron and Reid, 1982; Hagenmeier and Shaw, 1992):

$$r_{O_2} = \frac{([O_2]_e - [O_2]_i) * A^{fruit}}{R_{O_2}} \qquad [5.4]$$

where:

r_{O2} = rate of O_2 transfer between fruit and external atmosphere (cm^3 s^{-1})

$[O_2]_e$ = external O_2 concentration (mol mol^{-1})

$[O_2]_i$ = internal O_2 concentration (mol mol^{-1})

A^{fruit} = fruit surface area (cm^2)

R_{O2} = total resistance to O_2 diffusion (s cm^{-1})

Diffusive transmission rates of CO_2 were quantified in the same way by substituting their mole fractions in the internal and external atmospheres for those of O_2 in Eq. 5.4.

At steady state, rr_{O_2} and r_{O_2} are equal so that the steady state $[O_2]_i$ value can be calculated by rearranging the following equation and solving for $[O_2]_i$:

$$A^{fruit}\frac{([O_2]_e - [O_2]_i)}{R^{total}} = \frac{rr_{O_2}^{max}[O_2]_i}{(K_m + [O_2]_i)} \qquad [5.5]$$

which yields:

$$[O_2]_i = \frac{\left([O_2]_e - K_m - R_{O_2}^{total}\frac{rr_{O_2}^{max}}{A^{fruit}} + \sqrt{\left([O_2]_e - K_m - R_{O_2}^{fruit}\frac{rr_{O_2}^{max}}{A^{fruit}}\right)^2 + 4K_m[O_2]_e}\right)}{2} \qquad [5.6]$$

Since $[CO_2]_e = 0$, $[CO_2]_i$ can then be calculated as :

$$[CO_2]_i = \left(\frac{R_{CO_2}^{total}}{A^{fruit}}\right)RQ^{\infty}rr_{O_2}^{max}\left(\frac{[O_2]_i}{(K_m + [O_2]_i)} + \frac{10^{-10}}{([O_2]_i + a)^b}\right) \qquad [5.7]$$

Given values for $rr_{O_2}{}^{max}$, A^{fruit} and K^m, steady state predictions of the important gas exchange variables ($[O_2]_i$, $[CO_2]_i$, rr_{O_2} and rr_{CO_2}) were made using equations above.

For non-coated fruit, total resistance of fruit skin (R^{total}) comprises the effective resistance of the pores (R'^{pores}) and the cuticle (R'^{cut}) operating in parallel (Nobel, 1983). For such a system, the total resistance is the sum of the reciprocals of the

individual resistances, so that total resistance is given by:

$$R^{total} = \frac{R^{pores} * R^{cut}}{R^{pores} + R^{cut}} \qquad [5.8]$$

Application of a surface coating covers the cuticle and blocks pores on the fruit surface (Banks, 1984a; Ben-Yehoshua et al., 1985). Therefore, total resistance of a coated fruit (R^{total}) can be calculated using Eq. 5.8 by substituting the following:

$$R^{pores} = \frac{\left(R^{pores} + R^{coat}\right) * A^{fruit}}{A^{pores}} \qquad [5.9]$$

$$R^{cut} = \frac{\left(R^{cut} + R^{coat}\right) * A^{fruit}}{A^{cut}} \qquad [5.10]$$

R^{pores} = true resistance of pores (s cm^{-1})

R^{cut} = true resistance of the cuticle (s cm^{-1})

A^{pore} = total area of pores on fruit surface (cm_2)

A^{cut} = total area of cuticle on fruit surface (cm^2)

The fruit system model outlined above was used to predict the effects of blocking different proportions of pores on the internal atmosphere composition of a fruit with a surface coating. The coating had a differential resistance to oxygen and carbon dioxide (ratio of $R_{O_2} : R_{CO_2} = 4$; Hagenmeier and Shaw, 1992). The resistance of the fruit surface was calculated assuming that absolute $R^{pores}{}_{O_2} = 6$ s cm^{-1}, whilst the ratio of $R^{pores}{}_{O_2}$:$R^{pores}{}_{CO_2}$ = 4 : 5. Cuticular resistance to oxygen was set to 25000 s cm^{-1}

whilst the ratio of $R^{cut}_{O_2}$:$R^{cut}_{CO_2}$ was 30 to 1.

Fig. 5.1 shows the predicted relationship between $[CO_2]_i$ and $[O_2]_i$. As the effectiveness of the coating in blocking pores increased, so $[CO_2]_i$ increased and $[O_2]_i$ decreased. However, the slope was less than unity because the increase in R_{CO_2} was less than that of R_{O_2}. This phenomenon occurs because the resistance of a coated pore is so much higher than that of an open pore that coated pores make virtually no contribution to total fruit gas exchange. As more pores become blocked, so the cuticle contributes a greater proportion of the total gas exchange of the fruit. The more gas exchange is determined by cuticular resistance, the larger the difference between R_{O_2} and R_{CO_2} becomes because of the differential resistance properties of the cuticle, as outlined above. As more pores are blocked, the ratio of R_{O_2} : R_{CO_2} therefore changes from a ratio of about 4 : 5 (differential resistance of the pores) towards a value of 30 : 1 (differential resistance of the cuticle). Thus, the more pores are blocked by coating, the greater the difference in gradient for O_2 relative to that for CO_2.

This phenomenon can be explained mathematically, as outlined below. From Fick's law (Eq. 5.4):

$$[CO_2]_i = \frac{r_{CO_2} * R_{CO_2}}{A^{fruit}} \qquad \textbf{[5.11]}$$

where: R_{CO_2} = total resistance to CO_2 diffusion (s cm^{-1})

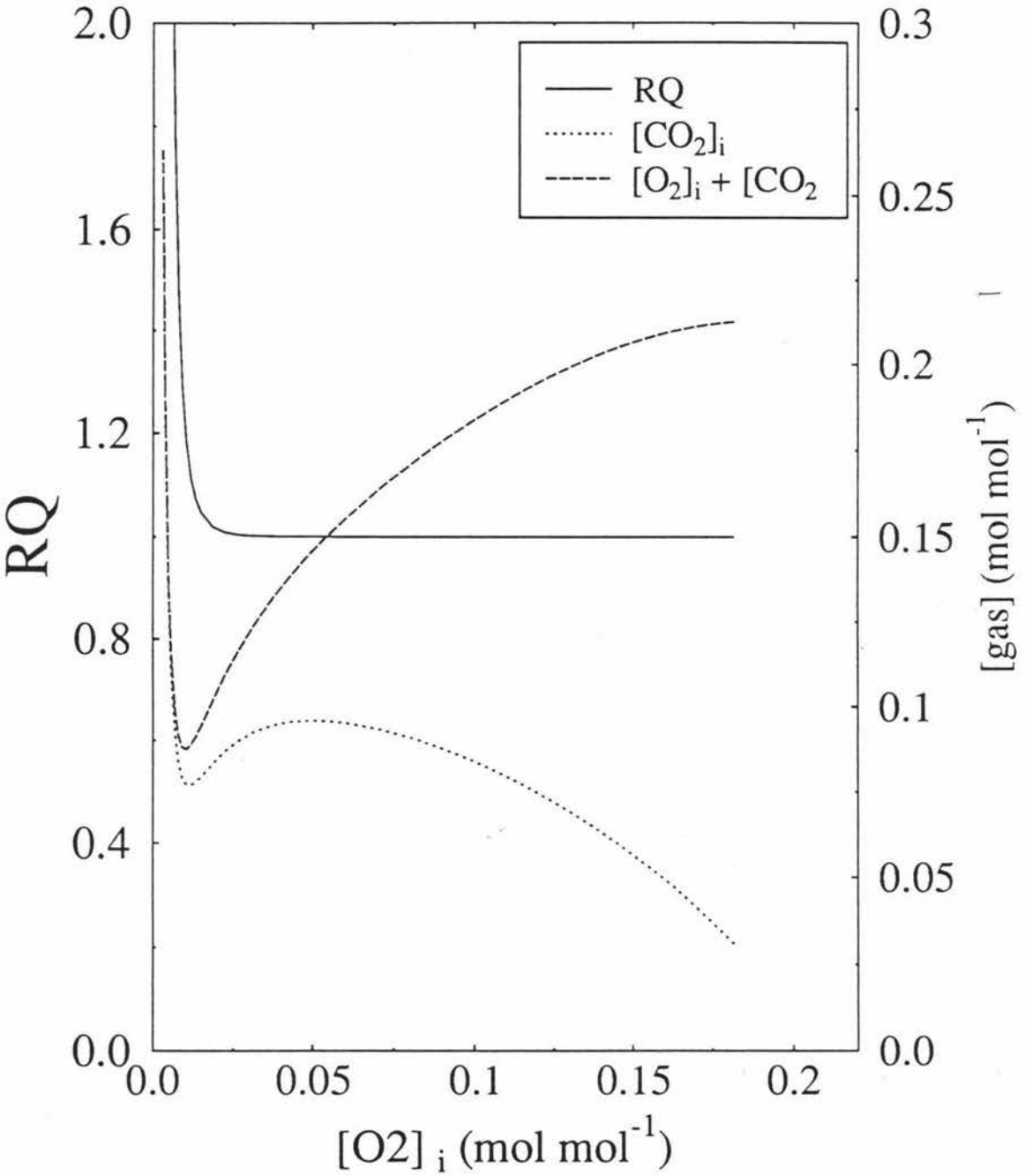


Figure. 5.1 Relationship of $[CO_2]_i$ + $[O_2]_i$, RQ and $[CO_2]_i$ with $[O_2]_i$ for a model system with $R^{cut}_{O2} = 30 \times R^{cut}_{CO2} = 2.5 \times 10^4 cm\ s^{-1}$.

Combining Eqs. 5.4 and 5.11, the sum of the internal concentrations of the respiratory gases can be calculated from:

$$[O_2]_i + [CO_2]_i = [O_2]_e - \frac{r_{O_2} * R_{O_2} - r_{CO_2} * R_{CO_2}}{A^{fruit}} \qquad \textbf{[5.12]}$$

For a fruit which is respiring only aerobically and RQ=1, ($rr_{O_2} = rr_{CO_2}$), this can be re-written as:

$$[O_2]_i + [CO_2]_i = [O_2]_e - \frac{\left(r_{O_2} * \left(R_{O_2} - R_{CO_2}\right)\right)}{A^{fruit}} \qquad \textbf{[5.13]}$$

Examination of this relationship illustrates why the sum of $[O_2]_i + [CO_2]_i$ declines with reducing $[O_2]_i$. The reduced $[O_2]_i$ arises because of increased R_{O_2} but, in a coated fruit, R_{CO_2} is not increased to the same extent as R_{O_2} (ie. the term $R_{O_2} - R_{CO_2}$ increases with increased R_{O_2}. Thus, $[O_2]_i + [CO_2]_i$ decreases as $[O_2]_i$ is reduced to an extent which depends upon the degree of differential resistance in the fruit cuticle and its initial porosity.

