POST-HARVEST MANIPULATION OF RIND COLOUR IN 'MAURITIUS' LITCHI (*Litchi chinensis* Sonn.) FRUIT

By

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Submitted in fulfilment of
the requirements for the degree of
DOCTOR OF PHILOSOPHY IN AGRICULTURE
in the Department of Horticultural Science
University of Natal
PIETERMARITZBURG
October, 1996

ABSTRACT

Litchi fruit are non-climacteric, and are able to endure relatively low storage temperatures compared to other subtropical fruits. Unfortunately however, the litchi rind is relatively thin and lacks a thick, durable cuticle. Consequently, post-harvest desiccation is a major factor, and rind colour changes rapidly from red to brown, unless counter measures are taken immediately after harvest. Presently, the South African industry uses sulphur fumigation to prevent browning, but sulphur treatment is undesirable in many respects, only partially successful, and some overseas markets have lowered the permissible level of sulphur to 10 $mg.kg^{-1}$ in the fruit flesh. Alternatives to sulphur fumigation were accordingly researched.

The author tested the hypothesis that, in order to preserve the desirable red rind colour, it was necessary to break down rind cell membrane integrity, so that the vacuole-bound anthocyanin pigments can be exposed to zero pH solution, which effects rind colour preservation. Thereafter, rind desiccation must be reduced.

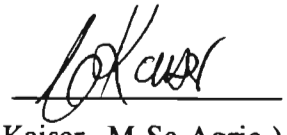
A 2 s steam (95°C) treatment followed by 4 min immersion in zero pH solution resulted in fruit which retained excellent red rind colour, with normal pulp characteristics and tasted similar to control fruit after 28 days storage at 1°C. Ultrastructural studies showed that 2 s steam (95°C) treatment resulted in rind cell membrane breakdown, and this was enhanced when used in conjunction with 4 min in zero pH solution. In addition, electrolyte leakage studies showed that rinds of untreated control fruit had lowest electrolyte leakage, while those of fruit subjected to 2 s steam (95°C) had highest electrolyte leakage, making the previously compartmentalized and vacuole-bound pigments available for preservation in the desirable red colour. Polyphenol oxidase in litchi rinds was strongly inhibited by 2 s steam (95°C), but even more so when fruit were subjected to 2 s steam (95°C) followed by 4 min in zero pH solution. Energy dispersive x-ray microanalysis studies found that chlorine concentrations were relatively high on both the inner and outer surfaces of fruit subjected to 2 s steam (95°C) followed by 4 min in zero pH solution. Similarly, sulphur concentrations were high in rinds of sulphur-fumigated fruit, but this element was also present at low concentrations in non-sulphur-fumigated fruit.

Rind colour of untreated control fruit lightened when stored at 30°C and hue changed from red to reddish orange. Rinds of fruit subjected to 2 s steam (95°C) only, lost colour rapidly and were a pale yellow hue 24 hr after treatment. The hue of fruit rinds subjected to 2 s steam (95°C) followed by 4 min in zero pH solution changed from reddish orange to red within 4 hr and then darkened up to 24 hr after treatment. Red colour was preserved in fruit held at 30°C for 72 hr, but lightened after 24 hr. HPLC of anthocyanin pigments found that the presumed cyanidin-3-rutinoside, pelargonidin-3-glucoside and pelargonidin-3,5-diglucoside all decreased in untreated fruit over 5 days storage at 30°C. Concentrations of presumed cyanidin-3-rutinoside in fruit subjected to 2 s steam (95°C) followed by 4 min in zero pH solution increased immediately after treatment, peaked 24 hr later, but then decreased to about double the concentration of fruit treated on the day of harvest after 4 days at 30°C. Furthermore, no copigmentation or self-associations of anthocyanins took place in rinds of fruit subjected to 2 s steam (95°C) followed by 4 min immersion in zero pH solution.

Semi-commercial trials showed that the steam: acid dip treatment is feasible, and has the potential to replace sulphuring as a fungicidal treatment. It also has the advantage of more permanently preserving the desirable rind colour, and in a more intense red colour.

DECLARATION

I hereby certify that the research work reported in this thesis is the result of my own original investigations, except where otherwise acknowledged.

Signed 

(Clive Kaiser, M.Sc Agric.)

ACKNOWLEDGEMENTS

The author wishes to express his sincere appreciation to the following people and organizations :-

1) Professor B.N. Wolstenholme for his continued interest, invaluable advice and support during this study.
2) Professor P. Allan for useful discussions and encouragement.
3) Professor S.E. Drewes for help with identification and interpretation of HPLC work.
4) The University of Natal, South African Litchi Growers' Association and Foundation for Research and Development for financial assistance for the duration of this study.
5) Dr D.L. Milne of Merensky Technological Services for providing fruit and research facilities in 1995 and 1996, as well as for facilitating the exchange study trips in 1995 and 1996 to the Thailand Institute for Scientific and Technological Research, Bangkok, Thailand.
6) Jonathan Levin of the Medical Research Council, Pretoria for his biometrical conjuring which, never ceases to amaze.
7) Mrs Sing Ching Tongdee of the Thailand Institute for Scientific and Technological Research, Bangkok, for outstanding co-operation during exchange study trips and for initiating electrolyte leakage studies.
8) Vijay Bandu, B.J. White and Priscilla Donnelly for assistance with electron microscopy and photography.
9) Ivan, Candy and Shannon Kaiser, Joan Aldworth, Jeff and Jeanette Finnie, Rob Osborne, Steve Ansermino, Kathy Milford, Frieda Dehrmann, Phil Weller, Zac Bard, Renate Oberholzer, Matt Wright, Derek Askew, Teri Dennison, Jean Phillips, Harvey Dicks, the 1996 final year Horticultural Science students for friendship, encouragement and various other contributions.

Once again special thanks to my parents Lionel and Daphne for support, sustenance and encouragement at all times.

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INTRODUCTION

The litchi or lychee (*Litchi chinensis* Sonn.) is an evergreen tree of the Sapindaceae family, the latter deriving its name from the presence of saponine in the seeds of some of the non-edible species. Sapindaceous genera number at least 125 and there are more than 1000 species, all of which are widespread throughout most of the tropics and warm subtropics. Other commonly cultivated but more tropical fruit types in this family are rambutan (*Nephelium lappaceum* L.) and longan (*Dimocarpus longan* Lour.). The litchi is thought to have originated between latitudes 23° and 27° north in warm subtropical southern China, northern Vietnam and Malaysia (Tindall, 1996). Menzel (1992) maintained that there are three distinct subspecies of *L. chinensis*, viz. *Litchi chinensis* spp. *chinensis*, which now grows wild in China, Vietnam and Cambodia; *Litchi chinensis* spp. *philippinensis*, which is found only in the Philippines where it is widely distributed but rarely cultivated; and *Litchi chinensis* spp. *javensis* which is known to be cultivated only in West Java and Indochina. In South Africa the most widespread cultivar 'Mauritius' belongs to *Litchi chinesis* spp. *chinensis* subspecies.

Chandler (1957) noted that despite being one of the most delicious of fruits and one of the least cloying, the litchi has spread rather slowly from its centre of origin. Contributing factors have been low yields, alternate bearing and a lack of dependable cultivars. However, post-harvest problems also loom large. In the past decade, the potential of this crop has been re-evaluated, and considerable research, especially in subtropical Australia, Israel, Taiwan, and South Africa, has rekindled interest in commercial plantings particularly for export. In South Africa, peak flowering usually occurs in September and depending on the area and cultivar, fruit are usually harvested between November and late January (Fivaz et al., 1996).

Litchi fruit flesh is a true appendage of the seed coat and is enclosed by a rind derived from pericarp tissue consisting of exo- (or epi-), meso- and endocarp tissues (Steyn and Robbertse, 1992). At the time of harvest, rinds of most cultivars are an attractive light to dark red colour due to flavonoid anthocyanin pigments. However, rind colour (in the absence of corrective post-harvest treatments) quickly becomes less attractive as desiccation sets in. Within a few days rinds become dry and brittle, leading to cracking under pressure, leakage

of juices and consequent bacterial and fungal outbreaks. Also, rinds turn brown as a result of anthocyanin breakdown (Jurd, 1972; Underhill, 1989) and enzymatic browning caused by polyphenol oxidase (Underhill and Critchley, 1995).

The South African litchi industry currently produces in the region of about 5500 t annually, although this figure differs markedly from season to season, and further growth in the South African litchi industry is dependent on resolution of horticultural and post-harvest problems. Usually, more than half the fruit produced annually is exported mainly to lucrative European markets which are strongly influenced by cosmetic "eye-appeal". The remaining fruit are either channelled to the fresh market or processed, but the latter usually constitutes only about 10% of the total crop (Burelli, 1994). Post-harvest research has only enjoyed attention relatively recently and lags behind other domesticated trees. Where aesthetics of fruit are concerned, rind colour retention is of primary importance. Since the mid 1980's until now, gaseous sulphur treatments have been utilized by the South African industry to act firstly as a fungicide, and secondly to prevent browning reactions (Swarts, 1985).

Sulphuring of litchi fruit however, results in undesirable residues (Kremer-Köhne, 1993; Milne and Ahrens, 1993), aftertastes (Lonsdale and Kremer-Köhne, 1991), constitutes a potential health problem for asthmatics (Koeing et al., 1983), and is ineffective against some fungi, where resistance has appeared (Botha et al., 1988). Consequently, it is not surprising that some European countries have lowered the acceptable levels of sulphur from 20 $mg.kg^{-1}$ to 10 $mg.kg^{-1}$ (fresh mass) in the fruit flesh. In addition, sulphur-fixed anthocyanins are colourless thus rendering treated litchi fruit rinds yellow or pale green. Effects of sulphur on anthocyanin colour have been known since as early as the seventeenth century, where Boyle (1664) wrote that "Roses held over the Fume of Sulphur, may quickly by it be depriv'd of their colour..." (Wheldale, 1916). If left at room temperature, litchi fruit will at best turn a pale pink colour after several days of storage (Swarts, 1985). Zauberman et al. (1990; 1991) found that extraction of sulphur-fixed anthocyanins from litchi rinds in acidified methanol resulted in a red solution. Reversal of sulphur-induced anthocyanin decolourization using acid was first reported by Kastle (1905) and the first detailed account of anthocyanin reactions with sodium bisulphite was given by Wheldale (1916).

Nevertheless, the re-discovery that sulphur decolourization of anthocyanins could be reversed using acids led to substantial Israeli and Australian research on low pH treatments, much of which was concurrent with the present investigation. Zauberman et al. (1990; 1991), Underhill et al. (1992b, 1994) and Fuchs et al. (1993) all tried to acidify litchi rind anthocyanins *in situ* by dipping whole fruit in dilute acid solutions after sulphur fumigation. Where fruit were dipped only in low pH solutions, a red background was achieved but with unsightly brown patches. Since fruit are pliable after sulphur fumigation, Kaiser (1994b) hypothesized that sulphur treatments were solubilizing cell membranes and tonoplasts (vacuole membranes) thus enabling low pH solutions to act on vacuole-bound anthocyanins. Furthermore, he hypothesized that the elimination of post-harvest sulphur fumigation by using alternatives, which would achieve adequate membrane breakdown, could be followed by fixation of anthocyanins *in situ* using low pH dips.

The present study was initiated following an in-depth literature review on anthocyanin physiology (Kaiser, 1994a), where the author realised that active research worldwide had overlooked principles and physiology of sulphur fumigation and was inadvertently perpetuating, rather than eliminating sulphur fumigation. Objectives of this study were firstly, to review morphological and physiological aspects of litchi rind colour, browning and its prevention, and to further develop the author's original hypothesis. Secondly, alternatives to sulphur fumigation, in particular boiling water or steam followed by a dip in low pH solution, were investigated. Effects of steam and low pH on rind ultrastructure, mineral ion distribution, enzyme activity, electrolytic leakage and anthocyanin pigmentation were compared to sulphur-fumigated fruit and untreated control fruit. The prime applied objective of these anatomical and physiological investigations was to develop post-harvest treatments to retain an acceptable rind appearance in the marketplace, thereby eliminating a major limiting factor to wider commercialization of the litchi industry.

CHAPTER 1

LITERATURE REVIEW

1.1 FRUIT ANATOMY AND MORPHOLOGY

Botanically, the litchi (*Litchi chinensis* Sonn.) fruit has been described as a simple, unicarpellary, ovoid to heart-shaped drupe, following a sigmoidal type growth pattern (Joubert, 1986; Paull et al., 1984; Steyn and Robbertse, 1992). However, drupe by definition implies that the endocarp is stony (Smith, 1977) and this is not the case. When mature, the fruit consists of a single, central seed; a thick, fleshy, edible pulp (which is invariably called an aril in the scientific literature, although the work of Steyn and Robbertse (1992) would seem to contradict this interpretation - see below); a leathery, indehiscent 1-3 mm thick, bright red to purplish rind which is morphologically derived from the pericarp (Fig. 1). When mature, the rind may be smooth or scaly with conical, acute warty protuberances (Menzel, 1992).

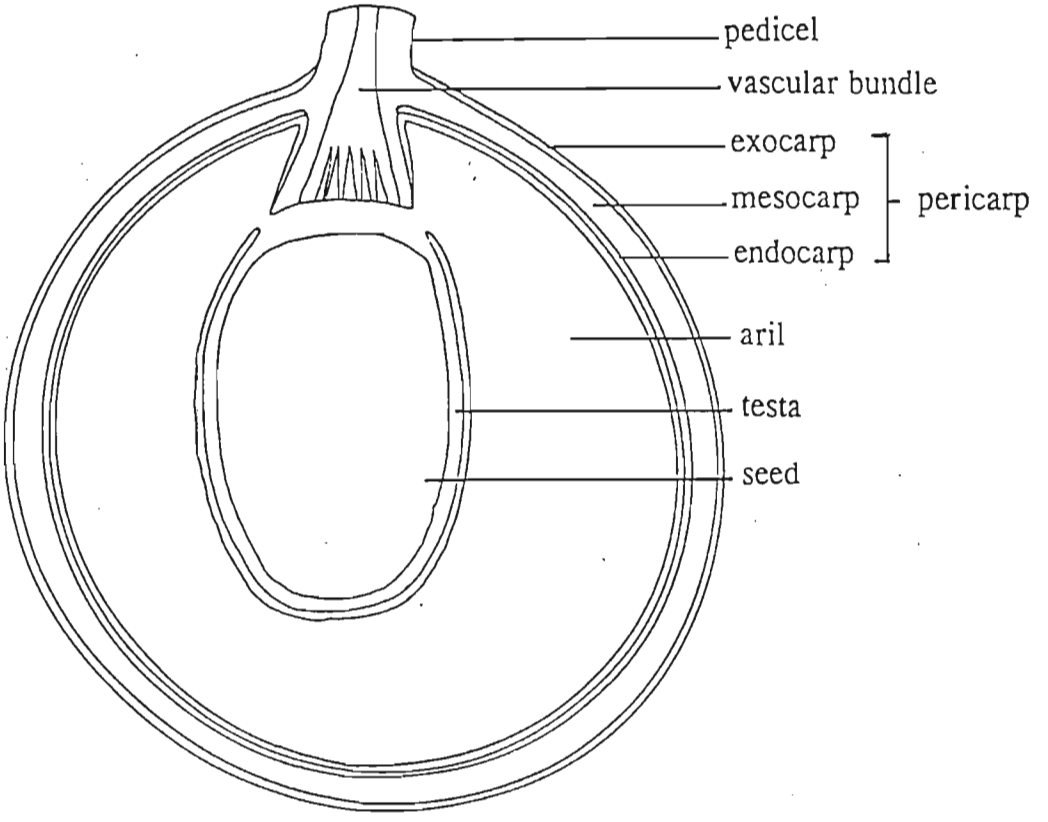

Figure 1. Longitudinal section of litchi fruit anatomy (x2)

The litchi fruit is considered non-climacteric with a declining rate of respiration during fruit growth. Some ethylene production occurs during initial stages of fruit growth, remains low during the stage of rapid fruit growth and increases slightly at harvest, although this was probably a wound response. Ethylene production increases however, during the final stages of fruit senescence (Akamine and Goo, 1973).

Joubert and van Lelyveld (1975) followed the ontogeny of the litchi fruit rind from flowering to fruit maturity. They found that at pollination, the ovary wall consisted of epidermal, vascular and undifferentiated parenchyma cells. Within a fortnight however, a distinct rind, consisting of an epicarp (exocarp), mesocarp and endocarp had differentiated. They maintained that initially the epicarp consisted of parenchyma cells and was covered by a simple layer of epidermal cells. Some of the parenchyma cells then differentiated and became lignified to form two or three layers of stone cells. Meanwhile, mesocarp parenchyma cells either expanded rapidly, resulting in large thin-walled cells with relatively large intercellular spaces, or differentiated into vascular tissue. The endocarp was distinguishable as two or three layers of small closely packed cells.

This study of the litchi rind was recently extended by Underhill and Critchley (1992; 1993) by ultrastructural studies at the electron microscope level. In both these studies they found that the differentiated epicarp consists of a continuous, waxy cuticle 1-3 μm thick, a uniseriate epidermis and a single layer of heavily lignified subepidermal macrosclereids. The mesocarp consists of some inter-connecting vascular tissues, but mostly of large thin-walled parenchyma cells and relatively large intercellular spaces. These parenchyma cells contain most of the photosynthetic organelles and also anthocyanin pigments. The endocarp comprises only small, thin-walled, unsuberized epidermal cells.

In following the early stages of fruit ontogeny, Underhill and Critchley (1992) maintained that development of the embryo and cotyledons of the seed was subsequent to rind (pericarp) differentiation. In addition, fruit flesh development was only observed some 62 days after anthesis. Robbertse et al. (1993) however, found under warm subtropical conditions (28°C day and 15°C night temperatures) that once cotyledons had formed, fruit flesh development began some 42 days after fertilization. Fruit flesh developed from a meristematic ring that

initiated from the rim of the outer integument and extended to the raphal side of the sessile seed. An earlier study by Steyn and Robbertse (1992) provided further conclusive evidence that the funiculus plays no role in the development of litchi fruit flesh. They concluded from that study that litchi fruit flesh is a true appendage of the seed coat. Clearly, these recent anatomical studies suggest that litchi fruit flesh, which has been traditionally referred to as an aril, by definition a funicular outgrowth (Smith, 1977), is strictly speaking not arillate. Henceforth, in this thesis, to avoid complications the edible pulp will be referred to as fruit flesh. After the early stages of fruit growth the flesh, along with the seed, continues to expand with the flesh eventually enveloping the seed and inducing a tangential stretching of rind (pericarp) cells (Underhill and Critchley, 1992). At maturity, fruit flesh is white and translucent and may account for up to 80% of the fruit mass (Menzel, 1992).

1.2 RIND COLOUR

1.2.1 Chlorophyll

Underhill and Critchley (1992) maintained that during the initial stages of fruit growth and development, green rinds were photosynthetic due to the presence of chloroplasts in the upper mesocarp, particularly in the inter-protuberance zones. Rind chlorophyll content increased up to 69 days after anthesis, but subsequently declined rapidly with only trace amounts being found in mature fruit. Concurrent with the decrease in chlorophyll content, anthocyanin concentrations increased from about 10 mg.100 g^{-1} rind (fresh mass) to about 60 mg.100 g^{-1} rind (fresh mass). Paull et al. (1984) also showed that chlorophyll content declined after a peak at about 30 days after anthesis. A significant increase in anthocyanin concentration was however, delayed in that study until about 50 days after anthesis. It is possible that differences in sampling position of fruit between the two studies may have been responsible for temporal differences in chlorophyll breakdown and anthocyanin biosynthesis, as fruit which are not exposed to direct sunlight remain green for longer, and when mature are not as red as fruit which are subjected to direct sunlight (Paull et al., 1984).

Table 1. Naturally occurring anthocyanidins and their substitution patterns (cf. Fig. 2) (adapted from Mazza and Miniati, 1993)

Anthocyanidin	**3**	**5**	**6**	**7**	**3'**	**4'**	**5'**
Common Structures							
Pelargonidin	OH	OH	H	OH	H	OH	H
Cyanidin	OH	OH	H	OH	OH	OH	H
Peonidin	OH	OH	H	OH	OMe	OH	H
Delphinidin	OH	OH	H	OH	OH	OH	OH
Petunidin	OH	OH	H	OH	OMe	OH	OH
Malvidin	OH	OH	H	OH	OMe	OH	OMe
Rarer Structures							
Apigeninidin	H	OH	H	OH	H	OH	H
Aurantinidin	OH	OH	OH	OH	H	OH	H
Capensinidin	OH	OMe	H	OH	OMe	OH	OMe
Europinidin	OH	OMe	H	OH	OMe	OH	OH
Hirsutidin	OH	OH	H	OMe	OMe	OH	OMe
6-Hydroxycyanidin	H	OH	OH	OH	OH	H	-
Luteolinidin	H	OH	H	OH	OH	OH	H
5-Methylcyanidin	OH	OMe	H	OH	OH	H	-
Pulchellidin	OH	OMe	H	OH	OH	OH	OH
Rosinidin	OH	OH	H	OMe	OMe	OH	H
Tricetinidin	H	OH	H	OH	OH	OH	OH

1.2.2 Anthocyanin Physiology

Anthocyanins (Greek *anthos*, flower and *kyanos*, blue) are the most important group of water-soluble, vacuole-bound plant pigments, emitting light in the visible spectrum (Strack and Wray, 1989). They are a large group of secondary plant metabolites (Mazza and Miniati, 1993) belonging to the most widespread class of phenolic compounds collectively known as flavonoids. They protect against ultra violet light (Schmelzer et al., 1988), act as antibiotics in plant defence responses (Lamb et al., 1989), and serve as attractants to pollinators in flowers, and seed dispersal agents in fruits (Harborne, 1967). Naturally occurring anthocyanins, numbering more than 240 (Hrazdina, 1982; Strack and Wray, 1989), are responsible for nearly all bright orange, pink, scarlet, red, mauve, violet and blue colours in flowers, fruits, leaves and stems of higher plants (Harborne, 1967). Notable exceptions are lipid-soluble lycopenes found in grapefruit (*Citrus paradisi*), deep red carotenoids found in tomato (*Lycopersicon esculentum*) fruit (Davies, 1980), and water-soluble betalains found in beetroot (*Beta vulgaris*) roots (Reznik, 1980).

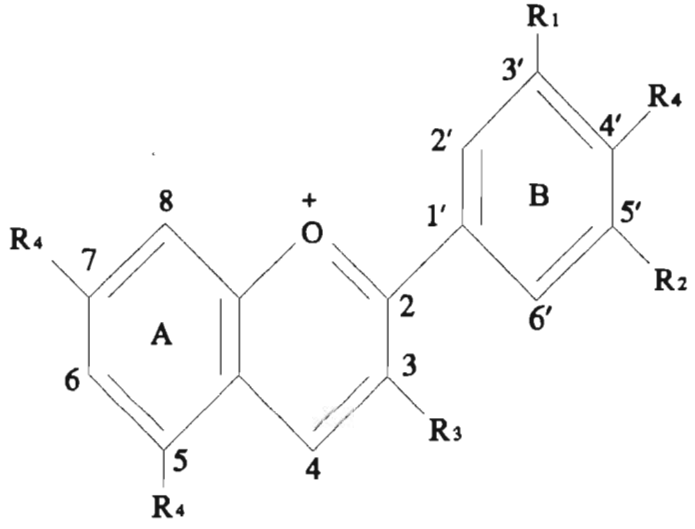

Figure 2. Flavylium cation. R_1 and R_2 are H, OH or OCH_3; R_3 is a glycosyl or H; and R_4 is a glycosyl or OH (from Mazza and Miniati, 1993)

Anthocyanins are all glycosides of polymethoxy and polyhydroxy derivatives of 2-phenylbenzopyrylium or flavylium salt, consisting of two 6-carbon rings, viz. A-ring and B-ring, connected by a 3-carbon bridge (Fig. 2). Individual anthocyanins differ in the number

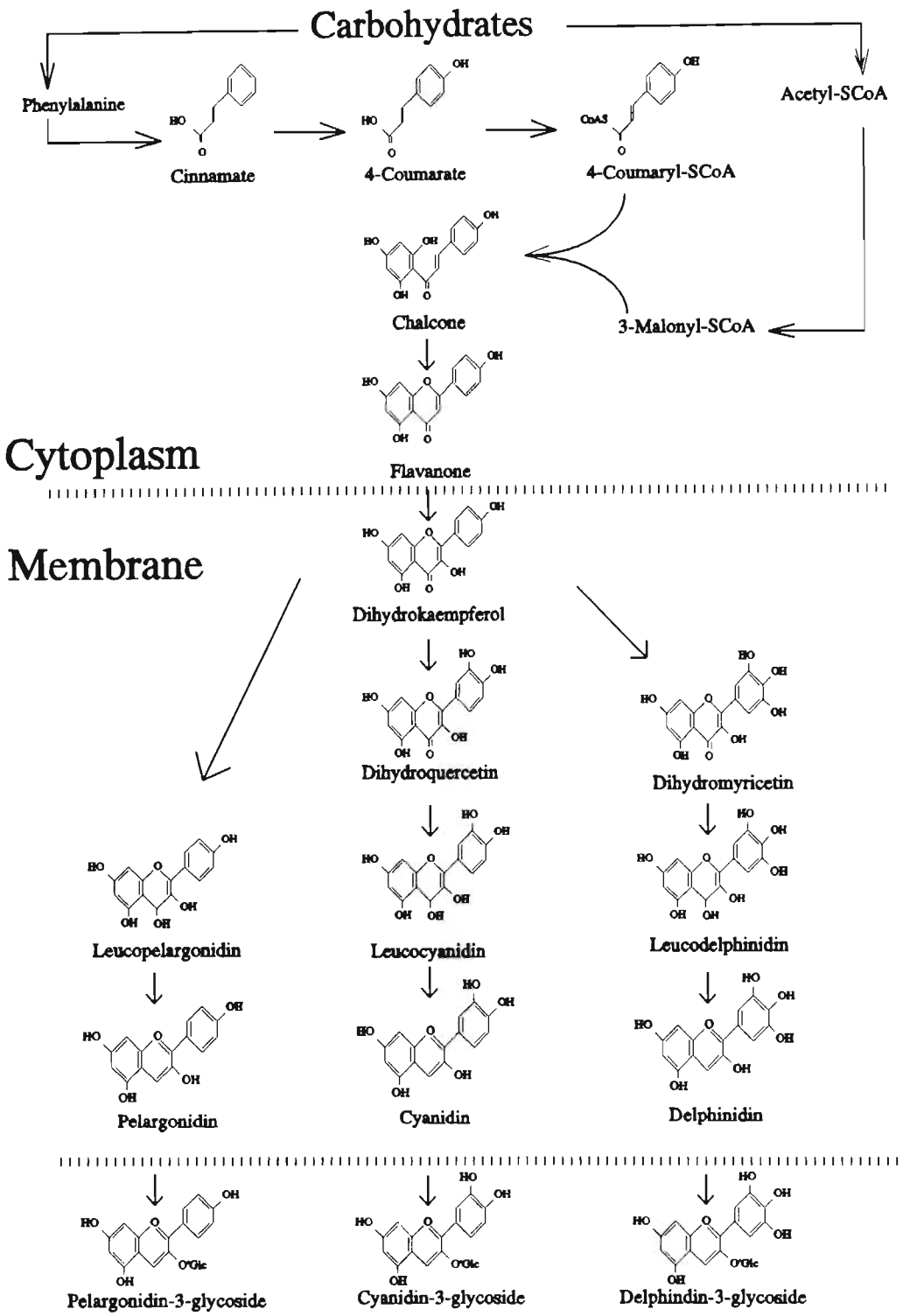

Figure 3. Putative biosynthetic pathway of anthocyanins (adapted from Grisebach, 1980; Lancaster, 1992; Holton and Cornish, 1995)

and position of hydroxyl and methoxyl groups, as well as the nature and number of glycosides, and aliphatic or aromatic acids attached to the glycosides (Mazza and Miniati, 1993). Glycosides are most important in stabilizing and solubilizing natural anthocyanins (Jurd, 1972). Should the glycoside be cleaved by hydrolysis from the anthocyanin structure, the resultant aglycone is known as an anthocyanidin. Several anthocyanidins have been identified in nature (Table 1), however pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidin occur most frequently in plants (Mazza and Miniati, 1993).

Lee and Wicker (1991a) identified only 3 polymeric anthocyanins in 'Brewster' litchi rind, viz. cyanidin-3-rutinoside (~67%), malvidin-3-acetylglucoside (~14%) and cyanidin-3-glucoside (~9%). However, Macheix et al. (1990) indicated that anthocyanin polymers of pelargonidin, viz. pelargonidin-3-rhamnoside, pelargonidin-3,5-diglucoside in addition to cyanidin-3-galactoside and cyanidin-3-glucoside were observed by Prasad and Jha (1978).

1.2.2.1 Biosynthesis

Biochemical and regulatory aspects of anthocyanin biosynthesis in apples and pears were recently reviewed by Viljoen and Huysamer (1995), while Lancaster (1992) also reviewed regulation of rind colour in apples. Other excellent reviews on genetic and biochemical aspects of anthocyanin and flavonoid biosynthesis include those by Hrazdina (1992), Martin and Gerats (1993), and Holton and Cornish (1995). Markham (1982) estimated that 2% of all carbon photosynthesized by plants was converted into flavonoids and related compounds. Flavonoids, of which anthocyanins are a constituent, are products of what Hrazdina (1992) coined as the "plant aromatic pathway" (Fig. 3). This pathway consists of three segments, viz. the shikimate, phenylpropanoid and flavonoid pathways.

The two aromatic rings, viz. A and B of the flavylium cation (Fig. 2) are derived independently of each other. A-ring polyketide synthesis occurs by head to tail condensation of three malonyl-SCoA molecules, which are formed by carboxylation of acetyl-SCoA, catalyzed by acetyl-SCoA carboxylase, in the presence of ATP (Ebel and Hahlbrock, 1982; Lancaster, 1992; Mazza and Miniati, 1993). In contrast, the B-ring and associated carbon atoms of the bridge are formed by shikimate and phenylpropanoid pathways (Gross, 1987;

Hrazdina, 1992). The shikimate pathway which takes place in chloroplasts and the cytoplasm, produces aromatic amino acids, phenylalanine, tyrosine and tryptophan. For more information on this pathway, the reader is referred to a recent review by Hrazdina (1992). The active precursor of the B-ring is 4-coumaryl-SCoA and this is formed by the phenylpropanoid pathway. Initially, phenylalanine is transformed into trans-cinnamate through the elimination of ammonia. The reaction is catalyzed by phenylalanine ammonia-lyase (PAL). Trans-cinnamate is then transformed by cinnamate-4-hydroxylase to form 4-coumarate, which in turn is transformed into the active form 4-coumaryl-SCoA by 4-coumarate-SCoA lyase (Ebel and Hahlbrock, 1982; Gross, 1987; Hrazdina, 1992; Kubasek et al., 1992; Lancaster, 1992) (Fig. 3).

Chalcone synthase catalyzes the condensation of the three malonyl-SCoA molecules and 4-coumaryl-SCoA to produce a yellow chalcone. The isomerization of chalcone into a colourless flavanone (naringenin or eriodictyol), precursor of all anthocyanins, flavones, flavonols and isoflavonols, proceeds spontaneously but is accelerated by chalcone flavanone isomerase (Dooner et al., 1991; Lancaster, 1992; Martin and Gerats, 1993). After the production of phenylalanine in chloroplasts, reactions up to and including the isomerization of chalcone into a flavanone are thought to take place in the cytoplasm. Flavanone is then thought to be translocated to the tonoplast (Grisebach, 1980; Hrazdina, 1992) where it is subsequently hydroxylated in the 3-position by flavanone-3-hydroxylase to form a dihydroflavonol (Martin and Gerats, 1993; Mazza and Miniati, 1993) thought to be dihydrokaempferol (Lancaster, 1992; Holton and Cornish, 1995) (Fig. 3).

It is known that petal limbs of petunia flowers are acyanic (Martin and Gerats, 1993) while corolla tubes do not normally produce pelargonidin. In addition, snapdragons and maize are incapable of producing delphinidin. Consequently, these phenomena implicate different pathways for cyanidin, pelargonidin and delphinidin synthesis. Indeed, specific enzymes have been identified which catalyze these different reactions. Dihydrokaempferol may either be reduced by dihydroflavonol 4-reductase to form leucopelargonidin, or hydroxylated by either flavonoid 3'-hydroxylase to produce dihydroquercetin, or by flavonoid 3',5'-hydroxylase to form dihydromyricetin. Flavonoid 3',5'-hydroxylase may however, also convert dihydroquercetin to dihydromyricetin. Dihydroquercetin and dihydromyricetin are then

reduced by dihydroflavonol 4-reductase to form leucocyanidin and leucodelphinidin respectively (Holton and Cornish, 1995). How leucoanthocyanidins are converted to form anthocyanidins, viz. pelargonidin, cyanidin and delphinidin is not clear (Martin and Gerats, 1993). Heller and Forkman (1988) and Martin and Gerats (1993) proposed two steps involving firstly a dioxygenase and secondly a dehydratase.

Anthocyanidins are then transported across the tonoplast into the vacuole (Grisebach, 1980) where they are glycosylated at the 3 position by UDP-glucose flavonol 3-*O*-glucosyl transferase to form anthocyanins, viz. pelargonidin-3-glucoside, cyanidin-3-glucoside and delphinidin-3-glucoside (Martin and Gerats, 1993; Holton and Cornish, 1995) (Fig. 3). Subsequently, anthocyanidin-3-glucosides may be modified by hydroxylation, methylation, glycosylation and acylation to yield other anthocyanins (Lancaster, 1992; Martin and Gerats, 1993; Holton and Cornish, 1995). A free hydroxyl group is essential at position 7, 5 or 4' where blue colours are concerned (Brouillard, 1982), whereas the presence of methyl groups leads to a decrease in blue colour (Mazza and Brouillard, 1990). Common classes of glycosides are monosides, biosides, triosides and 3,5-diglucosides, and the most common sugar moeities are glucose, galactose, rhamnose, arabinose and xylose. Most acylated groups are based either on 3,5-diglucosides but some have also been identified on 3-glucosides (Timberlake and Bridle, 1975; Strack and Wray, 1989). Acyl groups which occur on the sides of the flavylium cation may be either aromatic (phenolic) or aliphatic acids. Aromatic acids associated with anthocyanins include hydroxycinnamic, ρ-coumaric, caffeic, ferulic and hydroxybenzoic acids (Strack and Wray, 1989). Aliphatic acids found as acyl moeities of anthocyanins include acetic, oxalic, malonic, succinic and malic acids (Harborne and Grayer, 1988).

