Temperature and Atmosphere Composition Influence on Colour Change of Apples.

A dissertation presented in partial fulfilment of the requirements for a Masterate of Horticultural Science.

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Abstract

In apples colour is a major quality parameter used by consumers to determine apple maturity. A full understanding of the nature of the relationship between storage conditions and apple fruit colour change would be of advantage in formulating models to predict how changes to handling systems would affect fruit colour. While much is known in a general way about how environmental conditions affect colour change, little information is available to characterise the nature of the relationships between temperature, oxygen and carbon dioxide.

The postharvest change in colour was measured for two export apple cultivars; Cox's Orange Pippin and Granny Smith. Previous research on these and other apple cultivars has determined that colour change is from green to yellow. The colour of Cox's Orange Pippin and Granny Smith apples were measured by subjective and objective methods during experiments to investigate the effect of temperature and atmosphere composition on colour change. The objective methods used were: chlorophyll extraction and colour using a Minolta chromameter. The subjective method was colour matching for Granny Smith using the NZAPMB maturity colour charts. When related to changes in chlorophyll, the principal skin pigment, the colour parameters used had non-linear relationships. Lightness, hue angle and colour chart score all reflect pigment changes occurring as apples change colour from green to yellow. Lightness values were the least variable followed by hue angle then colour chart score. All methods used showed more sensitivity to changes in chlorophyll content when chlorophyll content was low compared to when chlorophyll content was high. The objective measurements were highly correlated with the subjective measurements and the conclusion was that the use of hue angle or lightness to follow colour change in the skin of Granny Smith and Cox's Orange Pippin apples is an accurate indirect measure of chlorophyll and other pigments.

The rate constant of colour change (k), measured using a declining exponential function, from green to yellow, at eleven temperatures over two seasons, two

harvests per season and several growers was investigated in order to characterise the relationship between yellowing and temperature. All the methods of colour measurement used had the same relationship with temperature which was described by a modified form of the Arrenhius equation. Re-worked published data also fitted the modified Arrenhius equation. The modified Arrenhius equation was used to generate ***k*** for the various colour parameters measured (chlorophyll, hue angle, lightness and colour charts score). The value of ***k***, as a function of temperature, increases slowly between 0°C and 6°C (the lag phase), increases exponentially between 6°C and 20°C and reaches a maximum at 25.3°C for Cox's Orange Pippin and 23.5°C for Granny Smith before declining. Pattern of response to temperature was the same for each cultivar although Granny Smith yellowed more slowly than Cox's Orange Pippin. For Cox's Orange Pippin apples more variation was accounted for by differences between growers than years or harvests within a year. For Granny Smith fruit most variation was accounted for by differences between years.

Sixteen atmospheres were used each year for Cox's Orange Pippin and Granny Smith apples from one harvest in order to characterise the relationship between yellowing and oxygen or carbon dioxide. Cox's Orange Pippin and Granny Smith apples differ in their response to oxygen. For Cox's Orange Pippin the value of ***k*** as a function of oxygen level increased slowly from 0% to 6% and thereafter increased exponentially from 6% to 19%. This function may be sigmoidal as the ***k*** values increase slows above 17% oxygen. The relationship for Granny Smith was poorly defined by this function, ***k*** values increased slowly as the oxygen level rose. This could be due to a fundamental physiological or biochemical difference between these two cultivars. Each cultivar had a similar response to carbon dioxide, described by a declining exponential function, with the relationship for Granny Smith being better defined than for Cox's Orange Pippin. The relationship of carbon dioxide with colour change was poorly defined as the effects of oxygen on colour change were not removed from the analysis.

Oxygen appears to have a greater influence on colour change than carbon dioxide. Atmospheres for Cox's Orange Pippin apples were not scrubbed for carbon dioxide in 1989 but were in 1990. The pattern of response to oxygen in the absence of levels of carbon dioxide above 1% in the atmosphere did not alter the sigmoidal relationship found. This may be evidence that the effect on yellowing by oxygen and carbon dioxide is by separate processes. Ethylene levels in the atmosphere appeared to have little effect on the rate of yellowing in all the atmospheres studied. The carbon dioxide and oxygen functions were combined into a single equation for use as a predictive model.

The temperature function, the modified Arrenhius equation, and the atmosphere functions were combined into one equation to which different environmental values were added. The use of such a model and other practical applications for the information gathered for this thesis are discussed and a chart drawn comparing the hue angle, lightness and colour chart score to chlorophyll level.

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List of Abbreviations

ΔH = change in enthalpy $J.(kg.mol)^{-1}$.

ΔS = change in entropy $J.(kg.mol)^{-1}.K^{-1}$

$A = K_a$

A_r = rate constant of the asymptote

A_o = chlorophyll concentration at time zero

A_t = chlorophyll concentration at time t

$B = E_a/R$

$C = \Delta S/R$

CO_2 = percent carbon dioxide

$D = \Delta H/R$

D_e = decay in specific rate constant of change

E_a = activation energy $J.(kg.mol)^{-1}$

k = rate constant of reaction

K_a = rate constant of a process if there is no inhibition

K_{CO2} = rate constant for carbon dioxide

O_2 = percent oxygen

R = gas constant 8314 $J.(kg.mol)^{-1}.K^{-1}$

t = time

T = temperature K

Y_o = mean value at zero concentration

$K_{o_2co_2}^{20^oC}$ = rate constant at 20°C for a controlled atmosphere

$K_{temp}^{20^oC}$ = rate constant at 20°C in air

K_{temp}^{n} = rate constant at a temperature of *n* in air

List of Formulae

Name	Formula	
Declining exponential	$A_t = A_o * e^{-kt}$	[2.1]
Arrenhius equation	$k = K_a e^{-E_a / RT}$	[4.1]
Boltzmann enzyme distribution function	$k = 1 + e^{\Delta S/R} e^{-\Delta H/RT}$	[4.2]
Modified Arrenhius equation	$k = \frac{K_a e^{-E_a / RT}}{1 + e^{\Delta S/R} e^{-\Delta H/RT}}$	[4.3]
Simplified modified Arrenhius equation	$k = \frac{A * e^{-B/T}}{1 + e^{C - D/T}}$	[4.4]
Maximum temperature	$T_{max} = \frac{D}{C + \ln(D/B - 1)}$	[4.5]
Gompertz growth function	$k = A_o \frac{e^{Y_o(1 - e^{-D_e O_2})}}{D_e}$	[5.1]
Carbon dioxide declining exponential function	$k = A_r + A_o e^{-K_{CO_2} CO_2}$	[5.2]
Combined Gompertz-declining exponential function	$k = A_o \frac{e^{Y_o(1 - e^{-D_e O_2})}}{D_e} e^{-K_{CO_2} CO_2}$	[5.3]

Temperature/atmosphere function

$$k = K^{20^{\circ}C}_{o_2 co_2} * \frac{K^{n}_{temp}}{K^{20^{\circ}C}_{temp}} \qquad [6.1]$$

Chapter 1.

Introduction

Fruit colour along with size, shape, freedom from rot and defects is used by consumers to assess the worth of fruit on sale. In the case of apples colour is a major quality parameter (Wills *et al* 1981) determining, in the eyes of the consumer, eating quality. To the consumer different cultivars of apples have different colour criteria, with redness being important in red skinned cultivars and yellowness important in green and red/green skinned cultivars. Additionally apple fruit quality has a different meaning to consumers of apples than to growers, handlers or retailers (Hedrick 1920). More sophisticated means of measuring fruit quality than by eye alone such as assessing fruit firmness and sweetness are not possible by apple consumers in retail outlets. A full understanding of the nature of the relationship between apple fruit colour change and methods used to maintain fruit quality would give apple marketers and handlers an advantage in prediction of how changes to handling systems would affect fruit colour. This is especially important for producers of high quality apples such as New Zealand growers in maintaining high quality standards.

Postharvest storage technologies such as storage at low temperatures and controlled or modified atmospheres are used extensively by the apple industry in New Zealand due to the large distances fruit are transported to export markets. The influence of the above storage technologies on apple colour change is well documented but the nature of the relationship is poorly defined. Specific quantitative information is scarce despite many publications mentioning the effect of various storage treatments on fruit colour.

In this thesis the changes in apple colour discussed are from green to yellow as earlier research has shown that production of red pigments depends on UV light

(Arakawa *et al* 1985, Chalmers *et al* 1973) and once harvested and placed into storage the red colour of fruit changes little compared to chlorophyll (Goldschmidt 1980). Change in colour from green to yellow for apples is result of chlorophyll breakdown with carotenoid biosynthesis playing a minor role (Gorski and Creasy 1977, Knee 1980a). Breakdown of chlorophyll represents the most conspicuous of a number of symptoms which together constitute the deteriorative process, known as senescence, that ends the functional life of plant cells (Ceppi *et al* 1987). In leaves and fruit, senescence involves many physical and metabolic processes including loss of structural integrity and progressive lessening of photosynthesis with increasing failure of synthetic chloroplast function. For example in tree leaves the saturating level of light intensity rises from 7000 lux in young leaves to about 21000 lux in fully expanded leaves and reduces to 8000 lux with increasing age (Richardson 1957). Chlorophyll content and photosynthetic rate do not necessarily follow one another closely. Even in the rapid senescence of seedling leaves, photosynthetic decline is not ascribable to chlorophyll loss, as the enzyme ribulose bisphosphate carboxylase/oxygenase (RUBISCO) is rapidly broken down in senescing leaves (Bathgate *et al* 1985).

These events are accompanied by a colour change, usually from green to yellow (Thimann 1980), but non-yellowing mutant grasses are known in which chlorophyll is retained throughout senescence (Osborne and Cheah 1982). Degradation of chlorophylls in aging plants is linked to changes both in chlorophylls themselves and other plant pigments (Hendry *et al* 1987). In millet, chlorophyll a and b concentrations decrease by about 83% while the concentration of carotenoids remain stable during senescence (Embry and Nothnagel 1988). Timing of leaf or fruit senescence appears to be controlled by extrinsic and intrinsic factors, the response being determined by events taking place in other parts of the plant and by genetic constitution of the leaf and the fruit. Disassembly of cell organelles is thought to be polygenically regulated, depending on a complex of tightly co-ordinated intracellular enzymatic agents (Ceppi *et al* 1987, Thomas and Stoddart 1980).

In recent years biochemical studies on chlorophyll catabolism have concentrated on the following lines of enquiry:

(i) Enzymatic.

(a) Chlorophyllase (EC 3.1.1.14) (Terpstra 1981, Shimokawa 1982).

Upsurge in chlorophyllase activity is found in ethylene treated citrus fruit and in senescing leaves (Sabater and Rodriguez 1978).

(b) Oxidative and peroxidative enzyme systems (Huff 1982, Martinoia *et al* 1982).

Model systems in which thylakoids fortified with linolenic acid rapidly degrade chlorophyll (Luthy *et al* 1984). The presence of an enzyme responsible for removal of Mg^{+} from the tetrapyrrole ring has also been investigated (Owens and Falkowski 1982).

(ii) Biochemical/biophysical changes.

(a) *In vivo* spectroscopy of senescing fruit to detect changes in biochemical/biophysical pigments associated with ripening and senescence (Gross and Ohad 1983).

(iv) Breakdown products, for example, 13^{2}-hydroxychlorophyll a as a breakdown intermediate (Schoch *et al* 1984, Maunders *et al* 1983).

Many attempts have been made to identify products of chlorophyll breakdown which remain elusive due to rapid disappearance of chlorophyll from senescing tissues. A similar lack of knowledge also applies to carotenoids of senescing tissue which undergo destruction before, during or after chlorophyll breakdown.

The following literature review outlines current knowledge and understanding of yellowing in plants and fruit in particular.

1.1 Structure and Location of Chlorophyll

1.1.1 Chlorophyll Structure

The structure of chlorophylls a and b are shown in Figure 1.1. Both chlorophylls are derivatives of dihyroporphyrin chelated with a centrally located magnesium atom, all contained an isocyclic ring. Chlorophylls are hydrophobic because of the C_{20} mono-unsaturated isoprenoid alcohol, phytol (which is esterified) with its double bond in the trans configuration (Schwartz and Lorenzo 1990). Chlorophyll is present in chloroplasts complexed with protein but the nature of binding is not well understood. Since chlorophylls are readily extracted with organic solvents, covalent linkages to other components are not present. Historically, a number of generic names for the chlorophylls and their derivatives have been accepted and are outlined in Table 1.1. Figure 1.2 indicates the relationship of the chlorophylls to their major derivatives. The central Mg atom is easily removed, particularly under acidic conditions, being replaced with hydrogen and thus forming the pheophytins.

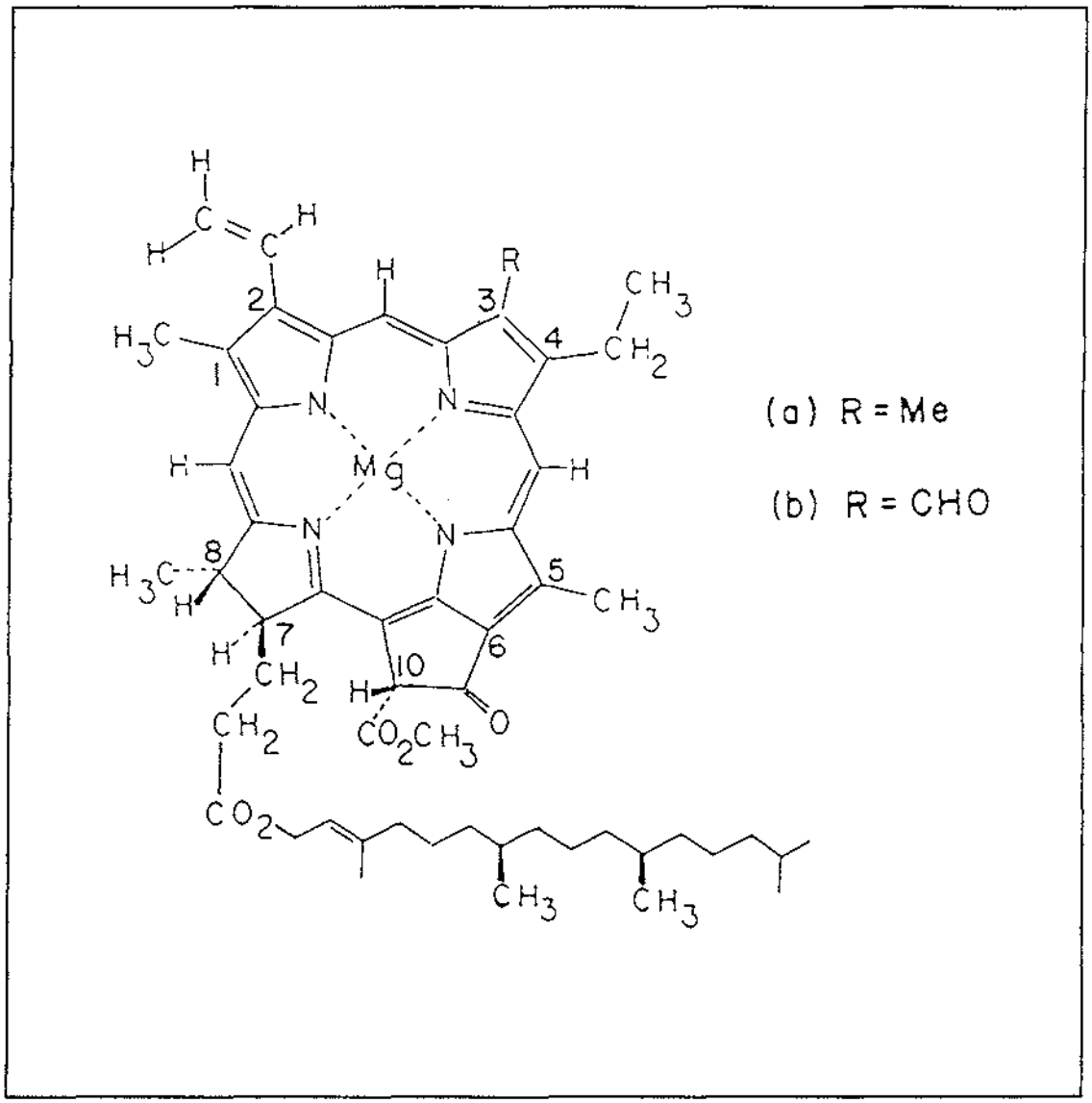
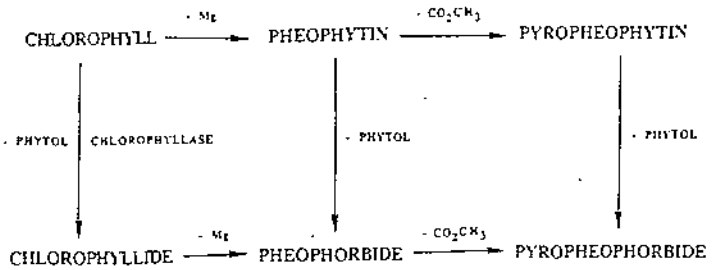


Figure 1.1 Structure of chlorophylls (Schwartz and Lorenzo 1990).



Phyllins:	Chlorophyll derivatives containing magnesium
Pheophytins:	The magnesium-free derivatives of the chlorophylls
Chlorophyllide:	The acid derivative resulting from enzymic or chemical hydrolysis of the C_7 propionate ester
Chlorophyllase:	The enzyme present in leaves which catalyzes hydrolysis of the C_7 propionate ester
Pheophorbides:	The products containing a C_7 propionic acid resulting from removal of magnesium and hydrolysis of the phytyl ester; the corresponding 7-propionate methyl (or ethyl) ester is, however, somewhat unsystematically named as methyl (or ethyl) pheophorbide
"Meso" compounds:	Derivatives in which the C-2 vinyl group has been reduced to ethyl
"Pyro" compounds:	Derivatives in which the C-10 carbomethoxy group has been replaced by hydrogen
Chlorins *e*:	Derivatives of pheophorbide *a* resulting from cleavage of the isocyclic ring; these are usually given a subscript number, e.g., chlorine e_6 specifies a product with six oxygen atoms (in three ester groups)
Rhodins *g*:	The corresponding derivatives from pheophorbide *b*

Table 1.1 Relationship of chlorophyll to some of its derivatives (Schwartz and Lorenzo 1990).

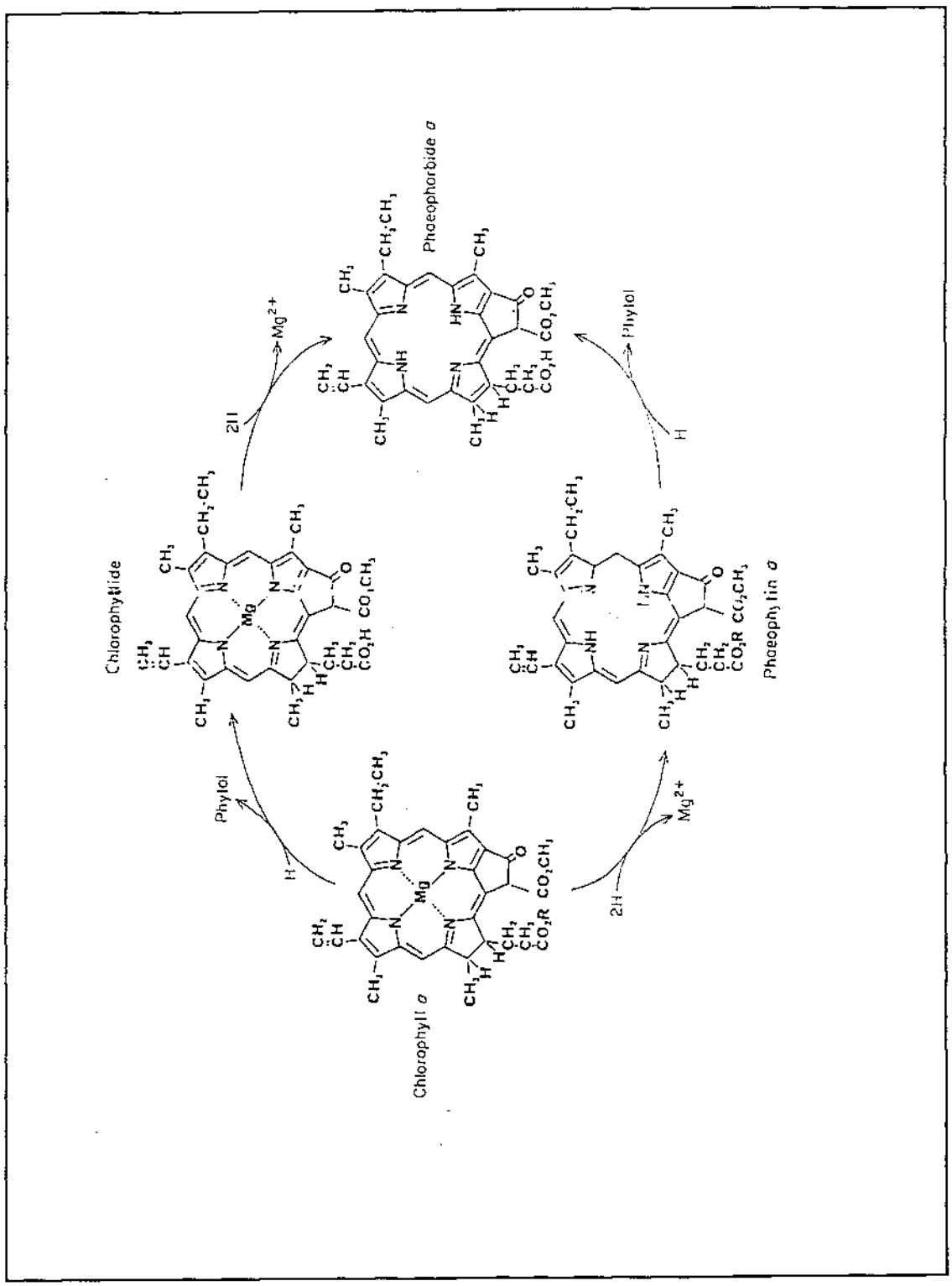


Figure 1.2 Formation of chlorophyll derivatives by demetalation and dephytolation (Hendry *et al* 1987).

1.1.2 Organelle Changes

Differentiation of chloroplasts into chromoplasts is a prominent part of senescence in mesophyll cells (Woolhouse 1984). It is an orderly process with all the features typical of developmental processes. At the organelle level chloroplast and endomembrane systems are susceptible to degradation by cytoplasmic agents (Thomas and Stoddart 1980, Hendry *et al* 1987). In leaf chloroplasts loss of plastid integrity is one of the earliest visible features of senescence and is presumably the same for fruit. The initial event in the sequence appears to be a change in characteristics of the envelope leading to separation of inner and outer membranes. Plastid disassembly appears to be mediated by agents synthesized in the cytoplasm (Duggelin *et al* 1988), and changes in envelope integrity are viewed as initial events in the transport of degradation agents into the chloroplast. Ingress of degrading enzymes may be a consequence of the decline or removal of envelope membrane components normally preventing access. It is known that enzymes associated with the outer surface of mature chloroplasts lose activity rapidly during early senescence (Davies *et al* 1990, Thomas 1977, Thomas and Stoddart 1980). Enzyme and structural protein lysis follow rapidly after envelope degradation. Chloroplast membrane proteins are rapidly degraded during yellowing.