In Fig. 5.1, $[O_2]_i + [CO_2]_i$ changed substantially as $[O_2]_i$ was depressed by coating until an abrupt upswing was reached at low $[O_2]_i$. The rate of decline in $[O_2]_i + [CO_2]_i$ became steeper with decreasing $[O_2]_i$, down to a minimum value of 0.09 mol mol^{-1} which occurred at an $[O_2]_i$ value of about 0.01 mol mol^{-1}. Below this level of $[O_2]_i$ the model predicted that $[O_2]_i + [CO_2]_i$ would increase as $[O_2]_i$ fell below the anaerobic compensation point (Boersig et al., 1988) and the fruit began to respire

through the anaerobic pathway. These effects were associated with an increase in $[CO_2]_i$ from 0.03 to 0.1 mol mol^{-1} as $[O_2]_i$ was reduced from 0.18 mol mol^{-1} to 0.05 mol mol^{-1}, a decline to 0.075 mol mol^{-1} as $[O_2]_i$ became severely limiting to respiratory CO_2 production followed by a dramatic rise $[O_2]_i$ as the fruit fell below the ACP (anaerobic compensation point) (Fig. 5.1).

As illustrated in Fig. 5.1, the optimum $[O_2]_i$ at which respiration rate is minimised without inducing anaerobic respiration lies just to the right of the point at which $[O_2]_i$ + $[CO_2]_i$ swings upwards; such plots could therefore be used to identify optimum $[O_2]_i$ in different fruit types in a range of conditions. I examined the possibility that this approach could be used to identify the optimum $[O_2]_i$ in squash. Three experiments were carried out. The first of these provided a preliminary screening of various coating materials to enable selection of a coating which was highly effective in reducing fruit weight loss but which did not interfere excessively with the fruit's gas exchange. The second was to identify the optimum coating treatment for squash for minimising weight loss without inducing adverse physiological effects. This was examined by applying different numbers of coatings to individual squash within a short period of time. In the third experiment, the effects of repeated coating on the internal atmosphere of individual squash were examined on fruit which were allowed to approach physiological equilibrium between each coating treatment.

5.3 Method

5.3.1 Experiment 1: Screening of coating materials

The following coatings were prepared at the stated concentrations in water: Tween 20 (2%; Atlas Chemical Industries Inc.), Semperfresh (sucrose esters of edible fatty acids, 2%; Semper Biotechnology, Reading, UK), PEG (Polyethylene glycol 4000, 5%; Bronson and Jacobs (NZ) Ltd, Auckland), Decco wax 227 (applied without dilution), Primafresh (applied without dilution; Johnson Wax, Racine, Wisconsin), Casein (2%; Dairy Research Institute, Palmerston North). Each solution was applied with a paintbrush to 6 fruit and allowed to dry; comparable control fruit were left untreated.

Fruit were kept in ambient conditions (at approximately 20°C, 50%RH). Weight of fruit was measured every 24 hours for 3 days to determine the rate of weight loss. A 1-ml sample of internal gas was withdrawn from the fruit cavity with a hypodermic syringe while fruit were temporarily submerged in water, and analyzed for O_2, CO_2 and C_2H_4, using gas chromatography (Shimadzu GC-8A).

5.3.2 Experiment 2: Effects of number of applied coatings

Batches of 5 fruit were given either 0 (no coating = control), 1, 2, 3, 4, 5 or 6 coatings with 'Primafresh'. Fruit were allowed to dry for 1.5 h between coatings. They were then stored in a plastic bag at 20°c with a continuous flow of humidified

air (90 %RH) and their rate of weight loss monitored at 24 h intervals.

Respiration rate was determined at 20°C by sealing individual fruit in a plastic bag (40cm x 30cm) with 1 cm diameter inlet and outlet pipes. Fruit were exposed to a continuous stream of air at 70% relative humidity from a water vapour generator (Dew Point generator LI-610 LI-COR Ltd) flowing at 500 ml min^{-1}. The outlet pipe was 1 m long; a septum port was attached at a distance 5 cm from the bag. Samples of the effluent air (1 ml) were withdrawn through the septum with a hypodermic syringe, and analysed for carbon dioxide by gas chromatography. A 1 ml sample of internal gas was withdrawn from the cavity of each fruit with a hypodermic syringe while fruit were completely submerged in water after 3 days at 20°C, 90% RH, and analyzed for O_2, CO_2 and C_2H_4 using gas chromatography.

Rate of CO_2 evolution (ml kg^{-1} h^{-1}) was calculated as follows:

$$r_{CO_2} = [CO_2]_{jar} * 60 * fr * \frac{1000}{wt} \qquad \textbf{[5.14]}$$

where :

$[CO_2]_{jar}$ = mole fraction of CO_2 in sampled gas

fr = air flow rate through the bag (= 500 ml min^{-1})

wt = fruit weight (g)

Carbon loss, the net rate of carbon loss from the fruit was calculated by multiplying the mass rate of CO_2 evolution by 12/44, which is the ratio of the molecular weights of carbon and CO_2.

Evaluation of sensory quality of fruit was investigated by Levin Research Centre in New Zealand. Three 3 cm thick slices (measured from the skin edge) were cut from each squash and trimmed to 4 cm in length by removing the top and bottom of the slice. Samples were taken from all five squash per treatment, giving a total of fifteen samples. Samples from different treatments were cooked separately by steaming in a sieve over rapidly boiling water for 20 minutes and then coded with three digit random numbers. They were served warm, under red lighting to mask colour differences, and a panel of 14 experienced panellists rated the samples for sweetness, flavour, texture (dryness) and off flavours.

5.3.3 Experiment 3: Effect of coating time on modification of internal atmosphere of squash

Two chambers were adhered to the fruit surface (Banks and Kays, 1988) of each of 12 squash and allowed to equilibrate at 20°C. Fruit with leaking chambers were eliminated, reducing the number of fruit in the study to 7. Equilibration time of the chambers with the internal atmosphere was established by determining chamber oxygen and carbon dioxide contents 48 h after sealing the chamber and then at 0, 1.5, 3, 4.5, 6, 7.5 h after flushing the chamber with air (Banks and Kays, 1988).

Three Primafresh coatings were applied to each fruit, with 2 days allowed between coating treatments to allow physiological equilibration before the following coating was applied. This procedure was expected to remove fruit to fruit variability which would be associated with using different fruit for each level of coating treatment.

$[CO_2]_i$ and $[O_2]_i$ were measured every day for each fruit by withdrawing a 0.1 ml sample of gas from each chamber with a gas-tight syringe followed by analysis for O_2, CO_2 and C_2H_4 using gas chromatography (Shimadzu GC-8a).

Equilibration times of the atmosphere inside chambers with the internal atmosphere of squash were estimated from CO_2 concentration taken from chambers at 1.5 h intervals up to 7.5 h as shown by Banks and Kays (1988). In the chambers adhered to squash kept at 20°C and 50% RH, 97.5% of the population would have achieved 99% equilibration with the internal atmosphere of squash in 30.2 h.

5.4 Results

5.4.1 Experiment 1

Fruit with Primafresh coating had significantly less weight loss than all other treatments (Tables. 5.1 and 5.2). None of the other surface coating materials reduced weight loss compared to the control. There were only slight effects of Primafresh coating on the internal atmosphere composition of the fruit, with a small decrease in $[O_2]_i$ and a small increase in $[CO_2]_i$. Neither PEG nor Casein had a discernible effect on internal atmosphere composition. In contrast, squash coated with Decco 227 had substantially lower $[O_2]_i$ and higher $[CO_2]_i$ than fruit in any of the other treatments ($P < 0.001$, Table 5.2). Intermediate effects were obtained for Semperfresh and Tween 20.

Table. 5.1 Internal atmosphere composition, and rate of weight loss in squash with different coatings. Weight loss was % loss out of initial weight for 5 days.

Material	Weight loss	Reduction % Wtloss	Internal O_2	Internal CO_2
Primafresh	0.0060 b	53	0.1797 a	0.0333 b
Casein	0.0126 a	3	0.1953 b	0.0250 a
PEG *	0.0134 a	+4	0.1875 a	0.0243 a
Contorl	0.0129 a	0	0.1906 b	0.0209 a

Table. 5.2 Weight loss, internal O_2 and internal CO_2 (mol mol^{-1}) in the squash fruit treated with several coatings for 5 days.

* Abbreviation: PEG , Polyethylene Glycol.

Material	Weight loss	Reduction % of Wt loss	Internal O_2	Internal CO_2
Primafresh	0.0085 b	28	0.17 ab	0.036 b
Decco 227	0.0131 a	+11	0.061 c	0.095 a
PEG *	0.0136 a	+14	0.183 a	0.026 cd
Semper fresh	0.0135 a	+14	0.166 b	0.041 b
Tween 20	0.0136 a	+15	0.178 ab	0.036 bc
Control	0.0118 a	0	0.184 a	0.024 d

5.4.2 Experiment 2

Weight loss of Primafresh-coated fruit was significantly less than that of non-coated controls ($P < 0.05$, Table 5.3). However, for coated fruit, there was no significant difference of weight loss associated with the number of coatings applied to the fruit surface. Weight loss of individual fruit within any given coating treatment was variable (Table 5.3). Overall, CO_2 production by coated fruit was significantly greater than those of non-treated fruit, and was highly variable both within and between the different coating treatments (Table 5.3, $P<0.05$).

Coating markedly depressed $[O_2]_i$ ($P<0.0001$), to an extent which depended upon the number of coatings applied ($P < 0.0001$ for the linear effect). However, $[O_2]_i$ was highly variable in individual squash within each coating treatment. Likewise, there were substantial effects of both coating and number of coatings on $[CO_2]_i$ ($P < 0.01$ in each case), although again the response of individual fruit was highly variable. $[C_2H_4]_i$ of coated fruit was significantly higher than that of coated fruit ($P < 0.05$). There was a significant trend that $[C_2H_4]_i$ of fruit increased as the number of coatings increased ($P < 0.01$ for the linear effect).