1.2.2.2 Regulation

Investigations on regulation of anthocyanin biosynthesis in litchi fruit are lacking and most studies have centred on flowers, particularly *Petunia* and *Antirrhinum*, and pome fruit colour. Lancaster (1992) maintained that regulation by endogenous and environmental factors differs between flowers and fruit. Consequently, only those factors affecting regulation of anthocyanin biosynthesis in fruit will be covered in this review. Environmental factors

include light and temperature, while endogenous factors are related to nutritional factors and phytohormones (Gross, 1987). Rootstocks and cultural practices such as pruning, thinning, and bagging also impact on anthocyanin biosynthesis, but their influence is almost definitely through affecting one or other of the environmental and/or endogenous factors (Saure, 1990). Recent reviews on the topic to which the reader is referred include those by Saure (1990), Lancaster (1992) and Viljoen and Huysamer (1995).

1.2.2.2.1 Light

Anthocyanin biosynthesis in rinds of apple fruit is fully dependent on light (Saure, 1990; Lancaster, 1992) where expression of photomorphogenic effects is the consequence of a sequence of events, initiated upon irradiation with light by photoreceptors, and completed once the response is expressed. However, there is some controversy surrounding the exact photoreceptors. Different studies have identified three distinct photoreceptors which may act singly or in combination :- (1) phytochrome (Fig. 4), which in photoresponses exists in two forms, viz. inactive phytochrome 660 and active phytochrome 730 (Mancinelli, 1985).

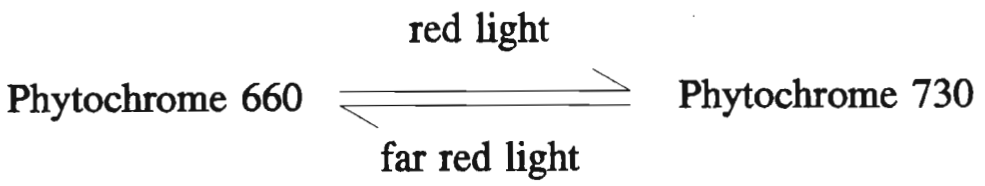

Figure 4. Inactive phytochrome 660 and active phytochrome 730 induced by far red light and red light respectively (adapted from Mancinelli, 1985)

Beggs et al. (1987) showed that anthocyanin was strongly induced by continuous far-red light and by both red light and red light pulses; (2) cryptochrome, which is activated by a combination of blue light (400-480 nm) and ultraviolet A light (320-400 nm) (Beggs et al., 1981), and (3) ultraviolet B light (290-320 nm) (Arakawa et al., 1985; Heller and Forkman, 1988). Induction of anthocyanin biosynthesis involves two known responses. Firstly, small amounts of anthocyanin are formed in response to short light exposures. This response is phytochrome-mediated displaying red light/far red light reversibility and reciprocity

(Siegelman and Hendriks, 1957). Secondly, there is the high irradiance response (HIR) where large amounts of anthocyanin are formed in response to prolonged irradiation of near visible and visible light (290-750 nm) at high fluence rates, where reversibility or reciprocity are not followed (Mancinelli, 1983; Schmidt, 1988).

Two radiation dependent phases can be distinguished for high irradiance responses in apple rinds. Firstly, there is an induction phase of approximately 20 hr where no anthocyanin production takes place and secondly, subsequent anthocyanin formation occurs at a rate directly proportional to time of constant irradiation (Siegelman and Hendricks, 1958a; Hahlbrock and Grisebach, 1979). Levels of irradiation are also critical and Proctor and Creasy (1971) found that no anthocyanins formed when energy levels were less than 0.5 $mW.cm^{-2}$ for 48 hr. They suggested a minimum energy requirement of 100 $J.cm^{-2}$ for initiation of anthocyanin biosynthesis. It should however, be noted that spectral sensitivity of anthocyanin production is different in different biological systems and can be markedly affected by fluence rates, duration and mode of application, i.e. continuous versus intermittent light, and physiological status of the biological system (Mancinelli, 1983; Saure, 1990). Consequently, differences in light regulation of anthocyanins between apple and litchi rinds are to be expected.

1.2.2.2.2 Temperature

Anthocyanin biosynthesis in apples is favoured by low temperatures (Harborne, 1980b; Gross, 1987; Saure, 1990; Shichijo et al., 1993). At average daily temperatures of greater than 21°C very little anthocyanin was synthesized (Uota, 1952), and red colouring was greater in fruit held at average night temperatures of 11°C compared to 22°C (Blankenship, 1987). Intense colouring of young apple fruitlets was thought to be associated with cool winter nights, and loss of colour during summer with increasing temperatures. Indeed, a combination of low day (12°C) and night (2°C) temperatures resulted in highest anthocyanin accumulation both on and off the tree (Gross, 1987; Macheix et al., 1990; Mazza and Miniati, 1993). Fruit maturity and cultivar also play a major role in determining response temperatures. Independent of picking date, 'Jonathan' apples needed night temperatures of 10°C and high day temperatures for maximum coloration. Conversely, early-picked 'Ontario'

apples needed night temperatures of 4°C while those picked later needed 8° to 10°C for maximum coloration (Saure, 1990). In contrast, litchi fruit mature and colour up during mid-summer, under high temperature conditions. Consequently, it is unlikely that low temperatures are implicated in litchi pericarp anthocyanin accumulation, although no evidence was found to this effect and further studies are warranted.

Several effects of temperature have been suggested. Siegelman and Hendricks (1958b) maintained that temperatures affected both the induction period and the subsequent rate of anthocyanin formation. Tan (1989) investigated PAL activity in relation to temperature and found more activity when temperatures were low. In support of this, Faragher (1983) maintained that high temperatures inactivated PAL as opposed to low temperatures stimulating enzyme activity. Lancaster (1992) suggested that low temperatures reduced respiration thus reducing photosynthate decline in apple rinds, resulting in increased substrate for anthocyanin biosynthesis.

1.2.2.2.3 Nutritional effects

Photosynthates, initial substrates for anthocyanin biosynthesis, have been shown to trigger anthocyanin accumulation (Pirrie and Mullins, 1976). In grape rinds, a close correlation between sucrose and anthocyanin concentrations was found. Here, an increase in anthocyanin concentrations followed an initial increase in sugar content (Pirrie and Mullins, 1977). In litchi rinds, Prasad and Jha (1978) detected high levels of rhamnose during ripening but none once fruit were fully coloured. From these results, Gross (1987) suggested that rhamnose was used during biosynthesis of anthocyanin glycosides.

Mineral nutrients nitrogen (N) and potassium (K) have a direct effect on anthocyanin biosynthesis (Gross, 1987; Saure, 1990). Excess N leads to poor apple fruit colour at maturity both directly and indirectly. Firstly, excess N leads to increased vegetative vigour, denser foliage and thus shading which results in less anthocyanin formation (Magness et al., 1940). Secondly, *in vitro* studies have shown that discs of apple rind fed with urea synthesized far less anthocyanin than untreated control discs (Faust, 1965). Furthermore, high N doses diverted photosynthates from carbohydrate accumulation to amino acid and

protein synthesis, thus limiting anthocyanin substrates. After reviewing the literature, Saure (1990) concluded that K effects on anthocyanin biosynthesis were negligible, but that a high K supply supplemented the positive effect of low N on anthocyanin biosynthesis. He also maintained that effects of other nutrients on anthocyanin biosynthesis were inconsistent as no clear correlations had been established.

1.2.2.2.4 Phytohormones

A pre-harvest ethylene (ethephon) spray has been shown to increase rate of anthocyanin biosynthesis in apples (Chalmers and Faragher, 1977) and grapes, although in the latter the response varied with time of application and cultivar (Weaver and Pool, 1971). Other fruits which responded to ethephon treatments include black currants, cherries, raspberries and olives but again responses varied with date of treatment, environmental conditions and cultivar (Gross, 1987). Ethylene was shown to stimulate activity of enzymes involved in anthocyanin biosynthesis, but this was only true when PAL was light-induced (Gross, 1987). If ethylene is applied to climacteric fruit close to maturity it may accelerate ripening (Lancaster, 1992). However, litchi fruit are non-climacteric and accelerated ripening is thus not a factor. Indeed, Sadhu and Chattopadhyay (1989) showed that a 2 500 mg.ℓ^{-1} post-harvest dip of ethephon for 5 min on litchi fruit resulted in uniformly red fruit but with some loss of eating quality. Consequently, pre-harvest ethephon sprays may well improve fruit colour of litchi fruit and further investigations are warranted.

Other plant growth regulators shown to increase anthocyanin concentrations include gibberellins in sweet cherries (Drake et al., 1978), cytokinins in olives (Shulman and Lavee, 1973), and daminozide (Schumacher et al., 1986) and paclobutrazol in apples. Saure (1990) however, maintained that effects of daminozide and paclobutrazol on anthocyanin biosynthesis are inconsistent, and were probably related to increased light interception as a result of decreased vegetative vigour. Likewise, abscisic acid has been shown to limit anthocyanin biosynthesis but again the effect was not consistent (Ebel and Hahlbrock, 1982).

1.2.2.3 Structural Changes

The chemical structure of anthocyanins has been shown to change *in vitro* with varying pH (Fig. 5). In neutral or acidic solution, four anthocyanin structures exist in equilibrium, viz. flavylium cation, quinoidal bases, carbinol bases and chalcone. At pH 1.0 anthocyanins are in the red flavylium cation form. At pH 2.0 to 4.0 the flavylium cation loses protons rapidly and yields blue quinoidal bases (Strack and Wray, 1989; Mazza and Miniati, 1993) which are unstable (Jurd, 1963). If pH increases to about 5.0 then more protons are lost from the flavylium cation. Subsequently, hydration of the flavylium cation will result in a colourless carbinol or pseudobase. With a subsequent increase in pH up to 6.0 the colourless carbinol will in turn equilibrate to form an open chalcone, which is also colourless (Strack and Wray, 1989; Mazza and Miniati, 1993). Chromenols are fairly stable at room temperature, but degenerate slowly with time to yield brown, phenolic acids (Strack and Wray, 1989). In addition, anthocyanin chromenols instantly regenerate the flavylium cation upon acidification (Jurd, 1963).

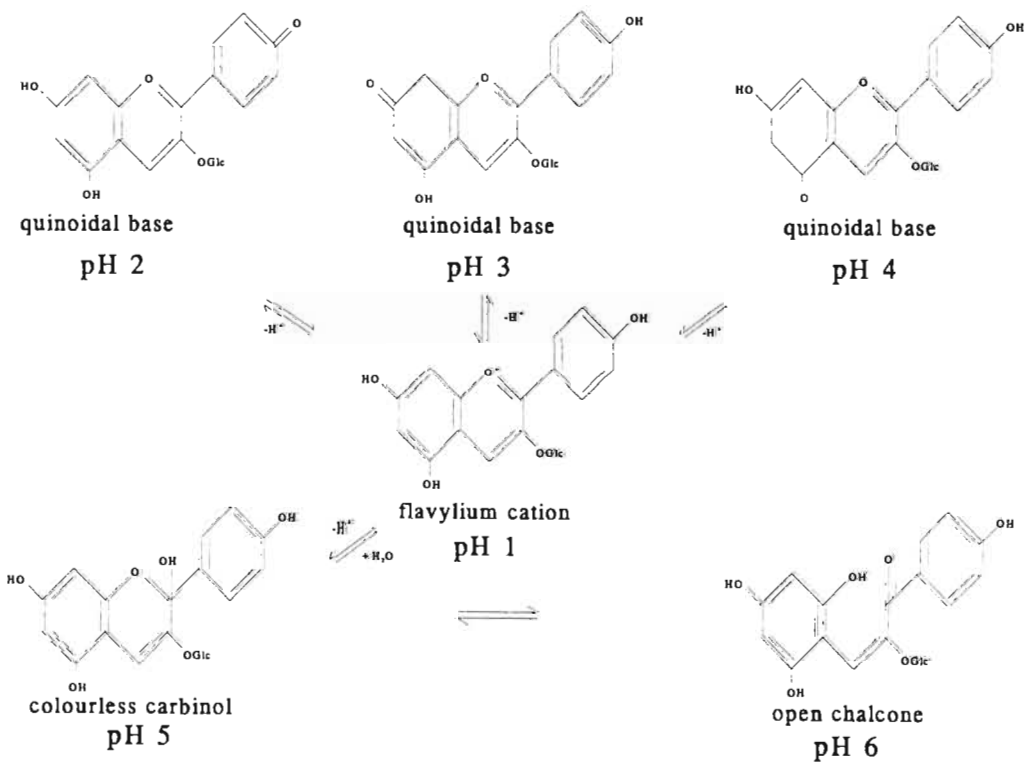

Figure 5. Anthocyanin (pelargonidin 3-glucoside) structural transformations in aqueous solution between pH 1.0 and pH 6.0 (adapted from Strack and Wray, 1989)

Macheix et al. (1990) however, showed that in an aqueous solution, glycosides of anthocyanins will be hydrolyzed rapidly to yield anthocyanidins and free sugar moeities. Electron deficient anthocyanidins are highly reactive, and rapidly undergo ring fission to yield an unstable α-diketone which is further decomposed to yield phenolic acids (Fig. 6) (Jurd, 1972). During litchi rind post-harvest senescence, membrane integrity is lost as a result of micro-cracking (Underhill and Critchley, 1993), resulting in mixing of vacuolar and cytoplasmic sap. The author suggests that this subsequent increase in pH in a free-standing aqueous solution results in hydrolysis of anthocyanins to yield anthocyanidins and free sugar moieties. Anthocyanidin molecules will then be broken down rapidly, which in turn explains visible browning observed during post-harvest storage of untreated litchi fruit. Anthocyanidin flavylium nuclei have been shown to break down on contact with air, and in the presence of ascorbic acid, sugars and amino acids (Pratt et al., 1954; Daravingas and Cain, 1968; Starr and Francis, 1968). The rate of anthocyanidin destruction is pH and temperature dependent, being faster at high pH and high temperatures (Markakis et al., 1957; Tinsley and Bockian, 1960).

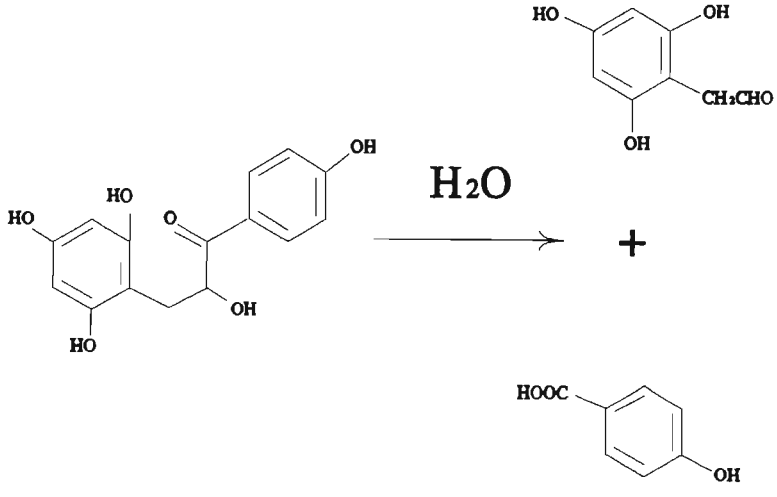

Figure 6. Decomposition of α-diketone (left) to yield benzoic acids (right) (from Jurd, 1972)

Studies of enzymatic reactions involving polyphenol oxidase and peroxidase enzymes in the presence of oxygen, have also shown that anthocyanidins may be irreversibly broken down into phenolic acids or melanin by-products (Pifferi and Cultrera, 1974; Joubert and van

Lelyveld, 1975; Huang et al., 1990). Polyphenol oxidase and peroxidase activity have however, been shown to be optimal at a pH of between 5.0 and 7.0, and at low pH, polyphenol oxidase activity is reduced (Underhill et al., 1992a; b). Clearly, it is the author's opinion that if the cytosol of senescent litchi rind cells is maintained at or below pH 1.0 anthocyanin and anthocyanidin molecules will remain in stable flavylium cationic forms, and degradation by enzymes such as polyphenol oxidase and peroxidase will be limited. This hyphothesis, *inter alia*, was tested in the research reported in this thesis.

1.2.2.4 Stabilization

Apart from pH effects, temperature and ionic strength of the medium, and structure and concentration of anthocyanin and anthocyanidin pigments, there are four possible stabilization mechanisms, which lead to tertiary structures of great intensity and stability i.e. are not easily decomposed or modified chemically. These are self-associations, intermolecular co-pigmentation, intramolecular co-pigmentation and, metal-complexing or chelation (Strack and Wray, 1989; Mazza and Brouillard, 1990; Mazza and Miniati, 1993; Viljoen and Huysamer, 1995). Interestingly however, Lancaster et al. (1994) found that an increase in rind darkness of apple fruit could be accounted for by increased anthocyanin concentration in more abundant, larger vacuoles, and not from co-pigmentation effects.

1.2.2.4.1 Self-associations

Self-association occurs when colour intensity of anthocyanins increases more than linearly with a linear increase in pigment concentration. Asen et al. (1972) showed that at pH 3.16 absorbance of cyanidin 3,5-diglucoside increased by more than 300 times when concentrations increased from 10^{-4} to 10^{-2} M. Brouillard (1988) suggested that anthocyanin solubility may be related to self-association but that a relationship between solubility, pH and self-association had not yet been investigated. At pH 7.0 using circular dichroism and ^{1}H-nuclear magnetic resonance techniques vertical stacking of chiral anthocyanin quinoidal bases was shown to occur due to hydrophobic interactions between aromatic nuclei (Goto and Kondo, 1991). However, at pH 4.0 or less the exact nature of the self-association complex formed remains unknown (Mazza and Miniati, 1993).

1.2.2.4.2 Intermolecular co-pigmentation

Intermolecular complexing may take place between anthocyanidins or anthocyanins and other non-covalently bound substances (Strack and Wray, 1989). Molecules which may act as co-pigments include amongst others, flavonoids, polyphenols, alkaloids, amino acids, organic acids, anthocyanins and their precursors (Jurd, 1967; 1969; Asen et al., 1972; Singleton, 1972; Scheffeldt and Hrazdina, 1978; Osawa, 1982; Mazza and Brouillard, 1990; Goto and Kondo, 1991; Davies and Mazza, 1993). Anthocyanins which contain an aromatic acyl group form much more stable co-pigment complexes with flavones (Asen et al., 1977b; Hoshino et al., 1980). Chen and Hrazdina (1981) suggested that hydrogen bonding between hydroxyl and carbonyl groups on the aromatic nuclei and sugar moeities was responsible for pigment-co-pigment associations. However, this can be ruled out as formation of an end to end complex does not prevent hydrolysis of the pyrylium ring (Brouillard, 1982). Recently, Goto and Kondo (1991), using circular dichroism and ^{1}H-nuclear magnetic resonance, showed that intermolecular co-pigmentation in aqueous solution is the result of vertical, hydrophobic stacking of aromatic nuclei in flavonoids and aromatic acids. Furthermore, stacking may be assisted by hydrogen bonding in hydrophillic sugar moeities superimposed on the stacks.

Intermolecular complexing results in firstly, a hyperchromic effect where colour intensity increases and secondly, a bathochromic shift where the wavelength of maximum absorbance shifts towards higher wavelengths and thus results in purple and blue colours (Asen et al., 1972; 1977a; Liao et al., 1992). Since hydration affects only flavylium cations, and co-pigmentation only results in increased absorbance in the visible range, Brouillard (1988) maintained that the co-pigment molecule partly prevents hydration of flavylium cations. Consequently, formation of the co-pigment complex competes with formation of colourless pseudobases. Furthermore, for pure anthocyanins obtained from Carl Roth (Karlsrubhe), intensity of intermolecular complexing depends on type of anthocyanin, degree of methoxylation and glycosylation, co-pigment concentration, and temperature of the medium (Asen et al., 1972; Osawa, 1982; Davies and Mazza, 1993). In addition, Chen and Hrazdina (1981) maintained that pH 3.5 was necessary for maximum co-pigment effect but that this varied slightly depending on the pigment/co-pigment system.

1.2.2.4.3 Intramolecular co-pigmentation

Several anthocyanins with planar acyl residues have been identified (Brouillard, 1988; Dangles et al., 1993; Figueiredo et al., 1996). These anthocyanins are stable in mildly acidic aqueous solution, whereas well known acylated mono- and diglucosylated anthocyanins are colourless under similar conditions (Figueiredo et al., 1996). Brouillard (1982) suggested for *Zebrina pendula* that two acyl moeities stacked above and below the pyrylium ring of the flavylium cation were preventing hydration. Similarly, Dangles et al. (1993) suggested that anthocyanins extracted from *Pharbitis nil* had undergone intramolecular co-pigmentation with caffeyl and sophorosyl and that the glycosyl unit was acting as a spacer. This sandwich-type stacking of acylated anthocyanins confers stability even when pH changes (Brouillard, 1982), but stability increases with increasing organic acid content and aglycone substitution (Mazza and Miniati, 1993). In support of intramolecular co-pigmentation, Figueiredo et al. (1996) maintained that nuclear magnetic resonance and mass spectroscopy data have clearly demonstrated the existence of linkage between folded aromatic acyl and chromophore protons.

1.2.2.4.4 Metal complexing

Anthocyanin anhydrobases, which are normally colourless at pH 4 to 6, have been shown, at these pH values to form remarkably stable coloured complexes, which had metal ions such as tin (Chandler and Clegg, 1970; Sistrunk and Cash, 1970), aluminium (Jurd and Asen, 1966; Asen et al., 1969) and iron (Asen et al., 1969; Chandler and Clegg, 1970) chelated with the B-ring. Brouillard (1988) maintained however, that only iron and aluminium are found in appreciable amounts in plants and that more abundant calcium, magnesium (Mg) and K do not form chelates with anthocyanins. Furthermore, he speculated that it was doubtful that metal complexing alone could account for colour *in situ*. In support of this, Goto and Kondo (1991) maintained that metal ions could be excluded from the metalloanthocyanin structure without colour loss, and that metal ions were simply co-ordinating copolymerization of several anthocyanin molecules. Mazza and Miniati (1993) maintained however, that quinoidal forms and flavylium cations were strongly stabilized and did not form colourless anhydrobases when dissolved in concentrated solutions of sodium

chloride and magnesium chloride. They suggested that colour stabilization in sodium chloride solution may be due to promotion of self-association of anthocyanins, while stabilization by magnesium chloride may have been due a reduction in concentration of free water by hydration of Mg ions.

1.3 RIND BROWNING

Once fruits are mature, litchi rinds will turn brown either in the field or after harvest, as a result of senescence or ageing (Menzel, 1984; Bagshaw et al., 1991; Underhill et al., 1992a; Underhill, 1994; Underhill et al., 1994). Although this natural senescence or browning of litchi rind tissues does not affect fruit flesh, it renders the fruit unsightly. This has serious economic implications as the litchi is a little-known fruit in export markets and an unblemished, well coloured fruit is needed to attract new consumers. Browning may either be a pre-harvest (Joubert and van Lelyveld, 1975; Joubert, 1986) or a post-harvest phenomenon (Nip, 1988; Zauberman et al., 1991; Underhill, 1992; Underhill et al., 1992b; Underhill and Simons, 1993). Several other factors which have been shown to cause pre-harvest rind browning include sunburn, wind scarring, insect attack, spray burn, high air temperatures, low humidity and low soil moisture (Menzel, 1984).

1.3.1 Pre-harvest

Pre-harvest browning of rinds was observed by Joubert and van Lelyveld (1975) approximately 4 weeks before fruit maturity and harvesting. Initially, rind mesocarp cells became necrotic but later the epi- and endocarp cells also degenerated. Plasmolysis and eventual collapse of the radial cell walls was observed while polyphenol oxidase activity, peroxidase activity and enzymatic oxidation of ascorbic acid were all higher in fruit with necrotic tissue than in healthy fruit. They suggested that pre-harvest browning may be the result of firstly, increased pressure on the rind as the result of continued fruit flesh growth; secondly, excessive temperature fluctuations during fruit ripening; and thirdly, excessive accumulation of moisture in the fruit flesh as a result of heavy rains during the final stages of fruit growth, thus causing tension swelling of fruit flesh and subsequent tension and damage to the rind cells. Menzel (1984) also maintained that inadequate soil moisture during initial stages of fruit growth result in rinds becoming hard and inelastic. Consequently, the

rind will crack when fruit flesh expands rapidly following irrigation. Later, Joubert (1986) implicated high temperatures and low relative humidity as causal factors most likely to induce pre-harvest browning, and suggested a minimum relative humidity:temperature ratio of 2:1 to prevent necrosis of the rind. A common thread in this type of browning appears to be inadequate cell division, due to high evaporative demand, to accommodate the subsequent "ballooning out" of flesh growth.

1.3.2 Post-harvest

Unless treated promptly, once fruit have been detached from the tree, post-harvest browning sets in. Underhill and Critchley (1995) examined anatomical litchi rind browning and found that it occurred first on the protuberance apices. Initially, surface browning was masked by anthocyanin pigmentation and could only be detected once anthocyanins were decolorized using sulphites. Subsequently, browning extended uniformly over the entire rind surface after being initially restricted to the upper epidermis. With further desiccation, browning extended into columns of collenchyma cells found between the epidermal sclerenchyma tissue as well as several layers of underlying mesocarp parenchyma tissue. Browning may be induced by several factors ranging from pigment breakdown through physical damage to the rind, to stress induced by harvesting and handling. Bagshaw et al. (1991) cited moisture loss and temperature as the two most common causes of post-harvest browning.

1.3.2.1 Moisture loss

Once harvested, litchi fruit lose moisture rapidly with most of the initial loss from the rind (Kuhn, 1962). Fruit flesh desiccation is somewhat delayed (Bagshaw, 1991), although it was later shown that micro-cracking occurs in the rind at harvest (Underhill and Critchley, 1993). Joubert (1986) maintained that up until that time the relatively thick epicarp and cuticle inhibited moisture loss. Underhill and Critchley (1993) subsequently showed that after fruit were harvested several cracks from 20 to 100 μm wide appeared rapidly on the surface of the rind. The micro-cracks extended through the sclerenchyma into the mesocarp parenchyma tissue, and proved to be primarily associated with dehydration and desiccation of the rind, but also served as sites of entry for fungal hyphae.

In a detailed study of mature red 'Kwai May Pink' fruit stored at 25°C and 60% relative humidity (RH) for 6 days, Underhill and Simons (1993) found immediately after harvest that rind micro-cracking first occurred radial to the rind protuberance apices. Subsequent inter-protuberance cracking was later observed. Initially, micro-cracks were up to 100 μm wide but increased with progressive dehydration of the rind. Eventually the micro-cracks formed a semi-continuous network which extended through the rind into the mesocarp parenchyma. Up to 12 hr after harvest, micro-cracking had not extended to the endocarp. Only after the entire rind had become desiccated (ca 48 hr) did the flesh and seed begin to desiccate. It was thus concluded that this selective dehydration resulted in limited movement of water between fruit flesh and the rind.

In identifying the causes of micro-cracking, Underhill and Simons (1993) found that micro-cracks formed as a result of desiccation rather than being the initiating factor. Although cuticle integrity was not maintained as a result of micro-cracking, cuticle thickness was unchanged during storage. Deterioration of the cuticle in the rind protuberances was observed but it was thought that this might be the result of mechanical abrasion associated with harvesting. However, small, squamous collenchyma cells which at harvest appeared as fine, white radial markings on the rinds were found disrupting sclerenchyma continuity and separated easily with initial rind dehydration resulting in micro-cracks. Underhill and Simons (1993) thus maintained that micro-cracking in the litchi rind is inevitable and is the major cause of moisture loss from the litchi rind. Bagshaw et al. (1991) maintained that post-harvest litchi rind browning began after the rind had lost about 20% of its moisture. In addition, the rate of browning was directly proportional to the amount of moisture lost, and browning was complete when the rind had lost between 60 an 70% of its moisture. Obviously ambient conditions will determine the rate and extent to which moisture is lost and thus the rate and extent of rind browning.

1.3.2.2 Temperature stress

Bagshaw et al. (1991) maintained that browning induced by temperature stress could be distinguished from that caused by moisture loss, as the former resulted in darker fruit with a more watery appearance. They also maintained that temperature stress induced by either

chilling or heat injury is least responsible for post-harvest browning. Where chilling injury is involved, temperatures of less than 5°C are known to cause necrosis and browning of rind tissues. Consequently, the Australian recommendation for litchi fruit storage is 5 to 7°C for up to 4 weeks. Where heat injury is concerned, Bagshaw et al. (1991) maintained that if a temperature of 50°C is exceeded or is maintained for more than 2 min (safe treatment time for hot benomyl dips) then fruit browning will occur.

Underhill and Critchley (1993) showed that immersion of fruit in hot water for 10 min at 60°C resulted in brown pigmentation throughout the rind. However, under ambient conditions, tissue browning was localized with brown pigmentation restricted to the exocarp. It was found that further desiccation of fruit at ambient temperatures did not affect brown pigmentation distribution.

1.3.2.3 Physiological changes

Underhill (1989) found that the loss of moisture from the litchi rind of fruit held under ambient conditions resulted in cell plasmolysis and eventually mesocarp cell death. Initially, desiccation led to an increase in pH of the cytosol as well as changes in membrane integrity and the subsequent release of polyphenol oxidases, peroxidase and other enzymes in their active forms. Bagshaw et al. (1991) noted that temperature stress also leads to increased enzyme activity.

1.3.2.3.1 Polyphenol oxidases

The relationship between physiological browning of fruit tissues and polyphenol oxidases was shown as early as 1951 by Joslyn and Ponting. A multitude of articles have since been published on the subject, with those by Mathew and Parpia (1971) and Mayer and Harel (1981) being two of the most comprehensive reviews to date. The discolouration that follows mechanical injury is rapid and intense and known as adventitious browning, while that which occurs during the normal life cycle of a plant or fruit is slow and known as functional browning. In both cases however, the browning reaction is the result of oxidation of phenolic compounds into *ortho*-quinones. The enzymes responsible for this oxidation are a large group

of enzymes known as the polyphenol oxidases, which may be divided into two main groups, viz. the laccases and the catechol oxidases. These two enzyme groups catalyze different reactions (Fig. 7), but catechol oxidases have been identified most frequently in fruit products (Mayer and Harel, 1981).

Phenolic compounds are known to occur throughout the plant kingdom. Those found in food products are mainly of the flavonoid type (Mathew and Parpia, 1971) of which anthocyanidins, the major rind pigment in mature litchi fruit, are a constituent. Pifferi and Cultrera (1974) working on sweet cherries, and Huang et al. (1990) on litchis, showed that polyphenol oxidase is the main enzyme involved in anthocyanidin breakdown during storage. Studies on subcellular localization of polyphenol oxidase in healthy cells of plant tissue have shown conclusively that polyphenol oxidase is a plastid enzyme (Vaughn and Duke, 1981; 1988; Bar-Nun and Mayer, 1983). Underhill and Critchley (1995) also provided circumstantial evidence to support this theory, claiming that polyphenol oxidase localization reflects chloroplast distribution. During the initial stages of fruit growth, polyphenol oxidase activity was found to be highest in the epicarp and upper mesocarp, the regions of greatest chloroplast density. Furthermore, polyphenol oxidase activity decreased with ontogeny and this was also accompanied by a decrease in chlorophyll content.

Flurkey and Jen (1978) identified polyphenol oxidase in the cytoplasm of degenerating or senescent tissues of ripening fruit. This is however, not a contradiction since breakdown of plastid membranes is associated with fruit ripening. Consequently, mixing of the cytoplasm and plastid contents would be expected. Indeed, polyphenol oxidase is apparently not activated until it crosses the plastid envelope (Vaughn and Duke, 1984a). Cytochemical and immunocytochemical studies by Vaughn and Duke (1981; 1984b) showed that polyphenol oxidase was synthesized on 80S cytoplasmic ribosomes, and then transported across the plastid membranes. They also showed that after incubation of mung bean (*Vicia faba*) plants, the radiolabelled anti-polyphenol oxidase only accumulated around the periphery of plastids, indicating that neither anti-polyphenol oxidase nor an inactive form of polyphenol oxidase is absorbed non-specifically to the plastid membranes.

Most studies of polyphenol oxidases have been on ripe fruit, but some have monitored polyphenol oxidase activity during the course of fruit development. Hobson's (1967) investigation of mango fruit showed that there was a rise in activity during fruit development, followed by a drop in activity in ripe fruit. Underhill and Critchley (1992) had similar results when they monitored polyphenol oxidase activity in the litchi rind during fruit ontogeny and development. Polyphenol oxidase activity increased during the first 48 days after anthesis, but declined to very low levels with subsequent seed maturation and fruit flesh expansion only to undergo a slight increase again with fruit maturity.

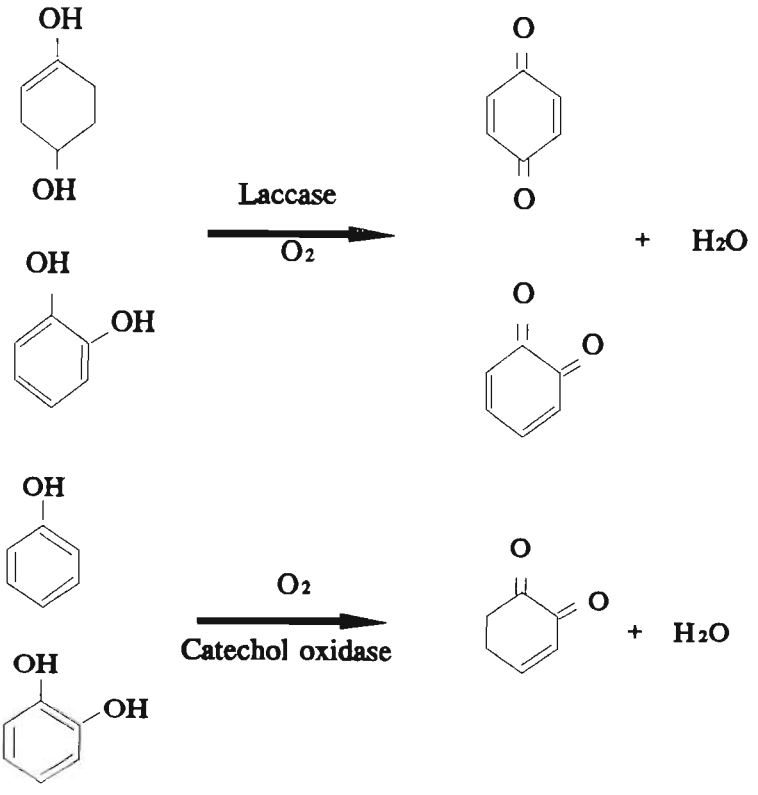

Figure 7. Reactions catalyzed by polyphenol oxidase enzymes, laccase and catechol oxidase (adapted from Mayer and Harel, 1981)

Litchi rind polyphenol oxidase activity was relatively low at maturity and Underhill and Critchley (1992) maintained that this suggested a reduced capacity to induce tissue browning. This result was however, not unusual as Mayer and Harel (1981) noted that previous studies on many different fruit types have shown that levels of polyphenol oxidase are highest during the early stages of development, but drop quite significantly after fruit maturity and during

ripening. They suggested that the decrease in activity during development of the fruit implied not only the cessation of enzyme synthesis but also the inactivation, inhibition or decomposition of the enzyme.