Cells become increasingly vacuolated with age, and surviving organelles are contained in a diminishing rim of cytoplasm. Changes in permeability of the tonoplast membrane, consequent upon degradation, might allow exposure to materials which lower cytoplasmic pH thus favouring the operation of hydrolases with acidic optima or, alternatively allow transfer of these enzymes from vacuole to cytoplasm (Thomas and Stoddart 1980).

Ultrastructural studies indicate that mitochondria persist in an intact state, except for some swelling or distortion of the cristae, throughout senescent breakdown. Advancing senescence is paralleled by considerable changes in composition and physical state of microsomal membranes (Thomas and Stoddart 1980). Changes

in leaf peroxisomes may result in a release of superoxide radicals which may be involved in further membrane breakdown (Rio *et al* 1989).

It is suggested that chloroplast disintegration involves action of two proteolytic systems, one acting on stroma enzymes and extrinsic membrane proteins and other degrading intrinsic thylakoid components, including chlorophyll. Thomas *et al* (1985), using a non-yellowing mutant of *Festuca pratensis*, a meadow fescue, found this non-yellowing character to be associated with a marked structural stability of chloroplast thylakoid membranes during senescence which was reflected in retention of thylakoid proteins and pigment protein complexes and of membrane lipids (Davies *et al* 1990).

1.2 Biochemistry of Yellowing

1.2.1 Chlorophyll Breakdown

Though some chlorophyll degradation in leaves may result from photooxidation of pigment the fact that mature leaves lose chlorophyll in the dark indicates that degradation *in vivo* is at least partially enzymatic. And treatments that inhibit or destroy enzymes such as low temperatures, anaerobic conditions, boiling or freezing and desiccation of leaves during incubation greatly reduce chlorophyll loss.

Occurrence of dephytylated forms of chlorophyll in senescent leaves indicates that chlorophyllase is responsible for the initial step of chlorophyll degradation. Data available suggests that chlorophyllase is located in plastids and thylakoids (Hirschfeld and Goldschmidt 1983, Tarasenko *et al* 1986) and that its activity in senescent leaves is correlated with loss of chlorophyll (Sabater and Rodriguez 1978). The enzyme appears to be present and potentially active in mature presenescent leaves. Extraction of chlorophyllase activity requires use of acetone powders, detergents or organic solvents (Holden 1961, Schoch and Brown 1987) indicating that under natural conditions the enzyme is inactive.

Although it is known that chlorophyllase catalyzed conversion of purified chlorophyll a does not occur, or occurs only slightly, in the absence of lipids (Terpstra and Lambers 1983) it is not clear how the contact between chlorophyll molecules, complexed with their apoproteins, and enzymes is achieved in a controlled fashion in senescent chloroplasts.

Rates of destruction for chlorophylls a and b are similar according to Jen and McKinney (1970) but other authors (MacKinney and Joslyn 1940, Schwartz and Lorenzo 1990, Schwartz and von Elbe 1983) suggest that chlorophyll a is destroyed faster than chlorophyll b. For example, chlorophyll a in aqueous acetone solution reacts with acid seven to nine times more rapidly than chlorophyll b (Figure 1.3). Measurements at various temperatures of chlorophyll loss indicate that the rate of chlorophyll degradation follows first order kinetics for spinach puree (Holden 1961) and canned kiwifruit (Robertson and Swinburne 1981).

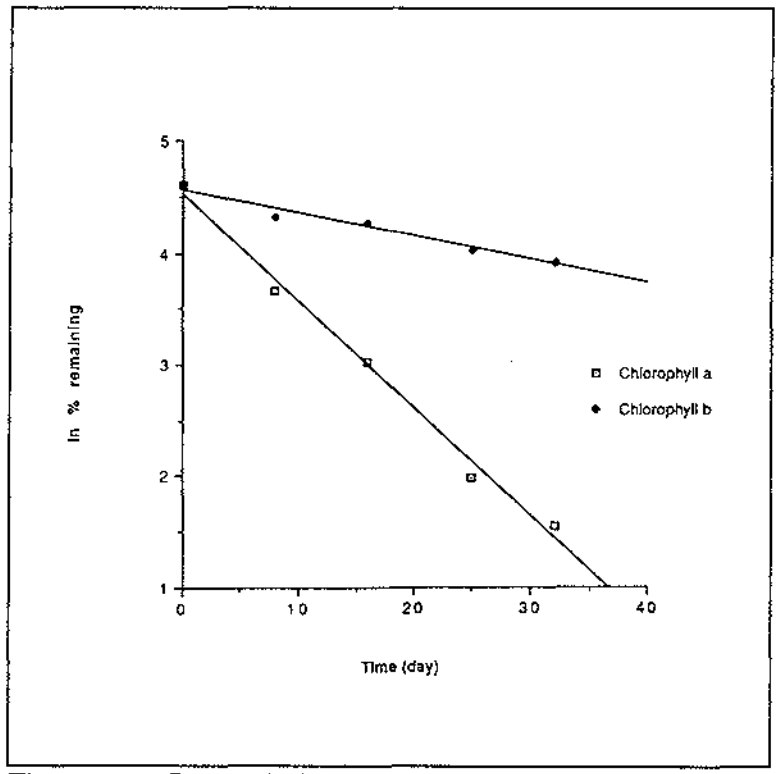


Figure 1.3 Degradation rate plot of chlorophylls a and b during storage of aseptically packaged spinach puree (Schwartz and Lorenzo 1990).

1.2.2 Proposed Pathway of Chlorophyll Breakdown

In recent years the biosynthesis of porphyrins and particularly of chlorophyll has been elucidated in great detail, but the mechanism of breakdown is largely unknown (Brown *et al* 1991). A reason for the lack of experimental data is that products of cleavage of the tetrapyrrole ring system remain undetected. Studies using non-yellowing mutants have established that synthesis and disassembly of chlorophyll-proteolipid complexes require close co-ordination of pigment and protein metabolism (Thomas *et al* 1989). Biosynthetic pathways of chlorophyll and chloroplast proteins have been intensively studied and possible points of regulatory interaction identified, but the routes where pigments and proteins are degraded are obscure (Hendry *et al* 1987). Recently more progress has been made towards establishing the pathway of chlorophyll catabolism (Matile *et al* 1987). It has been proposed that final products of chlorophyll degradation may be open-chain pyrroles and lipofuscin-like Schiff's base compounds accumulated in the cell vacuole (Hendry *et al* 1987) (Figure 1.4).

Several processes result in bleaching of chlorophyll in vitro have been described, but reaction products have not been analyzed or reactions identified unambiguously with those responsible for chlorophyll catabolism *in vivo* (Matile *et al* 1988). Chlorophyllase is the only enzyme which so far has been demonstrated to be involved in chlorophyll breakdown *in vivo* (McFeeters *et al* 1971, McFeeters 1975) as chlorophyll degradation in various systems is associated with the appearance of chlorophyllide and pheophorbide (Amir-Shapira 1987, Thomas *et al* 1989).

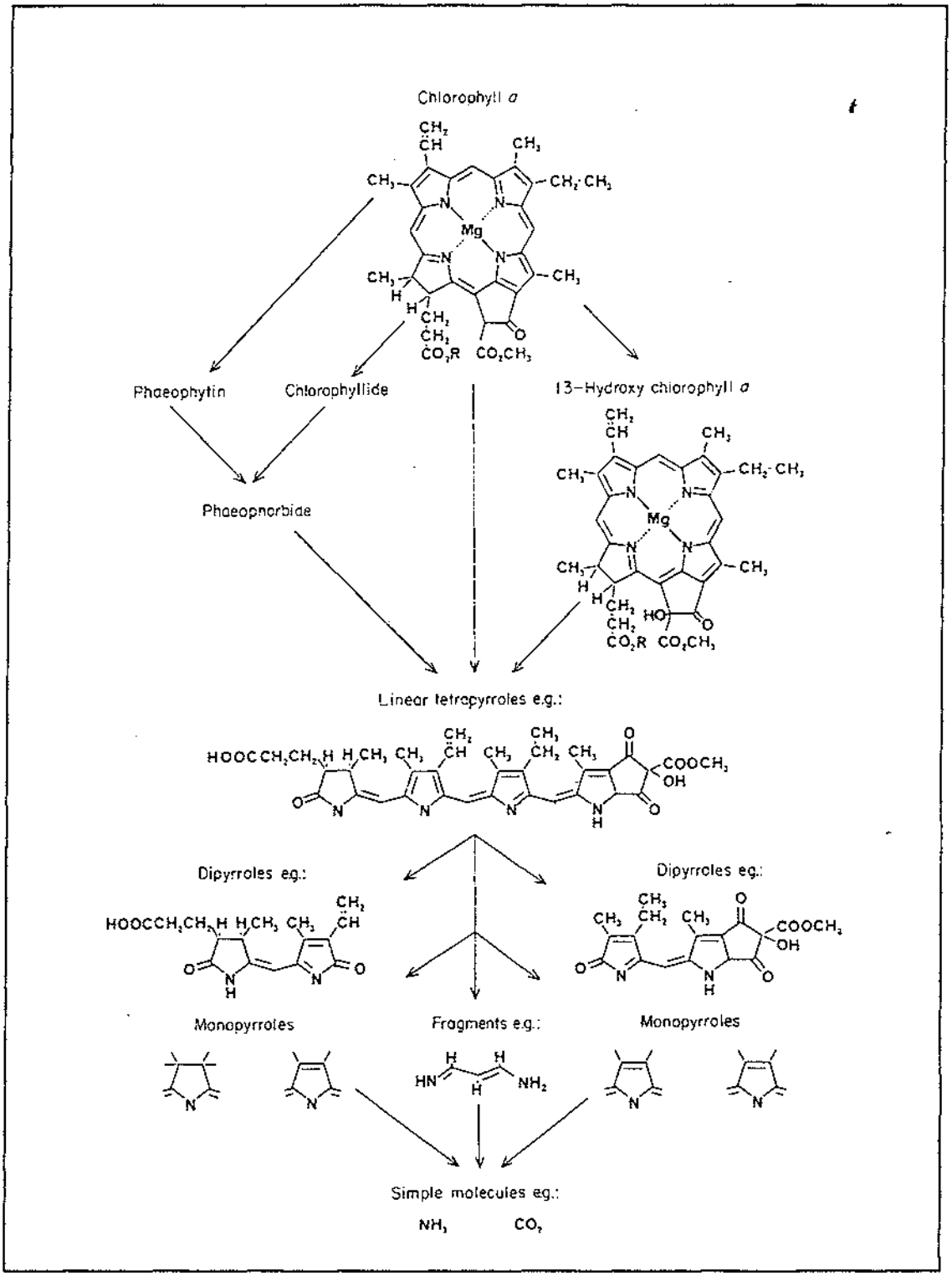


Figure 1.4 Possible model for degradation of chlorophyll (R = phytol) (Hendry *et al* 1987).

1.2.2.1 Dephytylation

Following release of chlorophyll from its protein complex is dephytylation or the removal of the C_{20} chain leaving the isocyclic ring intact (Schwartz and Lorenzo 1990) illustrated in Figure 1.2 chlorophyll a to chlorophyllide a. That dephytylation may be the initial step of chlorophyll breakdown can be inferred from findings with *F. pratensis* (Gut *et al* 1987). In isolated chlorophyll-protein-complexes phytol residues can be completely removed by action of purified chlorophyllase without release of free chlorophyllides (Schoch and Brown 1987). This agrees with the finding that chlorophyllide a accumulating in senescent leaves of the non-yellowing genotype of *F. pratensis* remains bound to the light harvesting complex of PSII (Thomas *et al* 1989). The reaction responsible for cleavage of the macrocyclic ring system seems to be dependent on oxygen (Thomas and Matile 1988). Whether this oxidative step takes place in the chloroplasts or in the cell sap after transport of free porphyrins from chloroplasts into vacuoles, is not known.

The decisive initial step of chlorophyll breakdown depends on oxygen as a polar form of chlorophyllide a is observed in normal yellowing leaves of *F. pratensis* subjected to anoxic conditions (Thomas *et al* 1989). In senescent leaves of barley anoxia does not result in the accumulation of chlorophyllide a but of pheophorbide a suggesting that in this species chlorophyllide may be readily dechelated (Matile *et al* 1989). Differences among species with respect to the appearance of polar forms of chlorophyll have been noted by Amir-Shapira *et al* (1987) in senescent parsley leaves. Chlorophyllide a appears in this species also if the leaves are treated anoxically. Whether or not polar forms of chlorophyll accumulate in senescent tissues may depend on subsequent reactions. It is this step which brings about colour changes associated with destruction of the tetrapyrrole ring of chlorophyll. All of the breakdown products described so far have an intact tetrapyrrole ring system and thus have green or brownish green colours, therefore degradation steps responsible for typical colour

changes associated with senescence and fruit ripening must involve demolition of porphyrin.

1.2.2.2 Porphyrin Breakdown

The first non-green putative catabolites of chlorophyll were discovered by comparison of TLC extracts from senescent leaves of yellowing and non-yellowing *F. pratensis* (Matile *et al* 1987). A number of coloured or fluorescent bands occurred that were present only in extracts from the yellowing genotype and they only appeared concomitant with chlorophyll breakdown. When the senescence process in barley leaves was hastened or delayed by various treatments including growth regulators and light, pink pigments turned out to be positively correlated with the rate of chlorophyll breakdown (Matile *et al* 1987,1988). Pink pigments occur in trace amounts, are unstable, thus difficult to isolate and purify, and chemical structures are not yet known.

Lipofuscin compounds (LLFC) found in yellowing and non yellowing leaves of meadow fescue may represent breakdown products of chlorophyll rather than products of lipid peroxidation (Duggelin *et al* 1988). Hendry *et al* (1987) has suggested that the fluorescent Schiff base compounds present in senescent plant tissues may represent -N=C-C=C-N- structures originating from the methine bridge carbon attached to two halves of pyrroles of the macrocyclic ring of chlorophyll. This hypothesis is supported by the positive correlations between the accumulation of LLFCs and the rate of chlorophyll breakdown in the wild type cultivar Rossa of *F. pratensis* (Duggelin *et al* 1988).

A number of additional compounds which probably represent products of porphyrin breakdown can be observed using TLC; the pink pigments and LLFCs may only represent a few of the total number of breakdown products (Matile *et al* 1989). The unambiguous identification of these compounds with catabolites of chlorophyll requires specific radiolabelling of chlorophyll in leaves prior to senescence. This has been achieved by feeding etiolated primary leaves of

barley with [4-C^{14}]5-aminolaevulinic acid (ALA). Both endogenous ALA synthesis and the flow of C^{14} into intermediary metabolism via glutamate were prevented by treatment with gabaculine, an inhibitor of the reversible ALA transaminase step in tetrapyrrole biosynthesis. About 80% of C^{14} incorporated during greening was recovered in chlorophylls accumulated in the mesophyll (Peiskes *et al* 1990). In the subsequent senescence period in permanent darkness, disappearance of green pigments was accompanied by accumulation of water soluble breakdown products. The chromatographic behaviour of this material corresponds with that of non-green putative catabolites of chlorophyll described earlier, but the resolution of monitoring radioactivity was insufficient for the establishment of the exact distribution of label among various compounds detected by their fluorescence and other properties. These results confirm earlier work by Shimokawa (1982) where radioactivity from C^{14}-chlorophyll was incorporated only into red fluorescence catabolites which may be the same as the pink pigments mentioned above. The solubility properties of catabolites found suggest that phytol residues responsible for the lipophilic property of chlorophylls are cleaved off during breakdown.

Green polar derivatives of chlorophyll arise in senescent chloroplasts, therefore non-green catabolites would also be expected in this organelle. Only trace amounts of pink pigments and LLFCs have been recovered in fractions of intact chloroplasts which were prepared from mesophyll protoplasts of senescent barely leaves. Most of these compounds appear to be compartmentalized in vacuoles (Matile *et al* 1988).

1.2.2.3 Photobleaching

The destruction of chlorophyll in solutions exposed to light or in leaves poisoned with herbicides which results in yellowing or bleaching of leaves is known as photobleaching. Compared to chlorophyll breakdown, photobleaching is a chaotic process and should not be confused with the controlled catabolism taking place during foliar senescence. The non-yellowing genotype of *F. pratensis* is

just as susceptible to photobleaching as the wild type when treated with methylviologen, a herbicide (Thomas and Matile 1988). Absence of previously mentioned chlorophyll catabolites in photobleached barley leaves also indicates that pathways of bleaching and natural yellowing are different. Little or nothing appears to be known about this process in fruit. The bleaching observed in Granny Smith apples is due to shading rather than high light intensities (Hirst *et al* 1990).

1.2.3 Breakdown by Peroxidases

Vacuoles are known as major sites of peroxidases (Matile *et al* 1988). They may be responsible for breakdown *in vivo,* for *in vitro* peroxidases are known to catalyse bleaching of chlorophyll (Huff 1982). Peroxidase (EC 1.11.1.7) bleaches chlorophyll in the presence of H_2O_2 and certain phenolics e.g. 2,4-dichlorophenol (Huff 1982, Matile 1980). Putative catabolites of chlorophyll localized in vacuoles appear to represent intermediary products of breakdown. A feature of thylakoid proteins from tissue of normal Rossa and mutant Bf993 *F. pratensis* at mid-senescence is appearance of a peroxidase band with low mobility. This component is absent from non-senescent and terminally degraded thylakoids. Cucumber cotyledons senesced in the presence of two ethylene induced peroxidases both of which were capable of degrading chlorophyll *in vivo* (Abeles *et al* 1988). A requirement for a phenolic compound in the peroxidative bleaching of chlorophyll has been demonstrated in chloroplasts (Martinoia *et al* 1982). An initial peroxidative cleavage of porphyrins could take place before degradation products are exported to vacuoles. The possibility that 'chlorophyll oxidase' (Martinoia *et al* 1982, Luthy *et al* 1986) or 'protophyrin oxidase' (Hougen *et al* 1982) are involved in breakdown can also be considered.

1.2.3.1 Mode of Action

Lipoxygenase (EC 1.13.11.12) mediates polyunsaturated fatty acid oxidation and produces free radicals which can oxidize chlorophyll, although this enzyme is

mainly present in non-green tissues (Kato and Shimizu 1985). Free radicals produced from phenolics can mediate chlorophyll destruction although it is not known if this process is involved in senescence.

Early results from experiments on peroxidative degradation of chlorophyll suggested that breakdown by peroxidases may be an alternative to breakdown by chlorophyllase (Matile 1980). A crude preparation of peroxidase from flavedo of *Citrus sinensis* can degrade chlorophyll *in vitro* (Huff 1982). Cucumber cotyledon senescence peroxidase levels increase 10 fold during the first 40 days of development and remain high for 65 days while total protein, chlorophyll, RNA, are degraded (Lewington *et al* 1967). Furthermore Abeles and Dunn (1989) found that it was not possible to inhibit peroxidase synthesis without blocking loss of chlorophyll. Since chlorophyll degradation is assumed to require protein synthesis (Thomas 1976) the effect described suggests that application of inhibitor's causes a simultaneous inhibition of chlorophyll degrading enzymes including peroxidase.

There is now clear evidence that peroxidase does not mediate chlorophyll degradation. The major proportion of peroxidase activity is associated with the vacuole and therefore is in a separate subcellular compartment to chlorophyll which suggests that chlorophyll's are inaccessible to peroxidase (Martinoia *et al* 1982). Also peroxidative activity shows an absolute dependence on H_2O_2 shown by a lack of activity in the presence of catalase with or without H_2O_2. In a dichlorophenol-peroxidase-H_2O_2 system chlorophyll and carotenoids were degraded and the chlorophyll a/b ratio decreased because of a preferential loss of chlorophyll a (Kato and Shimizu 1987). But the loss of carotenoids was more extensive than chlorophyll loss. Additionally the antibiotic tunicamycin, which inhibits protein glycosylation, inhibited both endogenous and ethylene induced peroxidase synthesis but did not inhibit chlorophyll loss (Handa *et al* 1985).

1.3 Protective Mechanisms to Photoinhibition Damage

Various reaction systems minimize photoinhibitory damage by scavenging or by preventing the formation of radicals or other reactive molecular species derived from O_2. For instance, carotenoids are known to deactivate triplet chlorophylls and transform singlet oxygen (O_2^*) to its triplet ground state (Cahuvet *et al* 1981, Harbour and Bolton 1978, Koka and Song 1978, Siefermann-Harms 1987). Superoxide dismutases together with ascorbate peroxidase seem to protect effectively against action of O_2^- and OH radicals and H_2O_2.

The destructive photooxidation of chlorophyll within the thylakoid membrane of chloroplasts in plants is thought to be prevented by carotenoids. Preventive modes of action by carotenoids and the mechanism of photooxidation of chlorophyll may be interrelated (Harbour and Bolton 1978).

The fact that leaves and fruit turn yellow rather than white indicates that carotenoids are more stable than chlorophylls. Carotenoids play both a protective role and a light harvesting role in chloroplasts (Koka and Song 1978, Siefermann-Harms 1987). Xanthophylls and ß-carotene represent accessory pigments of chlorophylls in photosystems and light harvesting complexes (Koka and Song 1978, Siefermann-Harms 1987). Their occurrence in photosynthetic pigment-protein complexes is important because carotenoids have the ability to scavenge singlet oxygen and protect the chlorophylls from photobleaching. Anderson and Robertson (1960) investigated an albino (carotenoid-less) mutant of corn and found that the chlorophyll of the mutant was unstable to high light intensities in the presence of oxygen. Under strictly anaerobic conditions chlorophyll of the mutant is stable to strong light. The conclusion is that carotenoids are required for the protection of chlorophyll from autophotodestruction.

1.4 Physiology of Yellowing

A major factor in colour change of fruit during ripening is transition of chloroplasts into chromoplasts (Rhodes 1980). The total chlorophyll per unit area is much lower in apples than in leaves and generally there seems to be little net fixation of CO_2; at most photosynthetic activity only just compensates for respiratory CO_2 production (Rhodes 1980). Leaf senescence occurs mostly in the mesophyll but each tissue type has its own pattern of senescence, not necessarily synchronized with that of the mesophyll (Thomas and Stoddart 1980). In xylem tissue, death of cell contents occurs in the primordial stage; and phloem elements too, undergo a process of autolytic degradation of cell contents during maturation. Little is known of senescence in epidermal tissue in leaves or fruit.

In fruit which yellow during ripening there is a change in background colour produced by rapid disappearance of chlorophyll and enhanced carotenoid biosynthesis, as chloroplasts are transformed into chromoplasts (Gross and Ohad 1983). Fruit colour depends on the pigment content of the skin and type of illumination. Pigments responsible for fruit skin colour are chlorophyll (green) as well as carotenoids, flavonoids (yellow) and anthocyanins (red). During ripening chlorophyll disappears making yellow pigments visible (Gorski and Creasy 1977, Hansen 1956).