Number of coatings had no significant influence on sweetness and flavour of squash fruit (Table 5.4). Fruit in the control treatment were wetter in texture than the coated squash ($P < 0.05$) but there was no consistent relationship between number of coatings applied and flesh texture. Coated fruit on average had a similar level of off-flavours to controls but there appeared to be a relationship between the incidence of

Table 5.3 Internal atmosphere composition (mol mol^{-1}), CO_2 production and rate of weight loss after 3 days in squash with different numbers of Primafresh coatings. Means of five replicates ± SE.

Number of coatings	Wt loss (%)	$[O_2]_i$ (mol mol^{-1})	$[CO_2]_i$ (mol mol^{-1})	$[C_2H_4]_i$ (mol mol^{-1})	CO_2 Prod mol kg^{-1} h^{-1}
0	0.68±0.23	0.195±0.0086	0.02±0.003	0.0002±0.000	44.2±17.5
1	0.41±0.10	0.156±0.018	0.063±0.018	0.0024±0.004	52.3±22.5
2	0.64±0.14	0.094±0.047	0.281±0.211	0.0125±0.012	58.5±43.7
3	0.50±0.09	0.129±0.011	0.092±0.012	0.0023±0.003	41.3±6.17
4	0.58±0.13	0.062±0.027	0.389±0.234	0.0211±0.014	46.3±17.5
5	0.52±0.08	0.046±0.009	0.430±0.149	0.0178±0.004	51.4±12.0
6	0.50±0.18	0.076±0.054	0.257±0.123	0.0223±0.023	62.1±13.9

off-flavours in a given coating treatment and CO_2 concentration inside the individual fruit (Figs. 5.2 and 5.3). This relationship is clarified in Table. 5.5: off-flavour ratings were highly, positively correlated with both $[CO_2]_i$ level ($P < 0.0001$) and negatively with $[O_2]$i level ($P<0.0001$). Individual fruit in the control treatment which

Table. 5.4 Sensory quality ratings of squash applied different number of coatings.

Number of coatings	Sweetness	Flavour	Texture	Off flavour
0	55.6	49.0	31.5	21.4
1	45.0	42.0	61.6	8.1
2	61.8	56.8	38.7	26.6
3	51.6	46.5	50.9	14.1
4	55.2	52.4	50.6	33.7
5	51.6	45.2	71.4	42.6
6	55.3	46.2	57.4	27.1
F test	ns	ns	$P < 0.0001$	$P < 0.0001$

had off-flavours but low $[CO_2]i$ also had internal rots when cut open, and these were associated with low texture ratings (indicating a wet, soggy texture).

Table 5.5 Correlation between sensory quality ratings and internal atmosphere of coated squash.

Internal atmosphere	Texture	Off-flavour
$[CO_2]_i$	ns	0.71 ($P < 0.0001$)
$[O_2]_i$	-0.39	-0.61 ($P < 0.0001$)

A plot of $[O_2]_i$ + $[CO_2]_i$ versus $[O_2]_i$ (Fig. 5.4) of individual squash rose gradually from 0.21 to approximately 0.24 mol mol^{-1} as $[O_2]_i$ declined from 0.21 down to 0.1 mol mol^{-1}, and then rose very steeply with further reduction in $[O_2]_i$.

5.4.3 Experiment 3

$[O_2]_i$ had decreased ($P<0.001$) 24 h after coating (Fig. 5.5a) but, after a further 24 h equilibration, it had recovered ($P<0.001$), but not to the level presented in a non-coated fruit ($P<0.001$). A similar pattern of change in $[O_2]_i$ was obtained after the second coating was applied, but the average $[O_2]_i$ level when it recovered after a further 24 h had declined further with the additional coating ($P<0.001$). This pattern and downward trend in $[O_2]_i$ was repeated with a third application of coating, but the recovery of $[O_2]_i$ 48 h after coating was not significant and was much smaller than that seen with the previous coatings ($P<0.01$).

These changes were associated with concomitant fluctuations (increases followed by decreases) in $[CO_2]_i$ (Fig. 5.5b). There was a significant rise in $[CO_2]_i$ ($P<0.001$) 24h after first coating, then $[CO_2]_i$ declined ($P<0.001$) after further 24 h of equilibration but not to the level present before coating ($P<0.001$). After the second coating, a similar pattern of change in $[CO_2]_i$ was repeated. Average $[CO_2]_i$ level at 48 h after each coating rose with each coating applied, whilst the decline of $[CO_2]_i$ level from 24 h after each coating application with a further 24 h equilibration was smaller after third coating than after the first and second coatings.

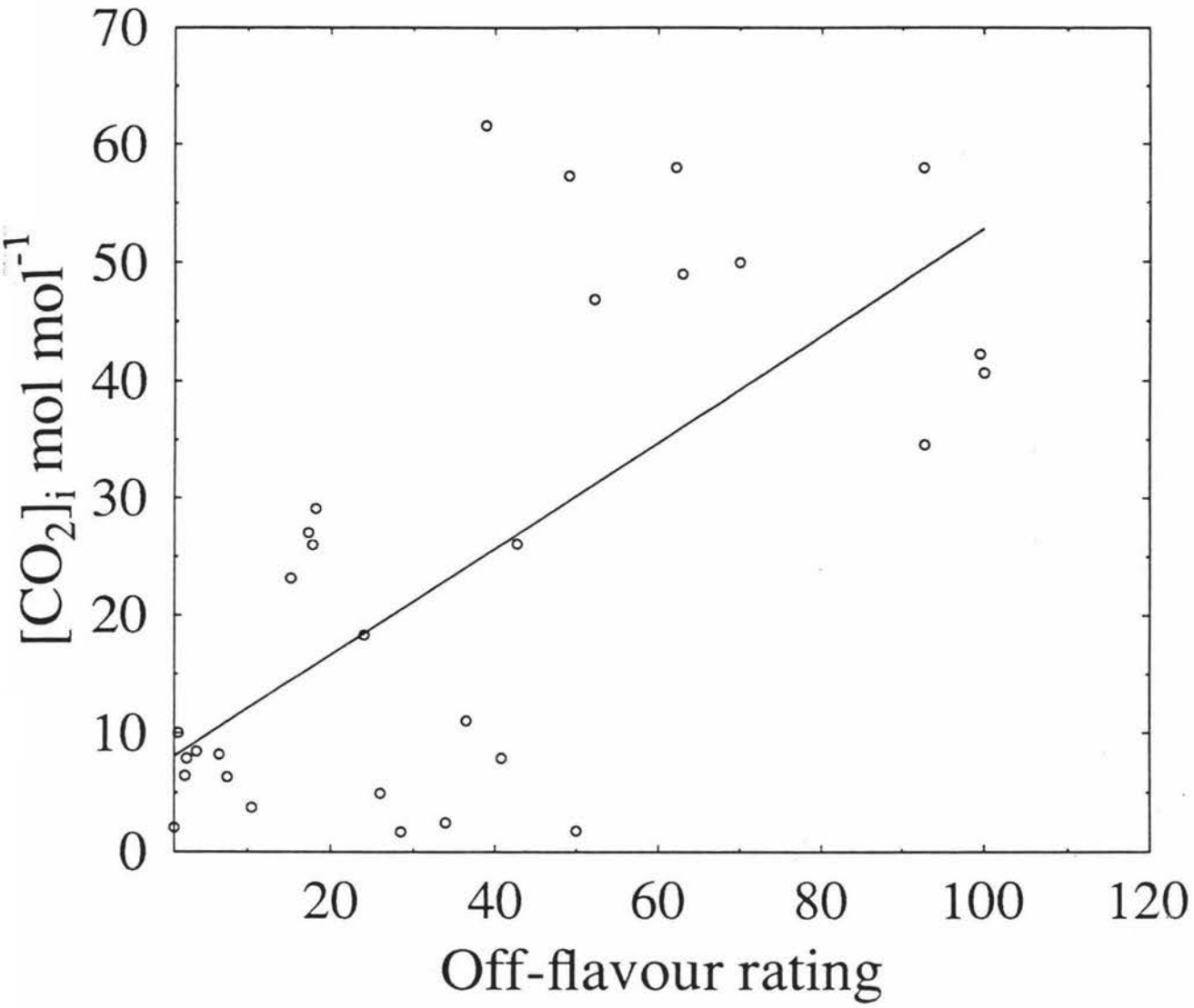


Figure. 5.2 Relationship between off-flavour score and internal carbon dioxide of individual squash coated with primafresh and kept at 20°C at 90 RH for 3 days. Fitted line shows the regression equation; $[CO_2]_i = 0.45211x + 7.5957$.