Akamine (1960) suggested that polyphenol oxidase was involved in post-harvest browning of the litchi rind and was associated with fruit desiccation. Subsequently, Tan (1989) also maintained that browning of the litchi rind was due to enzymatic browning caused by polyphenol oxidase. Lin et al. (1988) showed that polyphenol oxidase activity in the litchi rind increased rapidly after harvest and then peaked after 48 hr. In contrast, Zaubermann et al. (1990) observed little change in polyphenol oxidase activity during ambient storage of litchi fruit. Later, Underhill and Critchley (1993) found that polyphenol oxidase activity in the litchi rind decreased rapidly after harvest, observing a 3-fold reduction in activity within the first 24 hr after harvest. Subsequently, activity remained fairly constant but decreased slightly over time. Coupled with these results, Underhill (1992) and Underhill et al. (1992a) maintained that anthocyanin concentrations only decreased slowly after harvest, and that this implied that it is unlikely that degradation of anthocyanins by polyphenol oxidase plays a major role in litchi post-harvest browning under ambient conditions.

Based on the rate of anthocyanin degradation, distribution of brown pigmentation within the litchi rind, and polyphenol oxidase activity during storage, Underhill and Critchley (1993) suggested that it is unlikely that polyphenol oxidase plays a major role in rind browning under ambient conditions. In that study, initial red pigmentation loss was associated with mesocarp parenchyma cells becoming colourless while the epicarp (exocarp), which is not associated with anthocyanins, turned brown. Unfortunately, Underhill and Critchley's (1993) investigation made no mention of brown pigmentation distribution during later stages of fruit storage. The initial loss of colour in the mesocarp can be explained by the breakdown in cell organelles associated with natural post-harvest degradation of cells. The vacuolar sap, in which anthocyanin pigments are localized, is normally at very low pH (Smith and Raven, 1979). When the tonoplast degenerates, the vacuolar sap will mix with the cytoplasm, which fluctuates between pH 7.0 and 7.5 (Salisbury and Ross, 1992). Consequently, anthocyanin pigments will lose protons from their structure resulting in colourless chromenols, which although fairly stable will break down with time (Jurd, 1963). This last reaction is catalyzed

by polyphenol oxidase but previous work has shown that polyphenol oxidase is not active as a phenol oxidase in chloroplasts, where it is exclusively located in healthy tissue, but rather is limited as a phenol oxidase by latency or lack of substrate (Golbeck and Cammarata, 1981).

Latency in polyphenol oxidase activity can be overcome using one of several treatments, although most of these treatments mimic one or other aspect of senescence. Consequently, it is not surprising that polyphenol oxidase activity rises slightly after harvest, since later stages of fruit ripening would be associated with senescence. Treatments which are known to overcome latency of polyphenol oxidase activity include amongst others, detergents (Sato and Hasegawa, 1976), ethylene (Elstner et al., 1976; Sharon and Kahn, 1979) and heat (Underhill and Critchley, 1993). Detergents are known to break down cell and organelle membranes thus resulting in mixing of polyphenol oxidase and the cytoplasm and causing enzyme activation. Ethylene production is extremely low in litchi fruit in comparison to other fruit (Tongdee et al., 1982). Underhill and Critchley (1993) found that for mature 'Bengal' litchi fruit held at 25°C and 60% RH, ethylene production decreased significantly from the time of harvest, undergoing slightly more than a 3-fold reduction in concentration within the first 12 hr after harvest. They maintained that initial relatively high levels of 0.96 $\mu l.kg^{-1}.hr^{-1}$ when compared to fruit just prior to harvest, probably represented a wounding response. Nevertheless, browning of litchi fruit rinds was not associated with ethylene production but rather sensitivity of the tissue to desiccation caused by dehydration.

When fruit were immersed in hot water at 60°C for 10 min and then stored at 25°C for progressive assessment, polyphenol oxidase activity increased immediately after heat treatment, which corresponded with rapid browning of the rinds. Browning was seen throughout the rind, indicating that polyphenol oxidase was not restricted to specific areas within the rind. In contrast, they showed that browning which resulted in untreated control fruit held at 25°C and 60% RH was restricted to the epicarp. They postulated that the differences in browning localization observed between heat-treated and untreated control fruit may have been due to either a difference in localization of polyphenol oxidase activity or the presence of other browning enzymes such as peroxidase (Underhill and Critchley, 1993).

1.3.2.3.2 Peroxidase

Very few studies have been conducted on the role of peroxidase in litchi rind browning but, Zaubermann et al. (1991) concluded that peroxidase does not play a major role in rind browning in litchi fruit held at ambient temperatures. Subsequently, Underhill and Critchley (1995) maintained that rind browning which occurs during ambient post-harvest storage of mature red fruit is due to highly localized oxidative activity which is restricted to the epicarp and upper mesocarp. Using a nitrocellulose blotting technique adapted from Spruce et al. (1987), they maintained that both polyphenol oxidase and peroxidase were implicated in litchi rind browning, although the latter was only identified in the vascular traces of epicarp and mesocarp. Nonetheless, they suggested that peroxidase activity is of more importance than previously thought. Consequently, it is most likely that heat treatments resulted in disruption of cell and organelle membranes thus activating polyphenol oxidase and causing ubiquitous browning.

Despite the levels of polyphenol oxidase being lower during litchi fruit ripening than during fruit growth, the presence of the enzyme implies that provided the substrate is not lacking, it will still cause browning of the litchi rind albeit at a reduced rate. It is known that polyphenol oxidase which is localized in chloroplasts of healthy tissue, is only activated after degradation of these organelles (Vaughn and Duke, 1984a). During the latter stages of ripening and senescence, single unit membrane bound vacuoles, in which anthocyanins are localized, would presumably degrade before double unit membrane bound chloroplasts (Esau, 1977). Consequently, following degradation of vacuoles, oxidation of anthocyanins or colourless chromenols would be retarded. This may explain loss of red colour from the litchi rind during the initial stages of fruit storage at ambient temperatures, followed by subsequent browning of the epicarp and mesocarp tissues. Furthermore, because peroxidase activity is localized solely in the vascular traces of the epicarp and mesocarp tissues of the mature litchi rind, its role in physiological browning is dubious.

1.3.2.4 Control

Prevention or at least a reduction in post-harvest browning of the mature litchi rind may be achieved in one of two ways, viz. firstly, reduce or remove stress factors, and secondly, by interfering with the biochemical processes which result in browning. Initial stress on freshly harvested fruit may be reduced substantially with careful handling of fruit. Many different techniques have been examined in an attempt to achieve this. These include the adoption of forced air cooling to remove field heat rapidly (Bagshaw et al., 1991); hydro-cooling (Ketsa and Leelawatana, 1992); and heating of fruit to temperatures of between 30° and 70°C (Song and Kumar, 1996). Regular atmosphere refrigerated storage of fruit at temperatures ranging from 0°C (Huang and Wang, 1990), 1°C (Swarts, 1983), 2°C (Paull and Chen, 1987) and 5° to 7°C (Bagshaw et al., 1991; Jacobi et al., 1993) to reduce stress and prolong post-harvest life are recommended for different countries. In addition, surface coatings limiting moisture loss from individual fruit include polysaccharides and similar sucrose ester based coatings such as Semperfresh®, an antitranspirant (Duvenhage, 1993). These are preferred to polyethylene based waxes (pH greater than 7), which usually result in discolouration due to pH effect (Underhill, 1994).

Other methods which limit moisture loss from fruit include enclosing fruit in paper bags, polyethylene bags (Paull and Chen, 1987; Kremer-Köhne and Lonsdale, 1991) or plastic films (Campbell, 1959; Scott et al., 1982; Ahrens and Milne, 1993a); plastic liners in fibreboard cartons; punnets over-wrapped with plastic film (Bagshaw et al., 1991); and gas packaging (Lonsdale, 1993). Unfortunately, resulting condensation promotes unsightly fungal and bacterial growth, thus necessitating some form of chemical control. Furthermore, enzymes derived from fungi are thought to lead to pigment degradation (Nip, 1988; Lee and Wicker, 1991b) and it is thus imperative that fungal infection be controlled.

Chemical control using benomyl (1-(butyl carbamyl)-2-benzimidazole carbamic acid, methyl ester) has been shown to be very effective but a maximum residue of 10 $mg.kg^{-1}$ has been set for Australian conditions (Hargreaves, 1983). Scott et al. (1982) and Huang and Scott (1985) suggested a 0.05% benomyl dip at 52°C for 2 min. This recommendation was fine-tuned by Wong et al. (1991) who maintained that 52°C was too high for fruit of cultivars

'Tai So', 'Kwai May Pink' and 'Wai Chee'. Consequently, they recommended that the temperature be lowered to between 48° and 50°C. Furthermore they found that 'Tai So' ('Mauritius') was the least affected by the heat treatments. Bavistan® (methyl-2-benzimidazole carbamate) at 125 mg.ℓ^{-1} was also shown to give excellent protection of litchi fruit against fungal attack (Sandhu and Randhawa, 1992). In South Africa, Schutte et al. (1991) found that a room temperature dip treatment of benomyl (600 g.ℓ^{-1}) or Prochloraz® (250 g.ℓ^{-1}) proved effective for 4 weeks in preventing fungal attack of litchi fruit.

Many other selective and non-selective fungicides and bactericides have been investigated without success (Nip, 1988), but sulphur is a notable exception. Post-harvest sulphur dioxide (SO_2) fumigation of litchi fruit is used commercially in South Africa (Swarts, 1985; 1989), Israel (Zaubermann et al., 1990), Mauritius and Reunion (Menzel, 1990). Swarts (1985) found that sulphur dioxide fumigation in a closed container, achieved by burning 1 kg of chemically pure sulphur per 1600 kg of litchi fruit, provided adequate protection against fungal attack during storage of fruit. When dissolved in water, sulphur dioxide exists mainly as a mixture of the ionic species sulphite (SO_3^{2-}) and bisulphite (HSO_3^-) but the relative quantities of the ions depend on the pH of the solution. At pH 4.0 bisulphite is at its highest concentration (Green, 1976).

Duvenhage (1993) investigated the effects of a dip in sodium metabisulphite on its own, and in conjunction with a 5 min dip in 4% hydrochloric acid, followed by packaging with Semperfresh®, Vitafilm® and Freshpak® (Vitafilm® is a plastic product 13μm thick, while Freshpak® is a registered gas mix from Air Products South Africa). Although all treatments except sodium metabisulphite followed by Semperfresh® and the control led to less browning, levels of sulphur in the fruit as a result of sodium metabisulphite treatment were apparently not measured.

In addition to having fungicidal activity, sulphur reacts chemically with anthocyanin pigments (Jurd, 1972) and results in bleaching of litchi fruit rinds (Swarts, 1985; Zaubermann et al., 1991; Underhill et al., 1992b). Jurd (1964) showed beyond doubt, using spectrophotometry, that the flavylium cation of grape skins was the reactive species resulting in sulphite bleaching. Timberlake and Bridle (1967; 1968) furthered these studies and showed that

sulphite bleaching is an ionic reaction involving a nucleophilic attack by a negative ion of sulphurous acid on the flavylium cation to form what is probably chromen-4 (or -2) sulphonic acid (Fig. 8).

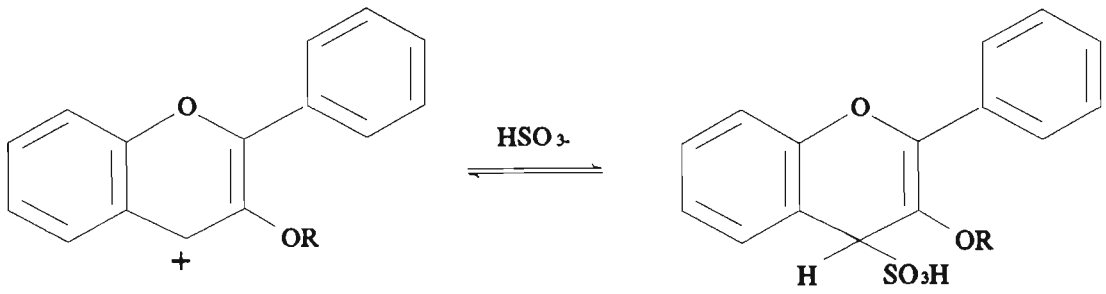

Figure 8. Ionic reaction resulting after sulphite bleaching (from Jurd, 1972)

Sulphured litchi rinds turn pale green or if fumigation was excessive, yellow. After treatment, rinds are pliable and do not split during handling. Rind colour is fixed and unlike untreated fruit, rinds do not turn brown during subsequent storage. Instead, rinds of pale green fruit subsequently turned a uniform pink colour after 3 to 5 days at room temperature of 22°C. Those fruit which were excessively fumigated remained yellow, although concentrations have been determined at which yellowing will not occur (Swarts, 1985).

$$[PPO] + HSO_3^- \text{-----} > [PPO]i\text{-}SO_3 < \text{-----} > [PPO]i + HSO_3^-$$

Figure 9. Proposed equilibrium reaction for inactivation of polyphenol oxidase by bisulphite. [PPO] stands for the initial concentration of the polyphenol oxidase, [PPO]i-SO_3^- for the possible complex formed between the inactivated polyphenol oxidase and HSO_3^-, and [PPO]i for the inactivated polyphenol oxidase. (adapted from Sayavedra-Soto and Montgomery, 1986)

In addition to fungicidal properties, Zauberman et al. (1991) showed that sulphur fumigation resulted in complete inhibition of litchi fruit polyphenol oxidase when compared to non-fumigated fruit. Mathew and Parpia (1971) stated that the exact mechanism of inhibition was

not fully understood. Sulphur dioxide could either be reducing oxygen and thus making it unavailable for oxidizing polyphenols, or reacting with quinones or other intermediates. Sayavedra-Soto and Montgomery (1986) however, found for polyphenol oxidase extracted from bananas, mushrooms and pears that at pH 5.0, levels of inactivation were correlated with concentrations of bisulphite, and that inhibition increased with time but had plateaued by 100 min. Where a solution of pH 6.0 was used, inhibition plateaued only after 24 hr. Consequently, they maintained that formation of a plateau and slow inactivation of the enzyme, coupled with electrophoretic investigations suggested an equilibrium (Fig. 9). Furthermore, inhibition of polyphenol oxidase activity by sulphite was complete and all attempts at regenerating activity were not successful.

Sulphur fumigation although partially successful and currently widely used commercially, does have drawbacks. Increased reliance on sulphur has led to ineffective control of post-harvest fungal decay (Swarts, 1989), and Botha et al. (1988) found that this was especially true for *Penicillium* spp.; fruit lose a considerable amount of moisture and thus mass (Kremer-Köhne and Lonsdale, 1991); overdosing results in unacceptable residues (Kremer-Köhne, 1993; Milne and Ahrens, 1993); there is a unpalatable aftertaste (Botha et al., 1988; Lonsdale and Kremer-Köhne, 1991); and residues constitute a health hazard in asthmatics (Koeing et al., 1983). Indeed, France has lowered the acceptable level of sulphur from 20 $mg.kg^{-1}$ to 10 $mg.kg^{-1}$ in litchi fruit flesh, and it can be expected that with greater environmental and health awareness in European markets, sulphur will be prohibited in the near future (Milne, 1996)[1].

Other treatments which interfere with the biochemical processes involved in litchi rind browning include the use of chemicals that are either oxidised in preference to polyphenol oxidase or complex with the enzyme; inhibition of polyphenol oxidase activity other than with sulphur dioxide; and manipulation of pH. Examples of these reactions were provided by Walker and Hulme (1965) who noted that polyvinylpyrrolidine competed with polyphenol oxidase, while Pifferi and Cultrera (1974) found that ascorbic acid was degraded *in vitro* in preference to anthocyanins. Early work by Akamine (1960) showed that dipping fresh litchi

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fruit into ascorbic acid (0.6 to 2.4 $g.\ell^{-1}$) had no effect on retaining red colour of the litchi rind. Bedrosian et al. (1959) showed that borates complexed with the oxidation site of polyphenol oxidase thus preventing apple browning. A temperature of ca 80°C for 10 min inhibited polyphenol oxidase of different fruit types. When subjected to boiling water (presumably 100°C) however, inhibition was complete by 3 min (Mathew and Parpia, 1971).

Manipulation of pH concerns both polyphenol oxidase, where low pH solutions have been shown to inhibit polyphenol oxidase activity (Mathew and Parpia, 1971; Zaubermann, 1991; Underhill et al., 1994), and anthocyanins and their corresponding anthocyanidins. Zauberman et al. (1990; 1991) found that extraction of sulphur-fixed anthocyanins from litchi rinds in acidified methanol resulted in a red solution. As a result of this finding, Zauberman et al. (1990; 1991), Underhill et al. (1992b, 1994) and Fuchs et al. (1993) all tried to acidify anthocyanins *in situ* on the day of harvest, by dipping whole fruit in dilute acid solutions between pH 0.0 and 0.5. Where fruit were dipped only in low pH solutions, rinds turned red but unsightly brown patches appeared several hours after treatment. Under similar conditions however, Underhill et al. (1992b) maintained that redder fruit were obtained when they were dipped in 1N HCl only, but a progressive colour loss was observed during storage. This was most likely due to an increase in the pH of the cell sap which accompanies moisture loss (Lukton et al., 1956). Under South African conditions, Ahrens and Milne (1993b) showed that after SO_2 fumigation at 600 $g.t^{-1}$, dipping of litchis in 4% HCl for periods of up to 5 min was very effective in regaining red rind colour. Phytotoxicity was observed when fruit were dipped for periods of 10 and 15 min. Consequently, a 2 min dip in 4% HCl was recommended after SO_2 fumigation at 600 $g.t^{-1}$.

1.4 DISCUSSION AND CONCLUSIONS

Anthocyanin pigments are responsible for rind colour of mature, red litchi fruit. These water-soluble, vacuole-bound plant pigments protect against ultra-violet light, act as antibiotics in plant defence responses, and serve as attractants to seed dispersal agents. Knowledge of the chemical structure and transformations of anthocyanins is imperative in understanding colour expression at harvest and subsequent post-harvest pigment breakdown. Attached to the flavylium salt, anthocyanins have a glycoside, which stabilizes natural anthocyanins. Should

the glycoside be hydrolysed, the resulting anthocyanidin will undergo a series of rapid chemical reactions resulting in either colourless compounds or brown phenolic pigments. Physiological browning of anthocyanins may also be affected by the enzyme polyphenol oxidase, which, in the presence of oxygen, causes the oxidation of anthocyanins into *ortho*-quinones.

Several mechanisms which stabilize anthocyanins have been identified in the pure chemical sciences. These include self-associations, inter- and intramolecular, and metal complexing. One such stabilizing mechanism is sulphur fumigation but sulphur is undesirable because of unpalatable aftertastes, unacceptable residues and health aspects. Furthermore, sulphur fumigation results in fruit with unsightly yellow rinds. Subsequent colouration of fruit rinds at room temperature, following sulphite bleaching, is probably the result of an equilibrium being attained between the flavylium cation and the chromen-n-sulphonic acid, a process in compliance with the disassociation of SO_3 from the pigment complex, proposed by Underhill et al. (1992a). Indeed, the fact that sulphite bleached litchis only attain a pinkish hue after an extended period of time, and that fruit dipped in low pH after sulphur fumigation, revert back to their original red colour (Zaubermann et al., 1990; 1991) is strong evidence for this.

Sulphur fumigation of litchi fruit is currently the only commercial post-harvest treatment, but the South African Litchi Growers' Association regards the elimination of sulphur fumigation as their number one research priority (Milne, 1996[2]). Consequently, alternatives to sulphur fumigation must be sought and the author hypothesizes that litchi rind anthocyanins can be fixed *in situ* using a low pH solution, while simultaneously avoiding the use of sulphur fumigation.

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[2] Dr D.L. Milne, *pers. comm.*, Chairman of South African Litchi Growers' Association Research Co-ordination Committee, Merensky Technological Services, Box 14 Duiwelskloof, Northern Province, South Africa

CHAPTER 2

RIND COLOUR RETENTION

2.1 INTRODUCTION

In South Africa, post-harvest treatment of litchi fruit has been restricted to sulphur-fumigation of the fruit at 600 g sulphur.t^{-1} for 30 min (Ahrens and Milne, 1993). Unfortunately, this results in bleached rinds as sulphur complexes with anthocyanin pigments. Sulphur does however, dissociate from pigments with time (Jurd, 1972) but the original red rind colour is never regained. Furthermore, sulphur results in an undesirable aftertaste, constitutes a health problem and is ineffective against some fungi. It is thus not surprising that some overseas markets such as France have imposed a residue limit of sulphur to 10 mg.kg^{-1} in the fruit flesh. Clearly, with greater consumer awareness sulphur-treated fruit will soon be unacceptable.

A promising alternative treatment reported on before initiation of this study was that of low pH dips following sulphur fumigation (Zaubermann et al., 1990, 1991; Underhill et al., 1992), where anthocyanin structure was manipulated to preserve red rind colour. These studies showed that immersion of litchi fruit in low pH solutions only, was ineffective in fixing rind colour. However, if fruit were immersed subsequent to sulphur fumigation, rind colour was a fixed red hue. Unfortunately, using this technology, sulphur fumigation was not avoided and sulphur residues remained a problem in the fruit flesh.

Prompted by these investigations, Kaiser (1994a) reviewed anthocyanin physiology and this led to the present hypothesis that sulphur fumigation can be avoided and rind anthocyanins fixed *in situ* without adversely affecting fruit flesh palatability. The author hypothesizes that sulphur fumigation effects solubilization of cell membranes and tonoplasts, thus allowing subsequent access to anthocyanins *in situ* using low pH solutions. Consequently, the aims of the investigations in this chapter were: firstly, to determine whether pH 0.0 is the optimal pH at which litchi rind anthocyanins are fixed; secondly, to identify alternative treatments which will achieve adequate membrane breakdown without affecting fruit flesh palatability;

thirdly, to quantify colour changes accurately, and fourthly to determine whether there were any differences in electrolyte leakage between control fruit and those subjected to heat.

2.2 pH DETERMINATION

2.2.1 Materials and Methods

Mature, red 'Three Months Red' fruit from the Northern Province were picked in the early morning in August 1993, placed in a coolbox and taken directly to the laboratory in Nelspruit, Mpumalanga Province. Since availability of these early season fruit was limiting, only four fruit were used per treatment. Fruit were immersed in distilled water, adjusted to a range of pH values from pH -1.0 and, pH 0.0 to 5.0 with intervals of 0.5 using 32% hydrochloric acid (HCl), for 2,3,4,5 and 10 min. Subsequently, fruit were left at room temperature together with untreated control fruit for 14 days, following which, differences in rind colour and texture were noted.

2.2.2 Results and Discussion

After 14 days at room temperature rinds of all fruit became thin and brittle. Rinds of untreated control fruit were brown, while fruit immersed for 2,3,4,5 and 10 min in distilled water, adjusted to pH 0.5 or more, were also brown after 14 days at room temperature. Likewise, fruit immersed for 2 min in zero pH solution, were also brown. Rinds of those fruit that were immersed for 3,4,5 and 10 min in zero pH solution, remained red for 14 days at room temperature, but had some unsightly brown marks and developed corky patches. Rinds of those fruit immersed for 2,3,4,5 and 10 min in distilled water, adjusted to pH -1.0, changed to mauve after 24 hr and retained this colour for 14 days. Furthermore, these fruit shrivelled after 14 days at room temperature. These results confirm that immersion of fruit in pH 0.0 for at least 2 min is necessary for fixation of litchi rind colour. Consequently, immersion of fruit for 4 min in pH 0.0 solution was considered optimal for fixation of litchi rind colour for all future trials.

2.3 MEMBRANE SOLUBILIZATION

2.3.1 Materials and Methods

Mature, red 'Three Months Red' fruit from the Northern Province were picked in the early morning in September 1993, placed in a coolbox and taken directly to the laboratory in Nelspruit, Mpumalanga Province. Four fruit were immersed in each of 3.5% NaOCl, 40% H_2O_2, 0.5% Triton X• 100 (a non-ionic detergent), 0.5% sodium dodecyl sulphate (SDS) (an anionic detergent) or 0.5% cetyldimethyl ammonium bromide (CTAB) (a cationic detergent) for periods of 5, 10, 15, 20 and 25 min. All fruit were then immersed for 5 min in 1% Safranine Fast Green, and subsequently rinsed in distilled water. Safranine Fast Green stains cytoplasmic contents, thus 100% staining of the fruit surface implies that all rind cell membranes are solubilized. Relative amounts of stain, expressed as a percentage of the total fruit rind surface area which took up stain were recorded for all the fruit and graphed over time (Fig. 10).

2.3.2 Results and Discussion

When fruit were immersed in 3.5% NaOCl or 40% H_2O_2 for up to 5 min, followed by immersion in 1% Safranine Fast Green for 5 min, rinds remained unstained. Some Safranine Fast Green was however, taken up by these fruit when immersed for up to 25 min in the same solutions, but no more than 12% of the total surface area of all fruit rinds was stained by Safranine Fast Green after 25 min immersion and a plateau seemed to have been reached after 15 min immersion in 3.5% NaOCl or 40% H_2O_2. When fruit were immersed in 0.5% Triton X• 100, 0.5% SDS and 0.5% CTAB for periods of 5, 10, 15, 20 and 25 min, followed by immersion in 1% Safranine Fast Green for 5 min, rinds in all three cases did not stain better when immersed for longer than 20 min. Fruit immersed in 0.5% Triton X• 100 for 20 min however, was most heavily stained, with slightly less than 50% of the entire surface area of all fruit rinds taking up Safranine Fast Green, when compared to ca 37% for SDS and ca 24% for CTAB respectively (Fig. 10).

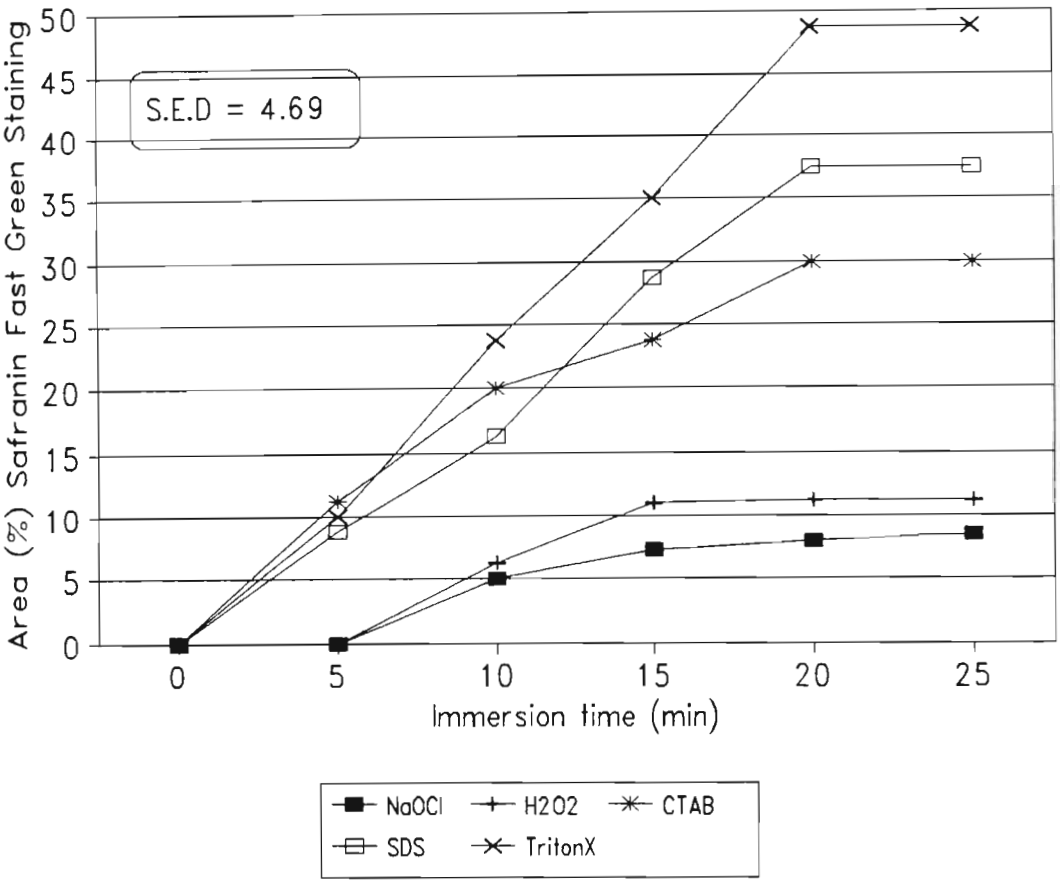


Figure 10. Area percentages for litchi rinds stained with 1% Safranine Fast Green after immersion in 3.5% NaOCl, 40% H_2O_2, 0.5% CTAB, 0.5% SDS and 0.5% TritonX100 for different lengths of time

Of all the potential membrane solubilizers investigated here, only the non-ionic detergent, Triton X® 100 showed any promise, as immersion for 20 min resulted in ca 50% area staining by Safranine Fast Green. When compared to the optimal 100% area staining, this coupled with the lengthy immersion time of 20 min deemed the treatment unsuitable. Consequently, the use of heat as a means of solubilizing rind cell membranes was investigated.

2.4 HEAT TREATMENTS

2.4.1 Materials and Methods

Mature, red 'Three Months Red' fruit from the Northern Province were picked in the early morning in October 1993, placed in a coolbox and taken directly to the laboratory in

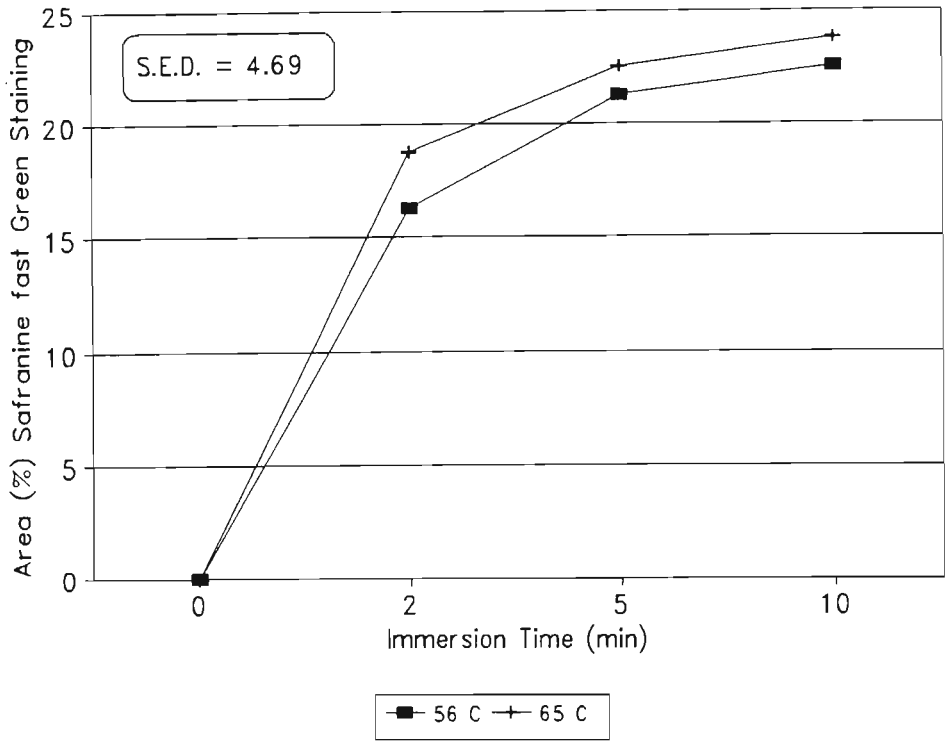


Figure 11. Area percentages for litchi rinds stained with 1% Safranine Fast Green after immersion in distilled water at 56° and 65°C for different lengths of time

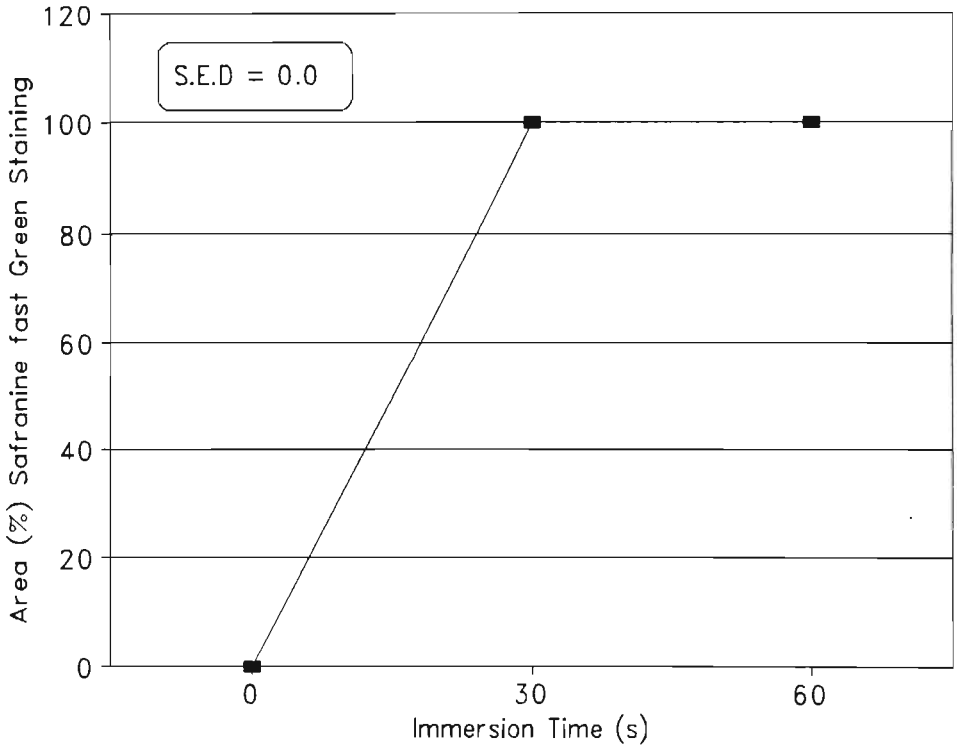


Figure 12. Area percentages for litchi rinds stained with 1% Safranine Fast Green after immersion in boiling water (98°C) for different lengths of time

Nelspruit, Mpumalanga Province. Four fruit were immersed in distilled water at 56°C or 65°C for 2, 5 or 10 min, and then for 5 min in 1% Safranine Fast Green or distilled water, adjusted to pH 0.0, ***hereafter referred to as zero pH solution***. Other fruit were immersed in boiling water (98°C) for 30 or 60 sec, and then for 5 min in 1% Safranine Fast Green or zero pH solution. Relative amounts of stain, again expressed as area percentage, taken up by those fruit dipped in 1% Safranine Fast Green were recorded and graphed over time (Figs 11 and 12), while the remaining fruit were halved, and together with untreated control fruit were either left at room temperature for 14 days or placed in regular atmosphere storage at 1°C, following which, differences in rind colour and texture were noted.