Degradation of chlorophyll is important in the citrus industry where fruit are considered fully mature and best eating quality once all chlorophyll is lost. Regreening, where fruit synthesise chlorophyll, is a major problem for the citrus industry. Changes in peel colour from green to orange in attached orange fruit during and after colour break is a result of the disappearance of chlorophyll, followed by a build up of carotenoids (Eilati *et al* 1975). The concentration of chlorophylls in the flavedo of very young fruit, 65 days after full bloom, averages 55 $\mu g.cm^{-2}$, similar to that of green citrus leaves, and diminishes throughout maturation. Chlorophyll content, expressed per fruit, increases for approximately 170 days after full bloom, the period of continuous fruit growth. Although fruit

continue to increase slightly in size after this period, the total amount of chlorophyll diminishes rapidly. The chlorophyll a/b ratio remains almost constant until approximately 200 days after full bloom, indicating a proportional decrease of both chlorophylls. Eventually the a/b ratio decreases due to the more rapid destruction of the major component, chlorophyll a. Gibberellin treatments delay the normal loss of chlorophyll in maturing Navel oranges and there is no change in the a/b ratio. In mature 'Valencia' oranges gibberellin caused regreening with an increase in both total chlorophyll and a/b ratio. The rind at the stem end which regreened first, also had the highest a/b ratio (Jahn and Young 1976).

Ethylene is known to induce senescence in citrus fruit and is used to overcome regreening. Pigment changes upon exposure to ethylene exhibit characteristic features (Eilati *et al* 1975). During the first 50 hours of degreening after ethylene treatment there is an initial rapid decrease in chlorophyll content to levels as low as 25 per cent of initial content regardless of the initial amount. This results in yellowing of the peel. Initial yellowing results from rapid loss of chlorophyll which masks the presence of yellow pigments followed by a marked decrease in carotenoids before typical carotenoid accumulation despite the fruit yellowing rapidly. This latter process was similar to that observed in attached fruit although the rate was enhanced.

Amir-Shapira *et al* (1987) used HPLC techniques to screen for breakdown intermediates in senescing citrus fruit peel and parsley leaves. At harvest citrus fruit had only a small portion of its pigment absorbance as polar dephytylated compounds. Ethylene treated fruit had an immediate drop in chlorophyll followed by the appearance of polar dephytylated derivatives. The same increase of polar dephytylated derivatives occurred in fruit held in air for five days. Chlorophyllase activity at harvest increased about five fold upon treatment with ethylene. In contrast to senescing citrus fruit, senescing parsley leaves did not show any increase in chlorophyllide a or other non phytylated chlorophyll derivatives.

In order to determine the pathway of chlorophyll breakdown, demonstration of an *in vivo* accumulation of large amounts of chlorophyllide a in senescing citrus peel is required. Dark senescing parsley leaves show accumulation of pheophytin a and other phytylated derivatives. This suggests a different degradative pathway in its initial steps involving phytylated ring-modified derivatives that are similar to the 13^2-hydroxychlorophyll identified in several systems (Schoch *et al* 1984). Similar differences in *vivo* breakdown intermediates have been detected also during heat stress induced chlorophyll degradation; whereas citrus accumulated chlorophyllide- and pheophobide-like materials, parsley revealed numerous phytylated derivatives (Amir-Sharpa *et al* 1987). It is considered that citrus and parsley represent different routes of chlorophyll breakdown in higher plants.

The appearance and appeal of many apple varieties depends on amounts of various pigments present in the peel tissue. Loss of chlorophyll has frequently been observed during ripening of apples (Knee 1972). For example during storage of Golden Delicious apples green fruit changes colour from green to yellow. Carotenoids do not change and chlorophyll levels fall continuously (Gorski and Creasy 1977). Chlorophyll levels decrease slowly in apples maturing on the tree and decline sharply at the onset of the climacteric. This may be due in part to increases in chlorophyllase activity (Looney and Patterson 1967, Rhodes and Wooltorton 1967). As chlorophyll disappears the yellow colour of carotenoids is unmasked (Gorski and Creasy 1977, Knee 1980a). Mussini *et al* (1985) studied chlorophyll breakdown of Granny Smith apples both on the tree and after harvest. During growth on the tree fruit showed a 36% decrease in chlorophyll on a per gram basis. This decrease may have been a result of fruit enlargement rather than any chlorophyll breakdown. In Passe-Crassane pears decrease in chlorophyll is exponential and chlorophyll a decreased more rapidly than chlorophyll b, as the ratio a/b decreases with maturity (Laval-Martin 1969). For example during ripening of Super Trevoux pears the a/b ratio decreases from 3.9 to 2.7 (Gross 1984). Mango fruit peel changes colour as a result of chlorophyll loss and carotenoid biosynthesis (Medlicott *et al* 1986a). There is an almost complete loss of peel chlorophyll during ripening; a/b ratios were found to

decrease from 2.2 in unripe fruit to 0.8 in ripe fruit indicating a preferential breakdown of chlorophyll a.

1.5 Effect of Temperature

Pigment metabolism and hence colour development has been shown to be temperature dependent in a number of fruits with chlorophyll loss being proportional to respiration rate (Fidler 1973, Laval-Martin 1969, Padfield 1969, Rhodes and Wooltorton 1967, Rhodes 1980). Optimum storage temperatures vary by orchard, maturity, delays in cooling, season etc but the variation is small (Table 1.2) (Padfield 1969).

Table 1.2 Flesh temperatures recommended for long storage of various New Zealand apples (Padfield 1969) (NZAPMB pers. comm.).

-0.5-0.5°C	0-1.1°C	1.1-2.2°C	2.2-3.3°C
Delicious Granny Smith Red Delicious Richared Delicious Democrat Gala Royal Gala Fuji Splendour Braeburn	Doughterty Golden Delicious Lord Wolseley Rokewood Stayman Winesap	Cleopatra Dunn's Favourite Frimely Beauty Rome Beauty Worcester Pearmain	Ballarat Bledisloe Cox Cox's Orange Gravenstein Jonathan Kidd's Orange Red Statesman Sturmer

The yellowing of several apple cultivars has been investigated and in general the lower the storage temperature the slower the rate of yellowing (Kidd and West 1933). Apples which are not promptly cooled after harvest and stored at low temperatures, 0°C to 3°C, yellow and soften more quickly during storage than those promptly cooled (Padfield 1969). Temperature effects have been noted for: Red Delicious apples which yellow more slowly at 5.6°C than 10°C or 14.8°C (Pai and Sastry 1990); Granny Smith apples are more yellow when stored at 4°C than when stored at 0°C (Little *et al* 1982) and have an accelerated rate of chlorophyll loss when removed from cold storage to 16-20°C (Mussini *et al* 1985); retention of chlorophyll in the peel of Idared apples is greater at 0°C

compared to 2°C or 4°C (Johnson and Ertan 1983); Filippa, Ingrid Marie and Lobo apples yellow faster at 12°C than 0°C, 4°C or 8°C and yellowing is proportional to temperature (Lanfald 1966); Bramely's Seedling apples yellow at almost twice the rate at 10°C (taking 14 weeks to yellow) than at 5°C (taking 23 weeks to yellow), while there is little difference in yellowing at 1°C (taking 24 weeks to yellow) compared yellowing at 5°C (Kidd and West 1930); Lane's Prince Albert apples at 1°C have a commercial storage life of 27 weeks before becoming yellow and rotten, at 4°C the apples have a storage life of 24 weeks and 14 weeks at 10°C (Kidd and West 1933); Cox's Orange Pippin apples stored at 1.1°C were less yellow than apples stored at 3.9°C at the end of commercial storage life (Kidd and West 1936) and were greener when stored at 0°C compared to 3°C (Watkins *et al* 1989); Grimes Golden apples yellow faster at 30°C than 20°C and have a shorter lag phase before yellowing starts and the actual rate of yellowing is similar (Workman 1964); temperatures above 7°C result in rapid chlorophyll loss at the time of the climacteric, while lowering the storage temperature to 1.1°C from 3.9°C further reduces chlorophyll loss but not so that the appearance of the fruit was affected in Bramely's Seedling apples (Knee 1975); chlorophyll loss in Golden delicious apples is accelerated at 38°C compared to 20°C (Lurie and Klein 1990).

Changes in storage temperature or other storage conditions do appear to have a subsequent effect on the rate on yellowing. For example, storage at 0°C does not affect the rate of yellowing at 20°C after 6 or 10 weeks at 0°C (Workman 1964). Granny Smith apples from different storage treatments yellow at a similar rate to each other when transferred to 20°C after 4 months storage at 0°C (Watkins *et al* 1991). Cox's Orange Pippin apples stored in different controlled atmospheres at 3.5°C to 4°C have approximately the same amount of yellowing when transferred to 10°C for 14 days in air irrespective of the previous storage conditions (Stow 1989).

Chlorophyll loss in pears is also proportional to temperature (Hansen 1956, Laval-Martin 1969). Chlorophyll loss for Passe-Crassane pears at 19°C is more

than twice the chlorophyll loss at 7°C with 12°C being intermediate (Laval-Martin 1969). Anjou and Bosc pears lose chlorophyll faster at 26.7°C than 10°C (Hansen 1956). Bartlett pears yellow more slowly at 2.8°C than at 0°C having almost twice the storage life (Allen and Claypool 1949). Packham's Triumph and Conference pears yellow faster at 2°C than at 0°C, with yellowing at these temperatures being faster than pears at -1°C after 14 to 16 weeks storage (Fidler 1973). The rate of yellowing of Conference pears is very slow at -0.25°C (Kidd and West 1942).

Loss of chlorophyll in orange fruit maturing on the tree is dependent upon day temperatures below 20°C (Goldschmidt 1980, Jahn 1976). Citrus fruit from the tropics remain green long after internal maturation has been attained, because of high temperatures prevailing all year round. Despite this, de-greening of citrus fruit is temperature dependant with higher temperatures accelerating the rate of chlorophyll loss. For example, oranges lose chlorophyll faster at 25°C than at 15°C so that after 2 weeks at 15°C many oranges are still noticeably green (Knee *et al* 1988). Hamlin oranges have been noted to lose chlorophyll faster at 30°C than 21°C (Jahn *et al* 1973). De-greening of Bearss lemons is faster at 21°C and 18°C than 15°C but 27°C inhibits de-greening (Jahn 1976). Storage at 8°C markedly inhibits chlorophyll changes compared to 20°C for Shamouti oranges (Eilati *et al* 1975).

Yellowing of other horticultural produce has been noted to be temperature dependent. Examples include: brussel sprouts which yellow faster at 15°C and 20°C than at lower temperatures in which yellowing is slower (Lyons and Rappaport 1958); cabbage remains greener at 0°C than when kept at 3.3°C or 7.2°C (Parsons 1958); and broccoli yellow faster at 7.5°C than 5°C (Lipton and Harris 1974).

High temperatures are known to inhibit yellowing and chlorophyll loss in bananas where degreening is found to be inhibited above 30°C (Blackbourn *et al* 1989, Yoshioka *et al* 1978). In tomatoes, chlorophyll breakdown occurs more rapidly at

33°C than at lower temperatures, although lycopene synthesis is inhibited at 33°C (Ogura *et al* 1975),and in mangoes, Hatton *et al* (1968) found that Florida varieties ripened at 26.2-32.2°C frequently possess a mottled skin indicative of inhibited degreening, although this does not occur in Keitt or Kent mangoes at 26.7°C. Similar results were shown by Medlicott *et al* (1986b) where degreening of Tommy Atkins mangoes was inhibited at 37°C, while no effect was shown on Kent or Haden mangoes. Temperature effects on pigment metabolism may also be dependent on variety. In apples high temperatures, 38°C for 4 days, have been shown to promote the yellowing of Anna and Granny Smith apples (Klein *et al* 1990).

1.6 Oxygen and Carbon Dioxide

Respiration rate of harvested horticultural produce is often regarded as an index of the rate of senescence and therefore potential storage life (Kader *et al* 1985). The latter can be extended by reducing temperature, oxygen availability or by increasing carbon dioxide concentration in the surrounding environment, the net effect of which is to correspondingly reduce respiration rate. The retention of the green ground colour over long periods is one of the chief characteristics of CA stored apples (Kidd and West 1936).

How oxygen levels may act to influence chlorophyll loss could be explained using information obtained in biochemical experiments. When a chlorophyll solution is exposed to light in the presence of O_2, it is irreversibly bleached (Jen and Mckinney 1970). This is due to the nature of chlorophyll, a photodynamic compound, which in the presence of oxygen and light give rise to singlet oxygen. Within thylakoid complexes photodynamic reactions are quenched by carotenoids reducing the potential damaging action of singlet oxygen which causes bleaching of chlorophyll, lipid peroxidation and collapse of membranes (Knox and Dodge 1985). The separation of chlorophyll from associated proteins and its further catabolism occurs after a dephytylation step (Thomas *et al* 1989). Decline in chlorophyll a and b concentrations is immediately halted on exposure to anoxia

and oxidized intermediates such as 13^2-OH-chlorophyll a are known to occur *in vivo* (Schoch *et al* 1984). The inhibitory effect of anoxia on senescence is usually ascribed to the requirement for respiratory ATP to drive active processes such as protein synthesis. Therefore the critical aerobic process in chlorophyll catabolism is an oxidation step.

Lowering oxygen or raising carbon dioxide levels using MA or CA reduces the rate of chlorophyll loss and hence yellowing (Geeson and Smith 1989). In apples yellowing may be proportional to oxygen level. For Cox's Orange Pippin apples the rate of chlorophyll loss reduces as the oxygen level declines to levels of O_2 of 1% where rates of chlorophyll loss are so low they are difficult to measure reliably (Knee 1980a). The O_2 concentration for half maximal rate of chlorophyll loss was found to be 3.9% O_2 for Cox's Orange Pippin peel (Knee 1980a). After a simulated marketing period Cox's Orange Pippin fruit from the 0.75%O_2 treatment were greener than fruit from other treatments (Stow and Genge 1990). Further reductions in oxygen levels from around 3% to below 1% increases the retention of chlorophyll and inhibits yellowing more. Examples include: Golden Delicious apples stored in low oxygen atmospheres of 2.5% to 1% in 1.8% CO_2 yellow less as the oxygen level declines (Lau 1985); chlorophyll content of Cox's Orange Pippin apple peel was higher in fruit from atmospheres of 1.25%O_2 than from 2%O_2 (Stow 1989); the mean concentration of chlorophyll in the peel of Idared apples stored in 1% or 2% O_2 was higher than those in air storage (Johnson and Ertan 1983). At such low levels of oxygen the rate of chlorophyll loss can be difficult to measure and some reports mention there is no differences between some atmospheres. As an example differences in background green colour of Granny Smith apples were slight over the range of atmospheres of 1%-3% O_2 and 0.75%-3% CO_2 (Watkins *et al* 1991) and Cox's Orange Pippin apples from atmospheres of 1%O_2 or less had the same levels of chlorophyll retention (Stow 1989). Increasing the level of oxygen in the atmosphere to above normal atmospheric levels accelerates the yellowing of Golden Delicious apples to a maximum at 50% O_2 and 1000 ppm ethylene at 20°C (Leblond 1961). However Workman (1964) found that raising oxygen above ambient levels did not hasten

chlorophyll loss of Grimes Golden apples when exposed to 60% oxygen at 20°C for 14 days.

Chlorophyll loss is delayed by increased CO_2 levels of up to 15% and higher (Burton 1982, Kidd and West 1933). Carbon dioxide inhibits the yellowing of Golden Delicious apples at 20°C even when the O_2 concentration is high (Leblond 1961). At 10°C the effect of 15% CO_2 is to nearly double the time taken to reach a given stage of yellowing for Bramley's Seedling apples compared to air stored fruit (Kidd and West 1930). The effect of carbon dioxide is as pronounced as oxygen, at 5% O_2 at 10°C Bramely's Seedling take about twice as long to yellow as in air (Kidd and West 1930).

The change in green ground colour in fruit stored in CA is retarded more than for control fruit in air, this result is attributed not only to an effect of CO_2 but also to O_2, the lower the concentration of O_2 the greater the retardation of yellowing (Kidd and West 1936). Cox's Orange Pippin apples yellow more slowly in atmospheres with 3-2% O_2 and/or 5% CO_2 than in air alone and CO_2 combined with O_2 seems to be more inhibitory than low O_2 alone (Fidler and North 1971). But at low oxygen levels the contribution of carbon dioxide to inhibiting yellowing is less than at high oxygen levels. At 1% O_2 and varying levels of CO_2 of 1, 3 or 5% there was no difference in colour after 9 months storage at 1°C for Delicious apples (Drake *et al* 1992). Cox's Orange Pippin apples ground colour after storage for 146 days at 4°C was the same for air, 5%CO_2 + 16%O_2 and 0.75%O_2 + <1%CO_2 (Stow and Genge 1990).

Reducing oxygen levels to 2.5% - 1% delayed yellowing and ripening of Bartlett pears, 1% O_2 increasing storage life by 75% at both 2.8°C and 0°C (Allen and Claypool 1949). Atmospheres containing 5% O_2 delay yellowing slightly and at 10% O_2 the rate of yellowing was similar to that in air (Allen and Claypool 1949).

Colour change in Shamouti oranges is hardly affected by 0.5% or 1.5% CO_2, but 5% CO_2 inhibits colour change (Apelbaum *et al* 1976). Storage of lemons in

atmospheres containing 5% or 10% CO_2 tends to suppress chlorophyll destruction (Biale and Young 1962). This may be due to CO_2 suppressing general metabolism rather than having a direct effect on chlorophyll degradation as oxygen may do. Carbon dioxide has a pronounced inhibitory effect on colour change in the presence of 0.1µl/l ethylene which is partially reversed by 0.5 and 1.5% CO_2 and completely counteracted 5% CO_2 (Apelbaum *et al* 1976).

Black currants have a reduced level of chlorophyll degradation at high CO_2 (10-30%) and 2% O_2 than in air (Agar *et al* 1991). Low oxygen storage is known to retard the yellowing of bananas (Wills *et al* 1982).

One of the most beneficial effects of controlled atmosphere storage of green vegetables is the decreased loss in green colour (Aharoni and Ben-Yehosua 1973, Groeschel *et al* 1966, Lebermann *et al* 1968, Lieberman *et al* 1954, Lyons *et al* 1962, Rhodes 1980, Smith 1940, Wang *et al* 1971). Lowering oxygen levels below 10% and increasing carbon dioxide levels up to 10% prevents colour change in several commodities (Table 1.3). Chlorophyll levels of green beans are higher after storage 7.2°C 90-95% RH than when the oxygen level is reduced from 21% to 5% and 2%; levels of CO_2 above 5% further inhibit chlorophyll loss but there seems to be little effect of increasing CO_2 above the 5% level (Groeschel *et al* 1966). The visual quality rating of Brussel Sprouts is almost the same at 0°C for atmospheres of O_2 ranging from 20% to 5% with CO_2 at 1% (Lyons and Rappaport 1962). When stored at 5°C or 10°C reductions in O_2 or increases in CO_2 reduce the change in visual quality rating (Lyons and Rappaport 1962). Low O_2 retards the yellowing of Broccoli curds and the lower the O_2 level the greater the effect (Lipton and Harris 1974). Gas effects of inhibiting yellowing are enhanced when the temperature is increased (Lipton and Harris 1974). Yellowing of broccoli is completely inhibited in the absence of O_2, while an atmosphere of 1% O_2 partially inhibits yellowing and has about the same effect as an atmosphere of 7-22% CO_2 and 10 to 21% O_2 (Lieberman and Hardenburg 1954). Increased carbon dioxide, 5% to 15%, and decreased

oxygen levels, 21% to 3%, reduced the rate of chlorophyll loss in asparagus at 1.7°C (Wang *et al* 1971).

Table 1.3 Ranges of oxygen and carbon dioxide levels which inhibit yellowing in controlled atmospheres for selected vegetables.

Crop	Atmosphere ($\%O_2$:$\%CO_2$)	Reference
Asparagus	3:5-15	Wang *et al* (1971)
Tomato	3:0	Rhodes (1980)
Brussel Sprouts	2.5:10-20	Lyons *et al* (1962)
Broccoli	2.5-10:10-15 0-1:0	Smith (1980) Lieberman *et al* (1954) Lebermann *et al* (1968)
Lettuce	14:3	Aharoni and Ben-Yehoshua (1973)
Green Beans	3:10	Groeschel *et al* (1966)

1.7 Plant Hormones

1.7.1 Ethylene

Ethylene is known to promote chlorophyll loss in some types of fruits but with a few exceptions most investigators have found it to have small or negligible effects on leaf senescence (Thimann 1980). Yellowing may be promoted by ethylene initiating the synthesis of chlorophyll degrading enzymes (Lewington *et al* 1967). For example chlorophyll synthesis is reduced if ethylene is applied to etiolated cucumber cotyledons in both the dark and light phase during chlorophyll synthesis (Abeles and Dunn 1989). On the other hand ethylene has no effect if present in only the light or dark phase. It appears that chlorophyll synthesis is reduced if events initiated by ethylene in the dark are allowed to continue in light.

Exogenously applied ethylene causes rapid degreening of citrus fruit rind (Jahn 1976). Without ethylene yellowing of citrus fruit consists of two phases,

degradation of chlorophyll and biosynthesis of carotenoids. Although both phases are promoted by ethylene, it is primarily used to remove chlorophyll, since carotenoid synthesis is sensitive to temperature, and carotenoid accumulation is reduced at degreening temperatures (Purvis and Barmore 1981). For example at 25°C all concentrations of applied ethylene caused faster chlorophyll destruction than in fruit with endogenous ethylene alone (Knee *et al* 1988). Ethylene not only enhances the destruction of chlorophyll but also promotes the subsequent build up of carotenoids (Apelbaum *et al* 1976). Detached mature green Shamouti orange fruit lose their chlorophyll rapidly. The change in colour which includes both chlorophyll destruction and carotenoid accumulation, shows a lag during the first 4 days after harvest, but the colour changes rapidly subsequently. Time to 50% rind chlorophyll destruction indicates that 5 days are required for control fruit but 3 and 2 days are required for fruits gassed with 0.1 and 5 µl/l ethylene, respectively.

Ethylene (1000 ppm) applied to Grimes Golden apples at 20°C induced an abrupt increase in respiration but only slightly increased yellowing rate (Workman 1964). The presence or absence of ethylene in the storage of Cox's Orange Pippin apples did not affect chlorophyll loss (Knee 1980a). In contrast, Cox's Orange Pippin apples sprayed with aminoethoxyvinylglycine (AVG), a synthetic inhibitor of ACC synthase and hence ethylene synthesis, were greener compared to apples from unsprayed trees 10 days after harvest (Child *et al* 1984). This may indicate that ethylene has an effect on yellowing but only at certain physiological stages of development.

1.7.2 Other Plant Hormones

Auxins are not generally effective in retarding leaf senescence (Thimann 1980). In oat leaves IAA retarded senescence only at concentrations around 500 times that of kinetin. A complication is that auxin, at levels only sightly above the physiological, stimulates the production of ethylene, which in some tissues promotes senescence.

Cytokinins are effective in preventing or at least delaying, senescence (Thimann 1980). All cytokinins that have been tested have this property; they delay proteolysis and chlorophyll loss in the dark. The action on leaves and fruit is generally paralleled by comparable effects in delaying senescence of flowers. Their action may be due to stimulation of chlorophyll biosynthesis (Ashtakaln *et al* 1989, Buschmann and Lichtenthaler 1982). There are a few plants whose leaves do not respond to cytokinins, and these respond instead to gibberellins. For leaves and fruit attached to the plant the effect of cytokinins are less marked. Such leaves and fruit senesce more slowly than when detached, so the differences from controls are smaller. The reason for this may be that attached leaves and fruit are receiving a supply of endogenous cytokinin from their roots.