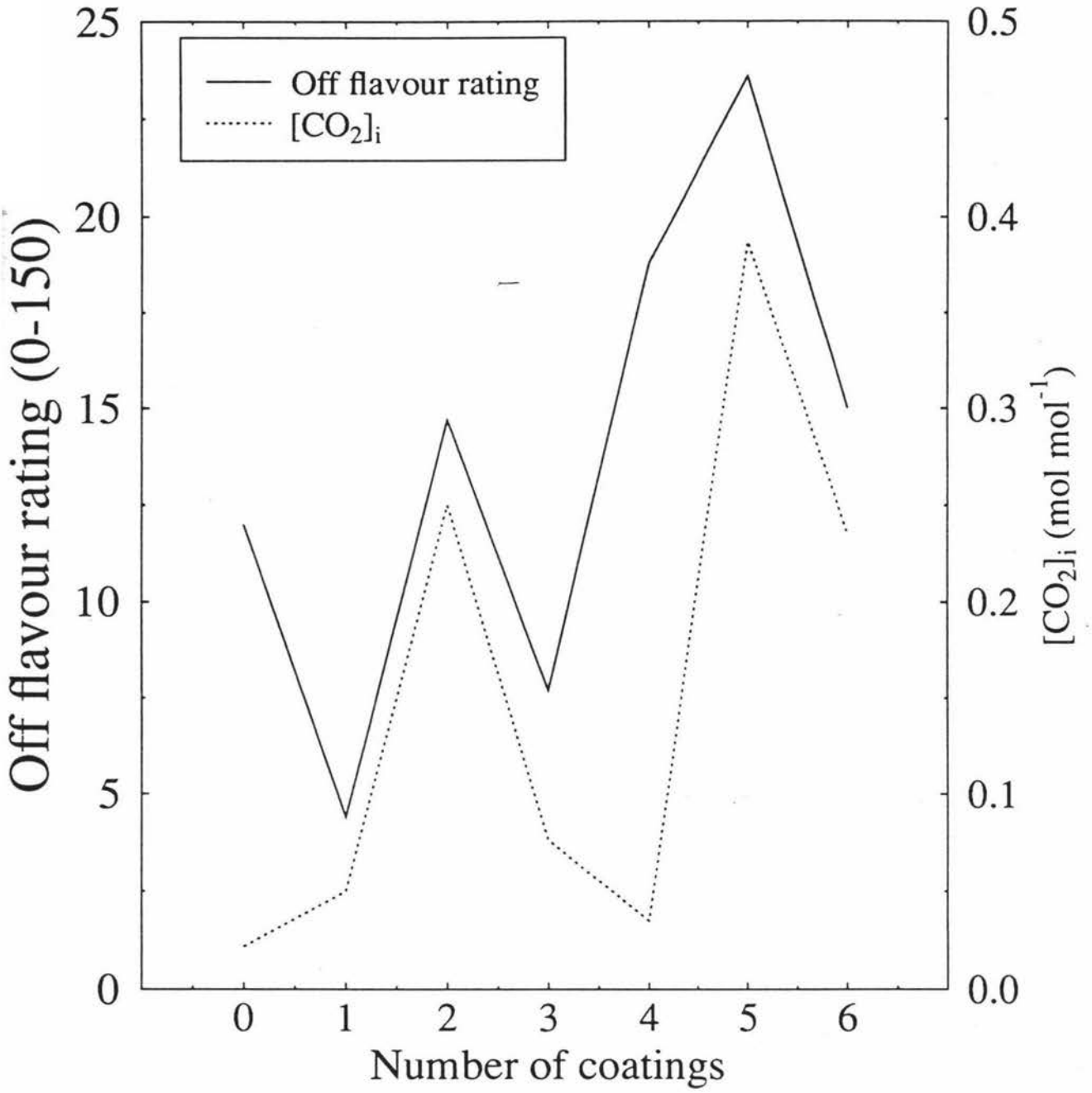


Fig. 5.3 $[CO_2]_i$ and off flavour ratings in groups of squash with different numbers of Primafresh coatings.

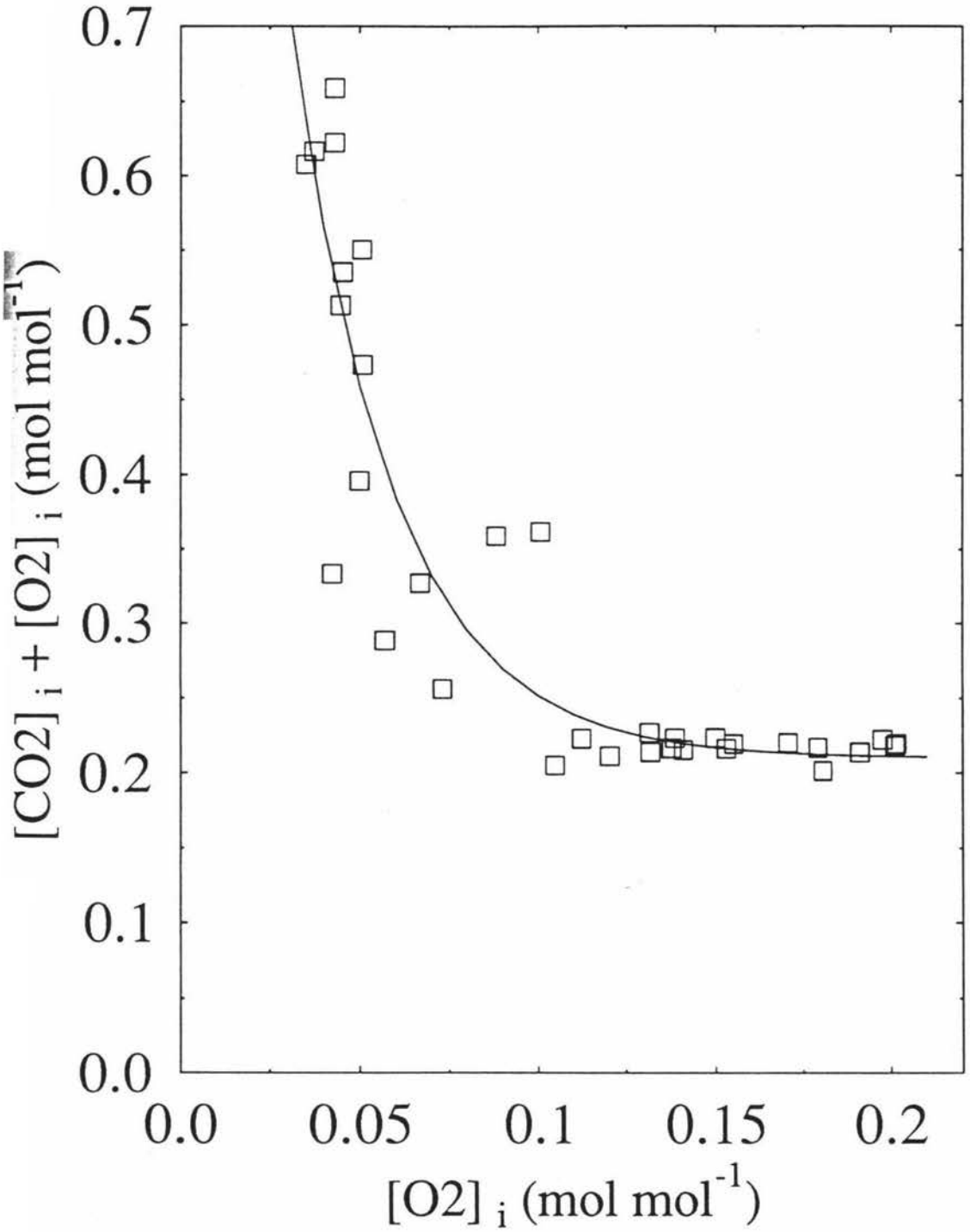


Figure. 5.4 Relationship of $[O_2]_i$ + $[CO_2]$i with $[O_2]_i$ of individual squash in experiment 2. Equation of fitted line: $y=0.21+1.486/\exp(35.7*[O_2]_i)$.

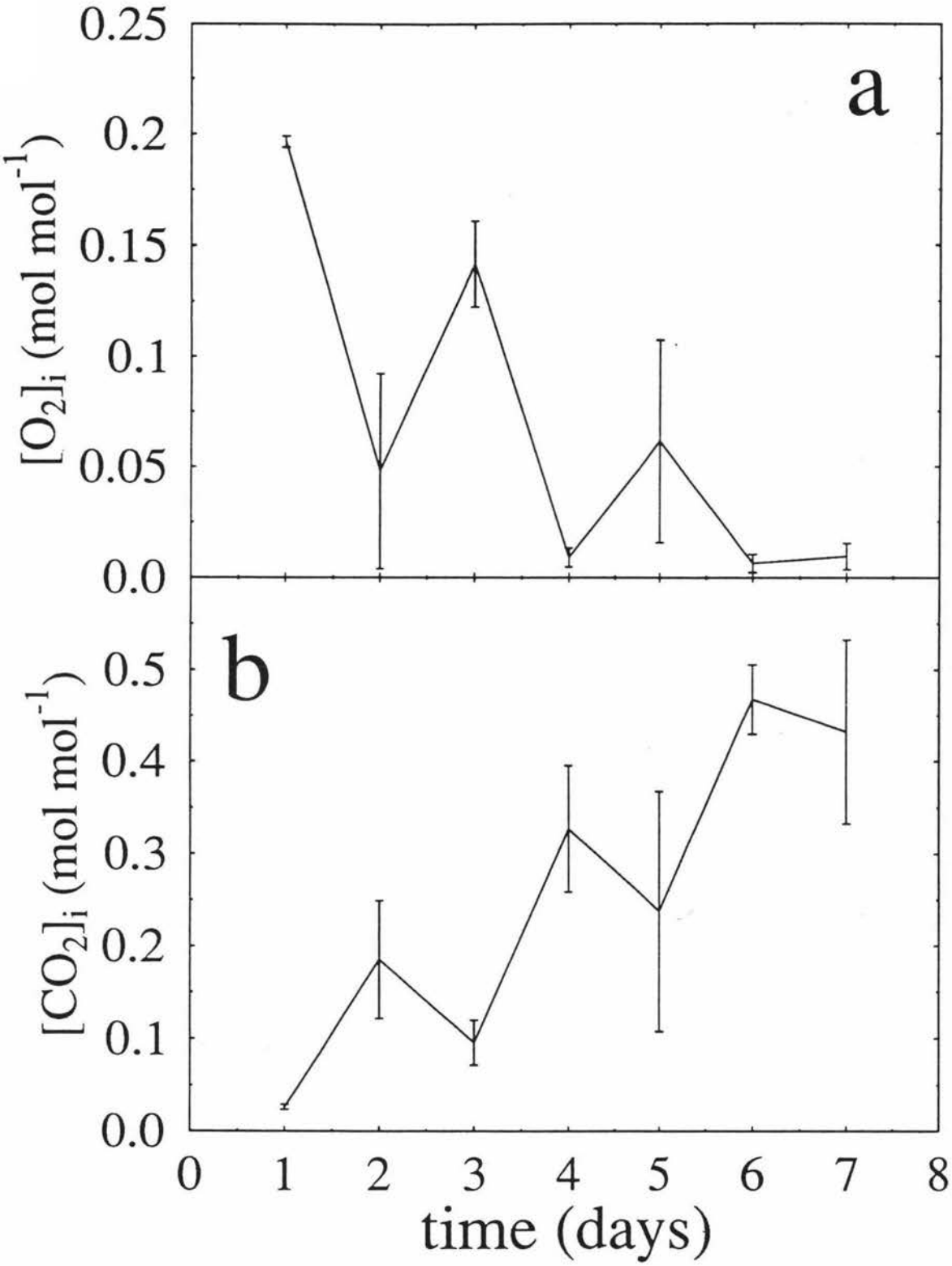


Figure 5.5 Effect of coating time on modification of internal atmosphere of squash. Fruit were coated on day 1, 3 and 5 after mesurement of $[O_2]_i$ and $[CO_2]_i$.

A plot of $[O_2]_i + [CO_2]_i$ versus $[O_2]_i$ of squash (Fig. 5.6) obtained over the duration of the experiment (7 days) rose gradually from 0.23 to 0.27 mol mol^{-1} as $[O_2]_i$ declined from 0.21 mol mol^{-1} to approximately 0.05 mol mol^{-1}. Below that level of $[O_2]_i$, $[O_2]_i + [CO_2]_i$ increased markedly.