2.4.2 Results and Discussion

Rinds of control fruit were brown by 14 days at room temperature. Rinds of all fruit which were immersed in water at 56°C or 65°C, followed by immersion in 1% Safranine Fast Green for 5 min, took up little stain, as less than 25% of the rind surface area was stained by Safranine Fast Green (Fig. 11). When other fruit were immersed in distilled water at 56°C or 65°C followed by 4 min in zero pH solution, fruit rinds changed to a pinkish colour, but were brown after 14 days at room temperature. An explanation for this was provided by Underhill and Critchley (1993) who published an article shortly after completion of this experiment, and showed that temperatures of 60°C result in rind browning because of heat injury. Rinds of those fruit which were immersed in boiling water (98°C) for 30 or 60 s, became colourless and pliable. When this treatment was followed by 5 min in 1% Safranine Fast Green, entire fruit rinds were stained by Safranine Fast Green ie. 100% staining (Fig. 12). Subsequently, when fruit were immersed in boiling water (98°C) for 30 or 60 s, followed by 4 min in zero pH solution, rinds of both treatments became pliable and changed from colourless to an even red colour within 60 min. Rinds of fruit immersed for 60 s in boiling water (98°C) were however, redder. When these fruit were left at room temperature for 14 days, rinds remained red and pliable, but rinds of fruit immersed for 30 s in boiling water (98°C) developed corky patches. Rinds of fruit immersed for both 30 and 60 s in boiling water (98°C) followed by immersion in zero pH solution for 4 min, remained an attractive bright red colour after 28 days in regular atmosphere storage at 1°C. The results showed clearly that litchi rinds may thus be preserved an attractive red colour for 28 days

of regular atmosphere storage at 1°C however, the effects on palatability had not been investigated. Consequently, the effects of immersing fruit in boiling water for different lengths of time on internal taste and appearance were investigated.

2.5 INTERNAL QUALITY

2.5.1 Materials and Methods

Seven 2 kg boxes of untreated, mature, red 'Mauritius' fruit obtained from Malelane in December 1993 were placed in a coolbox and taken directly to the laboratory in Nelspruit, Mpumalanga Province. One 2 kg box each of fruit was immersed for 60, 30, 20 and 10 s in boiling water (98°C), followed by immersion in zero pH solution for 4 min. Another 2 kg box of fruit was immersed in boiling water (98°C) for 30 s only. These fruit together with untreated control fruit and sulphur-fumigated (600 g sulphur.t^{-1}) fruit were stored under regular atmosphere storage at 1°C and their taste and appearance, both internal and external, compared to those of untreated control fruit after after 28 days of storage.

2.5.1 Results and Discussion

Rinds of sulphur-fumigated fruit varied from light green, yellow to salmon after 28 days of storage at 1°C (Fig. 13), while those of untreated control fruit were brown and infected with fungi (Fig. 14). Rinds of all fruit immersed for 60, 30, 20 and 10 s in boiling water (98°C), and then for 4 min in zero pH solution were pliable and changed to an attractive red colour within 24 hr and there were no obvious differences in rind redness. Rinds of those fruit treated for 60 or 30 s remained red for 28 days in regular atmosphere storage at 1°C (cf. Fig. 15), whereas those immersed for 10 or 20 s in boiling water had faded by 28 days in regular atmosphere storage at 1°C. Furthermore, none of the fruit immersed in boiling water (98°C) were infected with fungi, which implies that boiling water (98°C) sterilizes the fruit surface. Consequently, an immersion time of 30 s in boiling water (98°C) prior to immersion of fruit in zero pH was regarded as optimal for rind colour preservation.

Fruit flesh of some of the fruit immersed for 60 s in boiling water (98°C) was stained pink after 28 days storage at 1°C, implying that this treatment was probably too severe in terms of membrane breakdown, probably allowing pigment leakage from the rind into the fruit flesh. Fruit flesh at the distal and proximal ends of those fruit which were immersed for 30 s in boiling water (98°C) irrespective of whether subsequently immersed in zero pH or not, remained white, although in the region of the pedicel the top few millimetres of fruit flesh was brown after 28 days in storage (Fig. 16). Browning of fruit flesh in the pedicel region may have been due to one of several reasons, viz. excessive heat transfer from the boiling water (98°C) causing instant death of the cells; excessive loss of moisture through the rind, resulting in cell desiccation; penetration of zero pH solution, causing cell desiccation; and polyphenol oxidase activity being stimulated in the fruit flesh. However, since immersion of fruit for 30 s boiling water (98°C) only resulted in browning of fruit flesh, this confirms that zero pH solution did not cause fruit flesh discolouration.

2.6 STEAM TREATMENTS

In view of the undesirable side-effects on fruit flesh resulting from boiling water (98°C) treatments, an alternative method of breaking down cell membranes had to be sought. The author hypothesized that steam would be an effective substitute for boiling water (98°C), based on the fact that the latent heat of steam is some three times that of boiling water (98°C) (Kane and Sternheim, 1988). Consequently, since steam is a vapour, intense energy will be localized only on the fruit surface provided treatment time is relatively short. The main objectives of this investigation was to preserve the red colour of fruit rinds without causing fruit flesh discolouration after 28 days storage at 1°C.

2.6.1 Materials and Methods

Mature, red 'Mauritius' litchi fruit were picked in the early morning in Malelane or Hazyview, Mpumalanga Province in December 1995, boxed and couriered to Pietermaritzburg, KwaZulu-Natal Province. Individual fruit from five boxes were held in a jet of steam (95°C), generated by a 1 kW element for 5,4,3,2 or 0 s followed by immersion for 4 min in the zero pH solution. In addition, individual fruit from another box were

Plate 1

Figure 13 Sulphur-treated (600 $g.t^{-1}$) 'Mauritius' litchi fruit after 28 days of regular atmosphere storage at 1°C

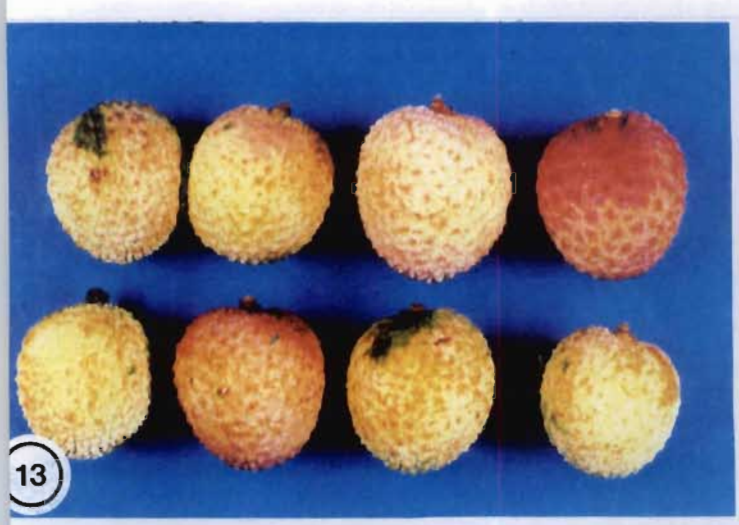
Figure 14 Untreated 'Mauritius' control fruit, after 28 days in regular atmosphere storage at 1°C

Figure 15 'Mauritius' fruit immersed in boiling water at (98°C) for 30 s followed by immersion in zero pH solution for 4 min, after 28 days in regular atmosphere storage at 1°C

Figure 16 Internal quality of 'Mauritius' fruit immersed in boiling water (98°C) for 30 s followed by immersion in zero pH solution for 4 min, after 28 days in regular atmosphere storage at 1°C

Figure 17 External appearance of 'Mauritius' litchi fruit treated with steam (95°C) for 2 s followed by 4 min immersion in zero pH solution, and subsequently dipped in 1% Vaporgard® solution, after 28 days of regular atmosphere storage at 1°C

Figure 18 Internal flesh of 'Mauritius' litchi fruit treated with steam (95°C) for 2 s followed by 4 min immersion in zero pH solution, after 28 days of regular atmosphere storage at 1°C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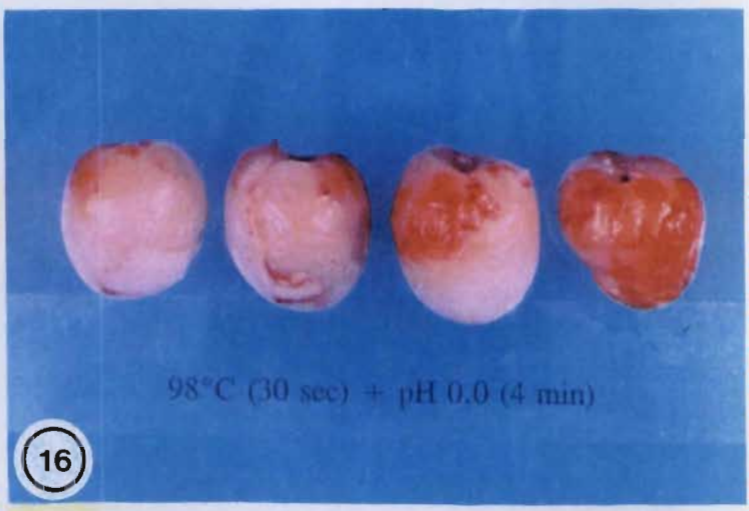

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subjected to 2 s steam (95°C), followed by immersion in zero pH for 4 min and then dipped in 1% Vaporgard• solution, a pinolene derivative, which acts as an antitranspirant, thus preventing potential moisture loss from the fruit. All these fruit together with a box each of sulphur-fumigated (600 $g.t^{-1}$) and untreated control fruit were stored at 1°C in regular atmospheric storage for 28 days, and the incidence of fungal infection was recorded. In addition, 20 fruits from each treatment were assessed by an independent panel of 10 people on a scale of 1 to 5 for taste, where 5 = good and 1 = poor; texture, where 5 = good and 1 = poor; juiciness, where 5 = juicy and 1 = juiceless; internal appearance, where 5 = clean white pulp and 1 = discoloured pulp; browning, where 5 = brown rinds and 1 = no browning of the rind; colour, where 5 = red rinds and 1 = brown or unsightly rinds; firmness, where 5 = firm fruit and 1 = spongy fruit. Frequency tables are calculated (Table 2) and data were analyzed using McCullagh's regression model for ordinal data (McCullagh & Nelder, 1989). After 28 days of storage, percentage total soluble solid (TSS) was measured for 20 fruits from each treatment using an OTAGO• hand-held refractometer and data were analyzed using a conventional General Linear Model.

2.6.2 Results and Discussion

Rind colour showed large differences between treatments, with steam treatments having high ratings and control and sulphur treatments low ratings (Table 2). In particular, McCullagh's model showed very strong effects of zero pH solution ($P<0.0001$) and when used in conjunction with steam, increased ratings markedly ($P<0.0001$). Vaporgard• however, when used in conjunction with 2 s steam (95°C), followed by 4 min in zero pH solution (Fig. 17) resulted in reddest fruit ($P<0.0001$). Where rind browning was concerned, there was an overwhelming difference between control fruit (with a 100% rating of 5) and other treatments (with a 98% rating of 1). Fruit firmness differed markedly, with controls and zero pH solution alone ($P<0.01$) scoring high ratings and in particular 4 and 5 s steam treatments scoring lower ratings ($P<0.0001$). Longer times in steam resulted in lower ratings ($P<0.0002$) (Table 2). Indeed, all fruit subjected to steam (95°C), with the exception of those subsequently dipped in 1% Vaporgard• solution were slightly shrivelled. Consequently, some form of protection is necessary for preventing shrivelling or desiccation of fruit following steam (95°C) treatments.

TABLE 2. Frequency tables for taste, texture, juiciness, internal appearance, incidence of browning, colour and fruit firmness of 'Mauritius' fruit after 28 days of regular atmosphere storage at 1°C. 0s 4m = 0 s steam (95°C) treatment followed by 4 min in zero pH solution; 5s 4m = 5 s steam (95°C) treatment followed by 4 min in zero pH solution; 4s 4m = 4 s steam (95°C) treatment followed by 4 min in zero pH solution; 3s 4m = 3 s steam (95°C) treatment followed by 4 min in zero pH solution; 2s 4m = 2 s steam (95°C) treatment followed by 4 min in zero pH solution; 2s 4m VG = 2 s steam (95°C) treatment followed by 4 min in zero pH solution and then dipped in 1% Vaporgard®. The ratings are on an ordinal scale, so strictly speaking one should not calculate a mean. Consequently the average is for summary purposes. The interpretation of the median is eg. Taste for 0s 4m "50% of the people rated the taste as 4 or better.

Taste

Score	1	2	3	4	5	Median	Average
Control	0	3	2	4	0	3	3.11
Sulphur	2	3	4	0	0	2	2.22
0s 4m	0	0	1	8	0	4	3.89
5s 4m	3	3	3	0	0	2	2.00
4s 4m	2	6	0	1	0	2	2.00
3s 4m	0	0	5	4	0	3	3.44
2s 4m	0	1	4	4	0	3	3.33
2s 4m VG	0	3	2	3	1	3	3.22

Texture

Score	1	2	3	4	5	Median	Average
Control	0	0	4	5	0	4	3.56
Sulphur	0	2	3	4	0	3	3.22
0s 4m	0	0	5	4	0	3	3.44
5s 4m	7	2	0	0	0	1	1.22
4s 4m	4	4	1	0	0	2	1.67
3s 4m	1	4	2	1	0	2	2.11
2s 4m	1	2	6	0	0	3	2.56
2s 4m VG	0	0	4	4	1	4	3.67

Juice

Score	1	2	3	4	5	Median	Average
Control	0	0	4	5	0	4	3.56
Sulphur	0	0	6	3	0	3	3.33
0s 4m	0	0	2	7	0	4	3.78
5s 4m	4	4	1	0	0	2	1.67
4s 4m	4	3	1	1	0	2	1.89
3s 4m	1	2	1	5	0	4	3.11
2s 4m	1	2	3	3	0	3	2.89
2s 4m VG	0	0	3	3	3	4	4.00

Internal Appearance

Score	1	2	3	4	5	Median	Average
Control	0	0	0	9	0	4	4.00
Sulphur	0	0	3	5	1	4	3.78
0s 4m	0	0	0	9	0	4	4.00
5s 4m	6	2	1	0	0	1	1.44
4s 4m	3	2	2	2	0	2	2.33
3s 4m	2	1	3	2	1	3	2.89
2s 4m	2	2	1	3	1	3	2.89
2s 4m VG	0	1	4	4	0	3	3.33

Browning

Score	1	2	3	4	5	Median	Average
Control	0	0	0	0	20	5	5.00
Sulphur	20	0	0	0	0	1	1.00
0s 4m	19	1	0	0	0	1	1.05
5s 4m	20	0	0	0	0	1	1.00
4s 4m	20	0	0	0	0	1	1.00
3s 4m	20	0	0	0	0	1	1.00
2s 4m	18	2	0	0	0	1	1.10
2s 4m VG	20	0	0	0	0	1	1.00

Colour

Score	1	2	3	4	5	Median	Average
Control	20	0	0	0	0	1	1.00
Sulphur	19	1	0	0	0	1	1.05
0s 4m	5	15	0	0	0	2	1.75
5s 4m	0	0	6	14	0	4	3.70
4s 4m	0	0	3	17	0	4	3.85
3s 4m	0	0	3	17	0	4	3.85
2s 4m	0	2	8	10	0	4	3.40
2s 4m VG	0	0	0	20	0	4	4.00

Firmness

Score	1	2	3	4	5	Median	Average
Control	0	0	0	0	20	5	5.00
Sulphur	0	0	6	13	1	4	3.75
0s 4m	0	0	0	16	4	4	4.20
5s 4m	0	6	14	0	0	3	2.70
4s 4m	0	0	20	0	0	3	3.00
3s 4m	0	0	19	1	0	3	3.05
2s 4m	0	1	13	6	0	3	3.25
2s 4m VG	0	0	7	13	0	4	3.65

After 28 days of storage, flesh of 4 and 5 s steam-treated fruit were brown internally, and McCullagh's model showed overwhelming evidence of a steam effect ($P<0.0001$). Meanwhile flesh of control and sulphur-treated fruit and those treated for 3 or 2 s with steam (Fig. 18), were unblemished after 28 days of storage. There was overwhelming evidence of effects of taste ratings of zero pH solution ($P<0.01$) and steam ($P<0.0001$) and some evidence of a sulphur effect which caused a low rating ($P<0.06$). Using zero pH solution led to an improved rating, while increased time in steam led to a lower rating. Furthermore, addition of Vaporgard• to steam-treated fruit improved taste ratings over sulphured fruit ($P<0.0001$). Texture was also affected by steam, with longer steam treatments resulting in lower ratings ($P<0.0001$). Addition of Vaporgard• however, significantly improved texture over those of steam-treated fruit alone ($P<0.001$). There was no evidence of any effect on flesh texture of fruit treated with sulphur or zero pH solution only. Juiciness was affected by 4 and 5 s steam treatments, which had lowest ratings ($P<0.004$) and addition of Vaporgard• significantly improved juiciness over steam and zero pH solution only ($P<0.001$) (Table 2).

TABLE 3. Average TSS percentages for 20 'Mauritius' fruit from each of 9 treatments after 28 days of storage at 1°C

TREATMENT	TSS	SED
Control	19.49	1.02
Sulphur	19.75	1.09
5 s steam + Zero pH solution	27.42	4.26
4 s steam + Zero pH solution	28.18	3.31
3 s steam + Zero pH solution	26.46	2.87
2 s steam + Zero pH solution	22.45	1.57
2 s steam + Zero pH solution + Vaporgard•	18.27	0.79

Very large treatment differences ($P<0.0001$) in % TSS in the fruit flesh could be broken down into a zero pH solution effect ($P<0.0001$) and a linear steam trend ($P<0.0001$). TSS levels increased from ca 22% to 28% with increased time in steam, while there was no evidence of any difference between control and sulphur treated fruit, which had low TSS

percentages of about 19% (Table 3). It is possible that longer times in steam resulted in increased rind membrane breakdown resulting in increased water loss, thus concentrating TSS levels. Circumstantial evidence for this can be seen as TSS percentages of fruit treated with Vaporgard• were not significantly different from control or sulphur-treated fruit but were significantly different from other steam-treated fruit (Table 3).

After 28 days of storage neither sulphur-fumigated (600 $g.t^{-1}$) fruit nor fruit subjected to steam (95°C) for any length of time were infected with fungi. However, untreated control fruit and those immersed in zero pH for 4 min only were completely infected by fungi (Fig. 19). This implies that steam (95°C) sterilized the fruit and that no reinfection occurred under the storage conditions of the trial, thus eliminating the need for using sulphur fumigation as a means of disease control.

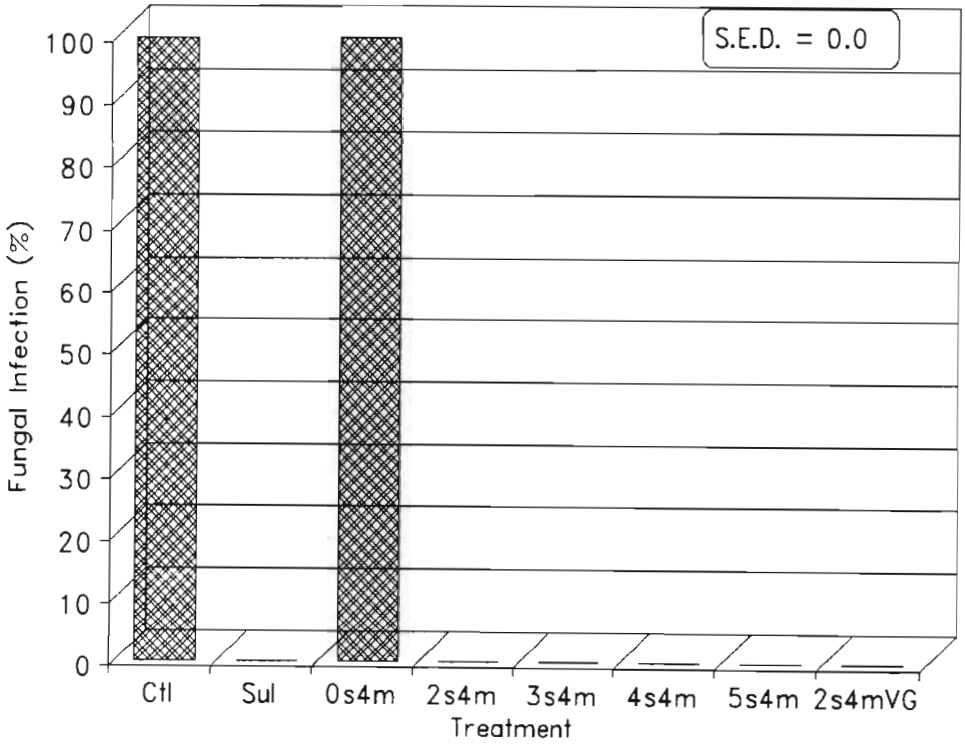

Figure 19 Percentage fungal infection of untreated control fruit (Ctl), sulphur treated fruit (Sul), fruit subjected to 0, 2, 3, 4 and 5 s steam (95°C) followed by 4 min in zero pH solution (0s4m, 2s4m, 3s4m, 4s4m and 5s4m respectively) and 2 s steam (95°C) followed by 4 min in zero pH solution and then dipped in 1% Vaporgard• solution

The results of this study showed clearly that rinds of fruit subjected to 2 s steam (95°) followed by immersion in zero pH solution and a subsequent dip in 1% Vaporgard• solution, resulted in excellent quality fruit after 28 days of regular atmosphere storage at 1°C. These fruit were firm, had red rinds, no external browning, were juicy, with good texture and taste and showed no signs of flesh discolouration when compared to sulphur-treated and control fruit. Furthermore, 2 s steam (95°C) sterilized the fruit of pathogens and no reinfection occurred under the storage conditions of the trial.

Questions that arise as a result of this study are firstly, how was colour affected by temperature and low pH; secondly, can membrane breakdown be quantified and thirdly, did steam cause inactivation of polyphenol oxidase enzyme. The aims of the succeeding investigations were to quantify rind colour and membrane breakdown, and to determine the effects of temperature and low pH on polyphenol oxidase activity.

2.7 COLOUR QUANTIFICATION

2.7.1 Introduction

Colour perception is extremely important in terms of consumer preference. Preconceived ideas of colour strongly influence product choice but in most cases this is subjective. Easily computable, quantitative measures may be obtained using a colorimeter. For each sample, these instruments generate a set of Cartesian co-ordinates, which pinpoint measured colour in a three-dimensional colour space. The Minolta• Chromameter CR-200 generates Commission Internationale de l'Eclairage (L^*, a^*, b^*) values, abbreviated to CIELAB, where the lightness coefficient, L^*, ranges from black = 0 to white = 100 (van Eck and Franken, 1994). Co-ordinates a^* and b^* locate colour on a rectangular co-ordinate grid, perpendicular to the L^* axis. At the origin ($a^* = 0$ and $b^* = 0$) colour is achromatic (grey). On the vertical axis, positive b^* measures yellow, and negative b^*, blue. On the horizontal axis, positive a^* measures red-purple, and negative a^* bluish-green (Fig. 20) (McGuire, 1992).

Lightness, L^*, of a colour is correctly reported without further manipulation. In contrast, a^* and b^* are merely co-ordinates and an indirect measure of chroma (degree of departure of a colour from grey towards pure chromatic colour) and hue (spectral colours) (McGuire,

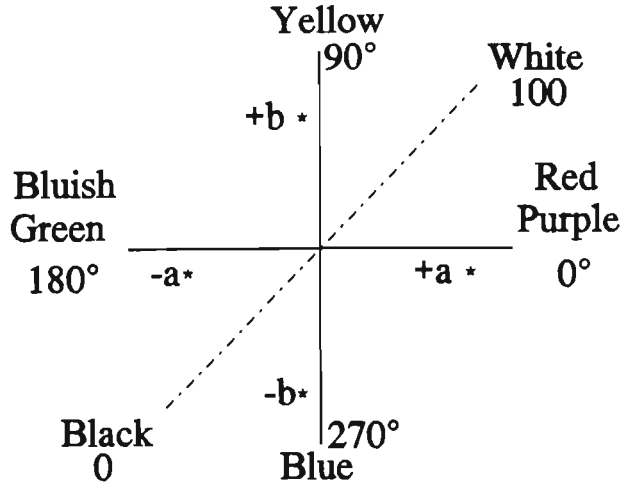

Figure 20 CIELAB colour space diagram. L^* indicates lightness of a colour, a^* indicates red:green colour ratio, and b^* indicates yellow:blue ratio (adapted from McGuire, 1992, and van Eck and Franken, 1994)

1992). Furthermore, a^* and b^* are not independent variables (Francis, 1980), and McGuire (1992) gave a case study proving that independent statistical analysis of these coordinates failed to detect a real change in postharvest colour of grapefruit. Consequently, hue angle and Chroma are better colour indicators. Hue angle (H°), defined as the angle between the hypotenuse and 0° on the a^* axis, is calculated from **ARCTANGENT (b^*/a^*)** and represents spectral colours (Fig. 21). Chroma (C^*), representing the hypotenuse of a right angled triangle created by joining points (0,0), (a^*,b^*) and (a^*,0), is calculated as $((a^*)^2 + (b^*)^2)^{1/2}$.

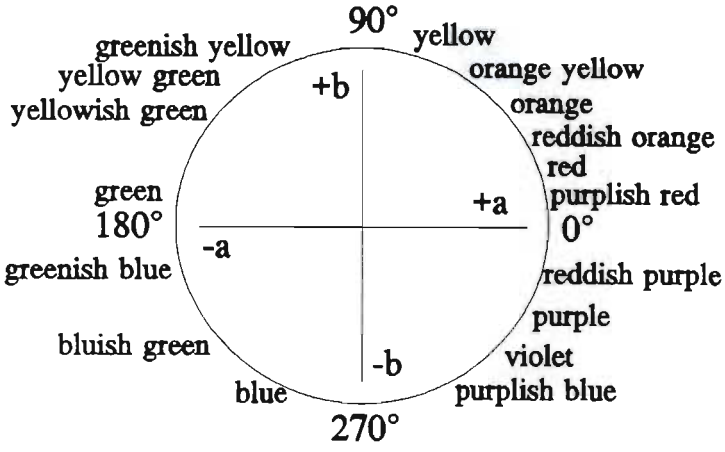

Figure 21 Hue sequence and hue-angle orientation on a CIELAB diagram (Voss, 1992)

The aim of this investigation was to quantify rind colour of 'Mauritius' litchi fruit on the day of harvest and again after storage at 30°C for specific times, and to compare this to fruit immersed for 4 min in zero pH solution only, fruit subjected to 2 s steam (95°C) only, and fruit subjected to 2 s steam (95°C) followed by 4 min immersion in zero pH.

2.7.2 Materials and Methods

Mature, red 'Mauritius' litchi fruit were obtained from Tzaneen, Northern Province in January 1996. On the day of harvest, chromameter readings were taken using a Minolta* Chromameter CR-200, for twenty untreated fruit from a box of control fruit, and again after storage at 30°C for 24 and for 48 hr. All other fruit were treated on the day of harvest. Firstly, 20 fruit were dipped in zero pH solution only for 4 min and then stored at 30°C for 24 hr after which chromameter readings were taken. Another 20 fruit were subjected to 2 s steam (95°C) only and chromameter readings were taken of these fruit immediately, and then again after storage at 30°C for 24 hr. A further 20 fruit were subjected to 2 s steam (95°C) followed by immersion in zero pH solution for 4 min. Chromameter readings were taken for these fruit after 0, 4, 8, 24, 48 and 72 hr. Chromameter readings a^* and b^* were transformed and an analysis of variance for lightness (L), hue (H°) and chroma (C^*) was performed. Averages and s.e.d.'s for all treatments are presented in Table 4.

2.7.3 Results and Discussion

All fruit were stored at 30°C since rind colour of red control fruit is known to degrade rapidly, changing to brown within 24 hr at this temperature (Underhill and Critchley, 1994). Analysis of variance showed highly significant treatment differences for lightness (L^*) ($F < 0.001$). As expected, rinds of untreated control fruit lightened with time (cf. Treatments 1 and 3), although rinds of control fruit were not significantly lighter after 24 hr (Treatment 2). Furthermore, after 24 hr, rinds of fruit immersed for 4 min in zero pH solution only (Treatment 4) were not significantly darker than those of control fruit (Treatment 2). Rinds of fruit subjected to 2 s steam (95°C) only, also lightened with time (cf. Treatments 5 and 6). Rinds of fruit subjected to 2 s steam (95°C) followed by 4 min in zero pH solution (Treatments 7 to 12) were lighter after 24 hr (Treatment 10), but subsequently darkened with

time until there were no significant differences in lightness between fruit treated on the day of harvest (Treatment 7) and those stored at 30°C for 72 hr (Treatment 12).

TABLE 4 Lightness (L^*), Hue (H°) and Chroma (C^*) for untreated 'Mauritius' litchi fruit and others subjected to zero pH solution, steam (95°C), and steam (95°C) followed by zero pH solution, and held at 30°C for different lengths of time (eg. *treatment 8 = 2 s 4 min 4 hr* represents chromameter readings taken after 4 hr at 30°C for fruit subjected to 2 s steam (95°C) followed by 4 min in zero pH solution). Letters "a" to "h" represent statistical significance at the 5% confidence interval

Number	Treatment	Lightness (L^*)	Hue (H°)	Chroma (C^*)
1	0 s 0 min 0 hr	36.09 e	30.43 d	35.72 e
2	0 s 0 min 24 hr	34.29 f	48.55 b	22.26 h
3	0 s 0 min 48 hr	42.87 a	60.59 a	27.74 g
4	0 s 4 min 24 hr	35.70 e	27.09 f	44.69 b
5	2 s 0 min 0 hr	35.49 e	31.91 c	33.19 f
6	2 s 0 min 24 hr	42.48 a	61.73 a	23.05 h
7	2 s 4 min 0 hr	37.69 d	30.34 d	48.01 a
8	2 s 4 min 4 hr	40.00 bc	27.48 ef	41.43 d
9	2 s 4 min 8 hr	39.36 c	26.30 f	47.38 a
10	2 s 4 min 24 hr	41.07 b	28.74 e	43.15 c
11	2 s 4 min 48 hr	39.60 c	27.48 ef	41.43 d
12	2 s 4 min 72 hr	38.05 d	28.03 e	45.35 b
	S.E.D.	1.08	1.47	1.12

There were also great treatment differences in hue angles (H°) of all fruit rinds ($F < 0.001$). According to the CIELAB diagram for hue sequence and hue-angle orientation (Fig. 21), rinds of control fruit changed from reddish orange (Treatment 1) to orange yellow (Treatment 2) after 24 hr and then to yellow (Treatment 3) after a further 24 hr. Rinds of fruit immersed for 4 min in zero pH solution only (Treatment 4) were red to reddish orange after 24 hr. Rinds of fruit subjected to 2 s steam (95°C) only, were reddish orange immediately after

treatment (Treatment 5) but changed to yellow within 24 hr (Treatment 6). In contrast, rinds of fruit treated with 2 s steam (95°C) followed by 4 min in zero pH solution were reddish orange immediately after treatment (Treatment 7), but this colour changed to a deep red within 4 hr (Treatment 8) and remained unchanged for up to 72 hr after treatment (Treatments 9 to 12). Interestingly, only fruit subjected to 2 s steam (95°) followed by 4 min in zero pH solution, after 4 hr (Treatments 8 to 12) were red, as opposed to the reddish orange rinds of control fruit on the day of harvest *proving that 2 s steam (95°C) followed by 4 min immersion in zero pH solution actually enhances red colour of litchi fruit (cf. Treatments 7 to 12).*

Chroma (C^*) of all fruit rinds also showed great treatment differences ($F < 0.001$). Colour intensity or chroma of control fruit rinds faded after 24 hr (cf. Treatments 1 and 2) and still further after 48 hr (cf. Treatments 2 and 3). Rinds of fruit immersed in zero pH solution for 4 min only were darker than control fruit after 24 hr (cf. Treatment 2 and 4), while chroma of fruit rinds subjected to 2 s steam (95°C) only, decreased substantially immediately after treatment to 24 hr later (cf Treatments 5 and 6). Chroma of all fruit rinds treated with 2 s steam (95°C) followed by 4 min in zero pH solution (Treatments 7 to 12) were relatively pure, when compared to those of control fruit rinds on the day of harvest (Treatment 1).

The results of this investigation showed clearly that rinds of fruit subjected to 2 s steam (95°C) followed by 4 min in zero pH solution were preserved a red colour and that this colour intensified with time after harvest. In contrast, rinds of control fruit, fruit subjected to 2 s steam (95°C) only, or fruit immersed for 4 min in zero pH solution all darkened, were less intense and changed to brown after 24 hr storage at 30°C.