Light delays the typical senescence syndrome in a manner superficially similar to the effect of cytokinins (Thimann 1980). Red light has been reported to delay senescence in rice leaves and far red to antagonize this effect. In the leaves of etiolated seedlings of radish protochlorophyllide is accumulated to a greater extent when cytokinins are present during growth (Buschmann and Lichtenhaler 1982). This leads to higher levels of chlorophyll. Perhaps the apparent retardation of senescence by cytokinins may be due to a stimulation of chlorophyll synthesis rather than inhibition of breakdown.

ABA has the effect of hastening senescence (Thimann 1980). The presence of ABA inhibits protein synthesis which is reversed by kinetin. ABA increases losses of protein and RNA from isolated leaves and leaf discs. As with cytokinins ABA is less effective on leaves attached to the plant. ABA concentrations higher than those of kinetin are required to affect a response although there is a wide variation in sensitivity among species and cultivars, and ABA can only act when the tissue is responsive i.e. at the right stage of development.

1.8 Colour Measurement

1.8.1 Perception of Colour

Consumers of fresh produce judge what to buy on the basis of all the senses at their command. They touch, taste, smell, and look at merchandise. In nearly every transaction, colour plays an important part (Judd and Wyszecki 1975). Colour aids not only in food identification but also is especially important for the identification of colour linked flavours such as orange, lime, cherry and grape (Christensen 1983). Christensen also found that colour significantly influences aroma judgements of foods; consumer perception was that the stronger the colour of a food, then the better the quality and the better the armoa. Colour controls the choice of a food sample in a pair but does not control the magnitude of the perceived differences between the coloured and uncoloured pair (Christensen 1983). Customers perceive colour as belonging to the merchandise or to the package; that is colour for consumers nearly always means object colour. For each package or each type of merchandise, consumers carry in their heads a memory of the colour or range of colours that is acceptable. Bread for which the crust is too dark may be passed by as probably burnt. Tomatoes and apples cannot have too green a colour or they may be rejected as unripe. Colour that is the wrong hue (such as orange instead of red) is equally unappealing.

The dependence of the customer on a mental colour standard is subject to various kinds of uncertainty. The spectral character of light under which the merchandise is viewed sometimes influences colour perception to an important degree. Some florescent lamps can give meat a greenish colour, a suit or necktie picked out under incandescent-lamp light may be returned the next day because its daylight colour is unsuitable. Colour of the surroundings influences the colour judged by successive contrast. For example adaption of the eye to blue colour adds yellow to the colour judged. By and large peoples eyes and

their ability to see colour belonging to objects work well regardless of a wide change in light and surroundings.

The use of lightness, saturation and hue terms to describe object colour experience is very common. Many people have organised their colour experiences along these lines, even though they have not given these specific names to the three variables.

Monitoring of apple quality requires methods of measuring quality to be accurate and sensitive. The most common method of measuring colour in the past has been a subjective assessment by the human eye. Very often colour differences perceived by the human eye, which can distinguish upwards of 10 million colours (Francis 1980), are very difficult to describe and quantitfy.

To prevent bias and to give a common base from which differences in colour can be compared (i.e. assign numbers to colours and colour differences), not only between different colour but also to bring to a common point different descriptions of the same colour by different observers. Describing colour objectively allows colour differences to be quantified more readily than can be determined by eye, this is especially important if comparisons of colour are made at different times e.g. daily. Visual assessment of apple colour by the use of colour charts is a common method used by growers to determine harvest maturity for some cultivars. Consumers assess colour by eye and use this as a basis for estimating ripeness. Therefore any models of environmental effects on colour change must use an objective measurement for which there is little ambiguity. In order to design a machine to achieve this an understanding of how the eye recognises colour and how light interacts with objects is necessary.

1.8.2 What is Colour?

The colour of apples is a function of how light is modified by striking the apple. Figure 1.5 describes different events possible when light strikes an object.

Light passes through two major events when it strikes an apple. Firstly a small proportion of the light is reflected back off the fruit without penetrating into the skin. This is known as specular reflection and gives rise to the glossy appearance of the fruit. The second event is where the major portion of light penetrates the surface of the skin. This light is refracted by pigment particles found in the epidermal layer. As light moves through the skin it is reflected at each surface. The entire process of multiple reflection and refraction diffuses the light so that when it reaches the surface of the apple it leaves in all directions. Light leaving the skin is known as diffuse reflection and the process by which it occurs as scattering.

The colour of fruit is caused by the passage of light through various pigment particles in the skin. As there is a mix of different pigments the colour of skin is a result of the most prominent pigment. The green pigment (mainly chlorophylls) absorb in the blue and red wavelengths and reflect green light. Other pigments present responsible for the yellow colour (carotenes and xanthophylls) absorb blue light but reflect green, yellow and red light. This process is known as selective absorption and is what defines the colours of all objects we see.

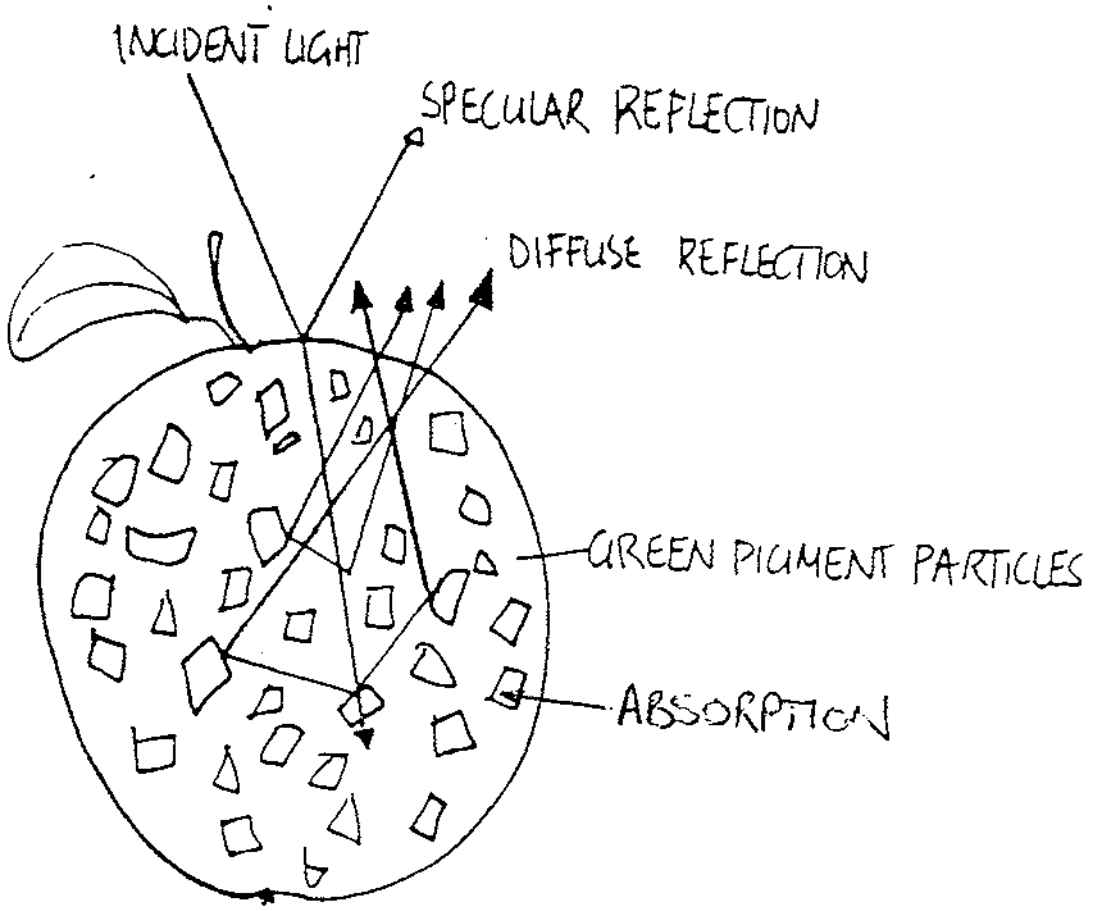


Figure 1.5 Events occurring when light strikes an apple fruit.

1.8.3 How the Eye Responds to Light

The human eye consists of several parts. Light first passes through the pupil, which is enlarged or reduced according to light intensity, and focused through a lens onto the back of the eye to an area known as the retina. Cells lining the surface of retina consist of two types; rods sensitive to light levels i.e. lightness and darkness, and cones sensitive to coloured light. The cones are divided into three types; red sensitive, green sensitive and blue sensitive. The cone cells require a certain light intensity to function, this is why moonlit objects appear to be in shades of grey, and so colour evaluations should be performed at the same light intensities.

The signals from the cone cells are combined or converted by a series of complex nerve areas to opponent colour signals which are yellow-blue and red-green. This results in the eye being able to distinguish more than three colours.

The design of instruments to measure colour have been based on the way the human eye responds. Shapes of the response curves of human cone cells have been duplicated in a series of filters which have the same transmission curves. This is the concept used in tristimulus colourimeters.

1.8.4 Instruments that Measure Colour

1.8.4.1 Spectrophotometer

These instruments have been used to measure the colour of pigments extracted from horticultural products in various solvents. Spectrophotometers measure the absorbance or transmittance of a solution at separate wavelengths and are commonly used to quantitfy pigment concentrations in solutions. Much of this information is useful and cannot be determined by eye. The limitation of this method is that it is destructive so measurements cannot be repeated on the

same object. The sensitivity to different pigments depends on the solvent system used and the process can be very time consuming to perform. Some instruments scan over a range of wavelengths giving spectra characteristic for some fruit depending on maturity and cultivar. Comparing changes in several spectra can show differences in colour. However differences in spectra are difficult to quantify as there are greater or lesser changes in the spectrum at different wavelengths from one spectrum to another (Francis 1952).

1.8.4.2 Tristimulus colourimeter

These instruments are designed to respond to the same wavelengths of light as the human eye. Very simply a tristimulus colourimeter consists of a set of X, Y and Z filters, designed to absorb light at the same wavelengths as pigments in the human eye, coupled to highly sensitive photocells. Light from a Xenon lamp is flashed into a mixing box inside the measuring head where it bounces around before passing out. This diffuses light from the lamp, so the chromameter will produce and measure diffuse light. For this reason the chromameter does not measure glossiness of an object or specular reflection. Light passes through the filters onto photocells which produce an electric current which can be measured and quantified. As the human eye sends colour data to the brain in a red-green pair and a yellow-blue pair of responses so most colourimeters are designed to report their output in this manner.

Colour as perceived by the human eye has three dimensions; hue, chroma and brightness. Hue and chroma are specified by the red-green and yellow-blue paired responses and define what would be commonly termed the colour of an object, i.e. the object is a yellow-green colour. The light passing through X,Y and Z filters is processed into x and y values which when plotted produce an ellipsoid shape. Consequently equal distances in x and y values do not represent equal distances in colour as perceived. The L*a*b* mode is concerned with the human sensitivity to colour. Equal distances in this system represent approximately equal distances in visual perception. As the hue or chroma do not describe a

specific colour completely, a brightness factor, Y, must also be added. The brightness factor defines how dark or light the colour is, a low value indicating a dark colour and a high value indicating a light colour. It is therefore possible to have two colours which differ only in their brightness. Most reports present data in the Hunter L*a*b* system which are transformations of the Y,x,y values. Very often the L*a*b* values are reduced from three to two values in order to make interpretation or calculation of colour differences easier.

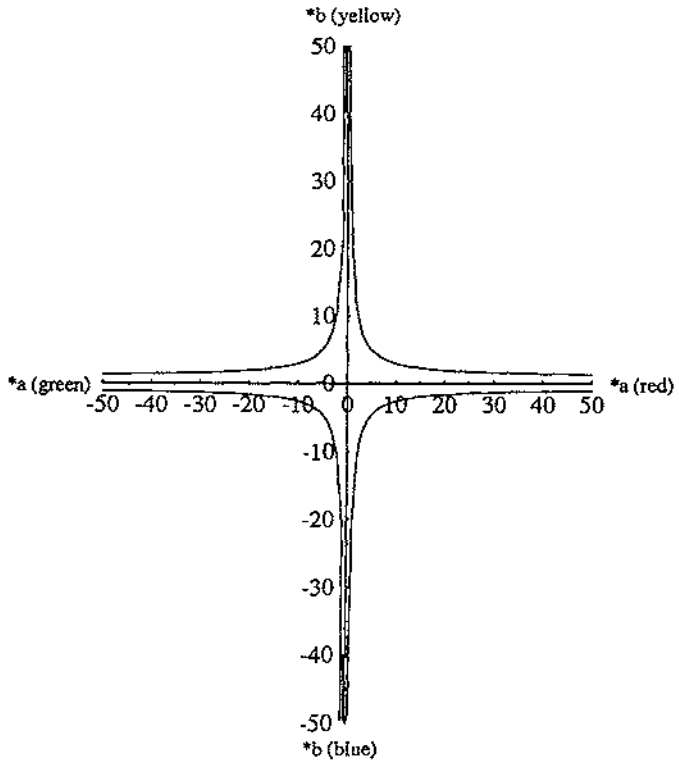


Figure 1.6 Graphical representation of the a*/b* ratio when b* is equal to 20 or -20 units. Note the tangential nature of the relationship.

The most common function used in the food industry has been the ratio of a*/b* which is a function of hue or the perceived colour. As the hue becomes more yellow the ratio approaches zero and as the hue becomes more green the ratio nears infinity and so on through the four quadrants. It is obvious from Figure 1.6 that the a*/b* ratio is not linear but tangential. If the ratio is near one where the angle of the ratio to the origin is approximately 45 degrees the departures from infinity are not too great. But if the a*/b* ratio is used in a portion of the colour solid where it approaches infinity, the concept breaks down. In such cases the actual angle the point makes with the vertical axis would be preferred. A rough rule of thumb proposed by Clydesdale (1975) is that if the a*/b* ratio is within 0.2 to 2.0, then it is probably satisfactory. If outside this limit then angle Θ ($\tan^{-1}$ b*/a*) should be used (Francis 1952).

1.8.5 Colour Charts

Comparing the colour of fruit to reference colours (British horticultural colour charts 1938, Yamazaki and Suzuki 1980) or a scale of reference is common in publications on fruit and vegetables and the horticultural industry; a few examples include: broccoli (Lebermann *et al* 1968), citrus (Apelbuam *et al* 1976), green beans (Groeschel *et al* 1966), apples (Anon 1967, Francis 1952, Knee 1980b, Magness *et al* 1926), asparagus (Kramer 1949) and Nashi (Yamazaki and Suzuki 1980). The NZAPMB also use colour chips to assess maturity for some cultivars such as Granny Smith. As this method is subjective and colour chips are often assigned to a scale there are errors in assessment by comparison with the colour chart between assessors as each individual persons concept of colour and personal experience of colour is different. In addition some scales may bias the colour measurement so that there is a greater range of values for the same difference in one colour compared to another colour. Using colour charts to measure colour means that glossiness is considered part of the colour. Unless the colour chip being used has the same glossiness there may be errors in colour perception. The above problems will lead to variability in measurements from different assessors.

1.9 Conclusions

Apples are stored in a range of environmental conditions during handling, storage and retailing ranging from low temperature storage in air to CA or MA storage to room temperatures in shop shelves. The different environmental conditions to which fruit are exposed, influence the yellowing of apples to a greater or lesser extent. Understanding the relationship between yellowing and levels of gases, principally oxygen and carbon dioxide, in the atmosphere would be of use in the apple industry to better predict the effect on yellowing of changing handling practices and to allow problems of yellowing to be anticipated in retail outlets. However there is a lack of information about the colour change of apples in particular the relationships between: colour change and temperature; colour change and atmosphere composition. Characterisation of these relationships for apples could be used to model the effect of different storage environments and changes in storage conditions. In order for such information to be used practically an understanding of the relationship of different methods of colour assessment is required. This would have the additional benefit of enabling some comparison to be made between the diverse range of colour measurements used in the literature.

In this study the relationship between yellowing and temperature was examined in order to characterise the responses observed. Additionally, yellowing was examined during; two seasons, two harvests within a season and with fruit from several growers, to identify the effect of possible sources of variation on the rate of yellowing. Yellowing in relation to oxygen and carbon dioxide concentrations in the external atmosphere during two seasons and using fruit from several growers was examined in order to characterise responses observed.

Chapter 2.

Materials and Methods

The effect of temperature and atmosphere on colour change was studied using apple (*Malus domestica* Borkh) cultivars Cox's Orange Pippin and Granny Smith size 125 (average weight 148g).

Measurements recorded both at harvest and at intervals over eight weeks were firmness, soluble solids, weight loss, colour using a Minolta Chromameter and/or colour charts and chlorophyll content of the skin.

2.1 Temperature Treatments.

Temperatures used were 0°C, 4°C, 6°C, 12°C, 20°C, 25°C, 30°C for Cox's Orange Pippin and 0°C, 6°C, 12°C, 20°C, 25°C, 30°C for Granny Smith apples during 1989. Experiments conducted in 1990 used the following temperatures; 0°C, 5°C, 10°C, 15°C, 20°C, 25°C, 35°C for both cultivars.

Fruit from different harvests were tested; one lot was taken early in the export harvest season (Harvest 1) and one lot taken towards the end of the export harvest season (Harvest 2), 8/2/89 and 22/2/89 for Cox's Orange Pippin apples and 1/4/89 and 18/4/89 for Granny Smith apples respectively. Harvest dates were 18/2/90 and 20/2/90 for harvest 1, 6/3/90 for harvest 2 of Cox's Orange Pippin apples and 10/4/90 and 16/4/90 for harvest 1, 6/5/90 and 9/5/90 for harvest 2 of Granny Smith apples during 1990.

After being weighed apples were stored in plastic bags to maintain a high humidity. To prevent a modified atmosphere occurring each bag was perforated with six 1 cm diameter holes. Ten bags for each harvest, each containing seven fruit were used for each temperature treatment. Five bags contained fruit from each of the growers supplying each harvest. Thus half the fruit in any one temperature treatment were from one grower.

Bags were placed into apple cartons and stored in modified refrigerated cabinets (Skope model CV100) which control temperatures to within 0.5°C of settings and 80% RH. These were used for temperatures 4°C, 6°C and 12°C in 1989 and 5°C, 10°C and 15°C in 1990. Fruit stored at 0°C ± 1°C were in a 2m x 4m coolstore at 80% RH. Fruit stored at 20°C or 25°C were in rooms where temperature was controlled using air conditioning and refrigeration and were maintained to an accuracy of ± 1°C and 60% RH. Fruit stored at 30°C or 35°C were in a Gallenkamp IH-270 model cooled incubator which maintained the temperature at ± 1°C and 40% RH.

2.1.1 Cox's Orange Pippin

2.1.1.1 1989

Fruit from Harvest 1, growers D265 and D389, were harvested on the 8/2/89, packed into cartons and kept overnight at ambient temperatures (approx. 16-18°C). The fruit were then sent to the Williams Street depot, Hastings, of the New Zealand Apple and Pear Marketing Board (NZAPMB) for inspection. Apples passed through inspection and were transported to Massey University on the afternoon of the 9/2/89. Fruit were stored overnight at ambient temperatures (approx. 16-18°C) packed into plastic bags on the morning of the 10/2/89, then placed into different temperatures.

Fruit from Harvest 2, growers D592 and D646, were harvested on the 22/2/89, packed into cartons and sent to the Williams street depot for inspection. The

fruit were collected the same day transported to Massey and placed into different temperatures.

2.1.1.2 1990

Grower D252 supplied apples for both harvests, grower D265 supplied fruit for harvest 1 experiments and grower D945 provided fruit for harvest 2 experiments. Fruit were harvested on the 10/2/90 and 16/2/90 for grower D265 and grower D252 respectively. Harvest 2 fruit, growers D252 and D945, were harvested on the 6/3/90. On each occasion the fruit was packed into cartons and sent to the NZAPMB Wakatu packhouse, Hastings, on the following day. The fruit were sent via courier after being inspected that day and were received in Palmerston North the following day. During this time the fruit were at ambient temperatures (approx. 16-18°C). The apples were packed and then stored the day of arrival.

2.1.2 Granny Smith.

2.1.2.1 1989

Fruit from harvest 1, growers D139 and D341, were harvested on 29/3/89, packed into cartons kept overnight at ambient temperatures (approx. 14-18°C) before sending to the NZAPMB Wakatu packhouse on 30/3/89. Cartons were collected from Hastings on 30/3/89 transported to Massey University and kept at 1°C ± 1°C for 24 hours before storage in different temperatures.

Apples from the same growers as for Harvest 1 were used for Harvest 2. Grower D341 supplied fruit from a different block (D069) to fruit supplied for harvest 1 experiments. Fruit were harvested on the 16/4/89 and 17/4/89 for grower D139 and grower D341 respectively, packed into cartons and sent to the Wakatu packhouse on the 17/4/89 and 18/4/89. Cartons were sent via courier to Palmerston North and received on the 18/4/89 and 19/4/89 during which time

fruit were at ambient temperatures. Apples were packed into plastic bags the same day, weighed and placed into storage at different temperatures.

2.1.2.2 1990

Fruit from growers D075 and D139 were supplied for both Harvest 1 and Harvest 2 experiments. Fruit were harvested on 10/4/90, harvest 1, and 9/5/90, harvest 2, for grower D075 and on 16/4/90, harvest 1, and 6/5/90, harvest 2, for grower D139. On each occasion fruit was packed into cartons and sent to the Wakatu packhouse at Hastings on the following day. Fruit were then sent via courier and were received in Palmerston North the following day. During this time fruit were at ambient temperatures (approx. 16-18°C). Apples were packed into plastic bags and placed into storage at different temperatures.

2.2 Oxygen and Carbon Dioxide Treatments.

Cox's Orange Pippin and Granny Smith apples from each grower at Harvest 1, in 1989 and 1990, were stored in a range of eight atmospheres at 20°C in the dark for up to eight or sixteen weeks. Ten fruit from each atmosphere were sampled weekly for Cox's Orange Pippin and biweekly for Granny Smith. Fruit from each grower were sampled on consecutive days. Oxygen, carbon dioxide and ethylene levels in storage chambers were monitored, daily for the first week then twice weekly and prior to sampling using gas chromatography.

One ml samples of gas, taken using a disposable 1 ml syringe fitted with a hypodermic needle (Monoject Tuberculin 1 ml syringes with 15.9 mm (5/8") 25 gauge needles), was assessed for oxygen, carbon dioxide and ethylene. Carbon dioxide and oxygen were measured using a thermal conductivity detector at 60°C and 80mA current in a Shimadzu GC 8A gas chromatograph using an Alltech CTR I column (Alltech cat no 8700) at 30°C with hydrogen at a flowrate of 30 mls/min as carrier gas. The Alltech CTR I column is a dual column, outer column 1.83m x 6.35mm packed with an activated molecular sieve, the inner

column 1.83m x 3.18mm packed with porapak mixture. Ethylene was measured using a flame ionization detector at 150°C, the column at 100°C in either a Varian 3400 or PYE 104 gas chromatograph fitted with a F-1 grade, 80/100 mesh activated alumina in a 1.83m x 3.18mm column. Nitrogen at a flowrate of 30 mls/min was used as the carrier gas. The flame was maintained with hydrogen (30 mls/min) and air (300 mls/min).

