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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Table of contents

List of Figures

List of tables Page

List of abbreviations

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_{CO_2}	=	Resistance to carbon dioxide diffusion (s cm^{-1})
r_{CO_2}	=	Diffusive transmission rate of CO_2 (cm^3 s^{-1})
$R_{C_2H_4}$	=	Resistance to ethylene diffusion (s cm^{-1})
R^{cut}	=	Resistance to gas diffusion through cuticle (s cm^{-1})
$R^{cut}_{CO_2}$	=	Resistance to CO_2 diffusion through cuticle (s cm^{-1})
$R^{cut}_{O_2}$	=	Resistance to O_2 diffusion through cuticle (s cm^{-1})
$R^{fruit}_{CO_2}$	=	Total resistance of a fruit to CO_2 (s cm^{-1})
$R^{fruit}_{O_2}$	=	Total resistance of a fruit to O_2 (s cm^{-1})
r_{H_2O}	=	Rate of water loss from a produce (% fresh weight day^{-1})
R_{O_2}	=	Resistance to oxygen diffusion (s cm^{-1})
r_{O_2}	=	Diffusive transmission rate of O_2 (cm^3 s^{-1})
R^{pores}	=	Resistance to gas diffusion through pores (s cm^{-1})
$R^{pores}_{CO_2}$	=	Resistance to CO_2 through pores (s cm^{-1})
$R^{pores}_{O_2}$	=	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_{CO_2}	=	Respiratory CO_2 production (cm^3 s^{-1})
rr_{O_2}	=	Fruit respiratory oxygen uptake (cm^3 s^{-1})
$rr_{O_2}^{max}$	=	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:

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

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:

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

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

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

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.