2.8 ELECTROLYTE LEAKAGE

2.8.1 Introduction

Electrolyte leakage of cells may be empirically determined by immersing whole fruits in distilled water and recording the electrical conductivity after a set time. Fruit rinds are then autoclaved and electrical conductivity of the final autoclaved solution is again recorded. Electrolyte leakage after the set time is expressed as a percentage of the total electrical

conductivity (Tongdee, 1995[3]). The objectives of this study were to determine whether there were any differences in electrolyte leakage between control fruit and fruit treated with water at 56°C or 65°C, boiling water (98°C) or steam (95°C), thus providing accurate quantification of membrane breakdown.

2.8.2 Materials and Methods

Five 2 kg boxes of mature, red 'Mauritius' litchi fruit were obtained from Tzaneen, Northern Province in January 1996. On the day of harvest, fruit from four of the boxes were treated as follows: (1) immersed for 30 s in distilled water (56°C) followed by 4 min immersion in zero pH solution; (2) immersed for 30 s in distilled water (65°C) followed by 4 min immersion in zero pH solution; (3) immersed for 30 s in boiling water (98°C) followed by 4 min immersion in zero pH solution; (4) subjected to 2 s steam (98°C) followed by 4 min immersion in zero pH solution. Immediately after treatment, batches of 10 fruit from each box, together with untreated control fruit, were immersed in separate beakers of distilled water. Electrical conductivity of the different solutions was recorded after 5 min and again after 65 min immersion using a HANNA HI® 8733 portable conductivity meter. Fruit were then removed from the beakers and peeled. Rinds were returned to respective beakers and autoclaved using a HIRAYAMA® HA 3D autoclaver. Electrical conductivity was again recorded and electrolyte leakage was calculated by expressing electrical conductivity at 5 min and 65 min as a percentage of the total electrical conductivity after autoclaving.

2.8.3 Results and Discussion

Rinds of untreated control fruit had the lowest electrolyte leakage (ca 12%) and this remained unchanged after 1 hr immersion in distilled water. Electrolyte leakage of fruit rinds immersed in distilled water at 56° or 65°C, boiling water (98°C) and steam (95°C) were all significantly greater than those of control fruit 5 min after treatment (ca 16 to 18%), and increased to between 17 and 19% by 65 min immersion in distilled water. Both 5 min and

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65 min after treatment, electrolyte leakage of boiling water (98°C) and steam (95°C), although greater on average than the distilled water treatment (56° and 65°C) (between 17 and 19% compared to 16 to 18% respectively) was not significantly different (Fig. 22).

Clearly, all the heat treatments investigated here, viz. immersion for 30 s in distilled water at 56°C, 65°C and boiling water (98°C), and 2 s of steam (95°C) were equally effective in causing membrane breakdown in litchi rinds, as electrolyte leakage was significantly higher in these fruit than control fruit, both 5 min and 65 min after treatment. However, immersion for 30 s in distilled water at 56° and 65°C was ineffective when coupled with 4 min immersion in zero pH solution. Here, the work of Underhill and Critchley (1993) provides and explanation for this observation as they showed that temperatures of 60°C resulted in rind browning because of heat injury.

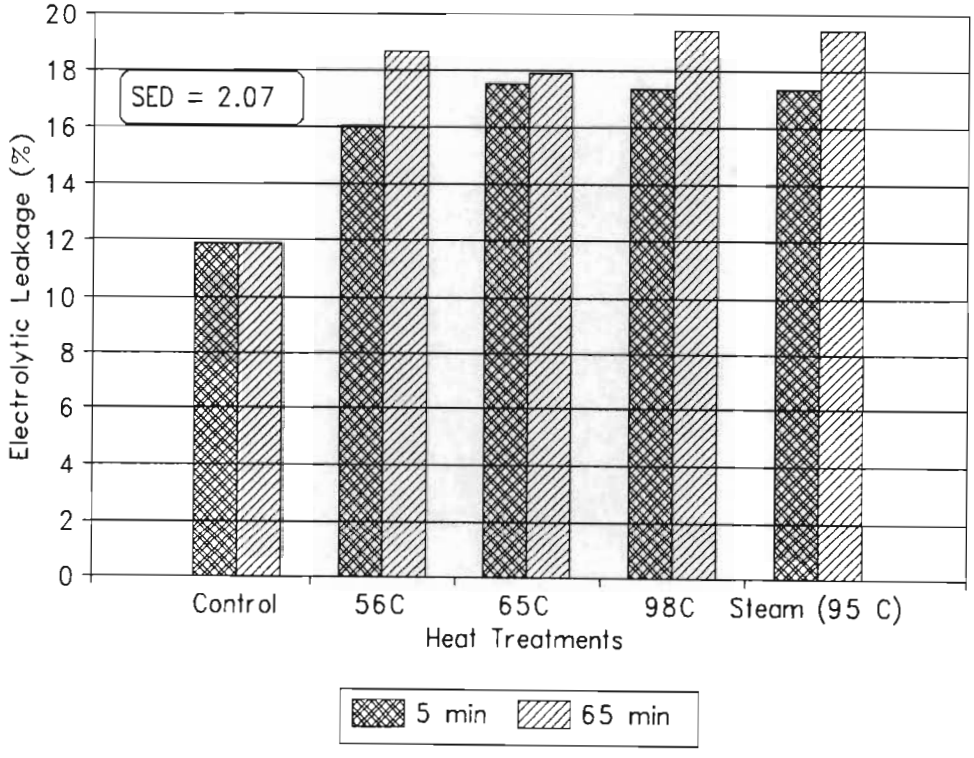


Figure 22 Electrolyte leakage (%) of 'Mauritius' litchi fruit rinds 5 min and 65 min after 30 s immersion in distilled water at 56° or 65°C, boiling water (98°C), or after 2 s steam (95°C) compared to untreated control fruit

2.9 POLYPHENOL OXIDASE ACTIVITY

2.9.1 Introduction

According to Underhill (1989), moisture loss from the litchi pericarp under ambient conditions results in cell plasmolysis and eventually cell death. Initially, desiccation led to an increase in vacuole pH, as well as changes in membrane integrity and subsequent release of polyphenol oxidases (specifically catechol-oxidase in the case of fruit according to Mayer and Harel (1981)), peroxidase and other enzymes in their active forms. Polyphenol oxidase has long been known to cause physiological browning (Joslyn and Ponting, 1951), and Huang et al. (1990) showed that polyphenol oxidase is the main enzyme involved in anthocyanidin breakdown in litchi rinds during storage. In healthy cells of plant tissue, polyphenol oxidase is a plastid bound enzyme (Vaughn and Duke, 1981; 1988; Bar-Nun and Mayer, 1983), and is only activated once it crosses the plastid envelope (Vaughn and Duke, 1984).

Underhill and Critchley (1992) found that rind polyphenol oxidase activity increased during the first 48 days after anthesis but declined to very low levels with subsequent seed maturation and fruit flesh expansion only to undergo a slight increase again with fruit maturity. They maintained that these low levels suggested a reduced capacity to induce tissue browning. However, Mayer and Harel (1981) maintained that previous studies on many different fruit types have shown that levels of polyphenol oxidase are highest during the early stages of development, but drop quite significantly after fruit maturity and during ripening. They suggested that the decrease in activity during fruit development, implied not only the cessation of enzyme synthesis but also the inactivation, inhibition or decomposition of the enzyme. The primary role of polyphenol oxidase is obviously during the initial stages of fruit growth, and is largely residual in ripe fruit where it causes unsightly browning.

Akamine (1960) suggested that polyphenol oxidase was involved in post-harvest browning of the litchi rind and was associated with fruit desiccation. Subsequently, Tan (1989) also maintained that browning of the litchi rind was due to enzymatic browning caused by polyphenol oxidase. Lin et al. (1988) showed that polyphenol oxidase activity in the litchi rind increased rapidly after harvest and peaked after 48 hr. In contrast, Zaubermann et al. (1990) observed little change in polyphenol oxidase activity during ambient storage of litchi

fruit. Later, Underhill and Critchley (1993) found that polyphenol oxidase activity in the litchi rind decreased rapidly after harvest, with a 3-fold reduction in activity within the first 24 hr after harvest. Subsequently, activity remained fairly constant but decreased slightly over time. Coupled with these results, Underhill (1992) and Underhill et al. (1992a) maintained that anthocyanin concentrations decreased slowly after harvest, and that it was unlikely that degradation of anthocyanins by polyphenol oxidase plays a major role in litchi post-harvest browning under ambient conditions. However, polyphenol oxidase is known to be limited by latency and substrate availability (Golbeck and Cammarata, 1981). Consequently, it seems most likely that polyphenol oxidase is important during the latter stages of post-harvest life. Furthermore, even at low concentrations, its presence indicates that it is still capable of causing browning reactions provided substrate is not lacking.

Bagshaw et al. (1991) suggested that browning could be prevented by either reducing stress or interfering with the biochemical processes involved. Both high (Song and Kumar, 1996) and low temperatures (Paull and Chen, 1987; Huang and Wang, 1990) have been shown to decrease browning, but Underhill and Critchley (1993) found that polyphenol oxidase activity was relatively high in fruit treated for 10 min at 60°C. In view of the above inconsistencies, the aim of this investigation was to determine the effects of post-harvest heat and low pH treatments on polyphenol oxidase activity in litchi rinds.

2.9.2 Materials and Methods

Four 2 kg boxes of mature, red 'Mauritius' litchi fruit were obtained from Malelane, Mpumalanga Province in January 1996. On the day of harvest, fruit from three of the boxes were treated as follows: (1) sulphur-fumigated (600 $g.ton^{-1}$ fruit); (2) subjected to 2 s steam (98°C) only and (3) subjected to 2 s steam (98°C) followed by 4 min immersion in zero pH solution. Entire rinds of eight fruit each of untreated, red 'Mauritius' litchi fruit, and fruit subjected to 2 s steam (95°C) followed by 4 min in zero pH solution and stored at 30°C, were sampled daily up to 5 days after harvest. Similarly, rinds of eight fruit each of sulphur-fumigated and fruit subjected to 2 s steam (95°C) only were sampled on the day of harvest and 24 hr after harvest. The technique was adapted from Golan et al. (1977) and Underhill and Critchley (1995), and conditions optimised for litchi rind polyphenol oxidase activity as

follows:- individual rinds were cryo-frozen using liquid nitrogen, and 1 g samples homogenized in 0.02M sodium phosphate buffer (pH 6.5) for 1 min using an ULTRA TURRAX•. The homogenate was extracted on ice for 1 hr and then centrifuged at 3000 r.p.m. for 15 min in a BHG HERMLE Z510• centrifuge. A 100 $\mu\ell$ aliquot of supernatant was added to 2 mℓ of 0.02M methyl catechol (pH 6.5) and absorbance read at 410 ηm over 30 s using a BECKMAN DU-65• spectrophotometer. Polyphenol oxidase activity (units.g^{-1} fresh mass) of the different treatments was determined based on the initial linear phase of colour change or reaction velocity, compared against a methyl catechol oxidase (Sigma• tyrosinase T-7755) standard curve, and plotted over time (Fig. 23).

2.9.3 Results and Discussion

Contrary to expectations, polyphenol oxidase activity was significantly highest in sulphur-fumigated fruit (ca 600 units.g^{-1} fresh mass) on the day of harvest and was still high (ca 580 units.g^{-1} fresh mass) at 30°C 24 hr after fumigation. Control fruit polyphenol oxidase activity at 30°C was also relatively high on the day of harvest (ca 390 units.g^{-1} fresh mass), increased significantly three days after harvest (ca 520 units.g^{-1} fresh mass) and reached ca 700 units.g^{-1} fresh mass 5 days after harvest at 30°C.

In contrast, rinds of fruit subjected to 2 s steam (95°C) only on the day of harvest, had significantly lower polyphenol oxidase activity (ca 200 units.g^{-1} fresh mass) than control fruit (ca 390 units.g^{-1} fresh mass). However, rinds of fruit subjected to 2 s steam (95°C) followed by 4 min in zero pH solution had significantly lowest polyphenol oxidase activity (ca 50 units.g^{-1} fresh mass). This implies that 2 s steam (95°C) resulted in decreased polyphenol oxidase activity but that in conjunction with 4 min of zero pH solution, activity was further reduced. Polyphenol oxidase activity in rinds of fruit subjected to 2 s steam (95°C) only, increased more than control fruit 24 hr after treatment (ca 510 compared to 320 units.g^{-1} fresh mass respectively), whereas polyphenol oxidase activity remained below 80 units.g^{-1} fresh mass up to 5 days after treatment at 30°C.

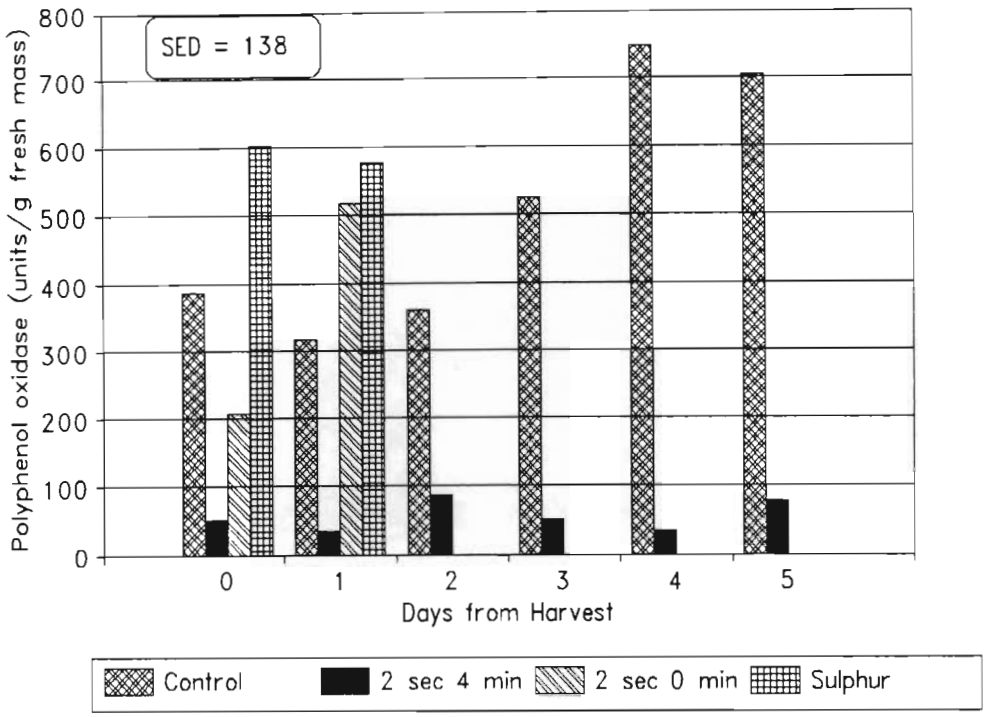


Figure 23 Polyphenol oxidase activity (units.g^{-1} fresh mass) in rinds of control fruit, sulphur-fumigated fruit, fruit subjected to 2 s steam (95°C) only, and fruit subjected to 2 s steam (95°C) followed by 4 min in zero pH solution 5 days after harvest

Finally, even though 2 s steam (95°C) followed by 4 min in zero pH solution significantly reduced polyphenol oxidase activity in litchi fruit rinds when compared to control fruit, and fruit subjected to 2 s steam (95°C) only, activity was still detectable at about 80 units.g^{-1} fresh mass. Consequently, it appears that physiological browning caused by polyphenol oxidase may be significantly inhibited by a 2 s steam (95°C) treatment followed by 4 min in zero pH solution, where low pH is known to inhibit enzyme activity (Underhill et al., 1992a; b).

CHAPTER 3

ELECTRON MICROSCOPY

3.1 RIND ULTRASTRUCTURE

3.1.1 Introduction

Kaiser (1994b) found on the day of harvest, that retention of pericarp (rind) colour of otherwise untreated 'Mauritius' litchi fruit was facilitated by dipping in a pH solution of 0.0 or less, and then only after immersion for a minimum of 2 min. He hypothesized that zero pH solution, being a relatively strong acid, was probably solubilizing pericarp cell membranes thus allowing anthocyanins to come into direct contact with zero pH solution (made by adjusting pH of distilled water to 0.0 using 32% hydrochloric acid). Subsequently, in an effort to achieve adequate membrane breakdown of pericarp cells, he tried diverse treatments including 3.5% NaOCl, 40% H_2O_2, hot water at 56°C and 65°C, and cationic (0.5% CTAB), anionic (0.5% SDS) and non-ionic (0.5% Triton X® 100) detergents. He found that immersion of fruit for 30 s in boiling water (98°C) resulted in excellent cytoplasmic staining of pericarp cells after 5 min immersion in 1% Safranine Fast Green. To reduce the unsightly flesh browning resulting from this treatment, boiling water was replaced by a 2 s exposure to steam (95°C), and this also resulted in excellent cytoplasmic staining following 5 min immersion of fruit in 1% Safranine Fast Green. However, staining of rinds by 1% Safranin Fast Green only provides circumstantial evidence of membrane solubilization. Consequently, the aim of this investigation was to determine the effects of the most promising treatments, viz. 30 s boiling water (98°C), 2 s steam (95°C), and 2 s steam (95°C) followed by 4 min immersion in zero pH solution, on cell membrane integrity and to compare this to sulphur-fumigated (600 $g.t^{-1}$) fruit and untreated control fruit.

3.1.2 Materials and Methods

Two 2 kg boxes of mature, red 'Mauritius' litchi fruit were obtained from Malelane, Mpumalanga Province in January 1996. On the day of harvest eight 'Mauritius' fruit were sampled per treatment. Pericarp (rind) samples 3 mm^3 in size were excised from untreated control fruit, as well as from fruit immersed in boiling water (98°C) for 30 sec, fruit

subjected to 2 s steam (95°C) only, fruit subjected to 2 s steam (95°C) followed by 4 min immersion in zero pH solution, and sulphur-fumigated (600 $g.t^{-1}$) fruit. Samples were fixed for 8 hr in 3% glutaraldehyde in a 0.05 M sodium cacodylate buffer (pH 7.1), and washed twice for 30 min in fresh sodium cacodylate buffer. Samples were then postfixed for 4 hr in 2% osmium tetroxide, washed in fresh sodium cacodylate buffer and dehydrated in a graded ethanol series (from 10% to 100%). Following postfixation, specimens were resin infiltrated using a graded series of ethanol and Spurr's resin (Spurr, 1969). Infiltrated specimens were immersed in fresh Spurr's resin, in small labelled aluminium dishes, and placed in an oven at 70°C for 16 hr to polymerize the resin. Once cool, specimens were cut out of resin blocks and stuck onto perspex stubs with Genchem® Superglue. Blocks were trimmed with glass knives on an LKB Ultratome III® ultramicrotome, and then ultrathin gold sections (between 60 and 70 nm thick) were cut using either a diamond or a tungsten-coated glass knife. Sections were expanded, using chloroform and picked up on 200 mesh copper grids. Double staining, with lead citrate and uranyl acetate (Reynolds, 1963), was followed by examination in a Jeol 100CX® TEM at an accelerating voltage of 80 kV. Specimens were photographed and noteworthy results are presented in Plates 2 and 3 as Figs 24 to 33.

3.1.3 Results and Discussion

Cuticles, cell membranes and cell organelles in the cytoplasm of epidermal (Figs 24 and 25) and rind mesocarp parenchyma cells of untreated control fruit were all intact on the day of harvest. In contrast, cuticles of fruit immersed in boiling water (98°C) for 30 s (Fig. 26) were severely disrupted. Epidermal (Fig. 26) and rind mesocarp parenchyma (Fig. 27) cell membranes were also disrupted and protein coagulates were clearly evident in all cells. In fruit treated with 2 s steam (95°C) followed by 4 min in zero pH solution, cell membranes of mesocarp parenchyma cells (Fig. 29) were similar in appearance to those subjected to 30 s boiling water (98°C). Similarly, cuticles were also disrupted (Fig. 28), although this was not as severe as with 30 s boiling water (98°C) (cf. Fig. 26). Rind cuticles of fruit treated with 2 s steam (95°C) only (Fig. 30), were disrupted when compared to untreated control fruit (Fig. 24). Disruption was intermediate when compared to those treated with 2 s steam (95°C) followed by 4 min immersion in zero pH solution (Fig. 28), implying that zero pH solution was complementing steam effects in cuticle breakdown. A 2 s steam (95°C)

Plate 2

Figure 24. Epidermal and cuticular layers of untreated 'Mauritius' litchi fruit rind on the day of harvest. C - cuticle, E - epidermal cell

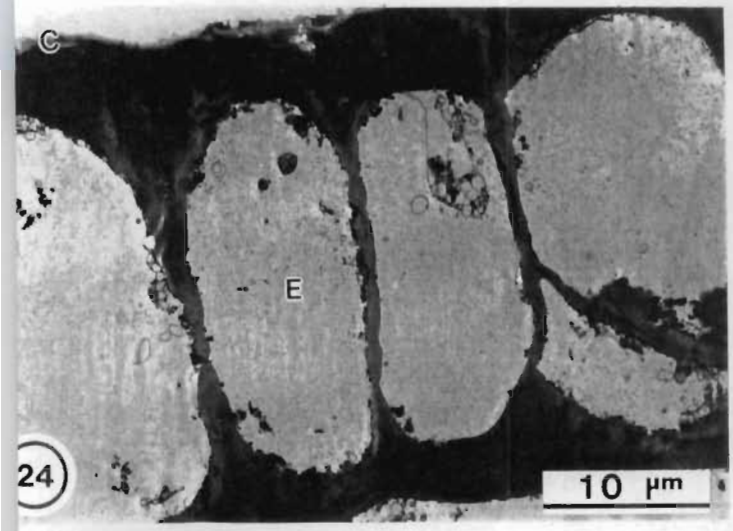
Figure 25. Intact cytoplasm of epidermal cell of untreated 'Mauritius' litchi fruit rind on the day of harvest. CY - cytoplasm

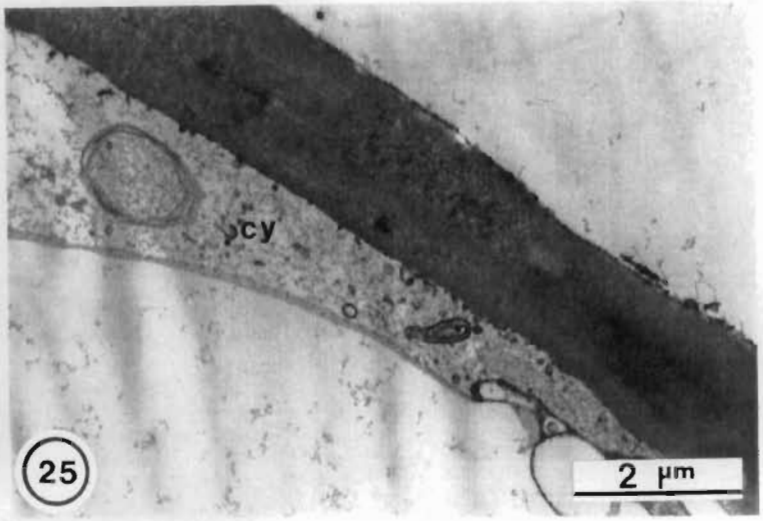
Figure 26. Epidermal cell of 'Mauritius' litchi fruit rind immersed in boiling water (98°C) for 30 s on the day of harvest. C - cuticle, E - epidermal cell

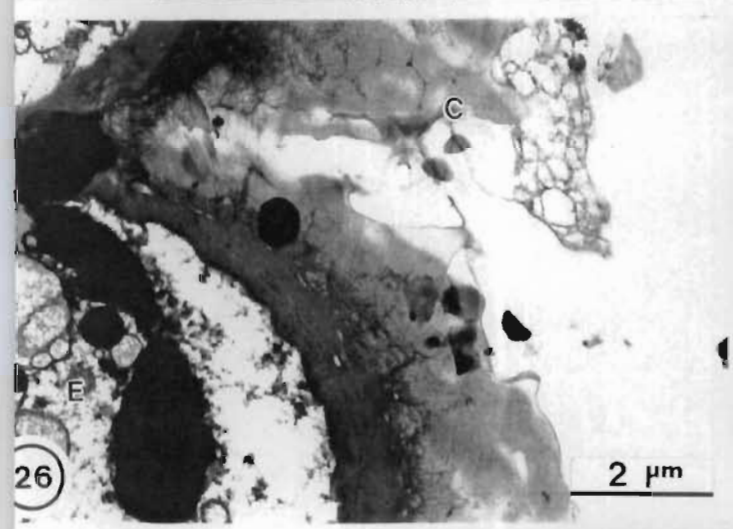
Figure 27. Mesocarp parenchyma cells of 'Mauritius' litchi fruit rind immersed in boiling water (98°C) for 30 s on the day of harvest. M - cell membrane, P - mesocarp parenchyma cell

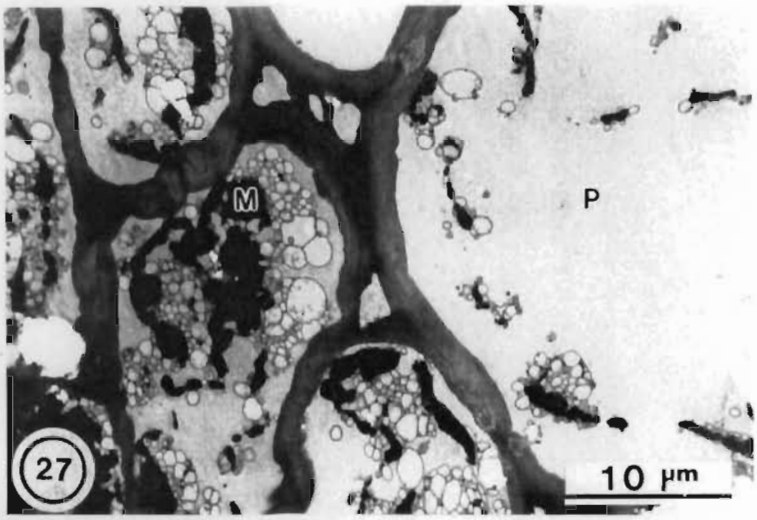
Figure 28. Epidermal cell of 'Mauritius' litchi fruit rind subjected to 2 s steam (95°C) followed by 4 min in zero pH solution on the day of harvest. C - cuticle, M - cell membrane

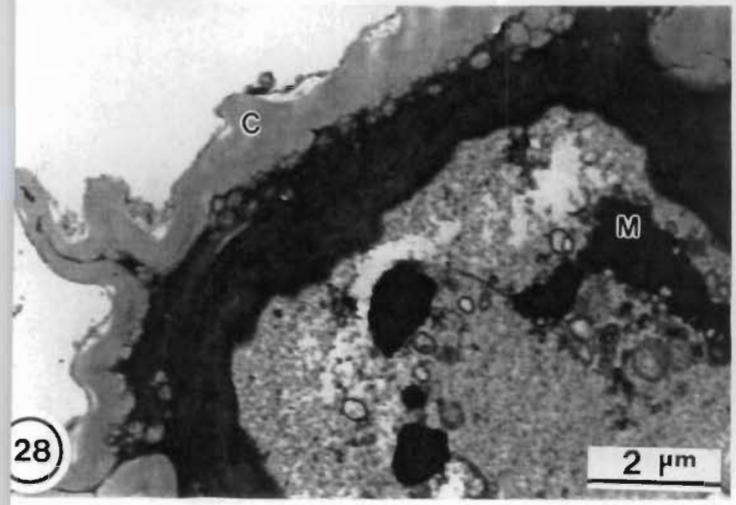
Figure 29. Mesocarp parenchyma cells of 'Mauritius' litchi fruit rind subjected to 2 s steam (95°C) followed by 4 min in zero pH solution on the day of harvest. M - cell membrane, P - mesocarp parenchyma cell


C
E
24
10 µm


cy
25
2 µm


C
E
26
2 µm


M
P
27
10 µm


C
M
28
2 µm

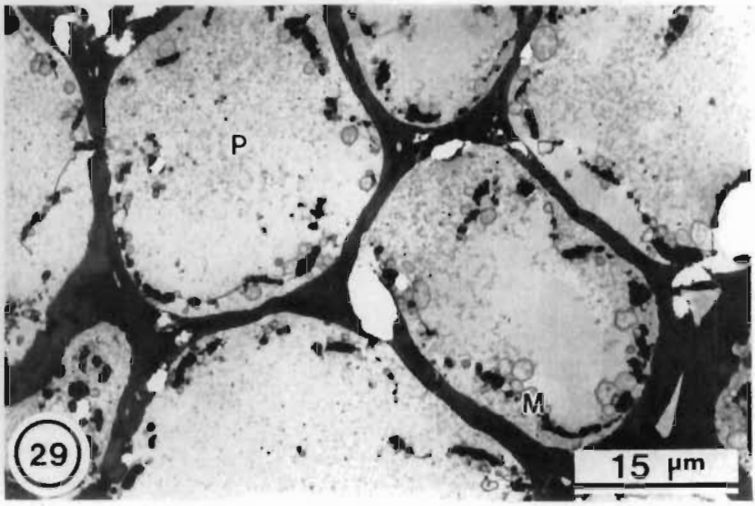

P
M
29
15 µm

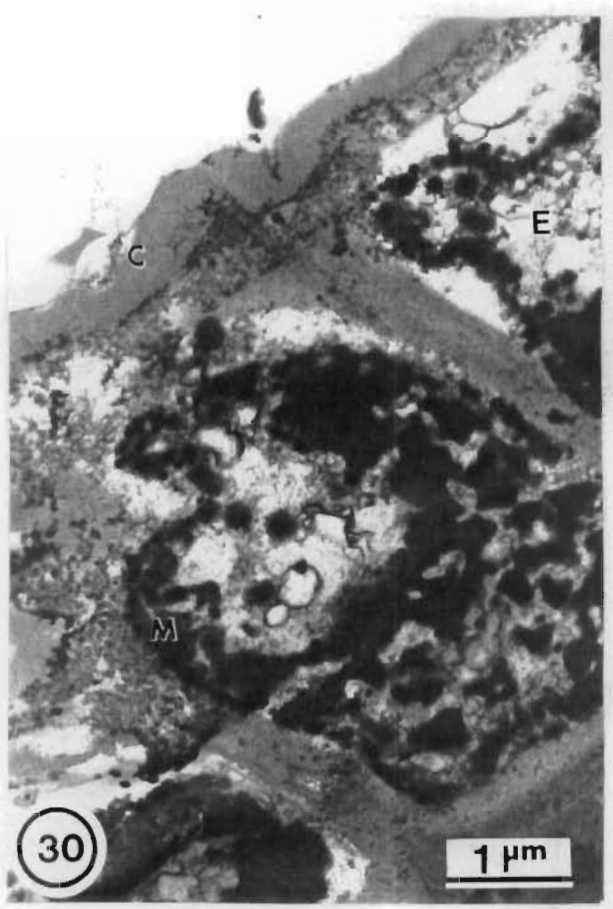
Plate 3

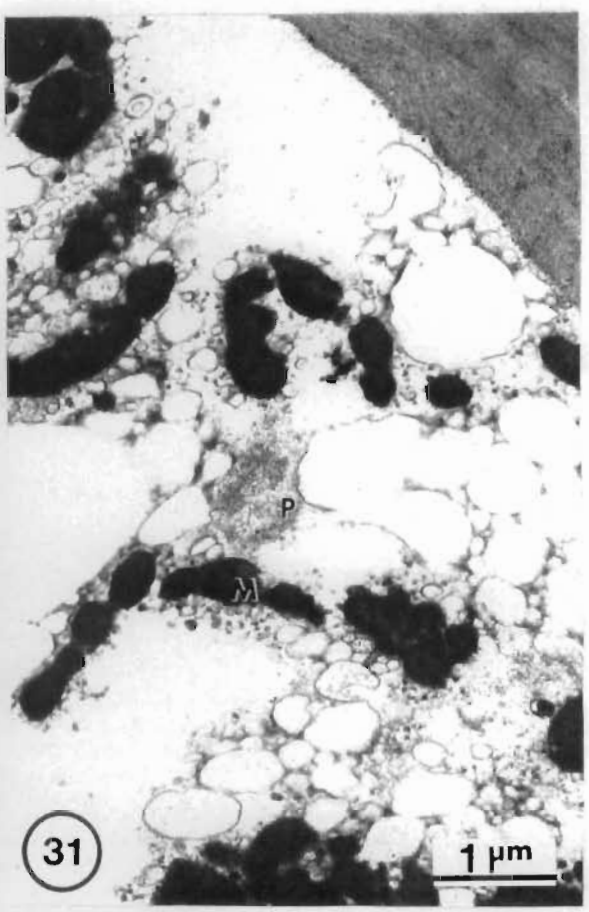
Figure 30. Epidermal cells of 'Mauritius' litchi fruit rind subjected to 2 s steam (95°C) only on the day of harvest. C - cuticle, E - epidermal cell, M - cell membrane

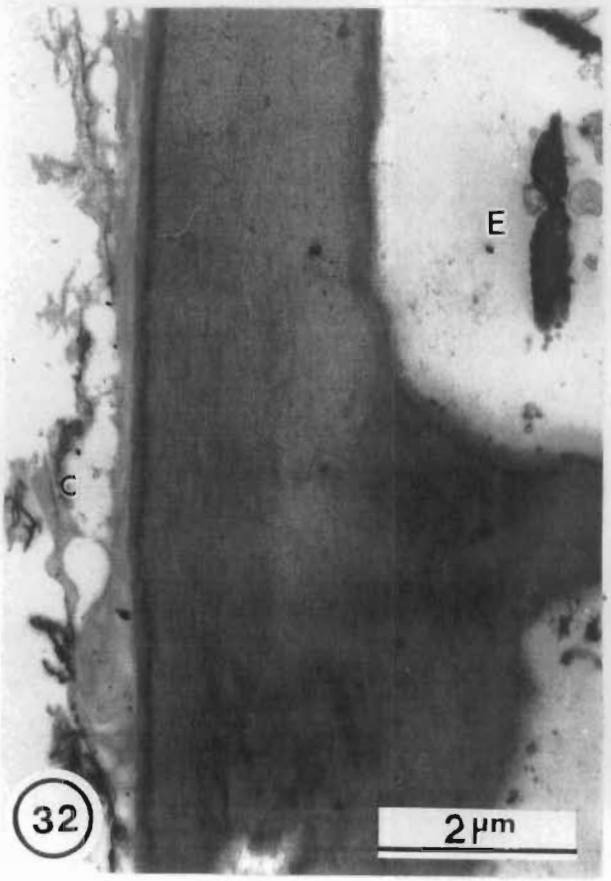
Figure 31. Mesocarp parenchyma cell of 'Mauritius' litchi fruit rind subjected to 2 s steam (95°C) only on the day of harvest. M - cell membrane, P - mesocarp parenchyma cell

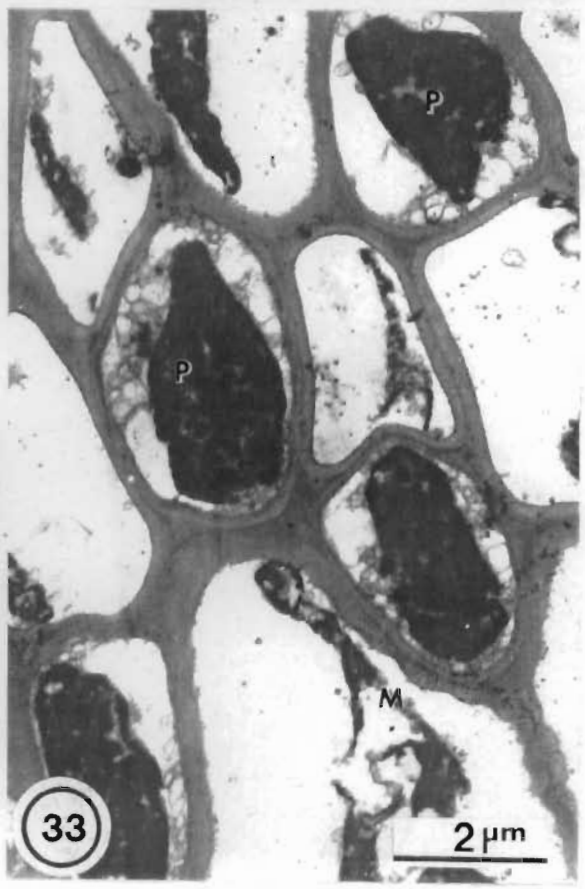
Figure 32. Epidermal cell of sulphur-fumigated 'Mauritius' litchi fruit rind on the day of harvest. C - cuticle, E - epidermal cell

Figure 33. Mesocarp parenchyma cells of sulphur-fumigated 'Mauritius' litchi fruit rind on the day of harvest. M - cell membrane, P - mesocarp parenchyma cell


E
C
M
30
1 µm


P
M
31
1 µm


E
C
32
2 µm


P
P
M
33
2 µm

treatment only was however, effective in causing membrane breakdown and protein coagulation in mesocarp parenchyma cells (Fig. 31).