Particular mixes of oxygen and nitrogen were obtained by mixing appropriate amounts of oxygen free nitrogen (<10 ppm oxygen NZIG) and air (from a compressor) using a gas mixing system developed and built at Massey University (Figure 2.1). For some atmospheres carbon dioxide was also added. Prior to being mixed, each gas (nitrogen, air and carbon dioxide) was humidified by bubbling through water then passed into a large manifold from which gas was drawn off to smaller manifolds where each component gas was metered to required flowrates before being mixed and passed to gas tight perspex chambers. A constant pressure in the system was maintained by excessive gas flow from each of oxygen free nitrogen, air and carbon dioxide venting to a chamber containing water to a height of half a meter. This creates enough back pressure to equalise pressure changes of the gas from high pressure storage cylinders from which the output pressure increases as the cylinder empties. The remaining gas was vented to the atmosphere.

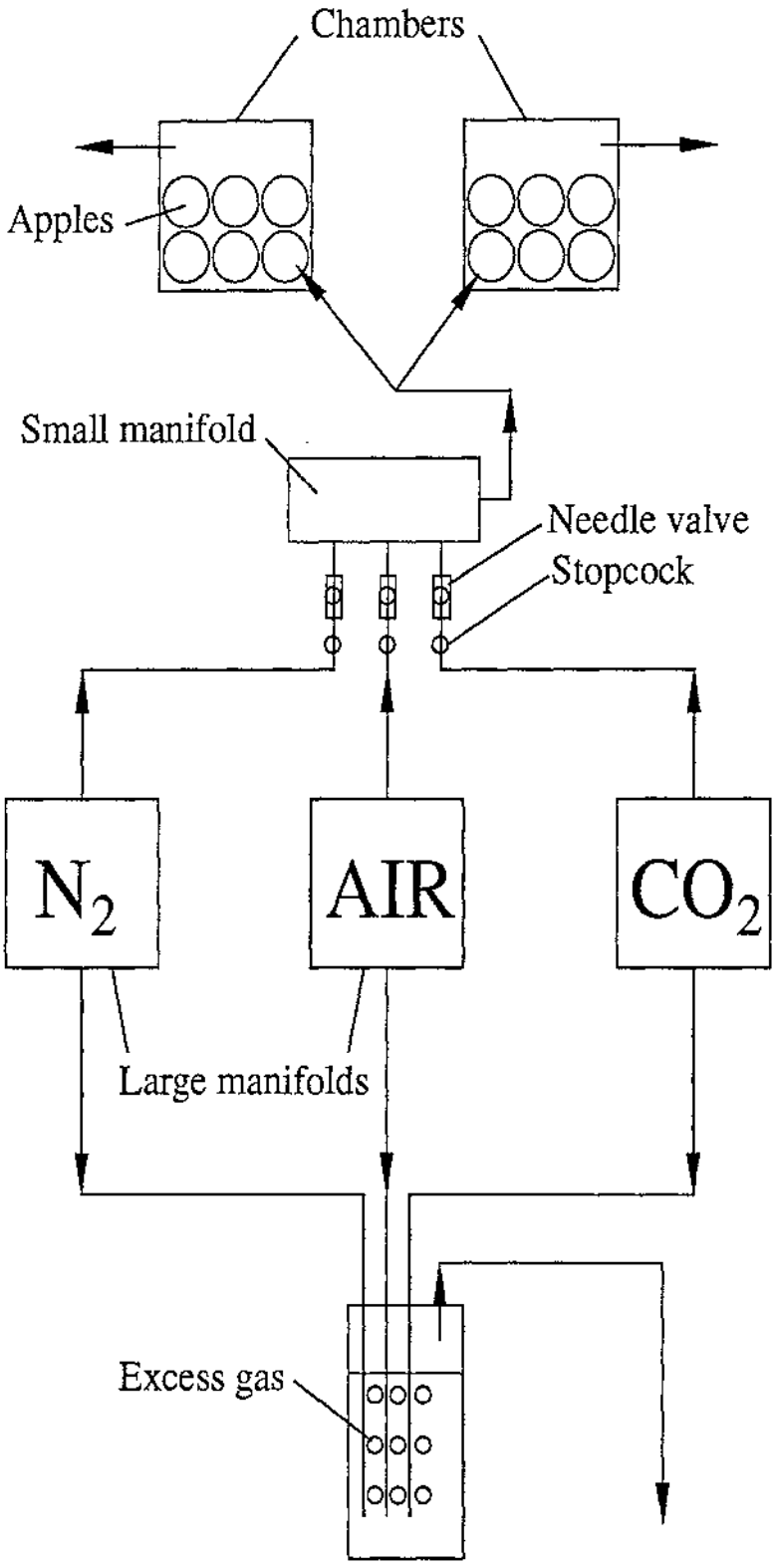


Figure 2.1 Schematic diagram of controlled atmosphere gas mixing system.

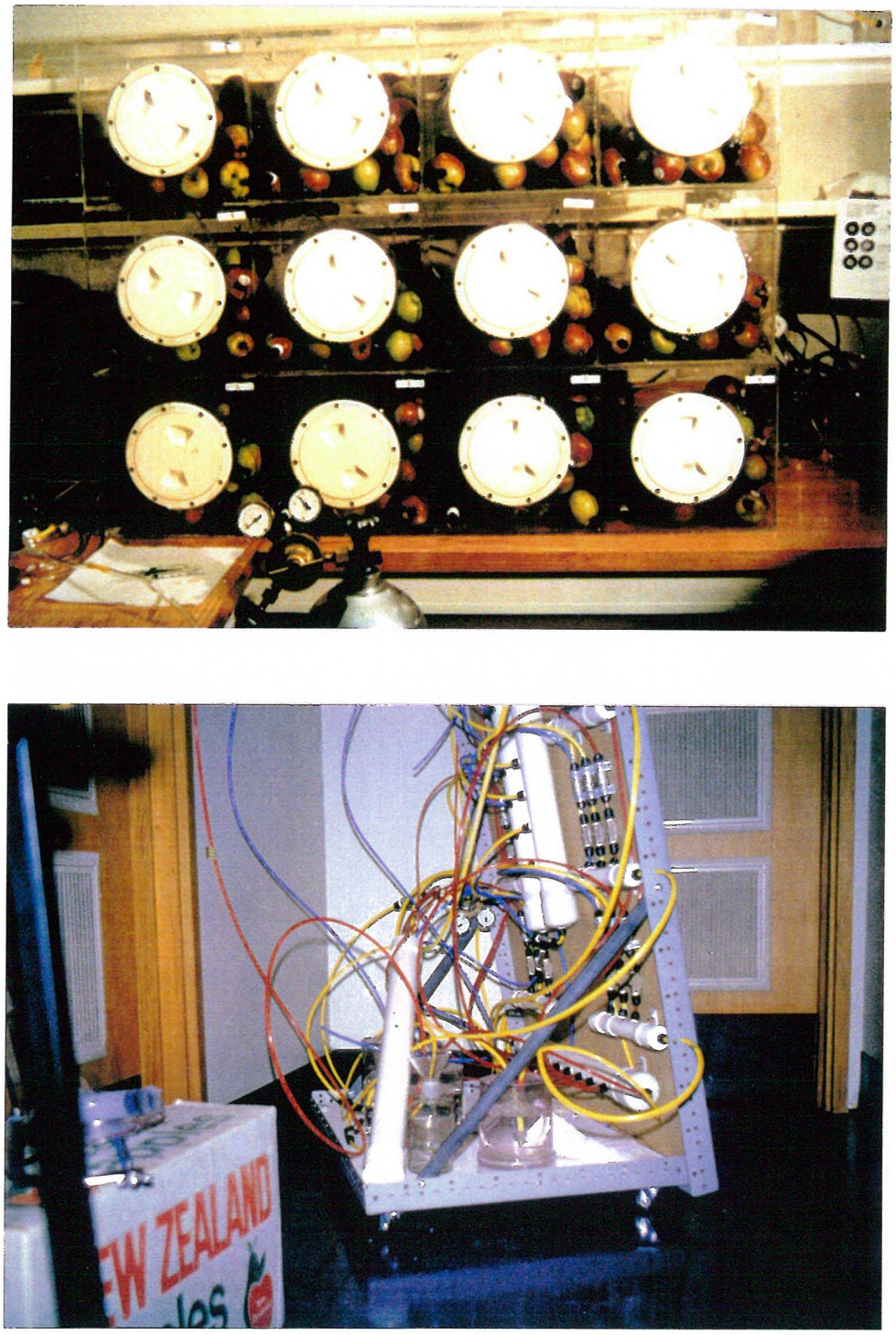


Figure 2.2 Controlled atmosphere system, chambers and gas mixing system.

The small manifolds were connected to needle valves and stopcocks. There was one needle valve, stopcock combination for each component gas; oxygen free nitrogen, air and carbon dioxide. Measurement of the flowrate of each component gas was made possible by using the stopcocks to turn off the gas flow from two of the three gas mix components. The manifolds gave good mixing of the gases used as long as they were added in the correct order. If not then a faster flowing gas would not allow a slow flowing gas to mix. An example is using carbon dioxide and nitrogen where a carbon dioxide flow rate may be one hundredth of the nitrogen flow rate. Therefore the gases must be added in order of fastest flowing to slowest flowing where the fastest flowing is at the far end of the manifold from the outlet.

Generation of atmospheres was carried out outside the temperature controlled room in which fruit was stored to facilitate replacement of gas cylinders. This did result in a problem with condensation of water in gas lines passing into the temperature controlled room when the room was cooler than the outside air as is common in February and March. To overcome this the gas lines had to be periodically emptied of water. Individual gas mixtures were divided into two each going to a separate perspex chamber. Each chamber received 5 $L.hr^{-1}$ of gas mixture, equivalent to one air change per three hours when the volume of fruit is excluded from the chambers. This was just sufficient to maintain the atmosphere at a constant oxygen level.

The effect of atmosphere on colour change of apples was investigated by using two series of atmospheres. The first used different concentrations of oxygen with carbon dioxide levels kept low (0.03% to 4%) while the second used different concentrations of carbon dioxide with oxygen maintained at a specific concentration, between 9% and 20% (see Appendix 3 for the composition of individual atmospheres). Each atmosphere was maintained at a constant level by having a continual stream of humidified gas mixture passing through the storage chamber at a flow rate of 5 $L.hr^{-1}$. All chambers were scrubbed for

ethylene using 100 grams of potassium permanganate impregnated onto alumina, a commercial preparation known as Purafil (sourced from Cambridge Engineering Services Limited, Cambridge New Zealand).

Seventy fruit from each grower were stored in each of eight 24.6L perspex gas tight chambers in which the oxygen level ranged from 21% to 1% and carbon dioxide from 0.05% to 30% levels at 20°C. A check was kept on the atmosphere composition using gas chromatography as described above. Ten fruit were sampled from each chamber once a week, up to eight weeks, for Cox's Orange Pippin and once every two weeks, up to 16 weeks for Granny Smith fruit. Atmospheres were re-established with in two hours of sampling.

The experiment was repeated in 1990 for Cox's Orange Pippin fruit incorporated the following changes. Carbon dioxide levels were maintained at a low level by the addition of a carbon dioxide scrubber, 100 grams of granulated sodium hydroxide (Carbosorb). The carbon dioxide enriched atmospheres were not scrubbed for carbon dioxide.

Carbon dioxide levels in both years for Granny Smith fruit were kept close to zero by addition to the chamber of 100 grams of sodium hydroxide (Carbosorb) a carbon dioxide scrubber. The carbon dioxide enriched atmospheres were not scrubbed for carbon dioxide.

2.3 Measurements Taken at Each Sampling.

Each of ten fruit from each treatment at each sampling time were assessed for the following:

2.3.1 Firmness.

Fruit at each sampling time were assessed for firmness using a hand held Effigi penetrometer. The skin on opposite sides of the fruit near the equator was

peeled using a potato peeler. A measuring head of 7/16" (10.9 mm) was used for Cox's Orange Pippin apples and a head 5/8" (15.7 mm) for Granny Smith apples. The average of each fruits firmness was used to compare treatments. The 1990 experiments used a 5/8" (15.7 mm) measuring head for both cultivars.

2.3.2 Soluble Solids.

Fruit at each sampling time were assessed for soluble solids using a hand held temperature compensating Atago N20 refractometer. Juice squeezed from pieces of flesh cut from each side of the apple was used for each measurement.

2.3.3 Weight Loss.

Fruit were weighed before and after storage on an Mettler electronic balance to two decimal places. The difference was used to calculate weight loss as a percentage.

2.3.4 Colour Measurement.

2.3.4.1 Minolta Chromameter.

Colour measurements were made with a Minolta DP100 portable chromameter. Fruit chromaticity was recorded in Commission Internationale d'Eclairage L*, a*, and b* colour space coordinates (Francis 1952). The meter was calibrated at illuminant condition C (6774K) with a white standard before use in 1989. A green standard was used in 1990 to allow the chromameter to be calibrated closer to the colour being measured.

At each sampling time ten fruit were measured four times around the equator. Cox's Orange Pippin fruit had two measurements taken of background green colour and two measurements of the red colour. Granny Smith apples background green colour was measured with care being taken to avoid blushed

or marked areas. The sites of the measurements were marked (sites of background green colour measurements only for Cox's Orange Pippin) so that identical areas on the fruit were measured by the chromameter, colour chart and tissue from this area used for chlorophyll extraction.

A chromameter records the composition of colour from an object by recording light reflected back from the object when illuminated by a pulse of light from a Xenon lamp through an 8 mm diameter measuring area. The results were expressed as Hunter L*a*b values describing a three dimensional position in a colour solid with L* being the difference between white and black (0 is black, 100 is white), a* is along the red/green axis, (positive values being red, negative values being green) and b* is the blue yellow axis, (positive values being yellow and negative values being blue). The values of a* and b* are not independent and should not be considered in isolation. Francis (1980) recommended that L*a*b* values be presented for analysis as two rather than three values. The first value is the L value which represents lightness and the second value is a function of hue or the visually perceived redness, greenness etc., which is calculated by a plot of Hunter a* vs b*. The line joining the a*, b* plot with the centre makes an angle, theta, with the horizontal axis.

The ratio of b*/a*, a measure of hue, is expressed as the Hue angle calculated by the following formula:

$$\text{Hue} = \tan^{-1}(b/a) + 90 \quad \text{if } a < 0$$
$$\text{Hue} = \tan^{-1}(b/a) \quad \text{if } a >= 0$$

2.3.4.2 Colour Chart.

Ten Granny Smith fruit at each sampling time were rated using the NZAPMB Granny Smith maturity index colour charts on a scale of 1 (green) to 9 (yellow). The colour of the chart was compared to the fruit colour at four locations around

the equator of the fruit and averaged. Two of the locations were identical to where the chromameter measurements were taken.

The colour of Cox's Orange Pippin fruit was not assessed by colour chart as there are no maturity index colour charts for Cox's Orange Pippin apples used by the NZAPMB.

2.3.5 Chlorophyll Content.

After chromameter and colour chart measurements had been taken a 10.04 mm diameter skin disk approximately 1 mm thick was cut from the identical location as measured with the chromameter and colour chart. Two disks of skin per fruit were taken corresponding to the location of the first two measurements. This was the least red positions or most background green colour for Cox's Orange Pippin and opposite sides of the fruit for Granny Smith. The skin disk was placed into labelled test tubes containing 3 mls of cold Analar grade N,N-Dimethylformamide (DMF) (Moran and Porath, 1980, 1982) then covered with tinfoil before being placed at 0°C ± 1°C. After 96 hours the contents of each test tube were decanted into a 1 cm glass cuvette. Absorbance readings were made at 710 nm, 665 nm, 647 nm using a Hitachi U2000 spectrophotometer. Scans of the absorbance of the chlorophyll solution from 750 nm to 400 nm indicated 665 nm and 647 nm were the wavelengths of maximum absorption for chlorophyll a and chlorophyll b. A measurement at 710 nm was used to quantify any turbidity of the solution being measured which was than subtracted from the absorbance measured at 665 nm and 647 nm. The concentrations of chlorophyll a and chlorophyll b were calculated using the differential equations of Arnon (1949) with later corrections for DMF of Porra *et al* (1989) (Appendix 1).

2.5 Calculating Chlorophyll Loss

In general the rate of decrease of a metabolite in any biochemical reaction is proportional to its concentration. Such a relationship between chlorophyll concentration and time has been observed in pears (Laval-Martin 1969).

Progressive loss of chlorophyll over time was described by a declining exponential curve (Bailey and Ollis, 1977), with rate of decline proportional to the amount of chlorophyll remaining. As levels of chlorophyll drop so does absolute rate of loss in chlorophyll. By transforming chlorophyll content to natural logs the relationship is converted from curvilinear to linear. Regressing this against time allows the rate constant of the reaction to be calculated and thus the effects of different temperatures and atmospheres on chlorophyll loss can be compared. The procedure outlined above is represented graphically in Figure 2.3.

The following formula was used for a declining exponential curve (Bailey and Ollis, 1973):

$$A_t = A_o * e^{-kt} \qquad [2.1]$$

Where; A_t = concentration at time t (ng/mm^2)
A_o = concentration at time 0 (ng/mm^2)
t = time (days)
k = rate constant ($days^{-1}$)

The units of k were determined by the following unit cancellations:

$$\frac{ng}{mm^2} = \frac{ng}{mm^2} * k * days$$

$$k = \frac{ng}{mm^2} * \frac{mm^2}{ng} * \frac{1}{days}$$

$$k = days^{-1}$$

2.6 General Observations.

Before evaluation for soluble solids and firmness, notes were made on the visual appearance of the fruit and of any rot and disorders, such as internal rot, bitterpit, core browning etc., that may have been present.

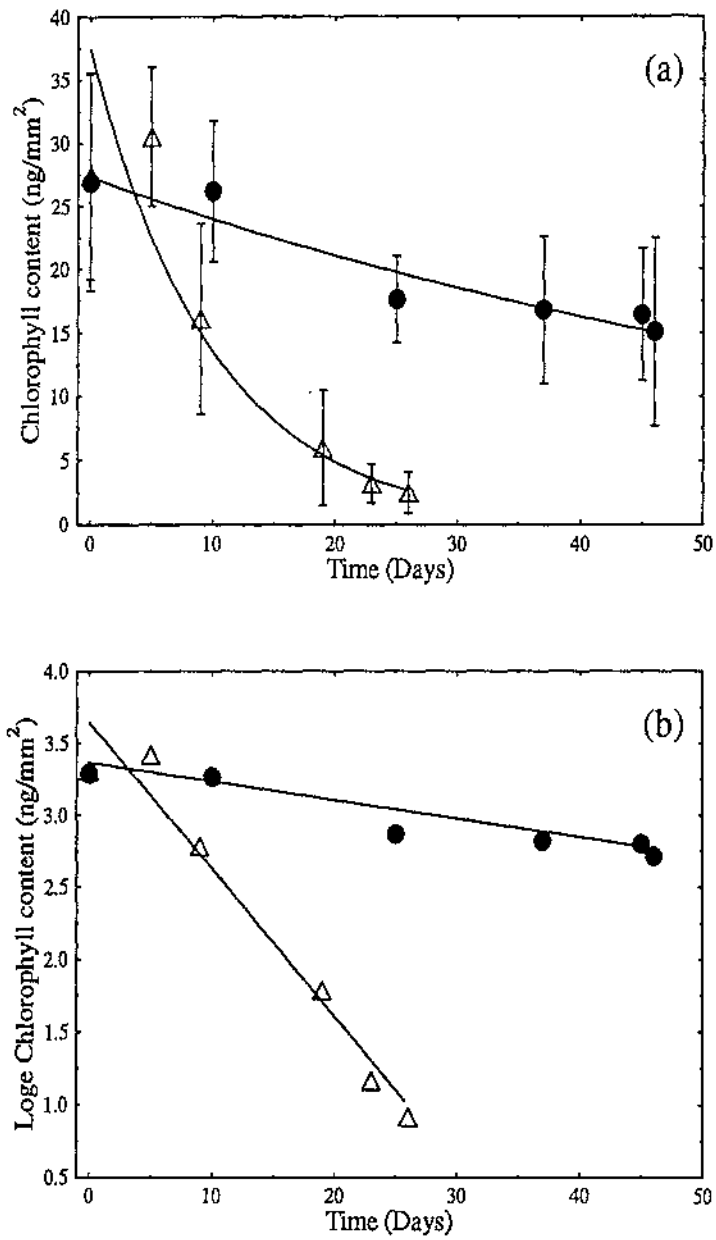


Figure 2.3 Change in chlorophyll a content over time for Cox's Orange Pippin apples at 4°C (●) and 25°C (△) showing (a) actual data and (b) $\log_e$ transformed data. For 4°C A_0 = 37.4 ng/mm^2; k = 0.013 days^{-1}; r^2 = 0.330; NS; for 25°C A_0 = 27.3 ng/mm^2; k = 0.102 days^{-1}; r^2 = 0.701; $p<0.001$.

Chapter 3

Comparison of Methods of Measuring Colour.

3.1 Introduction

In order to characterise changes in colour of apples under various storage environments rapid and accurate methods of estimating colour change are required. These methods must not only reflect physiological changes occurring in the skin of fruit but also accurately show changes as perceived by eye.

Many cultivars are harvested according to colour and many consumers have a preference for apples of a particular colour according to cultivar. For example Golden Delicious apples are yellow when fully ripe and Granny Smith apples green. Both Cox's Orange Pippin and Granny Smith apples yellow as they senesce and overripe fruit are characterised by their yellow background colour.

Over the past 50 or so years many methods have been used to assess colour and colour change of horticultural produce (Table 3.1). In physiological studies extraction and measurement of plant pigments such as chlorophylls and carotenoids is the most accurate and common method used. Most methods of measuring colour have been limited by available technology and many have been of little practical use outside a laboratory. With the advent of microelectronics experimenters are making increasing use of less expensive and portable tristimulus colourimeters, such as the Minolta chromameter, to describe colour changes (Meir *et al* 1992, Pai and Sastry 1990, Schwartz and Lorenzo 1990, Singha and Townsend 1989, Singha *et al* 1991, Stow 1989, Watkins *et al* 1991). Such instruments allow non-destructive measurement of fruit colour

during growth, maturation and postharvest life. With tristimulus colourimeters the most common mode of expressing colour of fruit and vegetables has been to use the L*a*b system. This system closely mimics the human eye, using the pairing of red-green (a*) and blue-yellow (b*) colours which is the same as the eye sees colour. It was originally developed by Hunter and associates in the 1940's to measure ripeness of tomatoes (Hunter 1975). The system converts the Commission Internationale d'Eclairage (CIE 1931) (Clydesdale 1975) chromaticity chart to an equally spaced perceived visual difference chart.

Analysis of data from tristimulus colourimeters is difficult as colour is measured as a point in a three dimensional colour solid. Accordingly researchers in colourimetry of food have recommended that the measurement of colour be condensed from three to two parameters to aid analysis of data. It has been suggested that the L*a*b* parameters should be transformed to the hue angle ($\tan^{-1}(b^*/a^*)$) and lightness values in which differences observed are of equal visual spacing (Francis 1952, 1975, 1980; Little 1975). Using only a* or b* values is not valid as they are not independent variables (as a* changes so does b*). A single axis value does not indicate how the visual colour is changing. An example of this would be bleaching of an apple. The perception would be that the colour is becoming lighter but the ratio of a*/b* may be unchanged indicating that the lighter colour has the same hue. If a* values were the only measure of colour considered the interpretation would be that the colour had changed, and was not just a lightening. The ratio of a*/b* or b*/a* is not linear; as either a* or b* become very small or very large the ratio increases very rapidly and is a tangential function (it approaches infinity as a* approaches zero or zero as b* approaches zero). Therefore the same differences between a*/b* ratios do not represent the same difference in perceived colour (Table 3.2). For example a difference of 0.039 in the a*/b* ratio when the a* value is -22.70 and the b* values is 44.51 is equal to a difference in hue of 1.90 degrees. The same difference in a*/b* ratio when the a* value is -6.11 and the b* value is 33.76 is equal to a difference in hue of 2.17 degrees. Francis (1975) recommended that if the a*/b* ratio was between 0.2 and 2 then difference between measurements

were approximately linear but if the values were outside these limits then the hue angle must be used. For these reasons the chromameter data reported as L*a*b* has been converted to Hue angles in this study.