5.5 Discussion

5.5.1 Experiment 1

Treatment effects on weight loss were not necessarily consistent with their effects on internal atmosphere composition. From the point of view of maximising reduction in water loss whilst avoiding modification of internal atmosphere composition, Primafresh was clearly identified as the material most suited to further testing. In contrast, squash treated with Decco 227 had the greatest modification of internal atmosphere composition with no obvious benefit for reducing weight loss. These data indicate that there may have substantial variation in relative resistance of the coating materials to diffusion of water vapour and the respiratory gases as noted by Hagenmeier and Shaw (1992) and Hagenmeier and Baker (1993).

Reduction of weight loss achieved in squash treated with Primafresh was 28% and 53% this early work. This indicates that the current amount of weight loss incurred during shipping to Japan (8%, Section 4.1) might be reduced to 5.3 - 3.8% by coating with Primafresh.

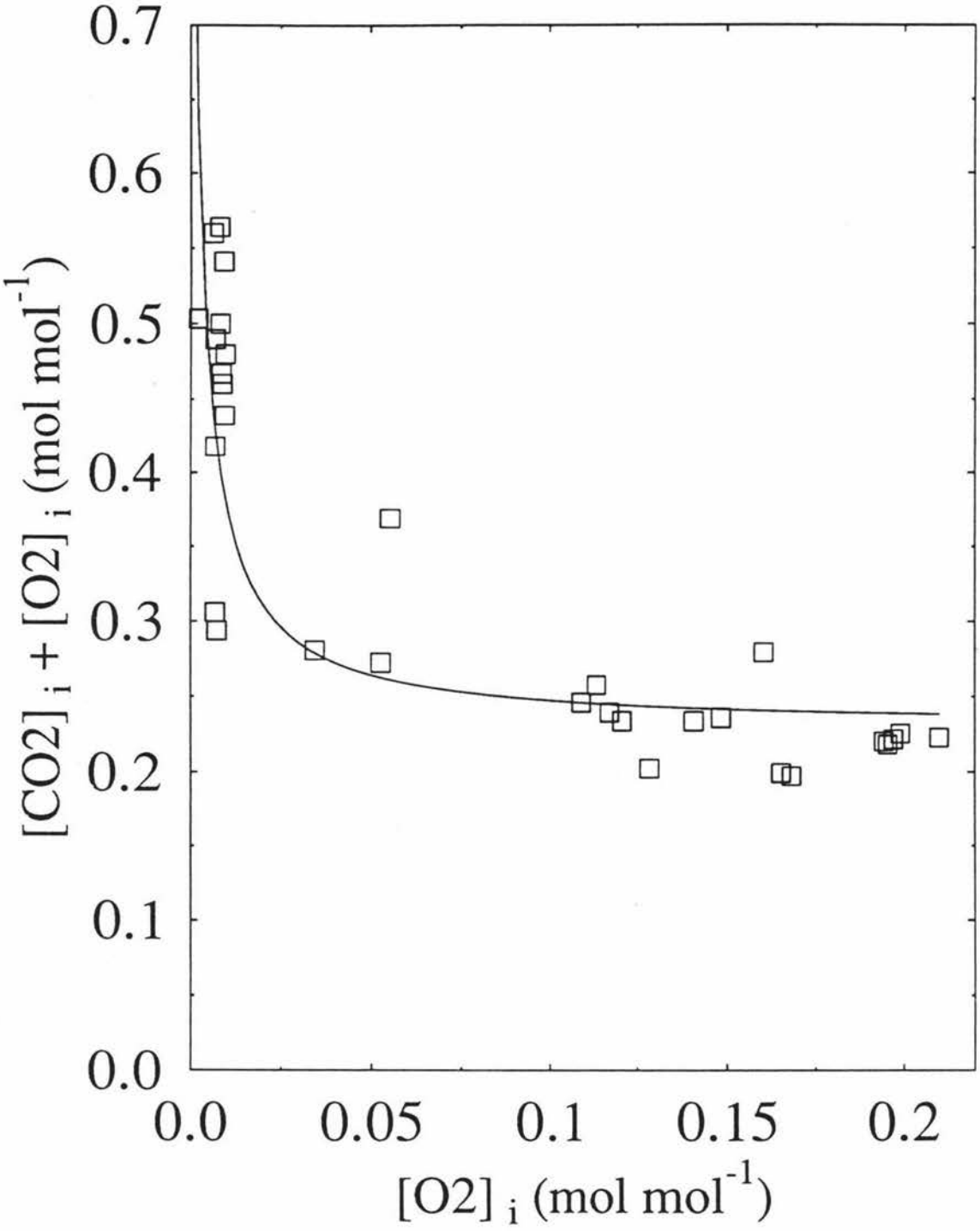


Fig.5.6 Relationship of $[O_2]_i$ + $[CO_2]_i$ with $[O_2]_i$ of individual squash in experiment 3. Equation of fitted line: $y=1-0.7703*[O_2]_i/(0.00235+[O_2]_i)$

5.5.1 Experiment 2

Coating squash reduced weight loss, an effect which was presumably at least in part due to increased resistance to water vapour diffusion and therefore decreased transpiration. However, this effect was not consistently related to the number of coatings on a given fruit. This may be related to the effects of coating on the internal atmosphere of the squash: many of the fruit were probably anaerobic and this would have increased their rate of carbon dioxide production through the process of fermentation (Cohen et al., 1990; Drake et al., 1987). Since total weight loss is the combination of weight losses due to the separate processes of transpiration and respiration, an increase in CO_2 production could have stimulated weight loss in some fruit with substantial alterations in internal atmosphere. In experiment 2, coatings reduced $[O_2]_i$ in order of increasing number of coatings but increases in $[CO_2]_i$ were not so consistent. This might be attributed to the proportion of anaerobic respiration in total respiration (aerobic and anaerobic respiration) in fruit, in that some fruit may have started to ferment whilst other may not. Once fruit began anaerobic respiration, $[CO_2]_i$ would have been elevated because of CO_2 production by fermentation regardless of change in $[O_2]_i$.

Respiration might generally be expected to be lower in coated fruit because of their modified internal atmosphere composition (low $[O_2]_i$ and high $[CO_2]_i$). However, in fruit which had become anaerobic, transpiration would comprise a greater proportion of total weight loss as the carbon loss associated with CO_2 production increased as anaerobic respiration increased. Therefore, the relative contribution of respiration to

total weight loss could be increased in anaerobic fruit.

Carbon loss is directly related to the respiration rate and therefore a function of temperature (Gaffney et al., 1985). The higher a relative humidity is, the larger proportion of carbon loss in total weight loss (Fig. 5.7), because transpiration is reduced whilst rate of respiration is not affected by relative humidity. For example, at 20°C, proportion of carbon loss out of total weight loss is 100%, 32% and 14% at RH 100%, 90% and 70% respectively (using an estimated CO_2 production of a squash fruit of 51 ml kg^{-1} h^{-1} at 20°C). The proportion of transpiration loss out of total weight loss is 0, 67% and 84% at RH 100%, 90% and 70% respectively (Fig. 5.7): even in saturated air, fruit may lose weight by carbon loss.

CO_2 production of fruit with different numbers of coatings was not consistent with coating number, and was highly variable between individual fruit even given the same number of coatings. Whilst CO_2 production levels measured in the stream of the air flowing over individual fruit were highly variable, repeated readings made on the same fruit over time were consistent. Therefore, high variability, rather than measurement variation, appears to be responsible for fruit to fruit variation. Some researchers have observed that application of coatings to fruit results in marked increase in variability of internal atmosphere concentration (Smith et al., 1987). Inherent variability in fruit resistance characteristics has been suggested as a cause of variable responses of individual fruit to coatings (Banks, 1985a). Small variations in proportions of blocked pores and variation of respiration rate of individual fruit resulted in the large degree of variation in $[O_2]_i$ (Banks et al., 1993).

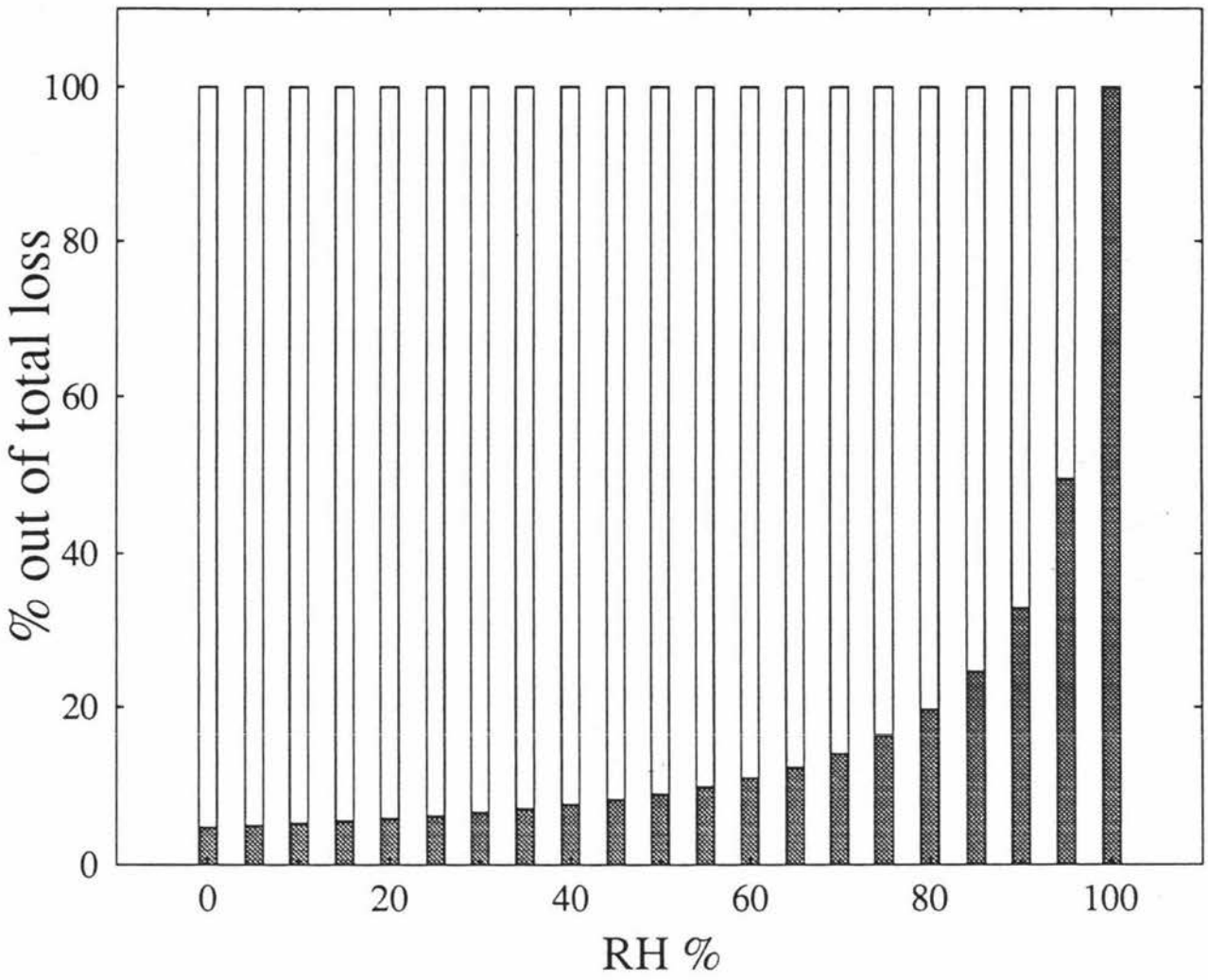


Fig. 5.7 Proportion of carbon loss to transpiration loss in a total weight loss in different relative humidities at 20°C. Grey: Carbon loss, White: Transpirtion loss.