Fuchs et al. (1993) found that after 20 min of sulphur fumigation electrolytic leakage in litchi rinds was slightly more than three times that of non-fumigated control fruit. Consequently, they suggested that sulphur fumigation significantly increased permeability of plasma membranes, thus enabling acid penetration of sulphur-fumigated fruit. The present investigation revealed that rind cuticles of sulphur-fumigated fruit (Fig. 32) were indeed disrupted. Here, cuticle disruption differed from that in fruit which were subjected to 30 s boiling water (98°C) (Fig. 26), 2 s steam (95°C) (Fig. 30), or 2 s steam (95°C) followed by zero pH solution (Fig. 28). Rind cuticles of sulphur-fumigated fruit separated from epidermal cells in waxy platelets of different thicknesses. Furthermore, although membrane breakdown of rind mesocarp parenchyma cells of sulphur-fumigated fruit was clearly evident (Fig. 33), cell disruption was not as severe as in those fruit subjected to 30 s boiling water (98°C) (Fig. 27), 2 s steam (95°C) (Fig. 31), or 2 s steam (95°C) followed by zero pH solution (Fig. 29).

Clearly, 30 s boiling water (98°C) (Fig. 26), 2 s steam (95°C) (Fig. 30) and sulphur fumigation (Fig. 32) all caused rind cuticle disruption when compared to untreated control fruit (Fig. 24), but boiling water (98°C) for 30 s was most severe (Fig. 26). A 2 s steam (95°C) treatment caused moderate cuticle disruption (Fig. 30), but the effects were enhanced when in conjunction with zero pH solution (Fig. 28). Cuticles of sulphur-fumigated fruit were also disrupted, as waxy platelets of varying thicknesses separated from the epidermis (Fig. 32). Cell membranes of mesocarp parenchyma cells were broken down and protein coagulates were evident in fruit subjected to 30 s boiling water (98°C) (Fig. 27), 2 s steam (95°C) only (Fig. 31), and 2 s steam (95°C) followed by immersion for 4 min in 'zero pH solution (Fig. 29). Cell membranes of sulphur-fumigated mesocarp parenchyma cells were also disrupted (Fig. 33) but some organelles were still intact indicating that sulphur fumigation was not as effective as either 30 s boiling water (98°C) or 2 s steam (95°C) in this regard. In addition, 2 s steam (95°C), and 2 s steam (95°C) followed by 4 min zero pH solution had a similar effect. Sulphur fumigation also resulted in cuticle and membrane

breakdown as suggested by Fuchs et al. (1993), but this was not as severe as 30 s boiling water (98°C) or 2 s steam (95°C).

3.2 ENERGY DISPERSIVE X-RAY MICROANALYSIS

3.2.1 Introduction

Scanning electron microscopy makes use of a focused beam of high energy electrons that systematically scans across the specimen surface (Postek et al., 1980). Electron beam-specimen interactions yield many different electron signals and electromagnetic waves at or near the specimen surface, as a result of elastic or inelastic scattering events (Fig. 34) (Morgan, 1985). Of these signals, secondary electrons of less than 50 eV are easily drawn to a positively biased detector system because of their low energy. Collected electron signals are eventually converted to an electronic signal and displayed on a cathode ray tube. The display, if photographed, produces a typical scanning electron micrograph of an enlarged specimen image (Postek et al., 1980).

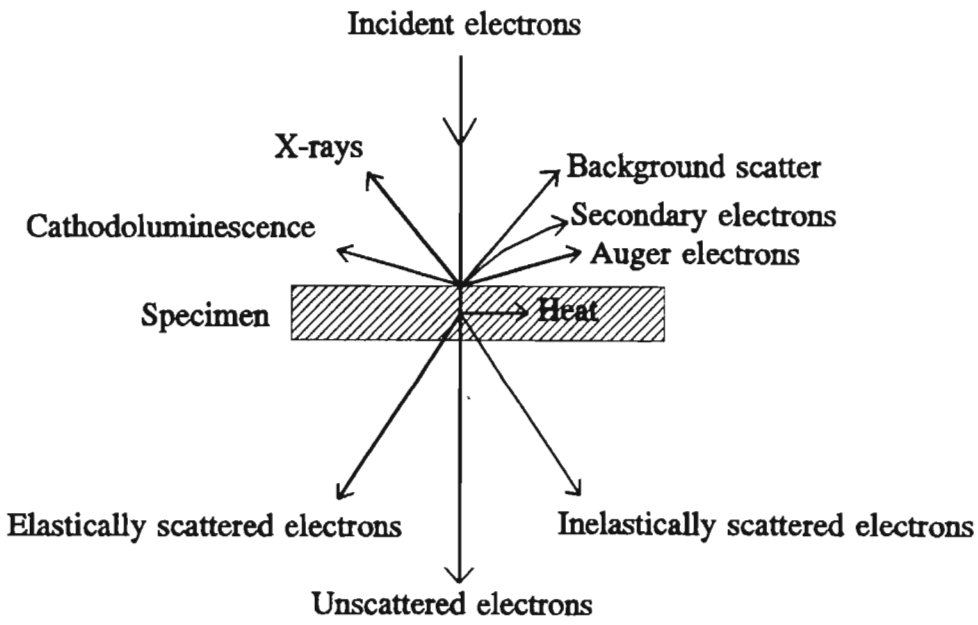


Figure 34 Schematic diagram of major interactions of high energy electron beams within a solid specimen (Redrawn from Morgan, 1985)

X-ray emissions, another of the signals, permit stabilization of atoms following ionization by the electron beam. An atom consists of a discrete nucleus surrounded by shells of orbiting electrons. These electrons have higher potential energy with increasing distance from the nucleus, but potential energy of electrons within a given shell varies discretely with individual atomic numbers. Consequently, energy differences between various shells change characteristically (Morgan, 1985). When an electron from an inner atomic shell is dislodged by the electron beam, an electron from an outer shell will fill the vacancy. Differences in energies between final states of transitional electrons may be emitted as X-radiation (Postek et al., 1980). X-ray emissions from ionized specimens may thus be detected by spectrometry allowing identification of individual elements (Morgan, 1985).

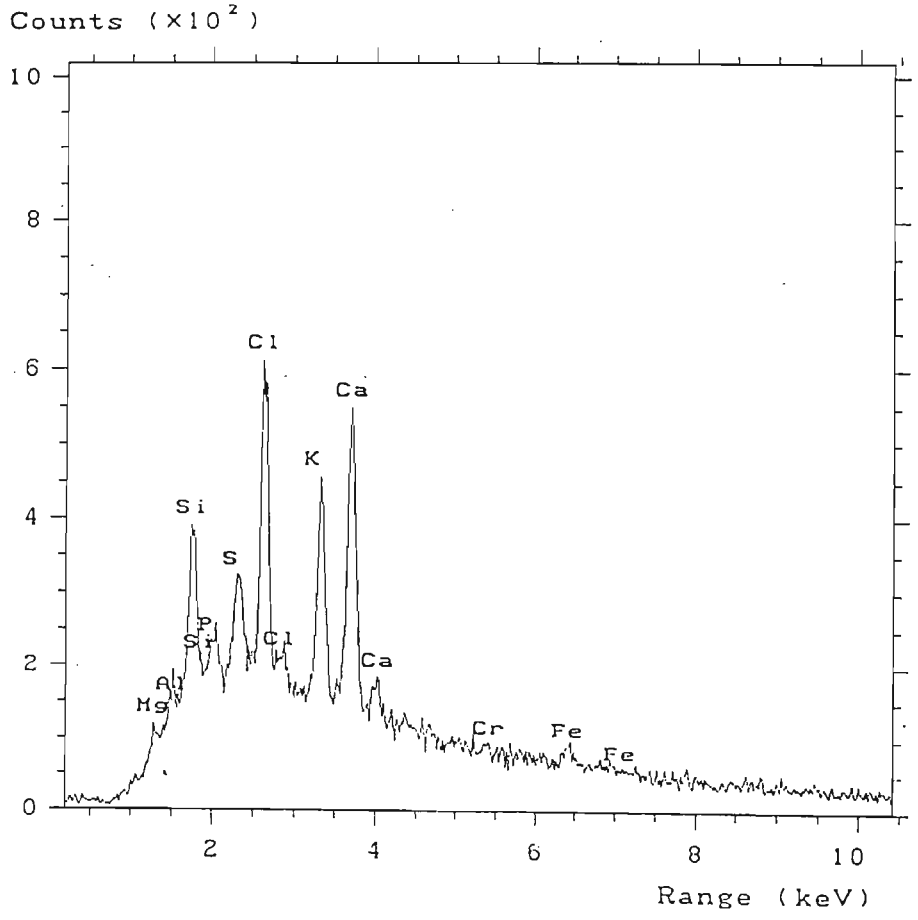


Figure 35 Typical X-ray spectrum of an exocarp peak of 'Mauritius' litchi fruit rind subjected to 2 s steam (95°C) followed by 4 min in zero pH solution

X-ray detection may either be by wavelength-dispersive spectrometry (WDS) or by energy-dispersive spectrometry (EDS). As implied by the name, only one band of X-rays can be analyzed by the former. Consequently, it is limited in that only one element can be analyzed per run. Energy dispersive spectrometry however, enables simultaneous display of all mid-energy (ca 1-20 eV) X-rays collected during a single analysis period, thus allowing identification of almost all elements present in the specimen. Notable exceptions are carbon and boron, however the latter may be detected if the beryllium window isolating the crystal from the microscope vacuum is withdrawn. The beryllium window stops crystal contamination by preventing deposition of microscope-derived products, and absorbs backscattered electrons (Morgan, 1985).

In addition to being pure, semiconductor detectors must be poor electrical conductors and bad insulators, although electrons must be able to pass through with some difficulty. Only man-made crystals of silicon and germanium have proven suitable as detectors, and inherent impurities in man-made crystals are counteracted using "lithium drifting". Here, lithium is evaporated onto the crystal at 400°C and at that temperature, some lithium diffuses into the crystal occupying crystalline interstitial sites resulting in a semiconductor crystal with effectively no impurities at all (Postek et al., 1980). Crystals, which are coated on both ends with a thin layer of gold, are cryogenically cooled with liquid nitrogen and held under vacuum to reduce lithium mobility and to limit electronic noise. When an X-ray strikes the active region of the crystal, energy imparted results in a series of internal collisions which, via electron excitation, ultimately results in a high voltage being maintained between the two gold contact layers. Voltage is amplified and digitized by a pulse-processor. Pulse numbers in each channel are entered and stored by a multichannel analyzer until a complete X-ray spectrum is accumulated, with energy on the horizontal axis and number of photons counted per energy interval on the vertical axis (cf. Fig. 35) (Morgan, 1985).

Energy dispersive X-ray microanalysis thus enables both semi-quantitative and qualitative analyses. Sulphur residues are known to occur in sulphur-fumigated fruit even after 28 days of storage at 1°C (Kremer-Köhne, 1993) however, the distribution of sulphur on the fruit surface and endocarp has not been determined. Likewise, it was thought that chlorine residues might be found on the rind surface of fruit immersed for 2 s in zero pH solution,

since zero pH solution is made using 32% hydrochloric acid. Consequently, the aim of this investigation was to examine the mineral ion concentrations on rind peaks and troughs, and the endocarps of sulphur-fumigated (600g.t^{-1}) fruit, fruit subjected to 2 s steam (95°C) followed by 4 min immersion in zero pH solution, and to compare these to untreated control fruit.

3.2.2 Materials and Methods

One 2 kg box of mature, red `Mauritius' litchi fruit was obtained from Malelane, Mpumalanga Province in January 1996. Eight fruit were subjected to 2 s steam (95°C) followed by 4 min in zero pH solution (hereafter referred to as "zero pH treated fruit") while a further eight fruit were fumigated with sulphur (600 g.t^{-1} fruit). Rinds of all these fruit as well as those of untreated control fruit were air dried and mounted, with double sided sticky tape, on numbered brass stubs and coated with carbon using an Edwards E306A® high vacuum coater. Specimens were elevated to 15 mm, tilted at 15° and viewed using an Hitachi S570® SEM at an accelerating voltage of 20 kV, and characterized with an OXFORD LINK eXLII® energy dispersive X-ray microanalyzer (EDX) fitted with a PENTAFET® semiconductor detector containing lithium drifted silicon crystals isolated from the microscope vacuum by a beryllium window. Eight readings of different exocarp peaks (protuberances) and troughs, and the endocarp (or inner surface of fruit rind) of individual fruit were recorded for both cultivars. This enabled an analysis of variance (Appendix 1) examining treatment differences and results are presented in Figs 36 to 44.

3.2.3 Results and Discussion

As expected, chlorine concentrations (Fig. 36) were higher in exocarp peaks and troughs of zero pH treated fruit ($P < 0.001$). Surprisingly however, chlorine concentrations were also high in endocarps of these fruit too ($P < 0.001$). This implies that zero pH penetrated the entire fruit rind, confirming that efficient membrane breakdown (section 2.8.3) took place throughout the rind, thus facilitating colour preservation (section 2.7.3). Furthermore, aftertastes in the fruit pulp were not an issue (cf. section 2.6.2).

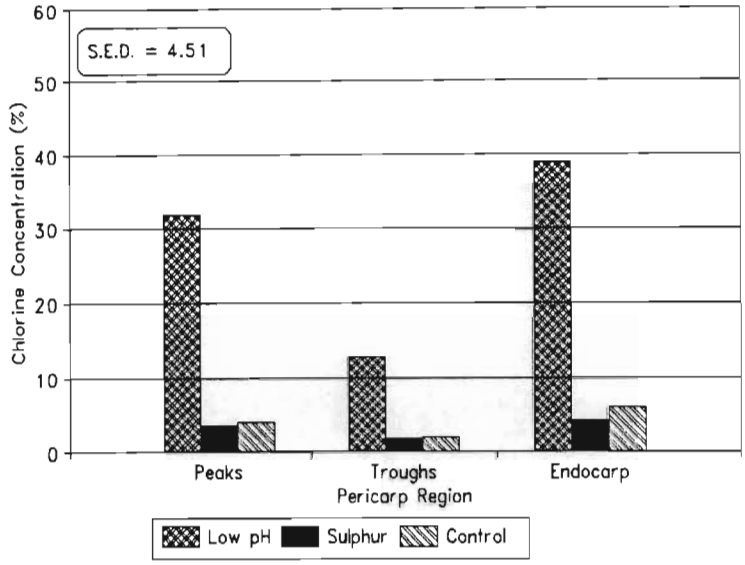


Figure 36 Chlorine concentration (% total mineral elements) of rind endocarp and of exocarp peaks and troughs of 'Mauritius' litchi fruit treated with 2 s steam (95°C) followed by zero pH solution, or sulphur (600 $g.t^{-1}$) compared to untreated control fruit

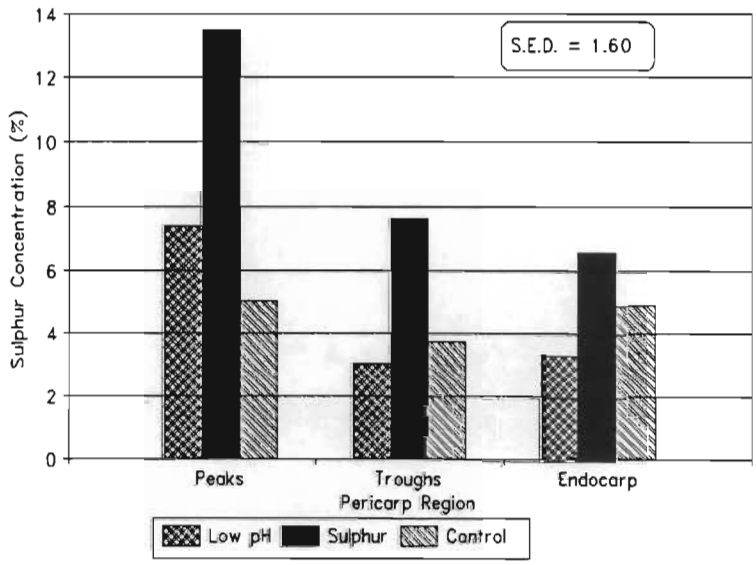


Figure 37 Sulphur concentration (% total mineral elements) of rind endocarp and of exocarp peaks and troughs of 'Mauritius' litchi fruit treated with 2 s steam (95°C) followed by zero pH solution, or sulphur (600 $g.t^{-1}$) compared to untreated control fruit

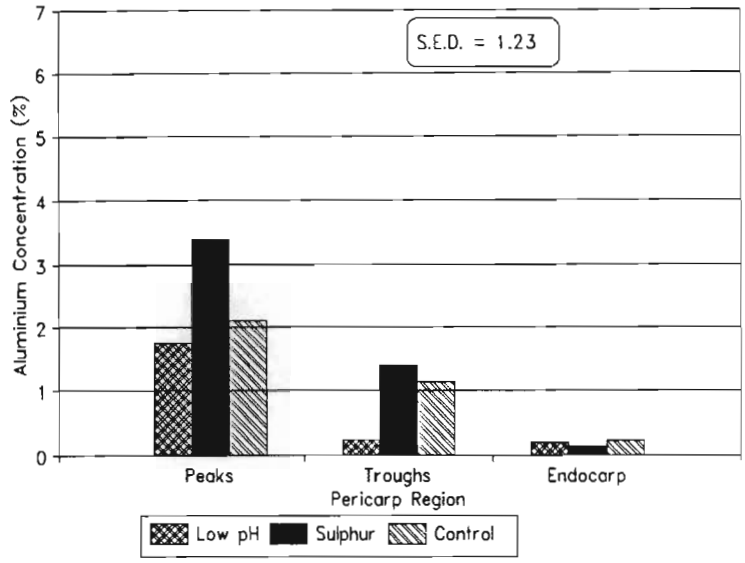


Figure 38 Aluminium concentration (% total mineral elements) of rind endocarp and of exocarp peaks and troughs of 'Mauritius' litchi fruit treated with 2 s steam (95°C) followed by zero pH solution, or sulphur (600 $g.t^{-1}$) compared to untreated control fruit

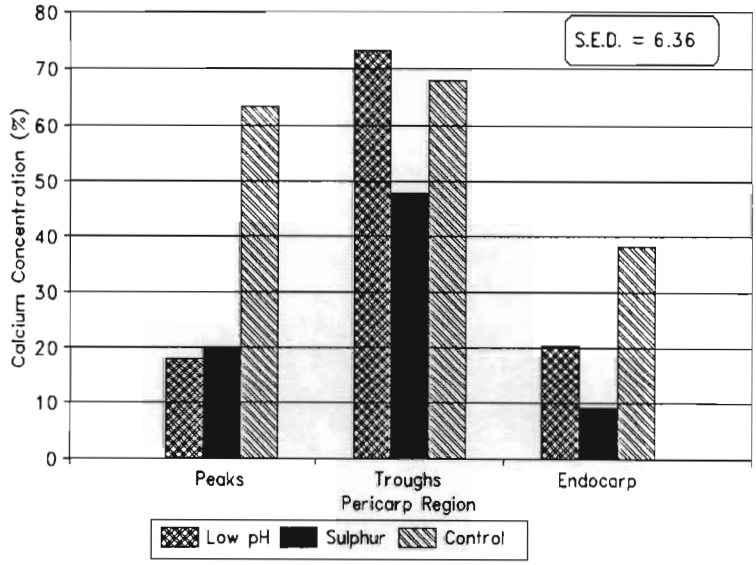


Figure 39 Calcium concentration (% total mineral elements) of rind endocarp and of exocarp peaks and troughs of 'Mauritius' litchi fruit treated with 2 s steam (95°C) followed by zero pH solution, or sulphur (600 $g.t^{-1}$) compared to untreated control fruit

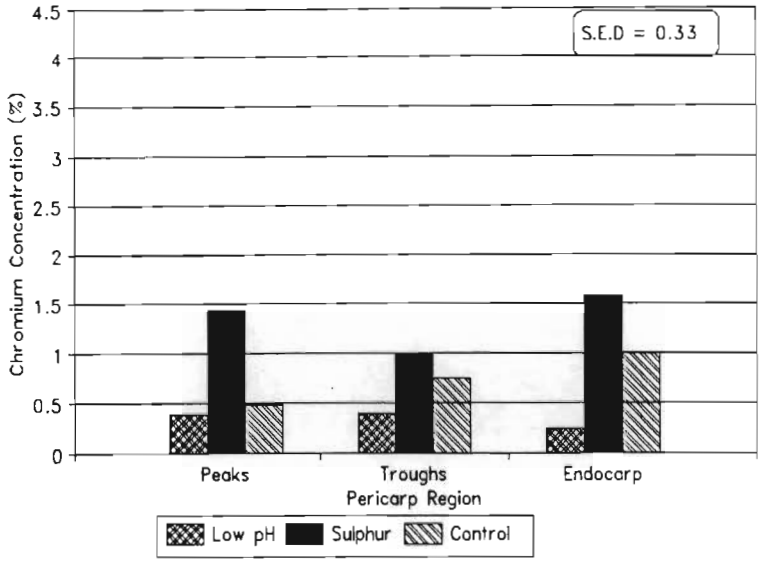


Figure 40 Chromium concentration (% total mineral elements) of rind endocarp and of exocarp peaks and troughs of 'Mauritius' litchi fruit treated with 2 s steam (95°C) followed by zero pH solution, or sulphur (600 $g.t^{-1}$) compared to untreated control fruit

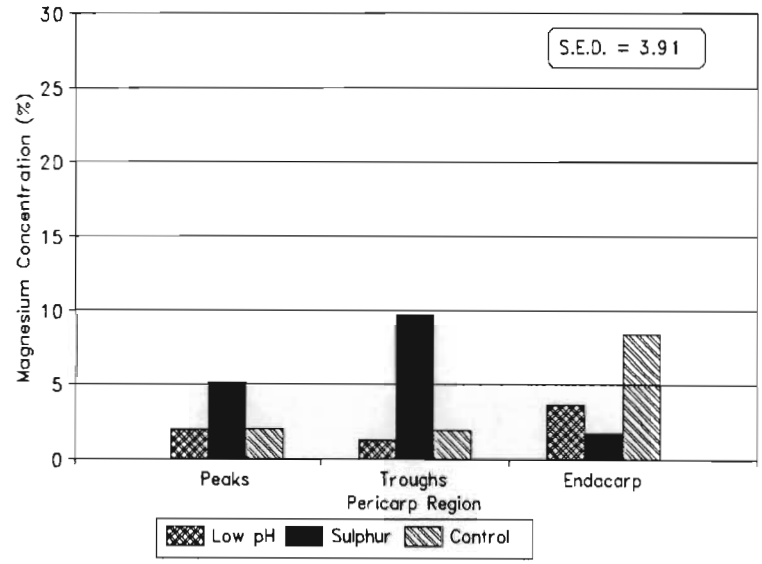


Figure 41 Magnesium concentration (% total mineral elements) of rind endocarp and of exocarp peaks and troughs of 'Mauritius' litchi fruit treated with 2 s steam (95°C) followed by zero pH solution, or sulphur (600 $g.t^{-1}$) compared to untreated control fruit

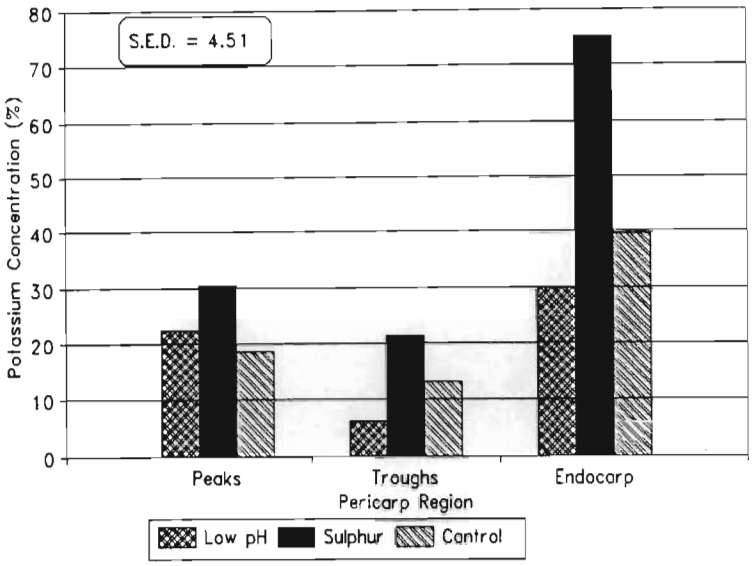


Figure 42 Potassium concentration (% total mineral elements) of rind endocarp and of exocarp peaks and troughs of 'Mauritius' litchi fruit treated with 2 s steam (95°C) followed by zero pH solution, or sulphur (600 $g.t^{-1}$) compared to untreated control fruit

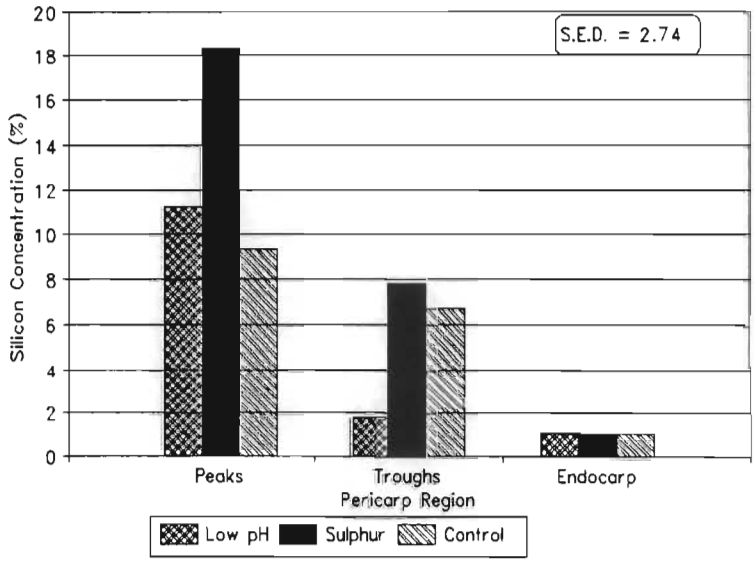


Figure 43 Silicon concentration (% total mineral elements) of rind endocarp and of exocarp peaks and troughs of 'Mauritius' litchi fruit treated with 2 s steam (95°C) followed by zero pH solution, or sulphur (600 $g.t^{-1}$) compared to untreated control fruit

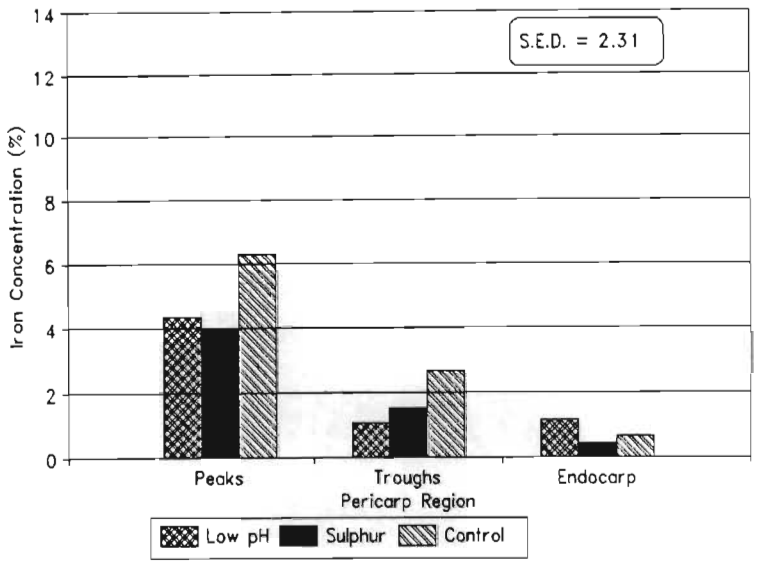


Figure 44 Iron concentration (% total mineral elements) of rind endocarp and of exocarp peaks and troughs of 'Mauritius' litchi fruit treated with 2 s steam (95°C) followed by zero pH solution, or sulphur (600 $g.t^{-1}$) compared to untreated control fruit

Sulphur concentrations (Fig. 37), although present in non-fumigated fruit, were consistently highest in endocarps and exocarp peaks and troughs of sulphur-fumigated fruit, implying that sulphur penetration occurred throughout the entire rind. Concentrations were however, only slightly more than double those found in untreated control fruit, and zero pH treated fruit ($P<0.001$), but it is possible that air drying of litchi pericarps allowed a greater concentration of sulphur to escape from pericarps by atmospheric diffusion.

Aluminium concentrations (Fig. 38) in all pericarp regions examined were less than 4% of total mineral elements while concentrations of exocarp peaks were highest and endocarps lowest. Sulphur-fumigated fruit had slightly higher average aluminium concentrations but treatment differences were not significant ($P=0.759$). When taking endocarps and the exocarp peaks and troughs into account, calcium concentrations (Fig. 39) were highest in untreated control fruit and lowest in sulphur-fumigated fruit ($P<001$). Calcium concentrations were however, highest (ca 70%) in troughs of zero pH treated fruit.

Potassium concentrations (Fig. 42) however, varied greatly from as little as 5% in troughs of zero pH treated fruit to as much as ca 75% in endocarps of sulphur-fumigated fruit. This is possibly because K^+ ions do not form part of the structural component of plant cells, and are mostly vacuolar bound thus being subject to leakage when membranes are disrupted (Salisbury and Ross, 1992).

Similarly, chromium (Fig. 40), magnesium (Fig. 41) and silicon (Fig. 43) concentrations were slightly higher in sulphur-fumigated fruit ($P<0.001$). Concentrations of all three pericarp regions examined were relatively low in the case of chromium (ca 1.5%), magnesium (< 10%) and silicon (ca 18%) thus overshadowing these differences. In contrast, potassium concentrations (Fig. 42) varied from as little as 5% in troughs of zero pH treated fruit to as much as ca 75% in endocarps of sulphur-fumigated fruit. Slight differences observed in iron (Fig. 44) concentrations between treatments were not significant ($P=0.095$).

Consistent differences in element concentrations between endocarps and exocarp peaks and troughs were not observed. In some cases endocarp element concentrations were highest eg. chromium (Fig. 40) and potassium (Fig. 42), but in others they were lowest, eg. aluminium (Fig. 38), silicon (Fig. 43) and iron (Fig. 44), and still others they were intermediate eg. magnesium (Fig. 41) and calcium (Fig. 39). Variability observed may simply be accounted for by the fact that one is dealing with a semi-quantitative technique which penetrates only a few cell layers into the specimen surface. Furthermore, concentrations are calculated as a percentage of the total quantity of mineral elements identified. Consequently, only large differences will show up clearly as was the case with chlorine (Fig. 36) and sulphur (Fig. 37).

CHAPTER 4

RIND ANTHOCYANIN BIOCHEMISTRY

4.1 INTRODUCTION

'Mauritius' litchi rind colour may be preserved for 28 days in storage at 1°C, following immersion of fruit in zero pH for 4 min (section 2.7). Anthocyanins, which impart red colour in litchi rinds, are normally very unstable in aqueous solution (Macheix et al., 1990) and fruit products (Markakis, 1982), and may undergo structural transformations or complexations (Mazza and Miniati, 1993) or decompose rapidly unless stabilized (Jurd, 1972). Consequently, since zero pH solution preserved the red colour of litchis, it was thought that rind anthocyanins may have undergone some form of molecular complexing. The aim of this investigation was to use high performance liquid chromatography, to separate anthocyanin pigments in litchi rinds of fruit subjected to 2 s of steam (95°C) followed by 4 min immersion in zero pH and to compare these pigment separation of untreated control fruit and fruit treated with sulphur, 2 s steam (95°C) only, 30 s in boiling water (98°C) and 4 min in zero pH only.