L*a*b* values have been presented in different ways in recent publications (Singha and Townsend 1989, Singha *et al* 1991, Watkins *et al* 1991), not following the recommendations of Francis (1975, 1980) and Little (1975). This has had the unfortunate effect of making comparisons or even determining differences between treatments difficult. A*/b* ratios have been published which are outside the linear range recommended by Francis or where only a* or b* values were analyzed (Watkins *et al* 1991).

Chlorophyll content of apple skin is an important visual indicator of fruit maturity for both growers and consumers (Olsen 1969). In this study three methods of measuring colour were used. Visual colour was measured using an objective measurement with a Minolta chromameter the measurements being expressed in terms of hue angle and lightness and a subjective measurement using the NZAPMB maturity colour chart. These were related then to chlorophyll content, the principle pigment which determines apple green skin colour.

A chromameter offers many advantages in measuring colour; in particular it is objective, quick and non-destructive. However before the chromameter can be used for colour assessment and the results obtained to develop predictive models there must be confidence hue angle and lightness are true measures of apple skin colour. To test this, subjective and objective assessments of skin colour were related to chlorophyll content for the identical area of skin measured. Thus comparisons could be made between different methods of colour assessment.

Table 3.1 Summary of methods used to measure colour and colour change in horticultural produce.

Method	Plant material	Description	Reference
Pigment Extraction	Asparagus	Extraction in 80% acetone.	Wang *et al* 1971, Kramer *et al* 1949
	Apple		Knee 1975, Knee 1972, Knee 1980a, Knee 1980b, Gorski and Creasy 1977, Mussini *et al* 1985, Francis *et al* 1955, Frenkel *et al* 1969, Workman 1964
	Pear		Gross 1984, Laval-Martin 1969, Frenkel *et al* 1969
	Strawberry		Woodward 1972
	Mangoes		Medlicott *et al* 1986a
	Green Beans		Groeschel *et al* 1966
	Citrus		Eilati *et al* 1975, Jahn and Young 1976, Knee *et al* 1988, Apelbaum *et al* 1976
	Broccoli		Lebermann *et al* 1968
	Ribes		Gross 1982
	Kiwifruit		Gross and Ohad 1983
	Cantaloupe		Gross and Ohad 1983
	Avocado		Gross and Ohad 1983
	Tomato		Gross and Ohad 1983
	Cherry Tomatoes	Extraction in 90% methanol.	Laval-Martin *et al* 1975
	Apple	Extraction in Dimethyl-formamide	Stow 1989, Johnson and Ertan 1983
	Citrus	Extraction in acetone then analysis by HPLC.	Amir-Shapira *et al* 1987
	Spinach		Yamauchi and Watada 1991

Enzyme Activity	Apple Banana Sugar Beet Leaves	Chlorophyllase activity measured after being prepared in acetone.	Rhodes and Wooltorton 1967, Looney and Patterson 1967 Looney and Patterson 1967 Holden 1961
Tristimulus Colour Meter	Apple Citrus Tomato	'Techwest' apple colour meter. Hunter colour difference meter. Gardener colour difference meter. Minolta chromameter. Hunter colour difference meter. Gardener colourimeter	Gorski and Creasy 1977 Francis *et al* 1955, Workman 1964, Stow 1989, Pai and Sastry 1990, Francis 1952 Child *et al* 1984 Watkins *et al* 1991, Singha *et al* 1991 Purvis and Barmore 1981 Pai and Sastry 1990
Light Transmittance	Citrus Asparagus	Light transmittance difference meter. Spectrophotometer.	Jahn 1976 Kramer *et al* 1949
Light Reflectance	Apples Citrus	Light reflectance meter.	Lott 1944, Johnson and Ertan 1983, Knee 1980b Jahn and Young 1976, Knee *et al* 1988
Colour Score	Green Beans Broccoli Asparagus Apple Oranges	Organoleptic panel scores. Visual score Colour chart Colour chart	Groeschel *et al* 1966 Lebermann *et al* 1968 Kramer *et al* 1949 Lau 1985 Francis 1952 Knee 1980b Apelbaum *et al* 1976

Table 3.2 Comparison of ratios of a*/b* to hue angle.

a*	b*	a*/b*	Hue angle	Difference in a*/b*	Difference in Hue angle
-22.53	40.64	-0.554	119.00		
-21.70	44.51	-0.487	116.03	0.067	2.97
-18.04	40.28	-0.448	114.13	0.039	1.90
-16.25	47.04	-0.345	109.07	0.103	5.06
-14.13	52.65	-0.268	105.01	0.077	4.06
-12.21	48.21	-0.253	104.21	0.015	0.80
-10.28	44.47	-0.231	103.01	0.022	1.20
-8.20	57.85	-0.142	98.09	0.089	4.92
-6.11	33.76	-0.181	100.26	0.039	2.17
-4.24	60.35	-0.070	94.08	0.11	6.16
-1.77	47.29	-0.037	92.18	0.033	1.90
-0.38	49.90	-0.008	90.30	0.029	1.88

3.2 Methods

The average chlorophyll content, hue angle, chroma and lightness and in the case of Granny Smith apples colour chart score were graphed (Figures 3.1 to 3.13), using the GLE graphics package, and regression equations determined, using the SAS statistical program, in order to characterise the relationship between different methods of colour measurement.

Graphs presented in this chapter were drawn using data from the experiment examining effect of temperature on colour change. Means of each sample time for each temperature, over both seasons from each cultivar, representing approximately 4000 data points or 2000 apples, have been used to determine relationships between the methods of colour measurement used.

3.3 Results

Apples used in all experiments changed colour from green to yellow over the course of the experiment as indicated by increase in lightness, decrease in hue

angle (indicating a shift towards yellow) and reduction in chlorophyll content (Figure 3.1). Colour chart score for Granny Smith increased (Data not shown). Lightness increased over time in an asymptotic pattern similar for both cultivars reaching about the same values by the end of the assessment period. Hue angle changed little over time for Granny Smith fruit but declined from 110° to 95° in a linear manner for Cox's Orange Pippin. Chlorophyll content declined in a pattern similar to a declining exponential. Although there was a higher chlorophyll content in Granny Smith apples the pattern of chlorophyll loss was the same as for Cox's Orange Pippin. There was a larger proportional decrease in chlorophyll for Cox's Orange Pippin compared to Granny Smith. The apparent rise in chlorophyll content for Granny Smith fruit after 1 and 2 days storage was not significant.

In Cox's Orange Pippin apples at 25°C for 30 days chlorophyll a declined faster (0.10 day^{-1}) than chlorophyll b (0.05 day^{-1}) (Appendix A2.1 and A2.3, Figure 3.2). The ratios of chlorophyll a/b decreased from 3.6 to 1.5 during this time as chlorophyll a declined faster than chlorophyll b. Granny Smith apples had slightly faster chlorophyll b loss (0.04 day^{-1}) than chlorophyll a loss (0.03 day^{-1}) at 25°C for 30 days (Appendix A2.2 and A2.4, Figure 3.2). The pattern of chlorophyll loss was similar for both cultivars.

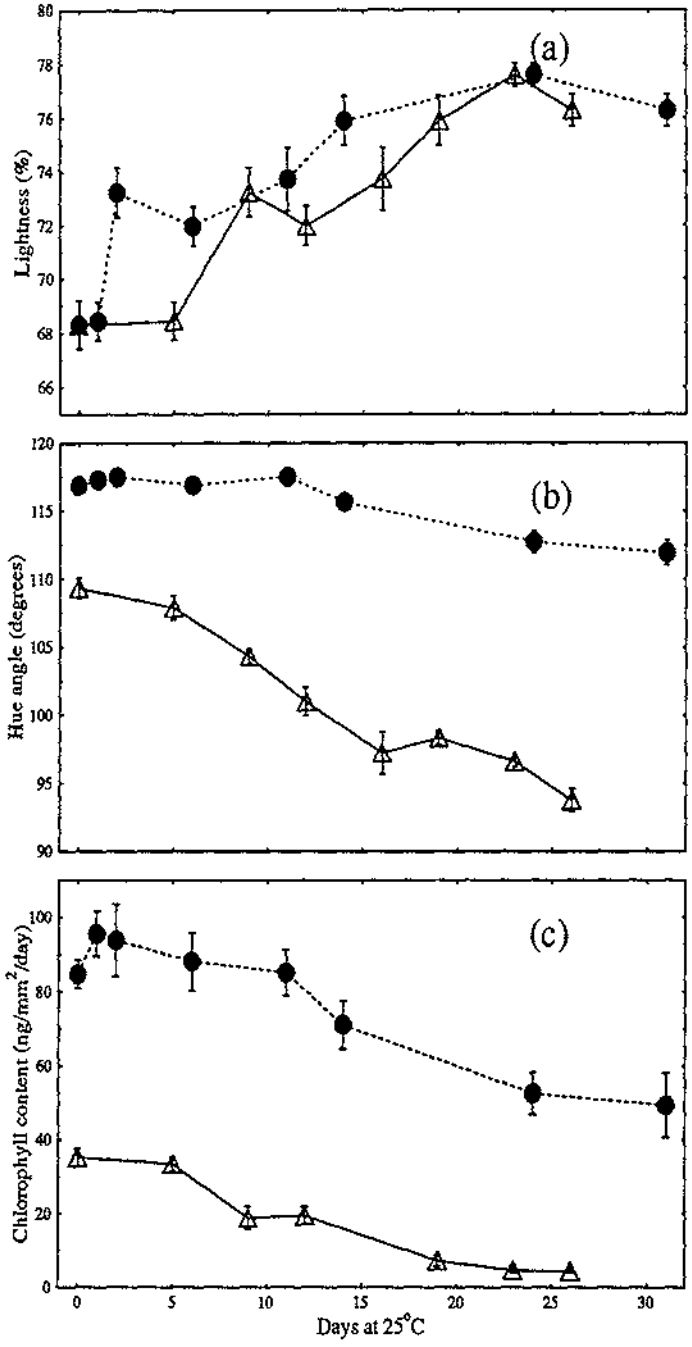


Figure 3.1 Comparison of change in (a) lightness, (b) hue angle and (c) chlorophyll at 25°C of Cox's Orange Pippin (△) and Granny Smith (○) apples early harvest, 1989.

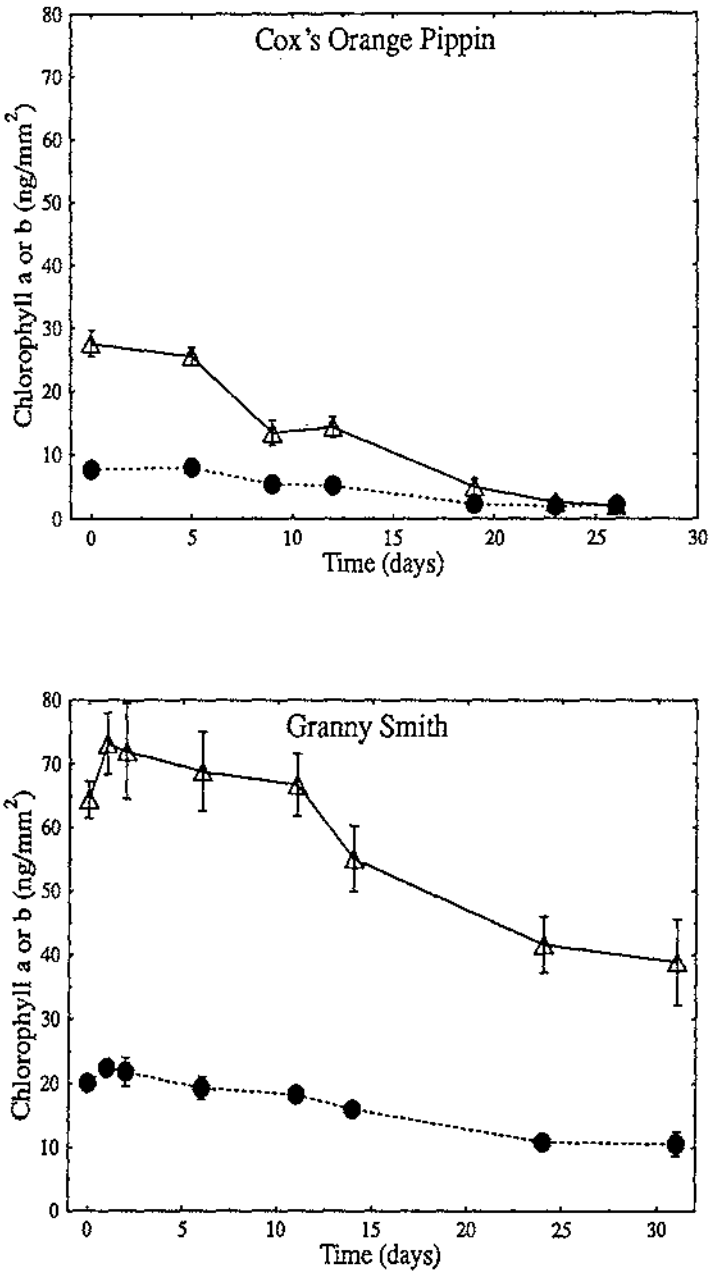


Figure 3.2 Chlorophyll a (△) and chlorophyll b (○) concentration in early harvested Granny Smith and Cox's Orange Pippin apples stored at 25°C, 1989.

3.3.1 Hue angle and Chlorophyll Content

Chlorophyll contents of Granny Smith ranged from 15 ng/mm^2 to 100 ng/mm^2 while contents for Cox's Orange Pippin had a much smaller range, 2 ng/mm^2 to 35 ng/mm^2. Chlorophyll content of the peel fell from 100 ng/mm^2 to 5 ng/mm^2, the hue angle changed from green-yellow (hue 120°) to almost yellow (hue 95°). This nonlinear relationship can be described by the asymptotic function: hue angle = loge(chlorophyll content/1.8719x10^{-04}))/0.11 (r^2=0.843 p<0.0001) (Figure 3.3). Applying this exponential regression equation to each cultivar's data indicated that the model gave a good fit (Figure 3.4). The regression equations for Cox's Orange Pippin: hue angle$_{COP}$ = loge(chlorophyll content/1.76x10^{-03})/0.088 (r^2=0.642 p<0.0001) and for Granny Smith: hue angle$_{GS}$ = loge(chlorophyll content/1.42x10^{-03})/0.093 (r^2=0.662 p<0.0001) also gave a good fit.

3.3.2 Hue angle and Lightness

As hue angle of the peel changes from green to yellow there is a nonlinear relationship between hue angle and lightness described by the equation: lightness = 158.767-(exp(0.03006*hue angle)+63.8063) (r^2=0.829 p<0.0001) (Figure 3.5). Applying this regression equation to data from each cultivar indicated that the model used fitted well (Figure 3.6). The regression equation for Cox's Orange Pippin was: lightness$_{COP}$ = 159.453-(exp(0.027*hue angle)+70.303) (r^2=0.448 p<0.0001); and for Granny Smith: lightness$_{GS}$ = 160.328-(exp(0.0306*hue angle)+63.483) (r^2=0.616 p<0.0001).

hue angle = loge(total chlorophyll/$1.87x10^{-04}$)/0.11 r^2=0.843

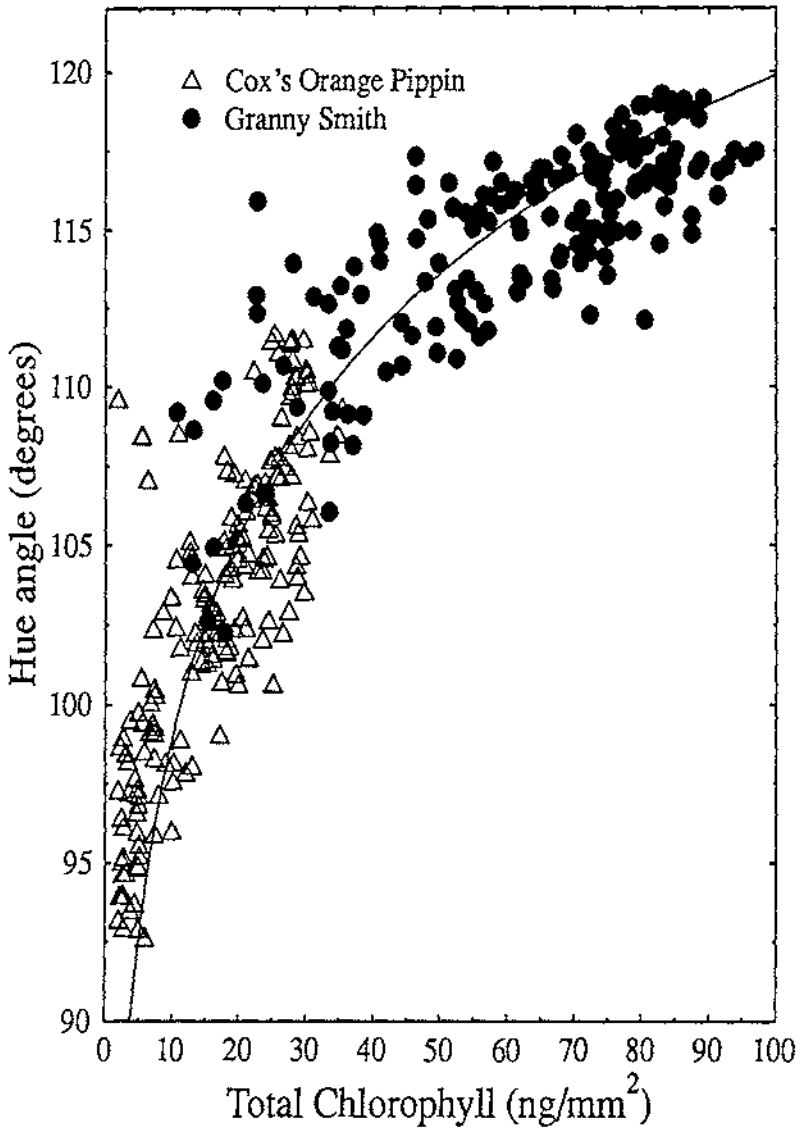
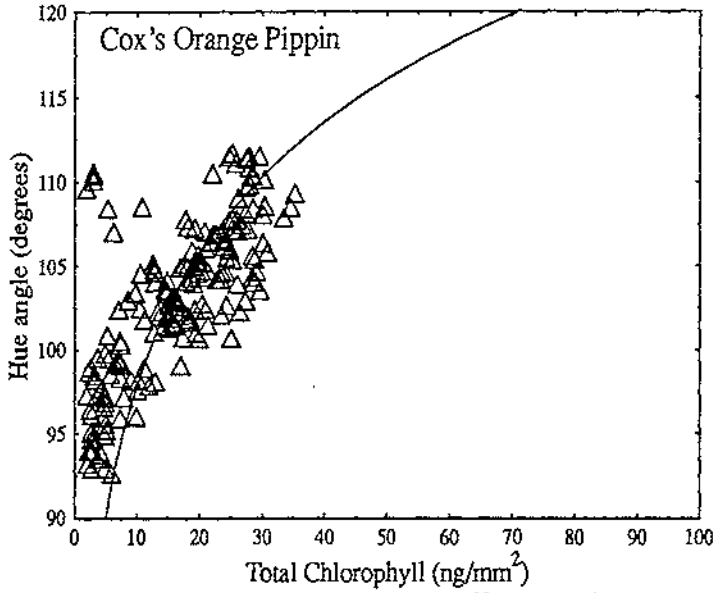


Figure 3.3 Hue angle as a function of total chlorophyll Cox's Orange Pippin and Granny Smith apples stored during 1989 and 1990.

hue angle = loge(total chlorophyll/1.76x10^{-03})/0.088 r^2=0.642



hue angle = loge(total chlorophyll/1.42x10^{-03})/0.093 r^2=0.662

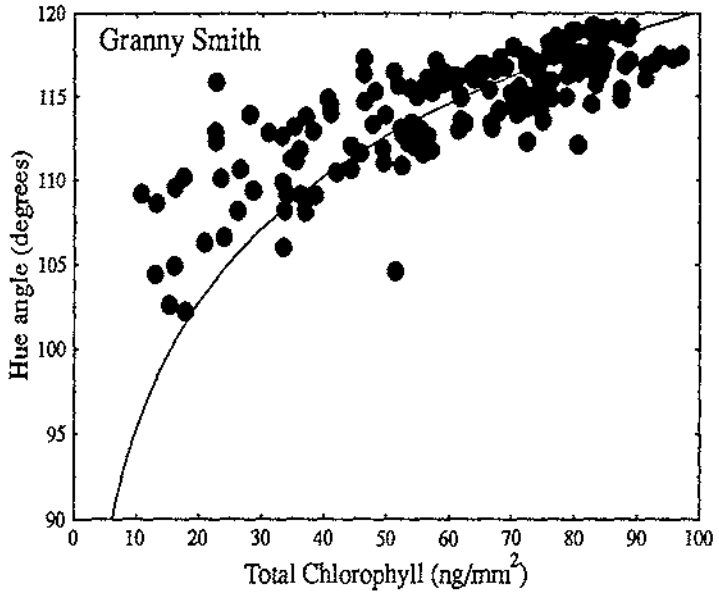


Figure 3.4 Hue angle as a function of total chlorophyll of Cox's Orange Pippin and Granny Smith apples stored during 1989 and 1990.