Hagenmeier and Shaw (1992) found that the commercial coatings used for fruits and vegetables had resistance to O_2 diffusion 2 to 8 times as high as that to CO_2 diffusion, and 40 to 20,000 times as high as that to water vapour. The effects of blocking the pores on a fruit surface with such a coating on the overall skin resistance to diffusion of the various gases have recently been explored using a mathematical model (Banks et al., 1993), assuming certain characteristics of the fruit surface and the applied coating material. Their predictions indicated that total skin resistance to water vapour in a coated fruit would be far less than resistance to respiratory gases. Thus, transpiration would be far less affected by coating than exchange of the respiratory gases. Internal respiratory gas concentrations could be markedly affected, leading fruit to begin anaerobic respiration at levels of coating which achieved worthwhile reductions of transpiration. In this situation, heavy coating treatments could actually enhance weight loss by stimulation of carbon loss from anaerobic respiration. Coatings may therefore in same cases have the opposite effect to my initial expectation that they would reduce weight loss. However, there may be some scope for reducing weight loss by a single thin coating using a material like Primafresh provided subsequent temperatures were not excessively high.

In Fig. 5.4, the sum of $[O_2]_i + [CO_2]_i$ rose continuously as $[O_2]_i$ levels were depressed towards zero, without any initial decline at $[O_2]_i$ levels between 0.20 and 0.02 mol mol^{-1} seen in Fig. 5.1. The reduction in $[O_2]_i$ would have been associated with increased proportions of blocked pores (Banks et al., 1993) but in the case of these squash, it appears that this did not lead to development of differential resistance in the fruit skin. Two potential mechanisms for this effect are discussed in section 5.6.

Another point of interest in these data is that the fruit appeared to become anaerobic (identified by the marked upswing the plot of $[O_2]_i + [CO_2]_i$ vs $[O_2]_i$) at quite high levels of $[O_2]_i$. One explanation for this is that the high level of CO_2 accumulated in the internal atmosphere of these squash stimulated fermentation (Kader, 1987).

High $[C_2H_4]_i$ accumulation occurred in the fruit with high $[CO_2]_i$, presumably because of increased skin resistance to gas exchange achieved by coating leading which would have reduced outward $[C_2H_4]_i$ diffusion. Generally high $[CO_2]_i$ is considered to suppress C_2H_4 evolution in fruit (Abeles et al., 1992; Yang, 1985), but any effect of the elevated $[CO_2]_i$ on ethylene production was overcome by reduced diffusion of C_2H_4 from the fruit.

Off-flavour ratings were related positively to [CO_2]i and inversely to $[O_2]_i$ except in control fruit, indicating that these off flavours may be attributed to metabolic abnormalities associated with the inhibition of O_2 and CO_2 exchange through the fruit skin, perhaps directly related to anaerobic respiration. Off-flavour was probably caused by elevation of ethanol and acetaldehyde as reported by Cohen et al. (1990) and Drake et al., (1987). Increased off flavour ratings with rise of $[CO_2]_i$ associated with multiple coatings suggests that more than one coating application carries excessive risk of flavour deterioration. Coating application on squash did not affect other aspects of taste quality such as sweetness and flavour in this experiment.

5.5.3 Experiment 3

In this experiment, increased skin resistance caused by coating application reduced $[O_2]_i$ and increased $[CO_2]_i$, to an extent which was largely determined by the magnitude of increase in resistance and initial fruit respiration rate. There was some recovery toward the initial internal atmosphere composition, presumably as respiration rate was affected by the lowered $[O_2]_i$, an effect which, interestingly, took longer to occur than initial physical aspects of equilibration. Thus, respiratory response of fruit to change of internal atmosphere by increased skin resistance was not instantaneous.

Eq. 5.4 from Fick's law used in section 5.2 can be rewritten as following

$$[O_2]_i = [O_2]_e - \frac{r_{O_2} * R_{O_2}^{fruit}}{A^{fruit}} \qquad \textbf{[5.15]}$$

When skin resistance was increased by coating, R_{O_2} increased and therefore lowered $[O_2]_i$, as $[O_2]_e$ and A^{fruit} are constant values. Fruit respiratory oxygen uptake (O_2 for being consumed by respiration rr_{O_2}) and r_{O_2} (O_2 diffusing into fruit) are the same at steady state as described in fruit system model (section 5.2). Therefore, reduced r_{O_2} was accompanied by lower CO_2 production and r_{CO_2} lowed by increase in $[O_2]_i$. From Eq. 5.11, increased R_{CO_2} achieved by coating increased $[CO_2]_i$ initially but then reduced respiration achieved by lowered $[O_2]_i$ resulted in reduction of $[CO_2]_i$.

The time dependence of this process is shown in Fig. 5.5 in the decreased $[O_2]_i$ and increased $[CO_2]_i$ on day 2, followed by the increase of $[O_2]_i$ and decrease of $[CO_2]_i$ on day 3 which presumably corresponds to be the moving towards a new steady state caused by elevated skin resistance by coatings. There was therefore a delay between achievement of physical effects and physiological effects of elevated skin resistance in these squash.

The fitted line $[O_2]_i + [CO_2]_i$ shown in Fig. 5.6 is similar to that in Fig. 5.4 in that there was no initial decline with depression of $[O_2]_i$ from 0.2 to 0.02 mol mol^{-1}. However, anaerobic respiration does not appear to have been induced until $[O_2]_i$ reached much lower concentrations (approximately 0.05 mol mol^{-1}) than in Experiment 2 (approximately 0.1 mol mol^{-1}). This was presumably because in Experiment 3 the fruit had an opportunity to reduce respiration in response to coating application before the next coating was applied, whereas in Experiment 2 coatings were applied in rapid succession.

5.6 Overall discussion

Wade and Graham (1987) showed that total mole fraction of respiratory gas in the internal atmosphere ($[O_2]_i + [CO_2]_i$) should always be approximately 0.21 mol mol^{-1} for non-coated fruit with RQ of 1 and in which skin is equally resistant to diffusion of respiratory gases. Likewise, $[O_2]_i + [CO_2]_i$ of non-coated squash was also approximately 0.21 mol mol^{-1} (Tables 5.1, 5.2 and 5.3). In contrast, coated fruit often have a total respiratory gas pressure of considerably less than 0.21 mol mol^{-1},

indicating that their skins are differentially resistant to O_2 and CO_2 (Ben-Yehoshua, 1967; Banks, 1985a,b; Nisperos-Carriedo et al., 1990; Dadzie, 1992; see also Fig. 5.1). However, the response of internal atmosphere composition of squash to coating was quite different.

The fitted lines for $[O_2]_i + [CO_2]_i$ in Figs. 5.4 and 5.6 were always more than 0.21 mol mol^{-1}, even when $[O_2]_i$ was clearly not severely limiting to respiration. This suggests that the skin resistance to CO_2 was at least equal to, or greater than that to O_2, even in coated fruit. This could arise because of two possible mechanisms. Perhaps $R^{cut}_{O_2}$ in squash is not substantially greater than $R^{cut}_{CO_2}$. Thus when pores became blocked, the increased proportion of total gas exchange occurring through the cuticle would not have resulted in differential resistance. Alternatively, if $R^{cut}_{O_2} > R^{cut}_{CO_2}$ then development of differential resistance could be avoided if absolute cuticular resistance was so great that cuticular contribution to total exchange remained low even when pores were blocked. These two scenarios have been investigated using the model described by Banks et al. (1993) and are presented in Figs. 5.8 and 5.9.

In the first of these graphs (Fig. 5.8), values input to the model included:

- $R^{coat}_{O_2} = 25000$ s cm^{-1}
- a differential resistance of coating to O_2 and CO_2 ($R_{O_2}:R_{CO_2} = 4$ Hagenmeier and Shaw, 1992)

- cuticular resistance to O_2 was set equal to that to CO_2 ($R_{O_2} = R_{CO_2}$ -absolute cuticular resistance (R_{O_2}) was 25,000 s cm^{-1}
- ratio of $R^{pores}{}_{O_2}$:$R^{pores}{}_{CO_2}$ was 4:5.

$[O_2]_i + [CO_2]_i$ gradually increased from 0.21 mol mol^{-1} to 0.26 mol mol^{-1} as $[O_2]_i$ dropped from 0.21 to 0.01 mol mol^{-1} and then rose dramatically as the fruit became anaerobic (about 0.01 mol mol^{-1} $[O_2]_i$).