Qualitative extraction of anthocyanins is difficult and most investigations have extracted anthocyanins by repeated maceration of plant material with a small amount of hydrochloric acid (0.1 - 1.0%), which acts as a stabilizer in methanol or ethanol (Harborne, 1980b; Francis, 1982; Strack and Wray, 1989; Macheix et al., 1990; Forni et al., 1993; Mazza and Miniati, 1993). Withy et al. (1993) stabilized raspberry anthocyanins with 50 $mg.kg^{-1}$ SO_2 by adding 1% potassium metabisulfite solution to raspberry extract. Lee and Wicker (1991a; b) extracted litchi anthocyanins by homogenizing litchi rinds with 50 mℓ acidified ethanol (1.5 N hydrochloric acid- 95% ethanol (15:85 v/v)). In the author's opinion, these extraction techniques may well give an indication as to which anthocyanins are present, however sulphur dioxide, hydrochloric acid, methanol and ethanol are all known to react with anthocyanins (Jurd, 1972), complicating qualitative analyses. Thus the use of other extraction

solvents, including butanol, ethanoic acid, water, formic acid and acetone in different combinations was suggested (Cowan, 1996[3]).

Quantitative extraction of anthocyanins from plant tissue has always been difficult, and it seems that there is no single completely satisfactory method. Grape skins extracted with 1% aqueous hydrochloric acid had 8% of the total anthocyanins in the fourth fraction. Qualitative analysis of each fraction showed that relative amounts of different pigments in each fraction were reasonably constant (Harborne, 1980b). Information about relative amounts may thus be obtained even though they have not been completely extracted from plant tissue. Thus, for the purposes of this investigation, litchi rinds were extracted four times and a 100 μl aliquot of the combined extracts withdrawn for use in high performance liquid chromatography (HPLC).

4.2 MATERIALS AND METHODS

Eight 2 kg boxes of mature, red 'Mauritius' fruit were obtained from Nelspruit, Mpumalanga province in January 1996. Rind tissue from eight untreated, red 'Mauritius' litchi fruit, and eight fruit subjected to 2 s steam (95°C) followed by 4 min in zero pH solution were each extracted, using an ULTRA TURRAX®, with methanoic acid containing 1% conc HCl. Extracts were centrifuged for 10 min at 15 000 r.p.m. in an HITACHI HIMAC® centrifuge and then chromatographed on Whatman® cellulose TLC plate using butanoic acid- acetic acid- water (4:1:5, v/v). Individual bands were excised from the TLC plate, resuspended in methanoic acid containing 1% conc HCl, and scanned between 510 and 540 nm using a Beckman® DU-65 spectrophotometer.

Initially, for HPLC extraction, 5 g of sulphur-fumigated litchi rind material was extracted on the day of harvest with methanol- 32% hydrochloric acid (99:1, v/v) (Strack and Wray, 1989; Macheix et al., 1990; Forni et al., 1993; Mazza and Miniati, 1993) or 1.5 N hydrochloric acid- 95% ethanol (15:85, v/v) after Lee and Wicker (1991a; b), but the colour

[3]Dr A.K.Cowan, *pers. comm.*, Department of Horticultural Science, University of Natal, Pietermaritzburg.

of both extracts became deep red within a few minutes indicating that a pH sensitive reaction was taking place. Furthermore, extraction with hydrochloric acid is not desirable since differences if any, would not be distinguishable between untreated control fruit, sulphur-fumigated fruit and those treated with zero pH solution. Consequently, butanol- ethanoic acid- water (4:1:5, v/v) was used, but again extract colour changed within a few minutes. Finally, water- acetone- formic acid (49:50:1, v/v) was used and the colour of the extract was unchanged after 1 hr at 21°C.

Untreated, mature, red 'Mauritius' litchi fruit, and fruit which had been subjected to 2 s steam (95°C) followed by 4 min in zero pH solution on the day of harvest were stored at 30°C for 5 and 4 days respectively. Each day 2 replicates of 5 g of rind material taken from eight fruit were homogenized in 20 mℓ water- acetone- formic acid (49:50:1, v/v) with an ULTRA TURRAX® and centrifuged for 10 min at 15 000 r.p.m. in an HITACHI HIMAC® centrifuge. This extraction was repeated four times and the resulting supernatant liquid combined for each replicate and a 2 mℓ aliquot passed through five WATERS SEP-PAK® cartridges in series. On the day of harvest a further 40 g of untreated rind material was frozen in liquid nitrogen and crushed to a powder, which was then stored at 30°C. Every 1.5 hr, 5 g of powder was extracted using the procedure described above for rinds of sulphur-fumigated fruit, fruit treated with zero pH solution only, fruit immersed in boiling water (98°C) for 30 s followed by 4 min in zero pH solution, fruit subjected to 2 s steam (95°C) only, and fruit subjected to 2 s steam (95°C) followed by 4 min in zero pH solution were extracted after 28 days of storage at 1°C.

In each instance, immediately after extraction, a 100 μℓ aliquot of pure extract was injected into a WATERS® HPLC supported by MILLENUIUM 2010® chromatography manager. Because of the unstable nature of anthocyanins, injections were not replicated as differences observed between samples after the run time of 80 min would have been the result of changes in chemical structure rather than differences in the biological system. Conditions were: a WATERS Nova Pak® C18 reverse phase column (3.9 x 150 mm) packed with dimethyloctadecylsilyl bonded amorphous silica (particle size 4 μm); a WATERS® guard column; gradient elution with 5% ethanoic acid/water (A) and 100% acetonitrile (B); flow rate 1.0 $m\ell.min^{-1}$, and detection at 520 nm using a WATERS® Programmable

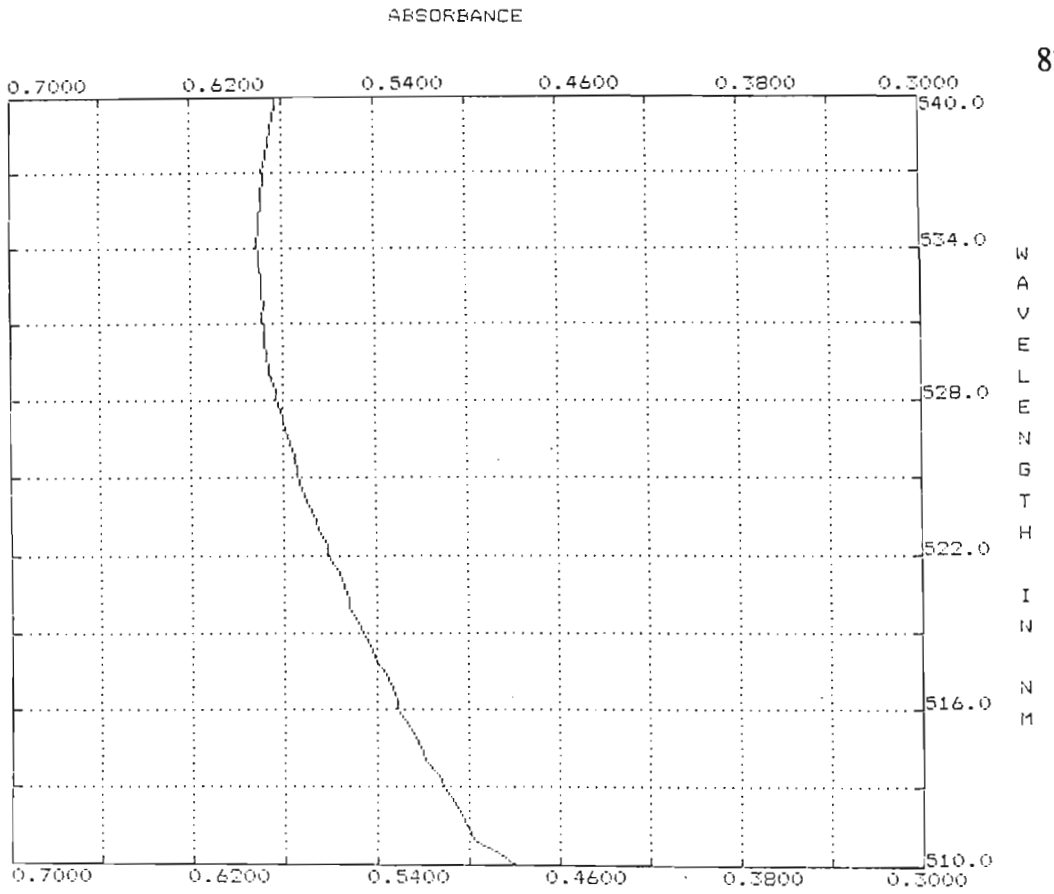


Figure 45 Spectrophotometric scan of red pigment separated by TLC using butanoic acid- acetone-water (4:1:5 V/V)

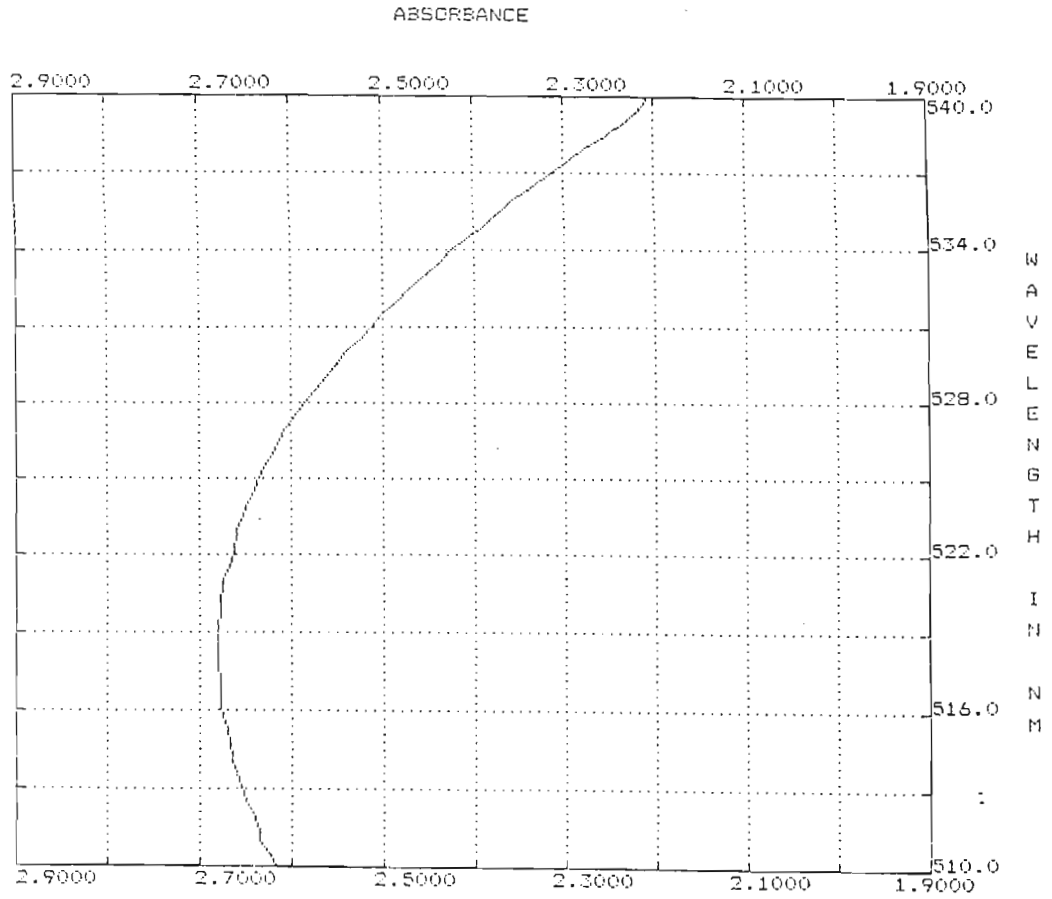


Figure 46 Spectrophotometric scan of mauve pigment separated by TLC using butanoic

Multiwavelength Detector. Initial solvent was 100% A and 0% B but this was changed linearly to 0% A and 100% B within 60 min and then returned to the initial condition. Due to the very unstable nature of anthocyanins, replication was not performed and results are thus purely indicative. Furthermore, it was not the intention of this investigation to determine the exact concentration of individual anthocyanins. Consequently, internal standards were not used to calculate percentage recovery and it was assumed that the peak area for each compound is an indication of comparative concentration in the rind (cf. Harborne, 1980b). Area under individual peaks separated by HPLC were however, integrated and presented as units in tables 5 to 8.

4.3 RESULTS AND DISCUSSION

Only two pigments were separated out by TLC using butanoic acid- acetic acid- water (4:1:5, v/v). Spectrophotometric scanning of these pigments in methanoic acid in 1% conc. HCl, revealed that they had wavelength maxima of 535 nm (Fig. 45) and 520 nm (Fig. 46), and when compared to visible wavelength maxima of (Harborne, 1980a) it was confirmed that they were cyanidin and pelargonidin. Lee and Wicker (1991a; b) identified cyanidin-3-rutinoside (rutinose is a disaccharide viz. 3-*O*-α-L-rhamnosyl-D-glucose according to Budavari et al. (1989)) and cyanidin-3-glucoside. In addition to these, Prasad and Jha (1978) identified pelargonidin-3-glucoside and pelargonidin-3,5-diglucoside. Based on these findings, the four peaks separated out by HPLC for 'Mauritius' litchi rinds in this investigation were tentatively assumed to be these four anthocyanins. The author is however, fully aware that the identification of the various sugar moeities was not carried out in a rigorous chemical manner, and is based on the earlier published findings as cited.

Cyanidin-3-rutinoside was by far the most abundant pigment in rinds of untreated, mature, red 'Mauritius' litchi fruit stored at 30°C for 5 days, but a 66% decrease was observed from the day of harvest to five days after harvest (Table 5). Pelargonidin-3-glucoside and pelargonidin-3,5-diglucoside concentrations had also decreased after 4 days but only by ca 18% and 2% respectively (Table 5). Cyanidin-3-rutinoside in fruit rinds which were crushed to a powder in liquid nitrogen on the day of harvest, and sampled every 1.5 hr also decreased with time and by 9 hr after crushing, decreased by ca 99% (Table 6). However,

TABLE 5 Area (units) of total anthocyanins from the day of harvest in rinds of untreated, mature, red 'Mauritius' litchi fruit stored at 30°C for 5 days.

Days	Cyanidin-3-rutinoside	Pelargonidin-3-glucoside	Pelargonidin-3,5-diglucoside
0	6689766	143499	27783
1	6523872	123666	29422
2	4952117	92232	86706
3	2133486	102368	55408
4	2471923	138579	24825
5	2258596	116612	27352

TABLE 6 Area (units) of total anthocyanins in rinds of untreated, mature, red 'Mauritius' litchi fruit crushed on the day of harvest and sampled every one and a half hours.

Time	Cyanidin-3-rutinoside	Pelargonidin-3-glucoside	Pelargonidin-3,5-diglucoside
1.5	8192810	119291	62338
3	6750351	338421	97178
4.5	2310164	241929	50152
6	1032880	241434	38638
7.5	365496	148548	59221
9	44341	111805	65235

concentrations of pelargonidin-3-glucoside and pelargonidin-3,5-diglucoside in the same extract remained fairly constant during this time (Table 6). It appears thus that cyanidin is less stable than pelargonidin in untreated control fruit, as the rate of breakdown was faster in all fruit stored at 30°C.

Rinds of fruit subjected to 2 s steam (95°C) followed by 4 min in zero pH solution and stored at 30°C for 4 days had consistently high concentrations of cyanidin-3-rutinoside, but no cyanidin-3-glucoside (Table 7). Cyanidin-3-rutinoside concentrations had almost doubled by 12 hr after treatment and then increased further to almost 3 times the inital concentration 24 hr after treatment. Concentrations then remained fairly constant up to 48 hr after treatment but then decreased after a further 24 hr to almost double that initially and this concentration remained constant up to 4 days after harvest. Pelargonidin-3-glucoside concentrations declined steadily during this time and had declined by 50% after 4 days storage at 30°C. Concentrations of pelargonidin-3,5-diglucoside however, remained fairly constant and had only declined by 20% after 4 days storage at 30°C (Table 7).

Only cyanidin-3-rutinoside, pelargonidin-3-glucoside and pelargonidin-3,5-diglucoside were found in untreated control fruit, and fruit subjected to 2 s steam (95°C) followed by 4 min in zero pH solution, stored at 30°C for 5 and 4 days respectively. Self associations, co-pigmentation or chelation of anthocyanins result in additional compounds being formed (Jurd, 1963; 1972). Consequently, if any of these stabilizations had taken place in the present study, additional compounds would have been separated by HPLC. However, in the case of zero pH treated fruit no additional compounds were separated out when compared to control fruit. Consequently, it seems that stability conferred on anthocyanins at zero pH must be a pH effect as described by Strack and Wray (1989), and explains why rind colour of zero pH treated fruit is preserved after several days at room temperature (section 2.7). Furthermore, after fruit were subjected to 2 s steam (95°C) followed by 4 min in zero pH solution, concentrations of cyanidin-3-rutinoside almost trebled by 24 hr after treatment and then declined by 4 days after treatment but was still about double that found in fruit rinds treated on the day of harvest, confirming that zero pH solution actually enhances rind colour (section 2.7.3). This increase may be as a result of either colourless precursors or chromenols being converted to cyanidin-3-rutinoside at zero pH.

TABLE 7 Area (units) of total anthocyanins in rinds of mature 'Mauritius' litchi fruit subjected to 2 s steam (95°C) followed by 4 min in zero pH solution, and stored at 30°C from the day of harvest to 4 days after harvest.

Days	Cyanidin-3-rutinoside	Pelargonidin-3-glucoside	Pelargonidin-3,5-diglucoside
0	4259781	123069	53520
0.5	8206006	122058	29469
1	11548974	117890	30733
2	11486455	65347	36887
3	8249381	60955	39127
4	7873592	68781	42811

TABLE 8 Area (units) of total anthocyanins in rinds of mature 'Mauritius' litchi fruit fumigated with sulphur, immersed in zero pH solution only, immersed in 30 s boiling water followed by 4 min in zero pH solution, or subjected to 2 s steam (95°C) only, after 28 days storage at 1°C

Treatment	Cyanidin-3-glucoside	Cyanidin-3-rutinoside	Pelargonidin-3-glucoside	Pelargonidin-3,5-diglucoside
sulphur	593014	2686197	23168	24152
zero pH only	3616281	2548341	51655	13401
30 s (98°C) + 4 min	5844192	6770770	41042	42109
2 s steam (95°C) only	411799	812006	77860	65276
2 s steam (95°C) + 4 min	0	4336826	108131	61604

Interestingly, after 28 days of storage at 1°C, cyanidin-3-glucoside was identified in rinds of sulphur-fumigated (600g.t^{-1}) fruit, fruit immersed in zero pH solution only, fruit immersed for 30 s in boiling water (98°C) followed by 4 min immersion in zero pH solution, and fruit subjected to 2 s steam (95°C) only, but not in fruit subjected to 2 s steam (95°C) followed by 4 min immersion in zero pH solution (Table 8). It is possible that 2 s steam (95°C) followed by 4 min immersion in zero pH may have stabilized cyanidin-3-rutinoside, ie. prevented or resisted chemical transformation of the pigment. For all other treatments, cyanidin-3-rutinoside may have been partially hydrolyzed to cyanidin-3-glucoside and rhamnose (Budavari et al., 1989), explaining the presence of cyanidin-3-glucoside.

After 28 days of storage, concentrations of cyanidin-3-glucoside and cyanidin-3,5-diglucoside in rinds of sulphur-fumigated fruit were relatively low when compared to the those in rinds of fruit immersed for in zero pH solution only, or fruit immersed for 30 s in boiling water (98°C) followed by 4 min immersion in zero pH. Concentrations of cyanidin-3-glucoside and cyanidin-3,5-diglucoside were however, lowest in fruit subjected to 2 s steam (95°C) only, confirming that steam caused breakdown of anthocyanin pigments. Concentrations of pelargonidin-3-glucoside and pelargonidin-3,5-diglucoside were relatively low in rinds of all these fruit when compared to fruit subjected to 2 s steam (95°C) followed by 4 min immersion in zero pH (Table 8).

GENERAL DISCUSSION AND CONCLUSIONS

The South African litchi industry has relied heavily on post-harvest sulphur fumigation of litchi fruit for several years. Initially, sulphur fumigation was intended as a fungicidal treatment but also prevented rind browning. Unfortunately however, rinds of sulphur-fumigated litchi fruit are yellow to pale green but change to a pink colour after 28 days in storage at 1°C. Resultant fruit have a low consumer appeal and this coupled with the facts that sulphur is ineffective against some fungi, which are now resistant; causes an aftertaste in the pulp, and constitutes a health hazard for asthmatics, has led to a limit of 10 $mg.kg^{-1}$ (fresh pulp mass) being set for some European markets. The South African litchi industry thus regards alternative post-harvest treatments to sulphur-fumigation as their number one research priority. Consequently, the main thrust of the research reported in this thesis aimed at finding a viable alternative to sulphur fumigation which would not only result in fruit with attractive, disease-free, red rinds but would also be palatable.

Litchi rind colour is initially a function of chlorophyll, which is a constituent of young actively growing fruit, and later anthocyanins, which confer the attractive red colour in mature fruit. Anthocyanins are synthesized during the latter stages of fruit development, and pre-harvest anthocyanin biosynthesis may be influenced by several factors including light, temperature, nutritional effects and phytohormones. It was not possible in the present study to manipulate or investigate pre-harvest factors affecting rind colour, as the author was entirely dependent on fruit donated by growers. It is however, important that pre-harvest factors are investigated, since pre-harvest anthocyanin accumulation influences the amount of colour in mature fruit, which in turn determines fruit cosmetic appeal. The latter is particularly important on export markets, where the fruit is still relatively unknown. It is well known, for example, that rind colour in more tropical and especially humid countries such as Madagascar, Mauritius, Reunion and Thailand is far more intensive and attractive than in the South African litchi growing areas. The author, supported by the South African Litchi Growers' Association, made several attempts to air freight fruit from Madagascar and Thailand to compare with local fruit, but these attempts were unsuccessful.

Red rind colour of litchi fruit is transient when compared to most fleshy fruits eg. red apple skins. The most likely reasons for this are the lack of a substantial protective rind cuticle to reduce desiccation and the presence of a relatively thin pericarp in litchis (Underhill and Simons, 1993). In apples, the juicy hypanthium tissue is continuous with the skin (Smith, 1977) and probably acts as a cushion against water loss, reducing desiccation and thus rind browning. Rind desiccation in litchi fruit leads to a rise in cell sap pH, which is strongly associated with the loss of red rind colour.

Underhill and co-workers in Australia, undertook several detailed investigations concerning litchi rind browning, during the late 1980's and 1990's, some of the work being concurrent with the author's study. Many of these studies were of an exploratory nature (Underhill, 1989; 1992; Underhill and Critchley, 1992; 1993; Underhill and Simmons, 1993; Underhill and Critchley, 1995) and shed new light on anatomical, biochemical and physiological aspects of litchi rind browning. Indeed, information drawn from some of these investigations provided the basis for the initial hypotheses investigated in this thesis, viz. localization of anthocyanins; browning reactions, including enzyme degradation and anthocyanin breakdown, and the importance of post-harvest micro-cracking.

Later investigations examined methods of preventing litchi rind browning (Underhill et al., 1992b; Underhill, 1994; Underhill et al., 1994), but these investigations were aimed at reversing the effects of post-harvest sulphur fumigation. Similarly, concurrent investigations by Zaubermann et al. (1990; 1991) in Israel, attempted to re-introduce red colour in litchi rinds using low pH dips following post-harvest sulphur fumigation. After an extensive literature survey of anthocyanin biochemistry, the author hypothesized that sulphur fumigation could be circumvented and the desirable anthocyanins fixed *in situ*, provided that anthocyanins could be accessed by solubilizing membranes by means other than the standard sulphur treatment.

Several attempts were made at solubilizing rind cell membranes (section 2.3), and 30 s immersion in boiling water (98°C) (section 2.4) resulted in good membrane breakdown (section 2.8). Unfortunately, fruit pulp browning resulted, and in retrospect a study examining the nature of pulp discolouration may have provided indications of alternative

methods of rind membrane disruption without discolouration. In any event, anthocyanin pigments were preserved in the desirable red form (section 2.7) using a 2 s steam treatment (95°C) followed by 4 min immersion in zero pH (section 2.6). Furthermore, this treatment proved to be a viable alternative to sulphur fumigation of 'Mauritius' litchi fruit in terms of fungal infection, as neither sulphur-fumigated (600 $g.t^{-1}$) fruit nor fruit subjected to steam (95°C) for any length of time were infected with fungi after 28 days of storage at 1°C. Untreated control fruit and those immersed in zero pH solution for 4 min only were however, completely infected by fungi, which implies that steam (95°C) sterilized the fruit, and that no reinfection occured under the storage conditions of the trial. This combination of one novel (a 2 s steam (95°C) and one established treatment (immersion of fruit in zero pH solution) represents the author's original contribution, and resulted in 'Mauritius' litchi fruit with red rinds and good internal quality after 28 days storage at 1°C. In this sense the author's contribution was facilitated by concurrent pioneering work in both Israel and Australia, and the three independent studies complementing each other.

In terms of anthocyanin biochemistry, spectrophotometry provided evidence that the pigments identified in the 'Mauritius' litchi rind were cyanidin and pelargonidin (section 4). The author accepts however, that the sugars attached to the anthocyanidins may not be what they are labelled and short of rigorous and time consuming chemical isolation of these compounds, in effect a Ph.D. in chemistry, there can be no certainty that assumptions drawn are absolute. After consultation with Drewes (1996[4]) however, it was decided that such an intensive study would not be justified.

Colour preservation of litchi rinds achieved in the present study as a result of fruit being subjected to 2 s steam (95°C) followed by 4 min immersion in zero pH solution was not the result of co-pigmentation nor self-associations. Should any of these stabilizations have taken place then new compounds would have produced distinct HPLC peaks, and this was not the case. Consequently, it appears that colour preservation in litchi rinds achieved in this study was solely a pH effect. A future, more basic study of the litchi rind anthocyanin chemical

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composition would however, be very interesting, although it would be unlikely to add to the purely applied aspects of post-harvest fruit treatments.

Polyphenol oxidase has been implicated in enzymatic browning of litchi rinds by several workers, but Underhill and Critchley (1993) suggested that it is unlikely that polyphenol oxidase plays a major role in rind browning under ambient conditions, since concentrations in harvested fruit are relatively low. The author believes that provided the substrate is not lacking, these lower levels of polyphenol oxidase will still cause browning of litchi rinds albeit at a reduced rate. Indeed the results of this investigation indicated that a steam treatment (95°C) significantly reduced polyphenol oxidase activity, and this was complemented when used in conjunction with zero pH solution. Underhill and Critchley (1995) maintained that peroxidase activity is more important in litchi rind browning than previously thought. Peroxidase activity has only been identified in the vascular traces of the exocarp and mesocarp of the rind, and it seems unlikely that peroxidase plays a major role in post-harvest rind browning. This hypothesis was not confirmed in the present study and future investigations could examine this aspect of litchi rind browning. In hindsight, the present study may have been improved if the author had better access to a larger number of fruit, over a longer harvest period. Unfortunately, all these constraints were beyond the author's control and despite co-operative research trips to Tzaneen in January, and Thailand in May of 1995 and 1996, the problems were not solved as adequate research facilities to perform detailed scientific investigations were not available at either of these centres.

Semi-commercial trials using 2 s steam followed by 4 min in zero pH solution as a post-harvest treatment for litchis were undertaken in 1995 and 1996 in Mpumalanga province, South Africa and in conjunction with the Thailand Institute for Scientific and Technological Research in Bangkok, Thailand, and proved most successful for 'Mauritius' and 'Hong Huay'. Some criticism has been levelled at steam treatment, as the general grower perception is that steam generators are costly, and are not justifiable for a crop that has a harvest season of at most one month for a given area in South Africa. Consequently, there is scope for future research aimed at solubilizing cell and organelle membranes by means other than steam. Techniques which should be investigated include ozonation, fluorine and chlorine gas, all of which are free radical scavengers and should effect rind cell membrane break down.

These chemicals are however, dangerous and precise application is imperative. Finally, with slight modifications the findings of this study may well be applicable to other litchi cultivars, and promising results were obtained for 'Three Months Red' and 'Hong Huay' but not 'Maclean Red', and indeed other subtropical and tropical fruits where red rind colour is imparted by anthocyanins and is short lived, eg. rambutans. Further investigations are recommended.

SUMMARY

Anthocyanins and their corresponding anthocyanidins are extremely unstable in litchi rinds, and if they are not treated after harvest, will undergo structural changes forming colourless compounds. Under extreme conditions they may even break down resulting in brown phenolic compounds. In South Africa, litchi fruit are fumigated with sulphur immediately after harvest to prevent rinds from turning brown. Sulphur is undesirable because of health reasons, residues, aftertaste, non-efficacy against some fungi, and it renders the fruit an unsightly yellowish green colour. Consequently, it was imperative that research aimed at eliminating sulphur fumigation be undertaken. Indeed this topic has been the South African Litchi Growers' Association number one research priority for the last 5 years (Milne, 1996[5]).

A detailed review of the literature led the author to the conclusion that anthocyanins could be fixed *in situ* thereby eliminating the necessity for sulphur fumigation. Several attempts were made at solubilizing or breaking down the functional integrity of rind cell membranes to expose anthocyanins to low pH solutions, which were shown by Zaubermann et al. (1990; 1991) to stabilize the desirable red rind colour. Initially, hydrogen peroxide, sodium hypochlorite and cationic, anionic and non-ionic detergents were used but were only partially successful. The use of heat distilled water at 56° and 65°C was also investigated, but these temperatures proved unsuccessful. Concurrent active research in Australia (Underhill, 1992; Underhill and Critchley 1993) showed that temperatures of up to 60°C for 10 min induced rind browning, probably by stimulating polyphenol oxidase activity. Consequently, the use of boiling water was investigated as it was hoped that this extreme temperature would inactivate browning enzymes. A 30 s dip in boiling water (98°C) resulted in excellent uptake of 1% Safranin Fast Green, which stains cytoplasmic contents, after 4 min, providing circumstantial evidence of membrane break down. Later, transmission electron microscopy confirmed that this treatment resulted in excellent membrane breakdown. When fruit were immersed for 4 min in zero pH solution, made by adjusting the pH of distilled water to 0.0

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with 32% hydrochloric acid, immediately after a 30 s dip in boiling water (98°C), red rind colour was still fixed after 28 days storage at 1°C. Unfortunately, some of the heat of boiling water was transferred to the top few layers of cells in the fruit pulp and resulted in death and subsequent unsightly browning of these cells.

The author then substituted steam (95°C) for boiling water (98°C) as it was hypothesized that the effects of steam, being a vapour, would be localized on the surface of fruit. Furthermore, the latent heat of steam imparted by condensation is some three times that of boiling water. Consequently, application of steam is an intense burst of energy on the fruit surface, provided treatment time is sufficiently short. This hypothesis proved correct and fruit subjected to 2 s steam (95°C) followed by 4 min in zero pH solution had excellent red colour, normal pulp colour, and tasted similar to control fruit after 28 days storage at 1°C. Ultrastructural studies showed that 2 s steam (95°C) resulted in membrane breakdown of rind mesocarp cells, but this was enhanced when used in conjunction with 4 min in zero pH solution. Further evidence of membrane breakdown was provided by electrolytic leakage studies which showed that rinds of untreated control fruit had lowest electrolytic leakage, while those of fruit immersed for 30 s in boiling water (98°C), and subjected to 2 s steam (95°C) had highest electrolytic leakage.

A treatment of 2 s steam (95°C) was required to break down rind cell membranes to allow access to anthocyanins. However, by shattering the cuticle and solubilizing cell and organelle membranes, the barrier between fruit and the atmosphere is removed. This allows moisture to escape from fruit rinds and flesh, which then shrivel. In addition, total soluble solids levels (TSS) of the fruit flesh increase in these fruit as a direct consequence of water loss. Consequently, some form of artificial rind covering was necessary to maintain moisture levels after treatment. Fruit which were dipped in 1% Vaporgard® after 2 s steam (95°C) followed by 4 min in zero pH solution were firm, and had similar flesh TSS levels to control fruit after 28 days storage at 1°C.

It was thought that 2 s steam followed by 4 min in zero pH solution might result in chemical residues on the fruit rind surface since zero pH solution is made using hydrochloric acid. Consequently, energy dispersive x-ray microanalysis was undertaken to compare chemical

element concentrations on the outer and inner surfaces of rinds of control fruit, sulphur-fumigated (600 $g.t^{-1}$ fruit) fruit and fruit subjected to 2 s steam (95°C) followed by 4 min in zero pH solution. Chlorine concentrations were relatively high in fruit subjected to 2 s steam (95°C) followed by 4 min in zero pH solution, but aftertastes were not an issue in the fruit pulp. Sulphur concentrations were high in rinds of sulphur-fumigated fruit, but were also present at low concentrations in non-sulphur-fumigated fruit.

Colour quantification of rind colour confirmed visual assessments as control fruit lightened when stored at 30°C and hue changed from red to reddish orange. Rinds of fruit subjected to 2 s steam (95°C) only, lost colour rapidly and were a pale yellow hue 24 hr after treatment. Hue of rinds of fruit subjected to 2 s steam (95°C) followed by 4 min in zero pH solution changed from reddish orange to red within 4 hr and then became darker up to 24 hr after treatment, indicating that 2 s steam (95°C) followed by 4 min in zero pH solution actually enhances red fruit colour. Red colour was maintained in fruit held at 30°C for 72 hr but became lighter after 24 hr. Monitoring of individual anthocyanin concentrations confirmed these findings as the presumed cyanidin-3-rutinoside, pelargonidin-3-glucoside and pelargonidin-3,5-diglucoside all decreased proportionally in control fruit over 5 days storage at 30°C. Concentrations of cyanidin-3-rutinoside in fruit subjected to 2 s steam (95°C) followed by 4 min in zero pH solution increased immediately after treatment but then decreased by 4 days after treatment at 30°C.

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APPENDIX 1

cf. accompanying computer disk

APPENDIX 2

Programme written by the author for measuring polyphenol oxidase activity using a Beckman® DU65 Spectrophotometer where change in absorbance is read over 30 sec at 410 ηm.