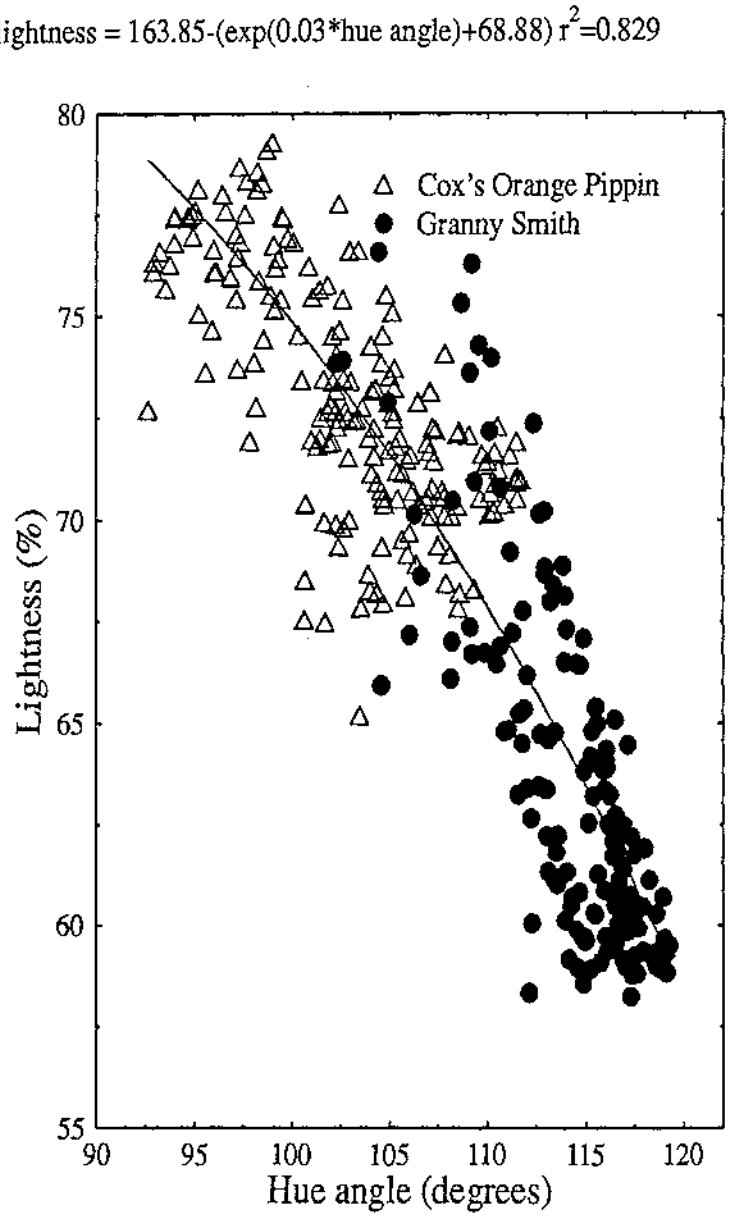
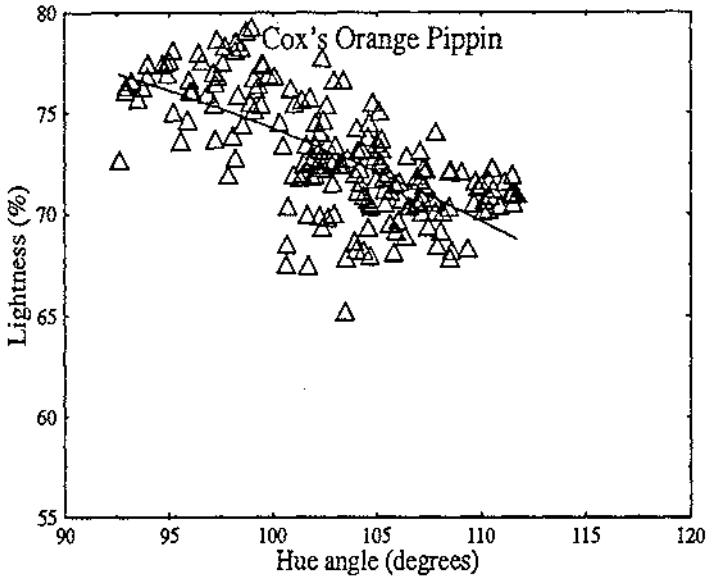


Figure 3.5 Lightness as a function of hue angle of Cox's Orange Pippin and Granny Smith apples stored during 1989 and 1990.

lightness = 159.45-(exp(0.027*hue angle)+70.30) r^2=0.488



lightness = 160.33-(exp(0.031*hue angle)+63.48) r^2=0.616

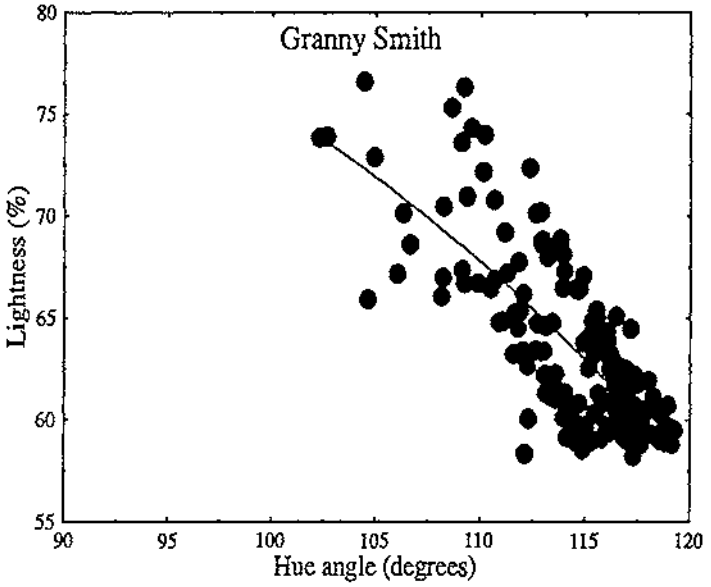


Figure 3.6 Lightness as a function of hue angle of Cox's Orange Pippin and Granny Smith fruit stored during 1989 and 1990.

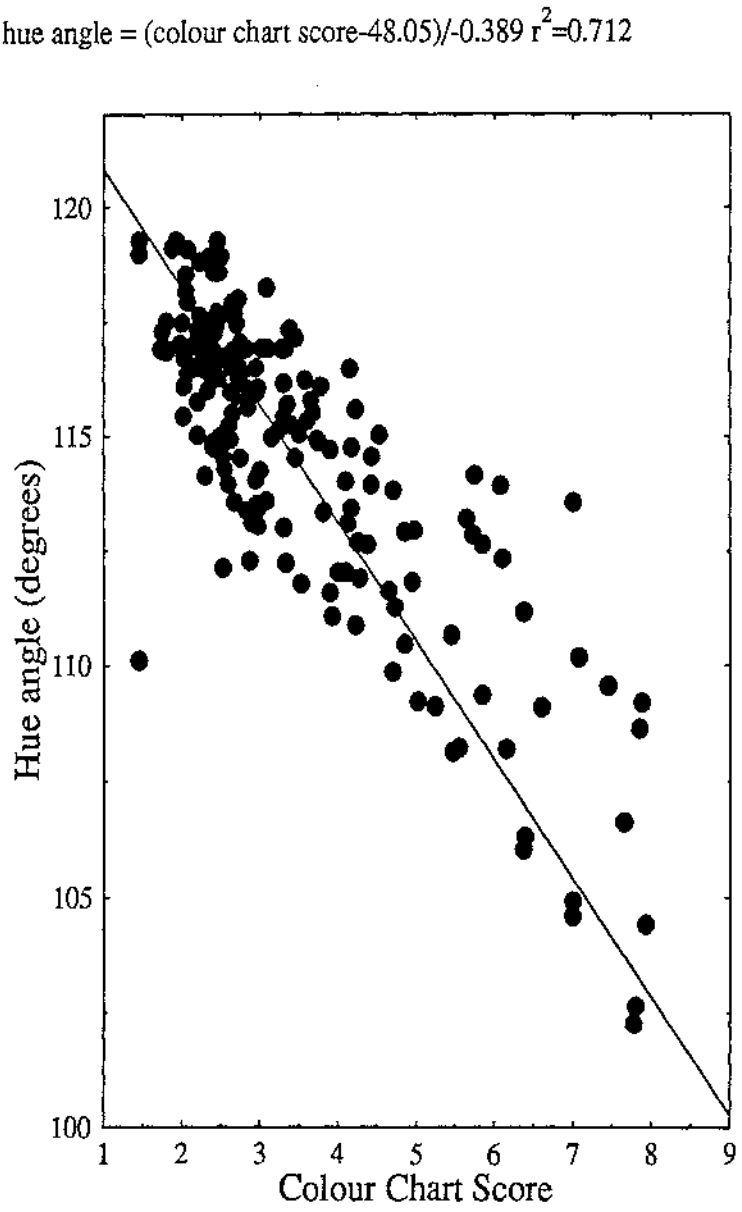


Figure 3.7 Colour chart as a function of hue angle for Cox's Orange Pippin and Granny Smith apples stored during 1989 and 1990.

The highest lightness values (75 to 80%) are found at the lowest hue angles (90° to 95°) and high hue angle values are associated with low lightness values. Therefore increases in hue angle occur along with decreases in lightness values.

Lightness values for Cox's Orange Pippin were recorded over a wide range of hue angles (90° to 115°). Granny Smith values were recorded over a similar range of hue angles but most data was clumped in the range of 110° to 120° hue and the data at lower hue angles had a wide scatter. This would indicate Granny Smith lightness values are more variable and don't always coincide with the same changes in hue angle. A 5° hue change for Cox's Orange Pippin represents a 2.5% change in lightness whiles the same change in hue angle is equivalent to a 4.5% change in lightness for Granny Smith apples.

3.3.3 Hue Angle and Colour Chart Score

As the hue angle of Granny Smith apples declined from 120° to 102°, the colour chart score rose from 1 to 9 in a linear manner (Figure 3.7). The differences in each colour chart score represents a similar difference in hue angle (2.5°) with the linear relationship: hue angle = colour chart score-48.046/-0.389 (r^2=0.712 $p<0.0001$).

3.3.4 Lightness and Chlorophyll Content

As apple skin colour darkens chlorophyll content increases (Figure 3.8) suggesting that lightness values give a good indication of chlorophyll content. When chlorophyll content is almost zero lightness is about 80% falling to about 57% at 95 ng/mm^2 chlorophyll. The relationship between lightness and chlorophyll content fits a curvilinear declining exponential function: lightness = 76.6878*exp(-0.00311*chlorophyll) (r^2=0.933 $p<0.0001$). Applying the model to data for each cultivar gave a good fit (Figure 3.9). The regression equation for Cox's Orange Pippin was: $lightness_{COP}$ = 76.8678*exp(-0.00311*chlorophyll)

(r^2=0.722 p<0.001) and for Granny Smith was: $lightness_{GS}$ = 75.3397*exp(-0.002841*chlorophyll) (r^2=0.872 p<0.001).

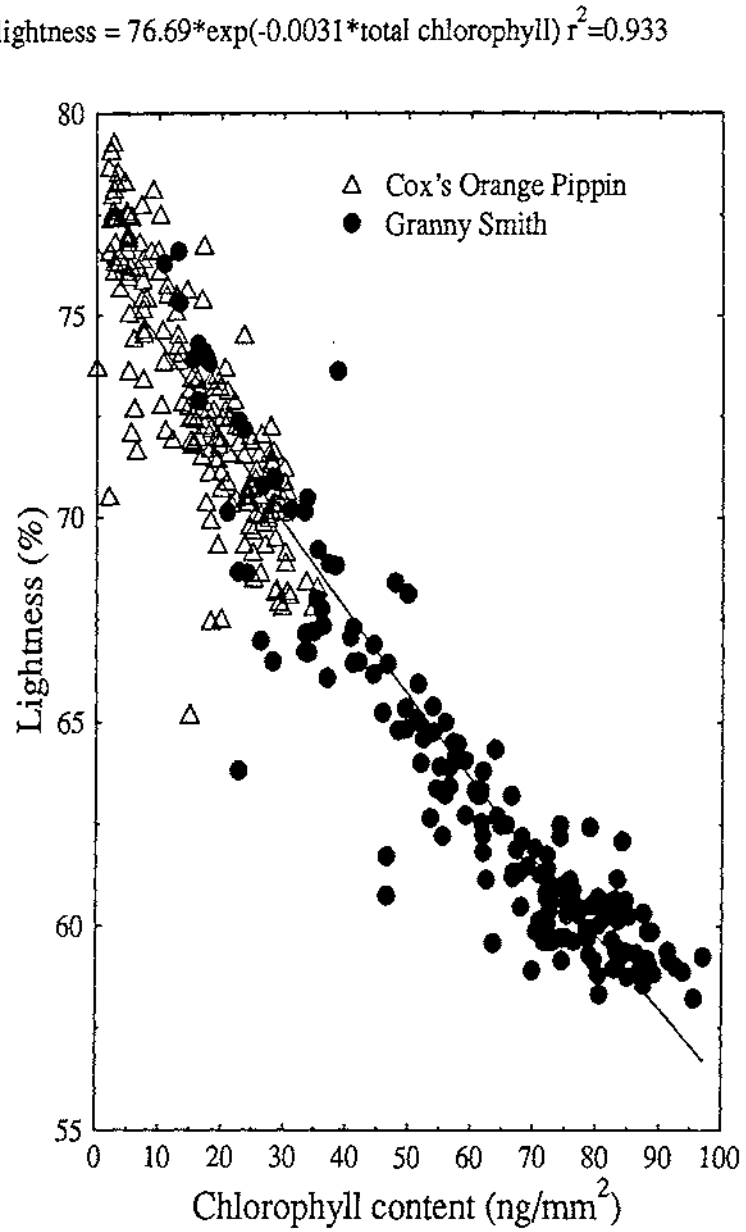
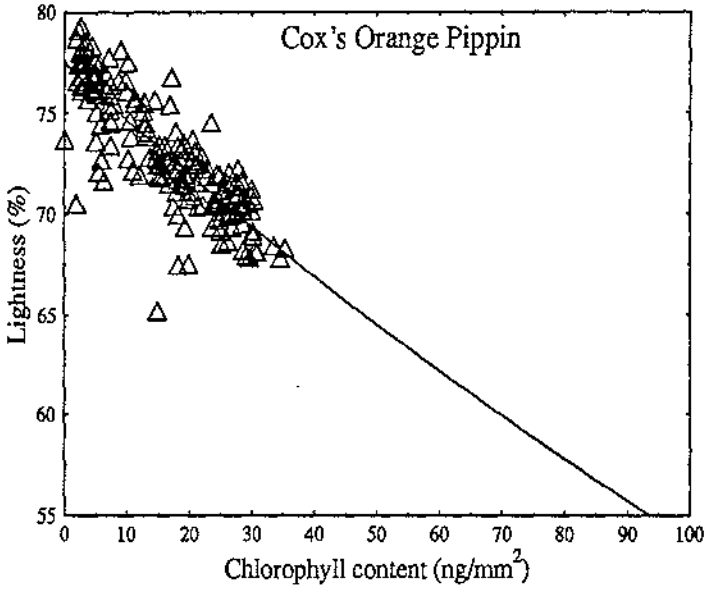


Figure 3.8 Lightness as a function of total chlorophyll for Cox's Orange Pippin and Granny Smith apples stored during 1989 and 1990.

lightness = 76.87*exp(-0.0031*total chlorophyll) r^2=0.722



lightness = 75.34*exp(-0.0028*total chlorophyll) r^2=0.872

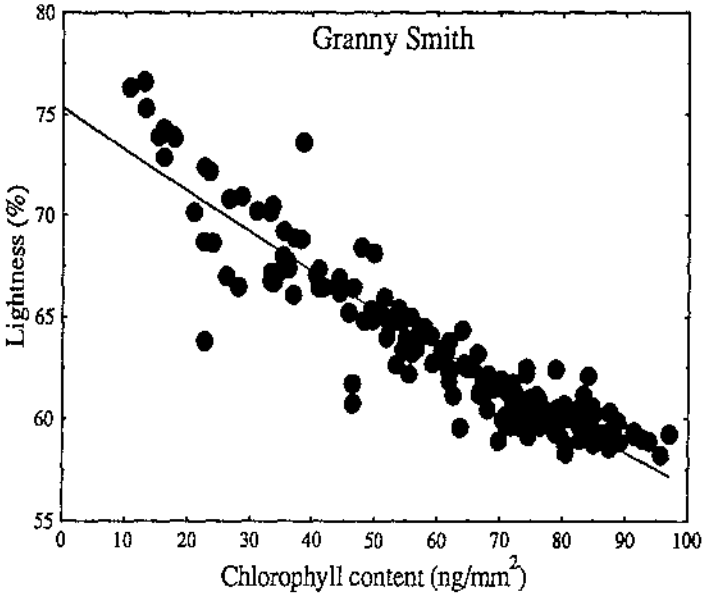


Figure 3.9 Lightness as a function of total chlorophyll of Cox's Orange Pippin and Granny Smith apples stored during 1989 and 1990.

3.3.5 Colour Chart Score and Chlorophyll Content

Colour chart score has a curvilinear relationship with chlorophyll content described by a declining exponential curve: chlorophyll = loge(colour chart score/9.099)/-0.01654 (r^2=0.721 $p<0.0001$) for Granny Smith (Figure 3.10). A change in colour chart score from 2 to 3 is equivalent to a chlorophyll change of 24.3 ng/mm^2 while a change in colour chart score of 7 to 8 is equivalent to a chlorophyll change of 6.4 ng/mm^2. These results are similar to the relationship between hue angle and chlorophyll content.

3.3.6 Lightness Values and Colour Chart Score

As colour chart score increases from 1 to 9 lightness increases in a linear manner which can be described by the following function: lightness = 54.4747+(2.551*colour chart score) (r^2=0.786 $p<0.0001$) (Figure 3.11).

3.3.7 Chroma, Hue angle and Chlorophyll Content

Chroma is a measure of the intensity of a colour; the more intense the colour the higher the chroma. For both cultivars there was a large variation in chroma when compared to hue angle or chlorophyll content (Figure 3.12). A broad trend of increasing chroma occurs as hue angle or chlorophyll content declines, but scatter of data was very high and no significant relationship existed.

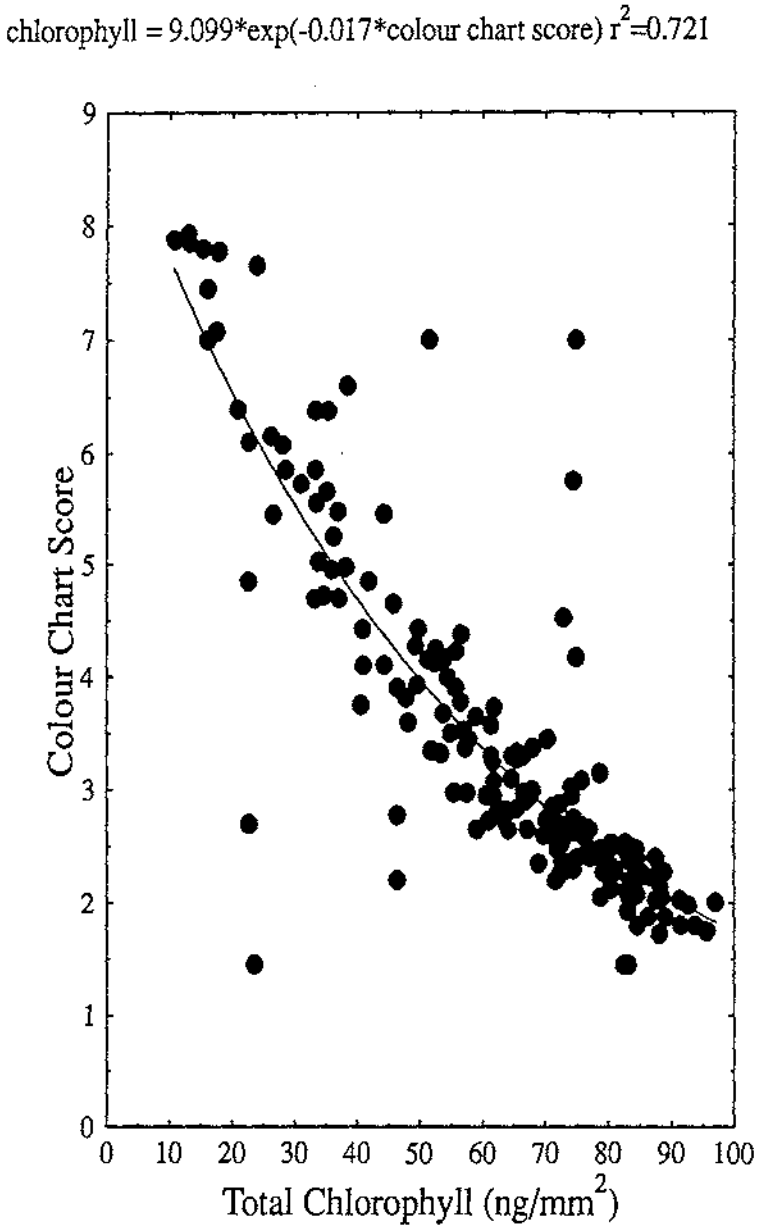


Figure 3.10 Colour chart score as a function of total chlorophyll of Granny Smith apples stored during 1989 and 1990.

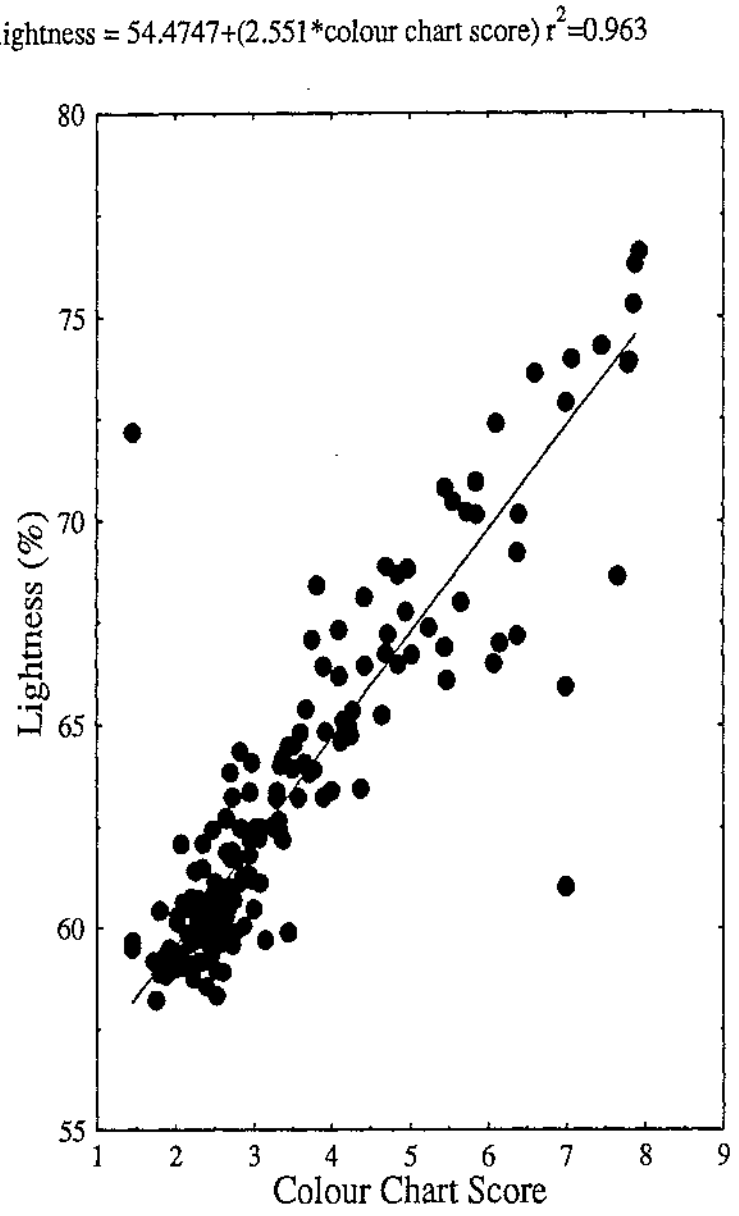


Figure 3.11 Lightness as a function of colour chart score of Granny Smith apples stored during 1989 and 1990.