The model predicted that as $[O_2]_i$ decreased so there would be an increase in $[O_2]_i + [CO_2]_i$. This would arise because of two effects. Firstly, the resistance of coated pores would be so much greater than that of open pores that coated pores would make virtually no contribution to total fruit gas exchange. In this case, the differential resistance of the coating material itself would have little impact on gas exchange characteristics of the fruit. Secondly, ease of diffusion of both gases through the cuticular route would be similar as the cuticle itself was equally resistant to them both; addition of the differentially resistant coating to the outside of the cuticle would have resulted in a combined $R^{cut}{}_{CO_2}$ about 60% of the value for $R^{cut}{}_{O_2}$. Since the diffusivity of O_2 in air is slightly greater than that of CO_2 then the term $R_{O_2} - R_{CO_2}$ in Eq. 5.13 would be a negative quantity, and $[O_2]_i + [CO_2]_i$ would gradually increase with progressive decreases in $[O_2]_i$ associated with pore blockage on the fruit surface. However, as $[O_2]_i$ declines further (as more pores become blocked), $[O_2]_i$ becomes more markedly limiting to aerobic respiration, and the associated decline in rate of CO2 production would reduce the slope in the relationship between $[O_2]_i + [CO_2]_i$

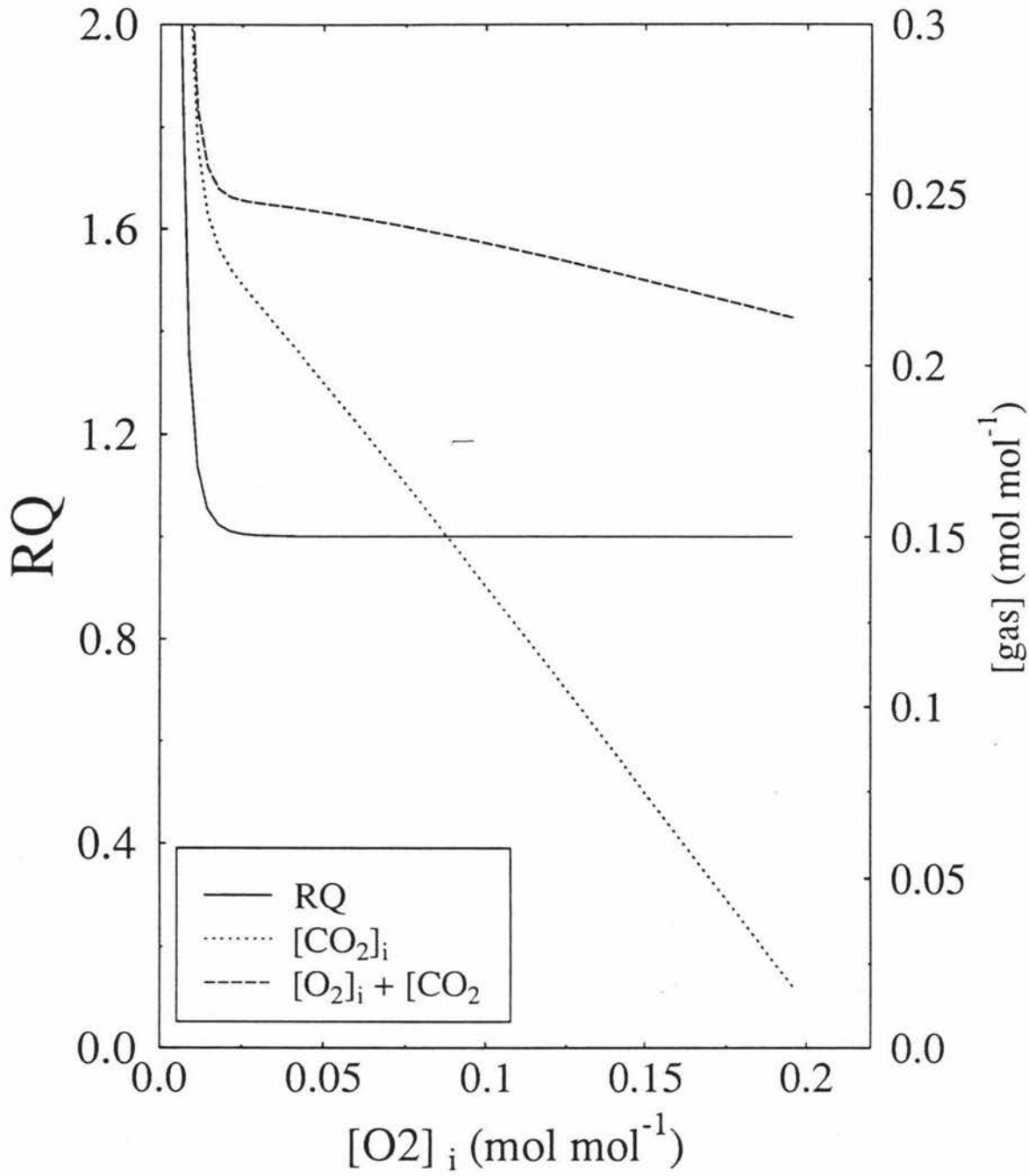


Fig.5.8 Relationship of $[O_2]_i + [CO_2]_i$, RQ and $[CO_2]_i$ with $[O_2]_i$ for a model system. $R^{cut}_{O2} = R^{cut}_{CO2} = 2.5 \times 10^4$ cm s^{-1}.

and $[O_2]_i$. Finally, the slope would increase dramatically as $[O_2]_i$ became severely limiting and the fruit began to ferment.

This same logic can be used to explain the increase in $[CO_2]_i$ in Fig. 5.8 from 0.02 to 0.23 mol mol^{-1} as $[O_2]_i$ was depressed from 0.19 to 0.01 mol mol^{-1} of $[O_2]_i$, then markedly increase due to anaerobic respiration at very low values of $[O_2]_i$. This linear rise in $[CO_2]_i$ contrasts with the pattern seen in Fig. 5.1 for a fruit with differentially resistant cuticle.

In the second graph (Fig. 5.9), values input to the model is

- ratio of cuticular resistance of O_2 : CO_2 is set for 30 : 1.
- absolute cuticular resistance(O_2) is 10^6 s cm^{-1}.
- an absolute value of $R^{coat}_{O_2}$ of 25,000 s cm-1 and a differential resistance of coating to O_2 and CO_2 (R_{O_2}:R_{CO_2} = 4; Hagenmeier and Shaw, 1992)
- ratio of $R^{pores}_{O_2}$:$R^{pores}_{CO_2}$ was 4:5.

A similar argument to that outlined above can be used to explain why $[O_2]_i$ + $[CO_2]_i$ increased as $[O_2]_i$ was depressed in Fig. 5.9 . However, in this case, the very high cuticular resistance would essentially eliminate contribution of the cuticular route to total gas exchange. Therefore, virtually all of the gas exchange of a coated squash would occur through the remaining open pores. Because ratio of R_{O_2} : R_{CO_2} in a total fruit skin resistance shifts from 4 : 5 towards 30 : 1 as more pores were assumed to be blocked, the ratio of increase in O_2 gradient between internal and external to that of CO_2 also shifted.

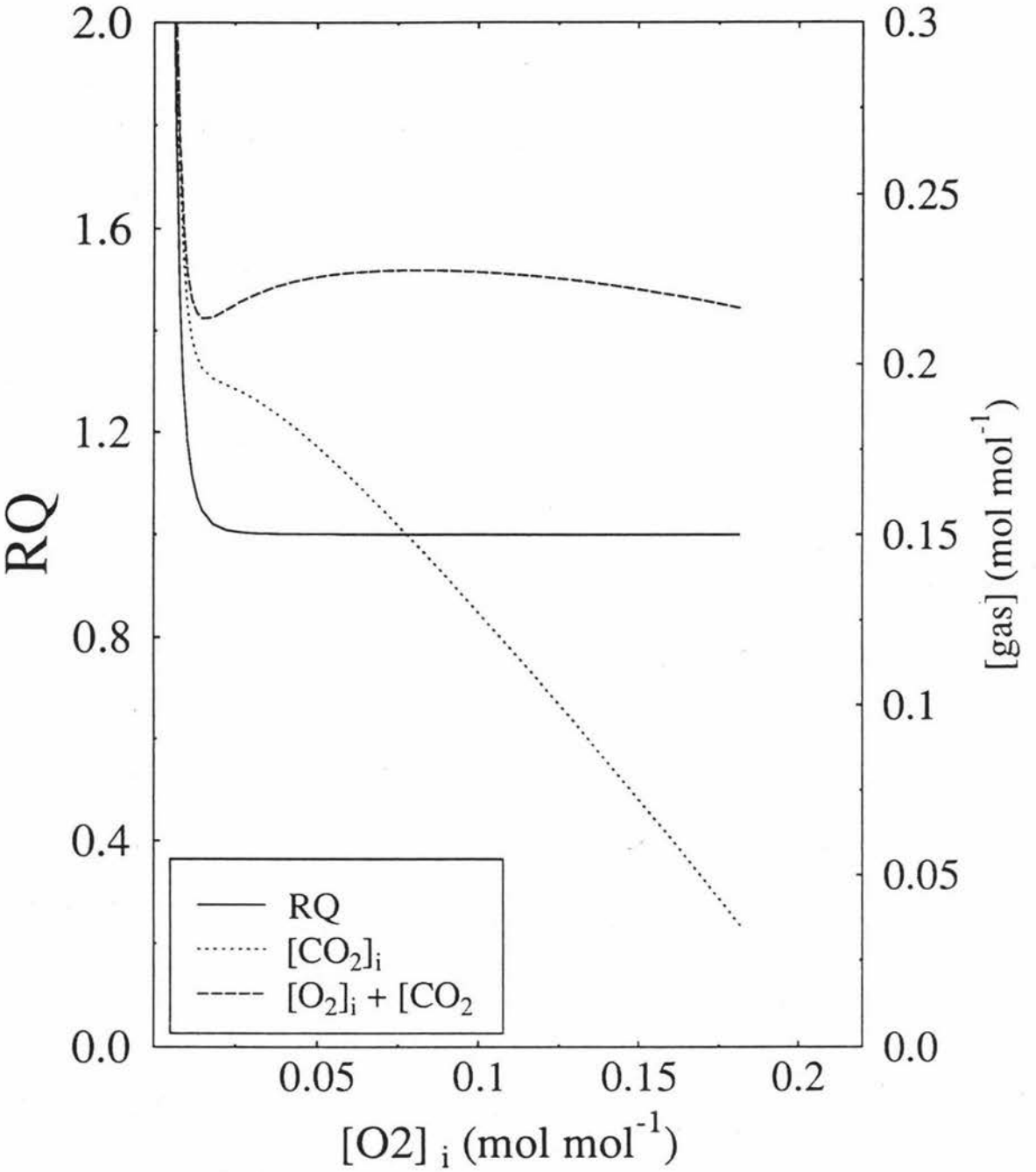


Figure. 5.9 Relationship of $[O_2]_i$ + $[CO_2]_i$, RQ and $[CO_2]_i$ with $[O_2]_i$ for model system. R^{cut}_{O2} = 30 x $R^{cut}_{CO2} = 10^6$ cm s^{-1}.