001: Strt
002: 410
003: LMDA
004: ABS
005: CALB
006: clrE
007: MSG cINS
008: MSG cERT
010: MSG cSAMP
011: MSG cLE
012: R/S
013: 0.000
014: STO 000
015: 36.000
016: CALL ONT
017: READ
018: RCL 000
019: E+
020: CALL POUT
021: XCHG
022: prt
023: XCHG
024: 5.000
025: +
026: STO 000
027: lbl B
028: CALL GETT
029: 5.000
030: x<=y
031: GOTO B
032: dec 001
033: GOTO A
034: lr
035: CALL CRLF
036: rtn
037: MSG c
038: rtn

APPENDIX 3

Plate 4

Figure 47 Chromatograph of rind anthocyanins of untreated, mature, red 'Mauritius' litchi fruit on the day of harvest. AU = absorbance units, tentatively cy-r = cyanidin-3-rutinoside, p-g = pelargonidin-3-glucoside, p-d = pelargonidin-3,5-diglucoside

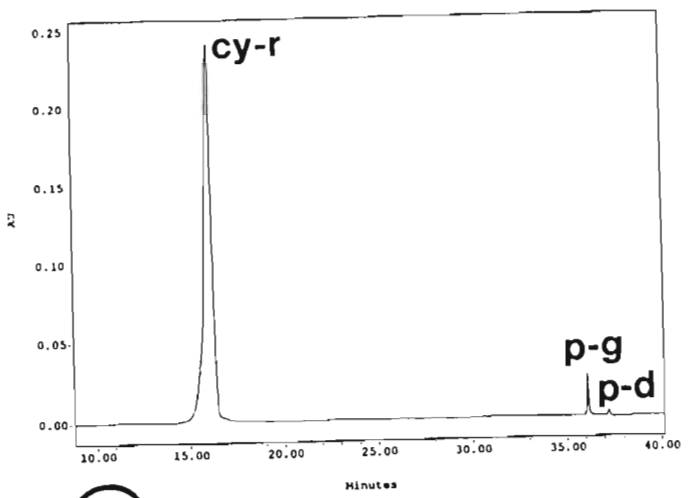
Figure 48 Chromatograph of rind anthocyanins of untreated, mature, red 'Mauritius' litchi fruit one day after harvest. AU = absorbance units, tentatively cy-r = cyanidin-3-rutinoside, p-g = pelargonidin-3-glucoside, p-d = pelargonidin-3,5-diglucoside

Figure 49 Chromatograph of rind anthocyanins of untreated, mature, red 'Mauritius' litchi fruit two days after harvest. AU = absorbance units, tentatively cy-r = cyanidin-3-rutinoside, p-g = pelargonidin-3-glucoside, p-d = pelargonidin-3,5-diglucoside

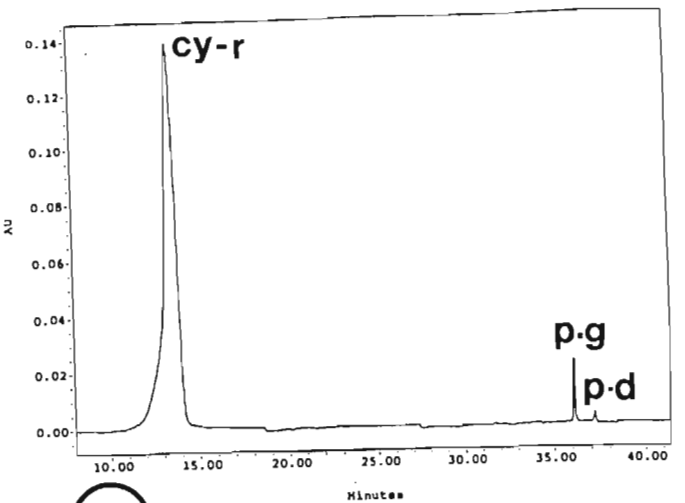
Figure 50 Chromatograph of rind anthocyanins of untreated, mature, red 'Mauritius' litchi fruit three days after harvest. AU = absorbance units, tentatively cy-r = cyanidin-3-rutinoside, p-g = pelargonidin-3-glucoside, p-d = pelargonidin-3,5-diglucoside

Figure 51 Chromatograph of rind anthocyanins of untreated, mature, red 'Mauritius' litchi fruit four days after harvest. AU = absorbance units, tentatively cy-r = cyanidin-3-rutinoside, p-g = pelargonidin-3-glucoside, p-d = pelargonidin-3,5-diglucoside

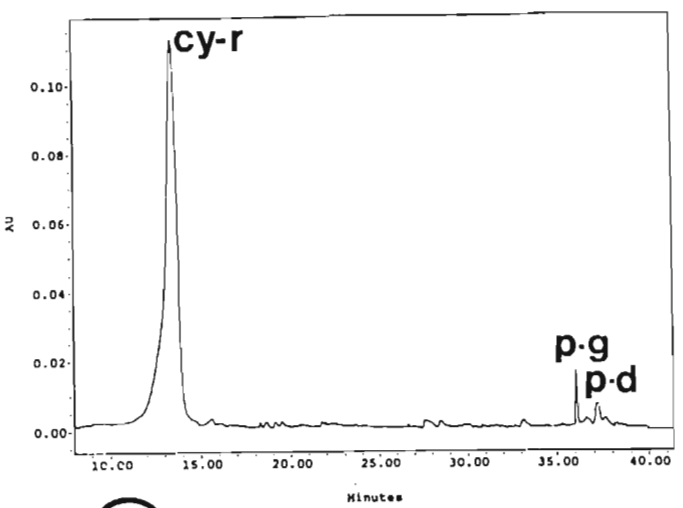
Figure 52 Chromatograph of rind anthocyanins of untreated, mature, red 'Mauritius' litchi fruit five days after harvest. AU = absorbance units, tentatively cy-r = cyanidin-3-rutinoside, p-g = pelargonidin-3-glucoside, p-d = pelargonidin-3,5-diglucoside



47



48



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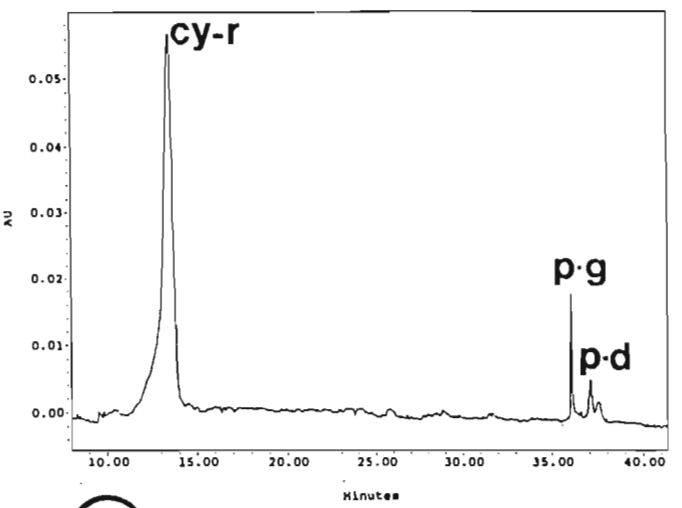

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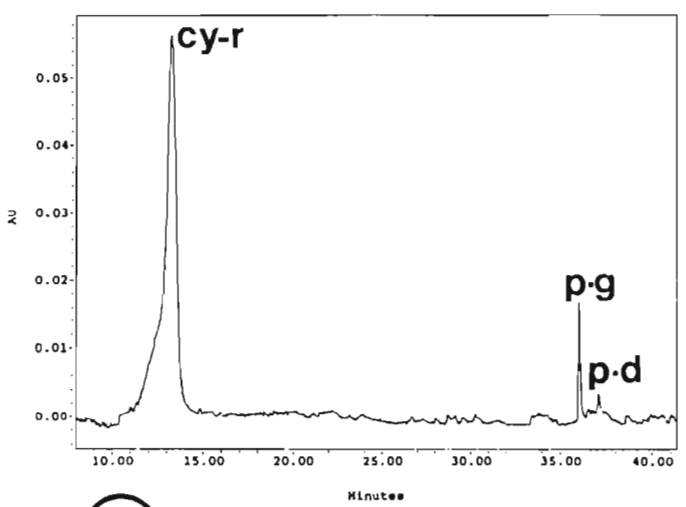
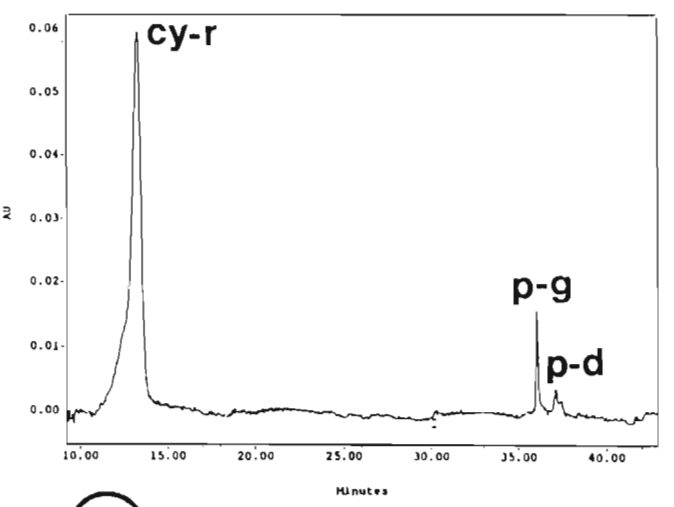


51



52

Plate 5

Figure 53 Chromatograph of rind anthocyanins of untreated, mature, red 'Mauritius' litchi fruit crushed on the day of harvest and sampled after 1.5 hr. AU = absorbance units, tentatively cy-r = cyanidin-3-rutinoside, p-g = pelargonidin-3-glucoside, p-d = pelargonidin-3,5-diglucoside

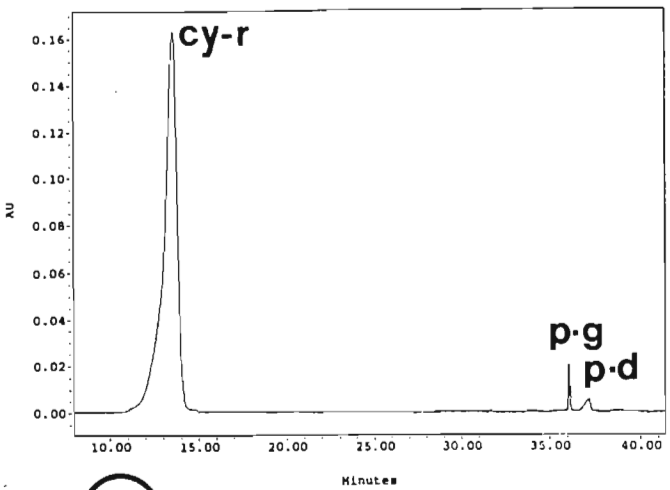
Figure 54 Chromatograph of rind anthocyanins of untreated, mature, red 'Mauritius' litchi fruit crushed on the day of harvest and sampled after 3 hr. AU = absorbance units, tentatively cy-r = cyanidin-3-rutinoside, p-g = pelargonidin-3-glucoside, p-d = pelargonidin-3,5-diglucoside

Figure 55 Chromatograph of rind anthocyanins of untreated, mature, red 'Mauritius' litchi fruit crushed on the day of harvest and sampled after 4.5 hr. AU = absorbance units, tentatively cy-r = cyanidin-3-rutinoside, p-g = pelargonidin-3-glucoside, p-d = pelargonidin-3,5-diglucoside

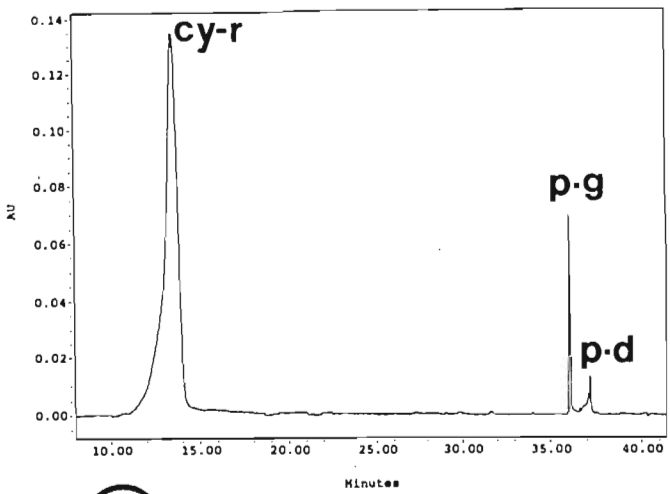
Figure 56 Chromatograph of rind anthocyanins of untreated, mature, red 'Mauritius' litchi fruit crushed on the day of harvest and sampled after 6 hr. AU = absorbance units, tentatively cy-r = cyanidin-3-rutinoside, p-g = pelargonidin-3-glucoside, p-d = pelargonidin-3,5-diglucoside

Figure 57 Chromatograph of rind anthocyanins of untreated, mature, red 'Mauritius' litchi fruit crushed on the day of harvest and sampled after 7.5 hr. AU = absorbance units, tentatively cy-r = cyanidin-3-rutinoside, p-g = pelargonidin-3-glucoside, p-d = pelargonidin-3,5-diglucoside

Figure 58 Chromatograph of rind anthocyanins of untreated, mature, red 'Mauritius' litchi fruit crushed on the day of harvest and sampled after 9 hr. AU = absorbance units, tentatively cy-r = cyanidin-3-rutinoside, p-g = pelargonidin-3-glucoside, p-d = pelargonidin-3,5-diglucoside



53



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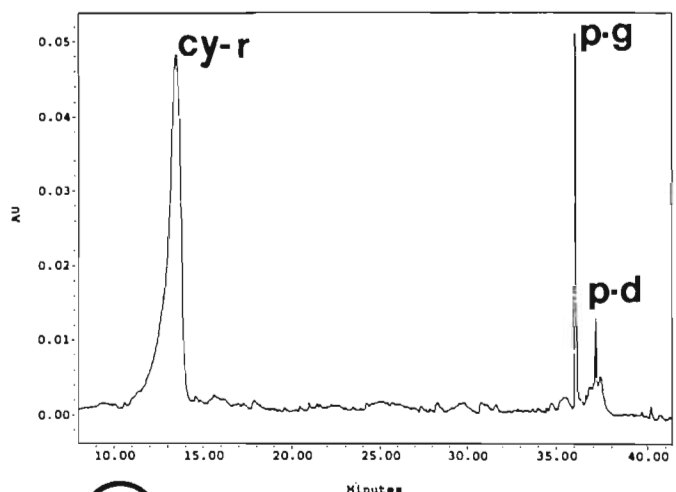

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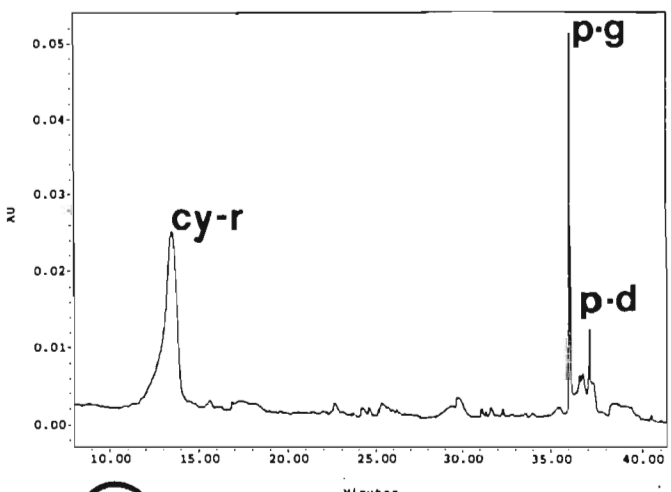

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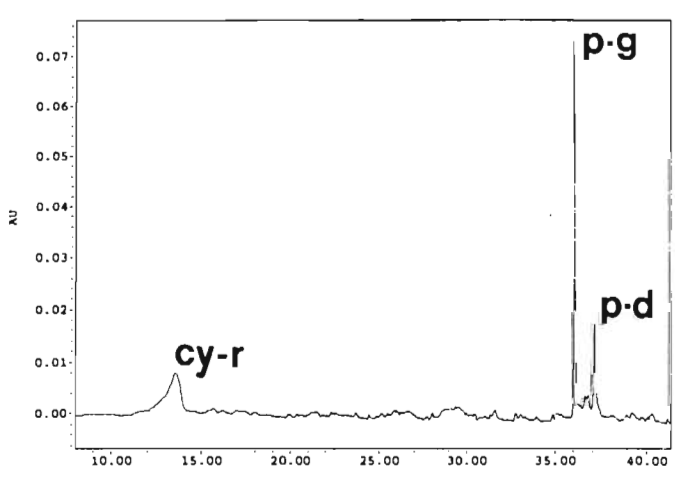

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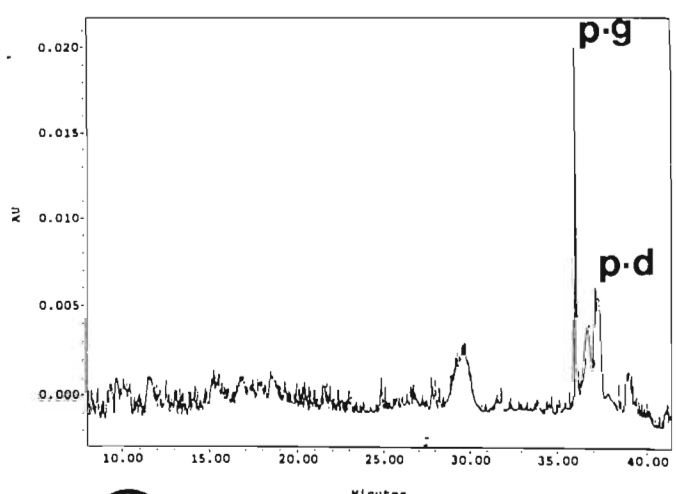


58

Plate 6

Figure 59 Chromatograph of rind anthocyanins of 'Mauritius' litchi fruit subjected to 2 s steam (95°C) followed by 4 min in zero pH solution on the day of harvest, immediately after treatment. AU = absorbance units, tentatively cy-r = cyanidin-3-rutinoside, p-g = pelargonidin-3-glucoside, p-d = pelargonidin-3,5-diglucoside

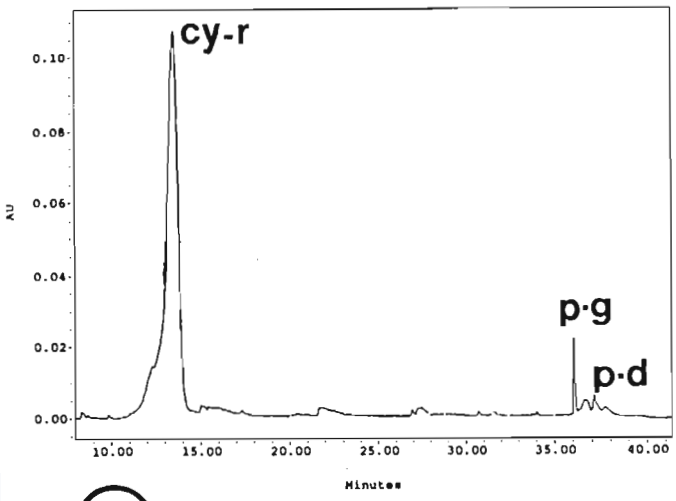
Figure 60 Chromatograph of rind anthocyanins of 'Mauritius' litchi fruit subjected to 2 s steam (95°C) followed by 4 min in zero pH solution on the day of harvest, 12 hr after treatment. AU = absorbance units, tentatively cy-r = cyanidin-3-rutinoside, p-g = pelargonidin-3-glucoside

Figure 61 Chromatograph of rind anthocyanins of 'Mauritius' litchi fruit subjected to 2 s steam (95°C) followed by 4 min in zero pH solution on the day of harvest, 24 hr after treatment. AU = absorbance units, tentatively cy-r = cyanidin-3-rutinoside, p-glu = pelargonidin-3-glucoside, p-d = pelargonidin-3,5-diglucoside

Figure 62 Chromatograph of rind anthocyanins of 'Mauritius' litchi fruit subjected to 2 s steam (95°C) followed by 4 min in zero pH solution on the day of harvest, 2 days after treatment. AU = absorbance units, tentatively cy-r = cyanidin-3-rutinoside, p-g = pelargonidin-3-glucoside, p-d = pelargonidin-3,5-diglucoside

Figure 63 Chromatograph of rind anthocyanins of 'Mauritius' litchi fruit subjected to 2 s steam (95°C) followed by 4 min in zero pH solution on the day of harvest, 3 days after treatment. AU = absorbance units, tentatively cy-r = cyanidin-3-rutinoside, p-g = pelargonidin-3-glucoside, p-d = pelargonidin-3,5-diglucoside

Figure 64 Chromatograph of rind anthocyanins of 'Mauritius' litchi fruit subjected to 2 s steam (95°C) followed by 4 min in zero pH solution on the day of harvest, 4 days after treatment. AU = absorbance units, tentatively cy-r = cyanidin-3-rutinoside, p-g = pelargonidin-3-glucoside, p-d = pelargonidin-3,5-diglucoside



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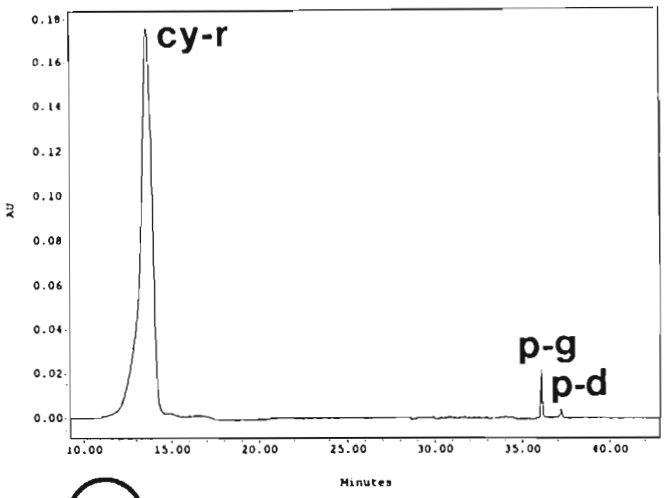

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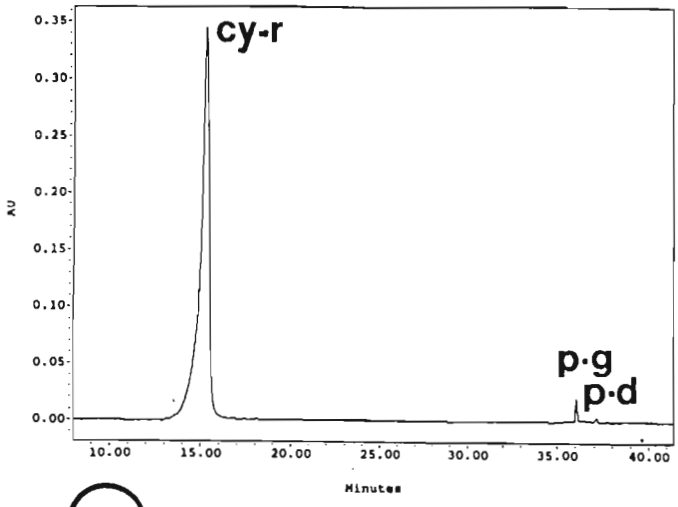

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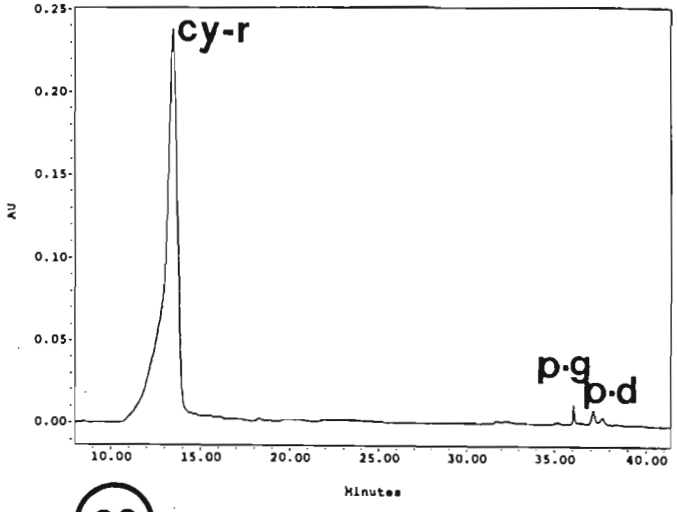

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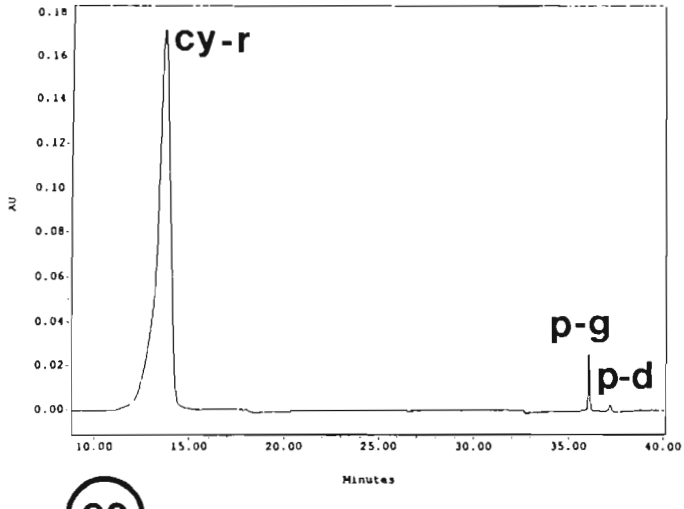

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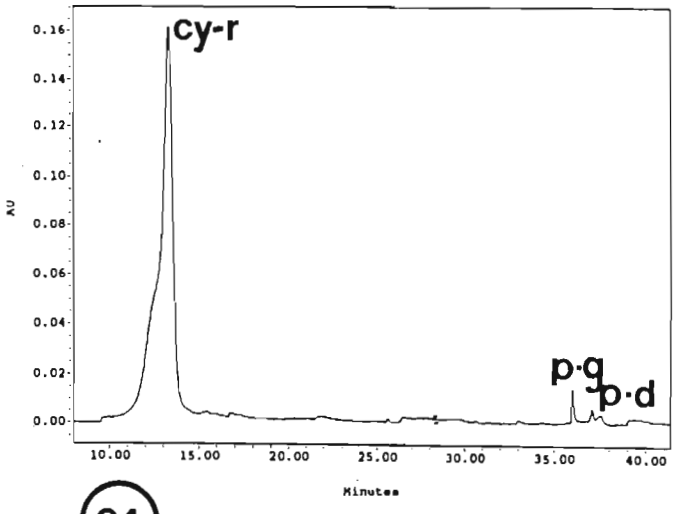


64

Plate 7

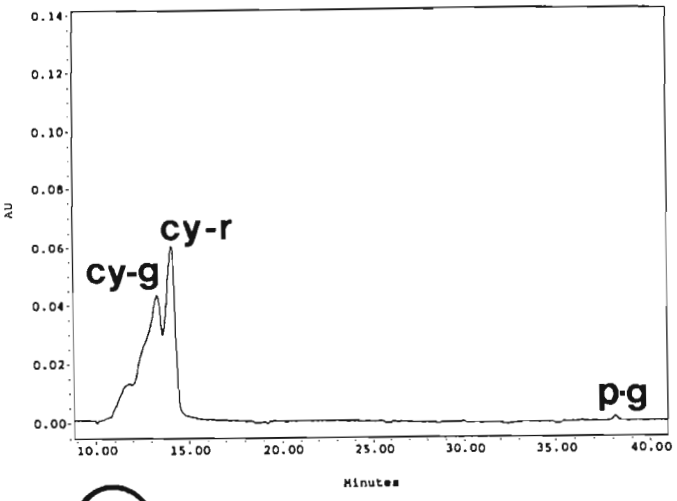
Figure 65 Chromatograph of rind anthocyanins of sulphur-fumigated ($600g.t^{-1}$) 'Mauritius' litchi fruit treated on the day of harvest, after 28 days of storage at 1°C. AU = absorbance units, tentatively cy-g = cyanidin-3-glucoside, cy-r = cyanidin-3-rutinoside, p-g = pelargonidin-3-glucoside

Figure 66 Chromatograph of rind anthocyanins of 'Mauritius' litchi fruit subjected to 4 min in zero pH solution only on the day of harvest, after 28 days of storage at 1°C. AU = absorbance units, tentatively cy-g = cyanidin-3-glucoside, cy-r = cyanidin-3-rutinoside, p-g = pelargonidin-3-glucoside

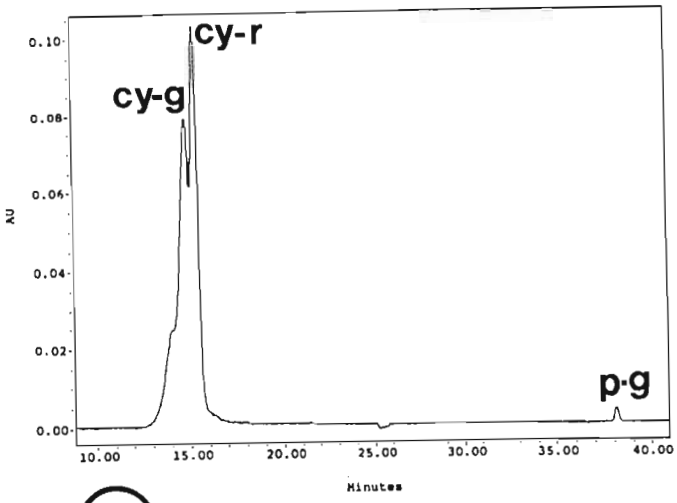
Figure 67 Chromatograph of rind anthocyanins of 'Mauritius' litchi fruit immersed in boiling water (98°C) followed by 4 min in zero pH solution on the day of harvest, after 28 days of storage at 1°C. AU = absorbance units, tentatively cy-g = cyanidin-3-glucoside, cy-r = cyanidin-3-rutinoside, p-g = pelargonidin-3-glucoside

Figure 68 Chromatograph of rind anthocyanins of 'Mauritius' litchi fruit subjected to 2 s steam (95°C) only on the day of harvest, after 28 days of storage at 1°C. AU = absorbance units, tentatively cy-g = cyanidin-3-glucoside, cy-r = cyanidin-3-rutinoside, p-g = pelargonidin-3-glucoside, p-d = pelargonidin-3,5-diglucoside

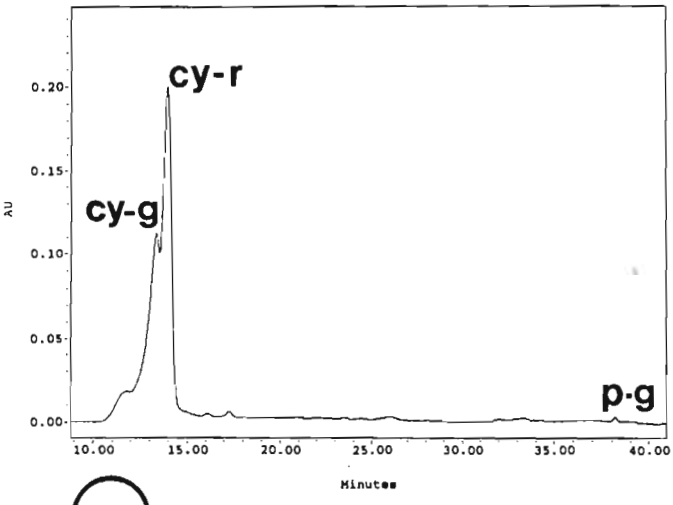
Figure 69 Chromatograph of rind anthocyanins of 'Mauritius' litchi fruit subjected to 2 s steam (95°C) followed by 4 min in zero pH solution, after 28 days of storage at 1°C. AU = absorbance units, tentatively cy-r = cyanidin-3-rutinoside, p-g = pelargonidin-3-glucoside, p-d = pelargonidin-3,5-diglucoside


cy-g
cy-r
p-g
AU
Minutes

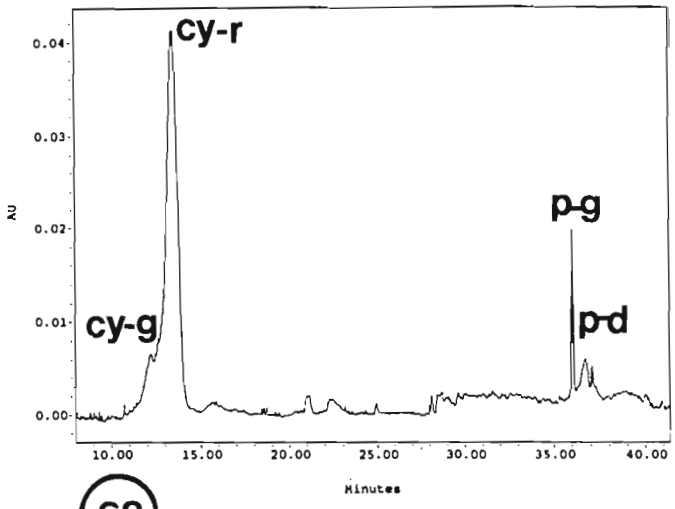
65


cy-r
cy-g
p-g
AU
Minutes

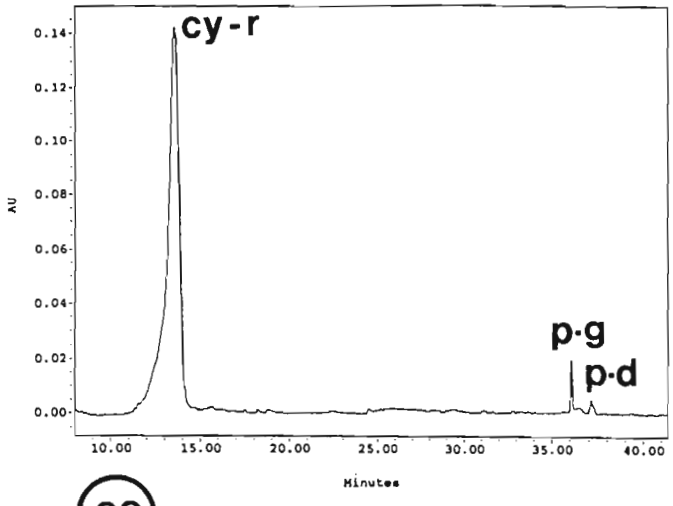
66


cy-r
cy-g
p-g
AU
Minutes

67


cy-r
p-g
cy-g
p-d
AU
Minutes

68


cy-r
p-g
p-d
AU
Minutes

69