From section 5.2, it can be deduced that a coated fruit respiring aerobically which has $[O_2]_i + [CO_2]_i$ less than 0.21 mol mol^{-1} has $R^{fruit}_{O_2} > R^{fruit}_{CO_2}$. On the other hand, if $[O_2]_i + [CO_2]_i$ of a coated fruit is approximately 0.21 mol mol^{-1}, or more than 0.21 mol mol^{-1}, then $R^{fruit}_{CO_2} \geq R^{fruit}_{O_2}$. The discussion above demonstrates that this could be due to the fruit having a cuticular resistance is too great to contribute to total fruit gas exchange or $R^{cut}_{O_2} = R^{cut}_{CO_2}$.

The similarity between the relationship between $[O_2]_i + [CO_2]_i$ and $[O_2]_i$ seen in Fig. 5.4 and Figs. 5.8 and 5.9 suggest that either cuticular resistance of squash must be equal to the two respiratory gases, or most of respiratory gas exchange of squash is through pores.

The results of experiment 2 indicated that coating fruit reduced weight loss but the degree to which coatings affected respiratory gas diffusion seemed to be much greater than their effects on water vapour diffusion. This is assumed to be due to different nature of squash from those fruits conforming to the predictions made in Fig. 5.1, as outlined below.

For the fruit in which total respiratory gas pressure is less than 0.21 mol mol^{-1} when coated, as in Fig. 5.1 (section 5.2), we could expect a certain degree of reduction in weight loss through reduction of carbon loss from decreased respiration, especially when relative humidity surrounding fruit is high (approximately in the range of 70-100%). In squash, the response to coating indicated that respiratory gas exchange probably occurs exclusively through the pores and the contribution of the cuticle to

total fruit gas exchange is negligible. Alternatively, there may be no differential resistance to the two respiratory gases through squash cuticles. Either way, coating substantially increased $[CO_2]_i$, leading to induction of anaerobic respiration as coating number increased (as more pores were blocked) without affecting transpiration markedly, because resistance of coatings to water vapour is far less than those to CO_2 and O_2 (Hagenmeier and Shaw, 1992), and water preferentially moves through cuticles where skin resistance to water vapour diffusion is much less than that to respiratory gas diffusion. This effect is enhanced because total area of cuticle on a fruit is much larger than that of pores. Therefore when the pores are blocked, the difference in the relative rates of diffusion between respiratory gases and water vapour would increase. Thus, we can little expect reduction of water loss by coating without changing internal atmosphere dramatically in squash. In fact, on the contrary, thick coatings have the potential for enhancement of weight loss from carbon loss by increasing anaerobic respiration.

Banks, (1984a) observed that coating on banana skin depressed $[O_2]_i$ at 0.02 to 0.06 mol mol^{-1} which suppressed the rate of aerobic respiration without a concomitant increase in level of $[CO_2]_i$ which could have proved toxic. For fruit such as apple shown in Fig.5.1 of section 5.2, $[CO_2]_i$ stays at a low level (0.08 to 0.1 mol mol^{-1}) at $[O_2]_i$ levels 0.1 to 0.02 mol mol^{-1}. This level of $[CO_2]_i$ may have little opportunity to cause any adverse effect on the fruit. Smith and Stow (1984) reported that there were no significant increases of absolute level of alcohol and physiological disorders found at $[CO_2]_i$ level 0.1 mol mol^{-1} of coated Cox's Orange Pippin apple fruit during

21 days storage at 18 °C. However, fruit shown in Fig.5.4 shows that $[CO_2]_i$ is more than 0.2 mol mol^{-1} when $[O_2]_i$ is just below 0.1 mol mol^{-1}. It may be expected that some harmful effect occurs in fruit at this $[CO_2]_i$ level. Kader, (1987) found that a significant increase in anaerobic respiration (ethanol and acetaldehyde accumulation) occurs at $[CO_2]_i$ level above 0.2 mol mol^{-1} and this can irreversibly damage the tissue. In the current study, sensory evaluation (section 4.3.2) showed that squash with $[CO_2]_i$ above 0.2 mol mol^{-1} had a high off-flavour rate. Paull and Chen (1989) showed an extreme example that $[CO_2]_i$ even 0.07 mol mol^{-1} in ripe papaya developed off-flavour.

Fig. 5.1 indicated that the degree to which coating decreases $[O_2]_i$ is crucial to successful modification of internal atmosphere of fruit. For this type of fruit, optimum $[O_2]_i$ can be estimated using a plot of $[O_2]_i + [CO_2]_i$ versus $[O_2]_i$ which indicates transition of respiration from aerobic to anaerobic processes. Ideal coating treatments could therefore be identified as those which generate internal atmosphere composition close to optimum $[O_2]_i$ and which achieve a satisfactory reduction of transpiration. However, in the case of squash given repeated coatings in rapid succession, the internal atmosphere modification achieved was qualitatively different from that shown in Fig. 5.1. Neither did coating with Primafresh achieve any marked reduction in weight loss. Optimum coatings for squash would therefore be difficult to identify on the that basis. One possibility for further work may be to seek a coating which is more resistant to water vapour diffusion but less resistant to respiratory gases than Primafresh, which could achieve greater reductions in water

loss without inducing anaerobic respiration.

Individual fruit wrapping is another potential method for reducing weight loss of squash without affecting respiratory gas exchange. This has been mainly used with citrus fruit for reduction of shrinkage and weight loss (Ben-Yehoshua, 1985; Grierson and Ben-Yehoshua, 1985). Ben-Yehoshua et al. (1983) calculated that coating increases only slightly (25 %) the resistance of fruit to water vapour, but raised the resistance to CO_2, O_2 and C_2H_4 by 140 %, 250 % and 100 % respectively. Conversely, wrapping (HDPE = high density polyethylene) was found to raise the resistance to water vapour, CO_2, O_2 and C_2H_4 by 1375 %, 72 %, 233 % and 125 % respectively, which suggested that individual fruit wrapping is more effective than coating in reducing water loss without changing internal atmosphere of fruit. This difference was thought not to be due to the film's differential resistance to gases but because it made a water saturated atmosphere between the fruit surface and the wrapping so that there was little water vapour pressure deficit to drive water movement from the fruit. Therefore, wrapping might be used to reduce water loss from squash without substantially hindering respiratory gas exchange.

Individual fruit wrapping is equivalent to the case of the loosely adhering coating modelled by Banks et al. (1993). If the coating only loosely covers a fruit, there is an opportunity for exchange of gases in the space between the fruit surface and the coating so that the effects of the coating are equivalent to those of individual fruit wrapping. On the other hand, most surface coatings probably adhere tightly to fruit

surface so that there is no opportunity for mixing of gases between fruit surface and coating (termed the tightly adhering coating by Banks et al., 1993).

Smith et al., (1987) reported that $[CO_2]_i$ and $[O_2]_i$ of fruit wrapped in sealed loose films (equivalent to a loosely adhering coating) are less variable than those found in coated fruit (equivalent to a tightly adhering coating). Variability in internal atmosphere modification would be expected to be less in fruit coated with a loosely adhering coating because the increased resistance achieved by the coating would be less dependent upon the initial characteristics of the fruit skin.

Individual fruit wrapping has been developed in the last two decades as a new technique for postharvest handling of fruit and vegetables (Ben-Yehoshua et al., 1981; Monselise, 1981; Hardenburg, 1971). On the other hand, although there are some investigations for utilization of coatings (Banks et al., 1993; Hagenmaier and Shaw, 1992), use of loosely adhering coatings has not yet been proved. Individual fruit wrapping may be a potential technique for reduction of weight loss of squash.

The main disadvantages to using wrapping are the condensation and excessively high humidity which can lead to decay (Ben-Yehoshua, 1985; Shirazi and Cameron, 1992). The pathogens, particularly dormant ones, can develop rapidly in the humid atmosphere (Ben-Yehoshua, 1978). For this reason, adequate decay control is of paramount importance in the use of individual fruit wrapping. However, postharvest use of fungicide is not recommended in Japan, and since Japanese eat squash without

peeling its skin, any additives (fungicides) applied after harvest might detract from the market acceptance of New Zealand squash.

Overall, there appears to be limited scope for overcoming the current problem of weight loss with a single treatment. Future investigations should examine the potential for combined benefits arising from improved temperature control, reduced water vapour pressure deficit through elevated storage humidity coupled with increased skin resistance to water vapour with modest applications of a suitable surface coating or wrapping.

Chapter 6

Concluding remarks

This thesis has examined the potential for improving retention of quality in harvested winter squash by use of surface coatings and by enhancement of fruit calcium status.

Application of sodium carbonate / olive oil mixture to the fruit during growth did increase calcium accumulation by the fruit and reduce rot development (Section 3.2). However, the relationship between fruit calcium concentration and rot incidence were not consistent between experiments and it seems that the success of the sodium carbonate treatment may have been as much due to its effects on pH of the fruit skin as to effects on calcium contents. Whilst there is therefore no immediate application for control of diseases from this work, it appears that further work on this type of treatment for control of rots may be justified.

In the case of coating, there was little benefit of coating application on weight loss. At this point, it is not clear if this was because of inadequate resistance of the coating material to water vapour transfer or due to stimulation of anaerobic respiration by the modification of the fruit's internal atmosphere. As suggested in Chapter 5 individual fruit wrapping may be one way to achieve improved weight loss without undesirable modification of the internal atmosphere. However, these benefits would have to be weighed against the extra costs of the treatment, coupled with the increased risks of rotting associated with the high humidity developing inside the

wrap.

Overall, the scope for achieving useful improvements in fruit quality by application of materials which influence the skin resistance to transfer of water vapour, oxygen or carbon dioxide appears to be limited. However, this study has shown a different type of relationship between the sum of $[O_2]_i + [CO_2]_i$ and $[O_2]_i$ from that recently published (Banks et al., 1993). This has provided some new insights into the potential mechanism by which the internal atmosphere of squash, and perhaps other fruits, is modified by application of surface coatings.

Japan, which is the only major market for New Zealand's squash exports, is expected to increase its access to overseas horticultural products in the immediate future due to further development of international free trade based on the recent success of the General Agreement on Tariffs and Trade. The main concern for horticultural products imported transoceanically into Japan is postharvest application of chemicals. Therefore, if further understanding in physiological response of squash to its storage conditions is achieved, and storage technology of squash for long duration with adoption of environmentally safer methods is established, this could create substantial benefits for New Zealand by establishing a reputation for safe horticultural products from New Zealand. This could justify the extra cost associated with any new procedures for improving the storage quality of squash, together with modified growing (preharvest) and storage (postharvest) techniques designed to minimize postharvest loss.

7. References

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