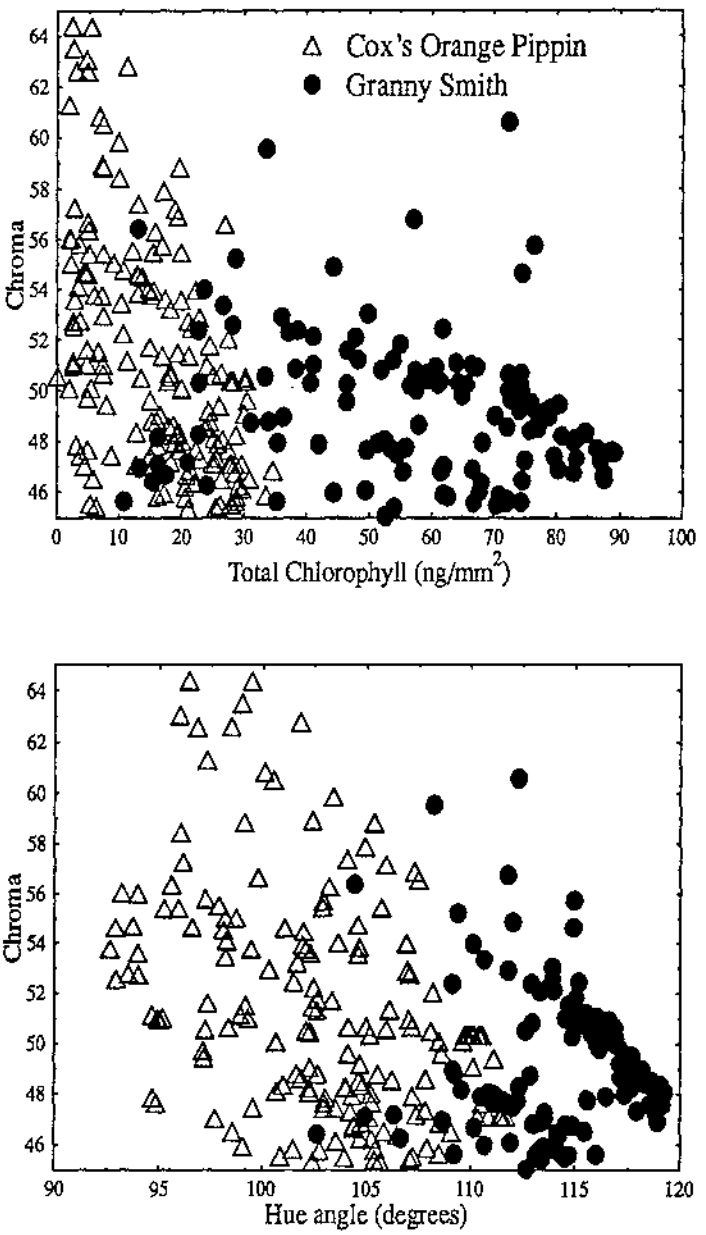


Figure 3.12 Chroma in relation to hue angle and total chlorophyll of Cox's Orange Pippin and Granny Smith apples during 1989 and 1990.

3.4 Discussion

To accurately estimate ***k*** of colour change for apples when exposed to various treatments reliable and precise methods of measurement are required. Each method must measure colour and colour change as would be seen by eye as well as accurately estimate pigment concentration in apple fruit skin. A chromameter offers a practical and straight forward way to measure yellowing of apples. But before the chromameter can be used with confidence to measure colour we must be certain the measurements taken relate accurately to changes in pigment concentration. To assess the validity of using hue angle calculated from L*a*b* values a comparison was made with the pigment content (chlorophyll) present, for both cultivars, and for Granny Smith apples the NZAPMB maturity colour chart.

Of considerable interest is the finding that a generalised equation could describe, the relationship between hue angle, lightness or colour chart to chlorophyll for both cultivars. This implies that any one of the methods used to estimate colour is equally valid and published results using one of these three methods can be compared. Although Cox's Orange Pippin and Granny Smith fruit differ in chlorophyll content these differences between cultivars are not due to the method of colour assessment used. This is in contrast to results obtained by Knee (1980b) who found the relationship between reflectance and chlorophyll content and other methods of measurement differed significantly according to cultivar, and even between individual batches of fruit. The reasons for this difference are not known.

3.4.1 Chlorophyll Content and Hue Angle

Using hue angle to measure colour change has a sound physiological basis as it correlates very well with chlorophyll content. Hue angle is a measure of total skin colour and therefore the predominant pigment colour. During senescence the predominant colour changes to yellow and the predominant pigments to

carotenoids. For example if chlorophyll content remains constant but yellow pigments increased the hue angle would change. It is known that yellow and red pigments change little during storage when compared to changes in chlorophyll content (Knee 1980a). The scatter in the data of Figure 3.3 may be due to varying amounts of yellow or red pigments in the skin. These were not measured although care was taken to measure areas of skin having background colour only. This was not always possible with Cox's Orange Pippin as some fruit had large areas of red or were speckled with red so that there was very little skin that was background colour only. In addition variation in hue angle may also be due to differences in surface features, such as waxiness of the skin (Knee 1980b, Francis 1952) which makes the light more diffuse coming from the apple, shifting the colour to the red.

Apples used in these experiments were of similar size (count 125, 148 g average fresh weight) and free of blemishes. Thus difference in size which has been reported to have a large influence on colour of apples (Francis 1952) was not a complicating factor. Each fruit was measured twice on its equator to obtain an average reading, this minimised differences due to different locations on each apple. Lightness values increased during the experiment showing that total pigment content fell. As both cultivars showed a substantial loss of chlorophyll during ripening (Figure 3.2) it is probable the level of yellow pigments remained constant or declined slightly.

There are two distinct populations of data in Figure 3.3 with Cox's Orange Pippin fruit having less chlorophyll and lower hue angles than Granny Smith fruit. Although chlorophyll content was higher in Granny Smith fruit there was only a small change in the hue angle as chlorophyll content decreased. A change in chlorophyll content in Cox's Orange Pippin resulted in a larger change in hue angle than was the case for Granny Smith apples as this was the portion of the curve where the slope was steepest. When chlorophyll content in the peel is low, changes in chlorophyll level measured by hue angle are greater than when chlorophyll content is high. A change in the hue angle from 90° to 105° resulted

in a change of 20 ng/mm^2 (0.75°/ng/mm$^{2)}$ in chlorophyll content whereas moving from a hue angle of 110° to 120° results in a change of 60 ng/mm^2 (0.17°/ng/mm^2). Therefore the hue angle measures changes in chlorophyll more sensitively at low than at high chlorophyll concentrations. As such the chlorophyll level can start to decline in fruit before changes can be visualised. This implies that when interpreting subjective assessments of colour and changes in colour especially when associated with physiological events such as the climacteric colour may be changing before any such changes are discernable by eye. Using a chromameter may be sensitive and consistent in determining if the background colour of an apple is changing during major physiological events before discernable changes are noticeable by eye.

3.4.2 Chlorophyll Content and Lightness

Lightness defines the brightness of a colour. Thus apple skins with high pigment contents would be expected to have a darker colour than apples with low pigment content. There is little scatter around the regression line making lightness a good predictor of chlorophyll content (Figure 3.8). This is similar to the relationship reported by Singha *et al* (1991) between anthocyanin content and lightness of Red Delicious apple skin and Meir *et al* (1992) for chlorophyll content and lightness of watercress leaves. Lightness is a useful indicator of apple skin pigment content, irrespective of the type of pigment, as the levels are determined by the concentration of the pigments present. Lightness increased as chlorophyll content decreased indicating that total pigment content fell as the fruit yellowed. This suggested that there was little or no increase in yellow pigment sufficient to replace lost chlorophyll. Knee (1980a) showed that yellow pigments rose only 3% in Cox's Orange Pippin during storage at 12°C while the chlorophyll content declined 90%.

3.4.3 Chlorophyll Content and Colour Chart Score

Colour chart score has a curvilinear relationship to chlorophyll content (Figure 3.10) due to the scale differences between each scores colour (Figure 3.13). The scale used for the colour chart is more sensitive to changes in low chlorophyll concentrations than for high concentrations as the differences between scores are less from 1-5 than 6-9. This is not surprising as the colour chart was developed from a DSIR study using a Minolta chromameter (I.F. Warrington pers comm.) and hence could be expected to vary in a similar manner to hue angle.

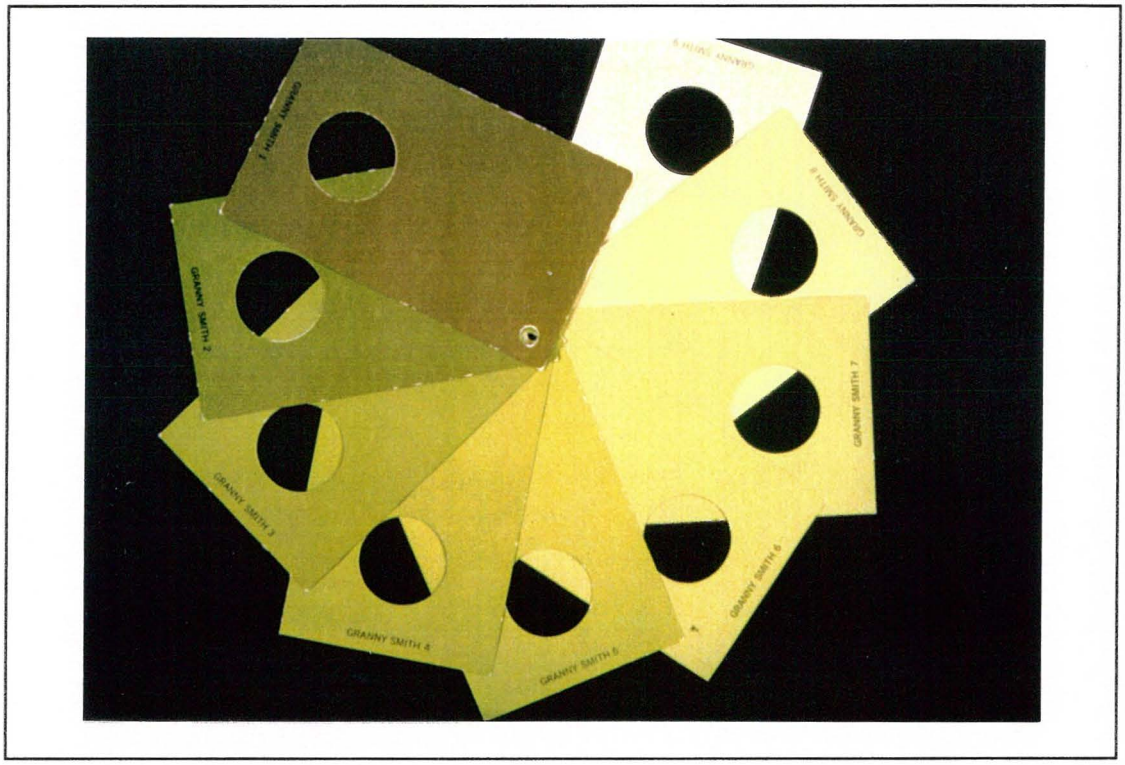
3.4.4 Hue Angle, Lightness and Colour Chart Score

The colour charts score has a linear relationship with lightness and hue angle which is an indication that hue angle is an accurate measurement of colour as seen by eye. Therefore hue angle or lightness could be used to estimate colour change. The large cluster of data points around colour chart scores of 1 to 4 with lightness and hue angle was due to few Granny Smith apples turning completely yellow during the course of the experiments.

3.4.5 Hue Angle and Lightness

Two fruit may have a similar hue angle but different lightness values as shown by the wide scatter of data in Figure 3.6. The fit of hue angle to lightness is good for each cultivar although better when the data was combined from each cultivar (Figure 3.5) This may reflect in part, the pigment composition of the skin and/or the total pigment content. One way to assess the level of pigment in the skin may be to look at chroma, the intensity of hue, the higher the concentration of pigments the more intense the colour. However, there was no clear relationship between chroma and chlorophyll content or hue angle (Figure 3.12). In fact the opposite of what was expected occurred. The less the chlorophyll

content, in some cases, the higher the chroma. This may have resulted from chroma representing the intensity of yellow pigment.



Colour Chart Score	1	2	3	4	5	6	7	8	9
Hue Angle	116.61	115.87	115.17	114.91	114.06	112.75	112.42	110.55	104.69
Lightness	51.22	56.62	62.67	65.25	68.32	71.93	71.47	77.25	83.08

Figure 3.13 New Zealand Apple and Pear Marketing Board Granny Smith maturity colour chips and table of hue angles and lightness of each score.

3.5 Conclusions

Colour values obtained by a chromameter, colour charts or pigment concentration in the skin of apples correlate highly with each other (Table 3.3). Therefore changes in colour, when measured using these techniques, are real and differences in colour do not depend on the type of measurement used. The lightness, hue angle or pigment content of the skin are all good indicators of colour and can be used with confidence to assess the effect of environmental conditions on colour change. Similarities in the relationships between different methods of colour assessment between Cox's Orange Pippin and Granny Smith indicates that the methods used should work well over a wide range of varieties.

Table 3.3 Correlation coefficients for chlorophyll relating to hue angle, lightness and colour chart score.

	Hue Angle	Lightness	Colour Chart Score
Cox's Orange Pippin	0.647***[1]	0.722***	
Granny Smith	0.662***	0.872***	0.721***
Combined	0.843***	0.933***	

1 Correlation coefficients are significant at $p<0.001$.

Therefore all three methods of analysis, hue angle, lightness or colour chart score could be used as predictors of chlorophyll content in the skin of Cox's Orange Pippin or Granny Smith apples. Of the methods of analysis lightness has the highest r^2, least scatter in the data and is the most linear over a wide range of chlorophyll contents. Therefore lightness would be the preferred choice for non-destructive analysis of Cox's Orange Pippin and Granny Smith colour changes.

Chapter 4

Temperature Effects on Colour Change.

4.1 Introduction

Low temperatures are the primary means by which postharvest technologists maintain quality for extended periods during storage of horticultural produce. Respiration and ripening of harvested fruits are dependent on temperature (Biale and Young 1962, Eskin *et al* 1971) and much is known of the affects of temperature during storage on respiration rate and quality parameters such as firmness changes. Fruit colour, whether yellow, green or red, is an important visual quality parameter affecting consumer perception of ripeness (Lockshin and Rhodus 1991, Olsen 1969). While the relationship between colour change and temperature has been well documented it has been poorly characterised. Many cultivars of apples and pears change background colour from green to yellow during storage and speed of change depends on storage temperature (Laval-Martin 1969). Establishing the relationship between colour change from green to yellow of apples and temperature would allow prediction of when apples become unmarketable due to excessive yellowing if storage temperatures are changed during handling. In order to develop a model of colour change additional detailed data is required for a wide range of temperatures corresponding to those used in current handling practices.

In general fruit ripening occurs in a narrow range of temperatures between 10°C and 30°C (Rhodes 1980). Temperatures above or below this range inhibit ripening and yellowing associated with ripening. Chlorophyll breakdown is not thought to occur from the action of heat alone but from the various enzymes involved and occurs at a rate dependent on temperature (Hendry *et al* 1987). Therefore it should be possible to model the rate at which apple fruit yellow at specific temperatures.

It has been proposed (Johnson and Thornley 1985, Feng *et al* 1990) that a model combining the Arrenhius equation with the Boltzman enzyme distribution function (Figure 4.1) can be used to accurately describe the rate of loss or increase in a physiological attribute expressed as a rate constant (***k***) with temperature. This has been used subsequently to estimate temperature dependent ***k*** in such diverse systems as growth rates of bacteria, leaf growth and shoot dry weight changes in spring wheat (Johnson and Thornley 1985, Feng *et al* 1990). It should be possible to use this model for colour change in apples.

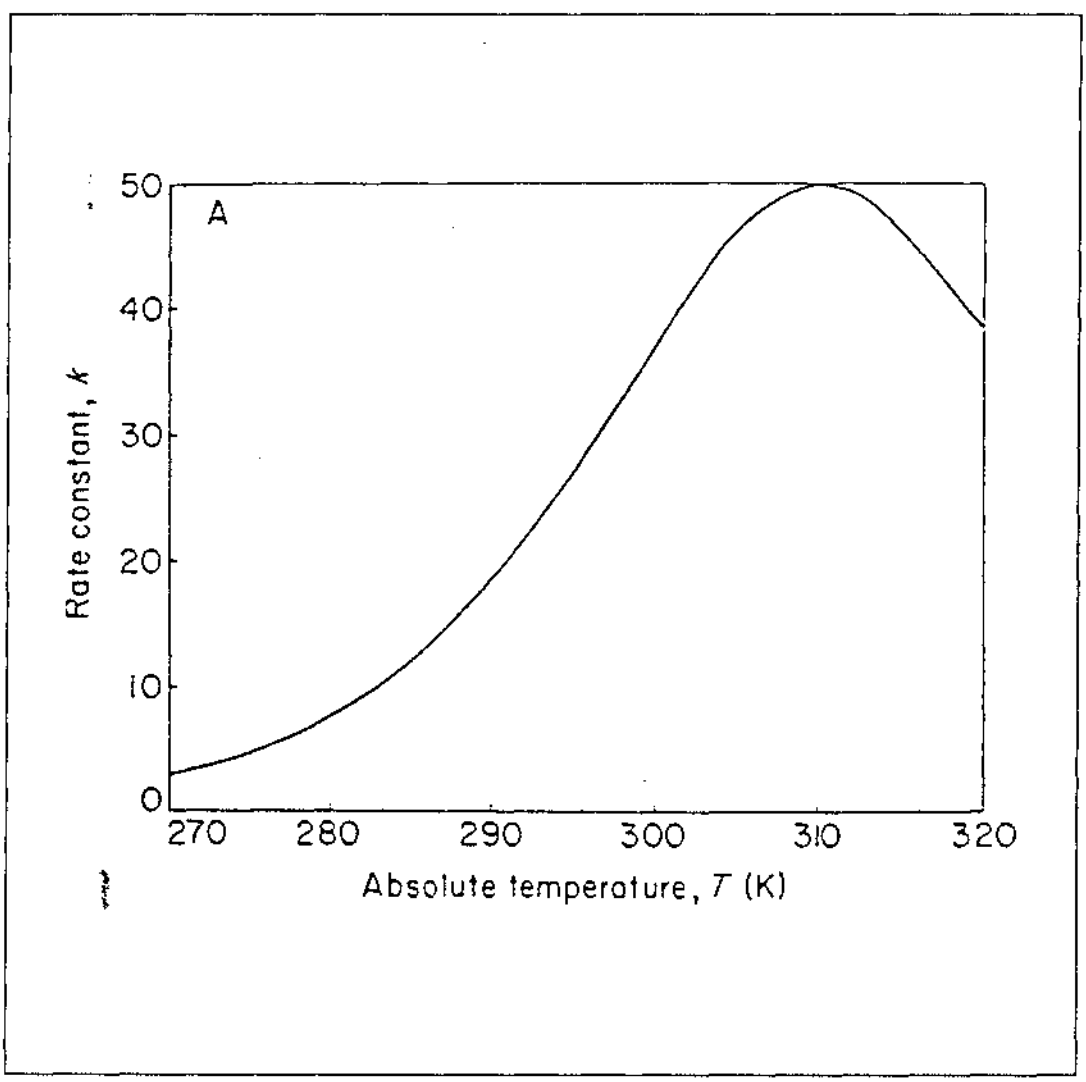


Figure 4.1 Graphical representation of the modified Arrenhius equation taken from Johnson and Thornley (1985).

The following formulae were used to develop the modified Arrenhius equation (Johnson and Thornley 1985) :

The Arrhenius equation: the initial exponential phase of the curve.

$$k = K_a e^{-E_a/RT} \qquad [4.1]$$

The Boltzman enzyme distribution function: the loss of activity at higher temperatures.

$$k = 1 + e^{\Delta S/R} e^{-\Delta H/RT} \qquad [4.2]$$

The modified Arrenhius equation: the combination of the two.

$$k = \frac{K_a e^{-E_a/RT}}{1 + e^{\Delta S/R} e^{-\Delta H/RT}} \qquad [4.3]$$

Where: k = rate constant of reaction, poorly described by [4.1] and [4.2] but well by [4.3]

K_a = rate constant of the process if there was no inhibition, i.e. constant of Arrenhius equation.

Ea = activation energy $(J.(kg.mol)^{-1})$

R = gas constant 8314 $(J.(kg.mol)^{-1}.K^{-1})$

T = temperature (K)

ΔS = increment of entropy $(J.(kg.mol)^{-1}.K^{-1})$

ΔH = increment of enthalpy $(J.(kg.mol)^{-1})$.

Equation [4.3] describes a reaction in which the enzymes involved are either in an inactive or active state in differing proportions according to temperature and

that there is a temperature optimum at which the process occurs at a maximum rate.

Parameters of the model were further simplified to the following formula (Feng *et al* 1990):

$$k = \frac{A * e^{-B/T}}{1 + e^{C - D/T}} \qquad [4.4]$$

Parameters are: $A = K_a$

$B = E_a/R$

$C = \Delta S/R$

$D = \Delta H/R$

The temperature at which ***k*** was maximum determined by equation [4.4], was calculated by the following formula (Feng *et al* 1990):

$$T_{max} = \frac{D}{C + \ln(D/B - 1)} \qquad [4.5]$$

Thus the optimal temperature for yellowing of apples can be estimated from the above equations. Knowledge of the relationship between temperature and yellowing of apples would be of practical benefit to growers and marketers of apples as it allows a sensible estimation of the effect that changes in temperature during handling will have on apple yellowing.

4.2 Methods

A sample of ten fruit was taken seven times during storage from each temperature treatment (ranging from 0°C to 35°C) and the chlorophyll content,

hue angle and lightness, and in the case of Granny Smith apples, colour chart score, measured according to the techniques described in Chapter 2. The experiment was conducted using fruit from several growers and two harvests within a season. The experiment was repeated over two seasons, 1989 and 1990. This represents a database of readings from 1960 fruit. The values obtained at each sample time were averaged and used to calculate ***k***, equation [2.1], for each storage temperature. Values of ***k*** and regression equations were obtained, using the SAS statistical program, and graphed (Figures 4.3 to 4.8), using the GLE graphics package (a general purpose graphics package produced by the DSIR Gracefield), . Differences between parameters, cultivars, seasons, harvests and growers were determined using analysis of variance and multiple analysis of variance procedures of the SAS statistical program, for chlorophyll levels, hue angle, lightness, colour chart score, firmness and total soluble solids.

4.3 Results

Cox's Orange Pippin and Granny Smith apples for all parameters of colour change ***k*** calculated from equation [2.1] showed good fits and exhibited no 'lack of fit' (the standard errors of parameters were not large being in general 10-15% of ***k***).

4.3.1 Colour change and temperature.

Values of ***k*** for chlorophyll, hue angle, lightness and colour chart score increased exponentially from 0°C to 20°C to reach a maximum at temperatures ranging from 21°C to 27°C (Figures 4.3 to 4.8) with an average of 23.7°C. Above 24°C, ***k*** declined. Below 5°C increases in ***k*** were slower than between 5°C and 24°C where the increase in ***k*** showed a doubling about every 7°C (Figures 4.3 to 4.8). The pattern described above was the same for each of the colour parameters measured and varied only in magnitude between fruit from different growers and harvests (Appendix 2, Tables A2.1 to A2.11). There were clear differences in appearance of the fruit on removal from storage, the fruit at low temperatures,

0°C to 6°C, remaining green and the fruit at warm temperatures, 15°C to 25°C, turning yellow (Figure 4.2)

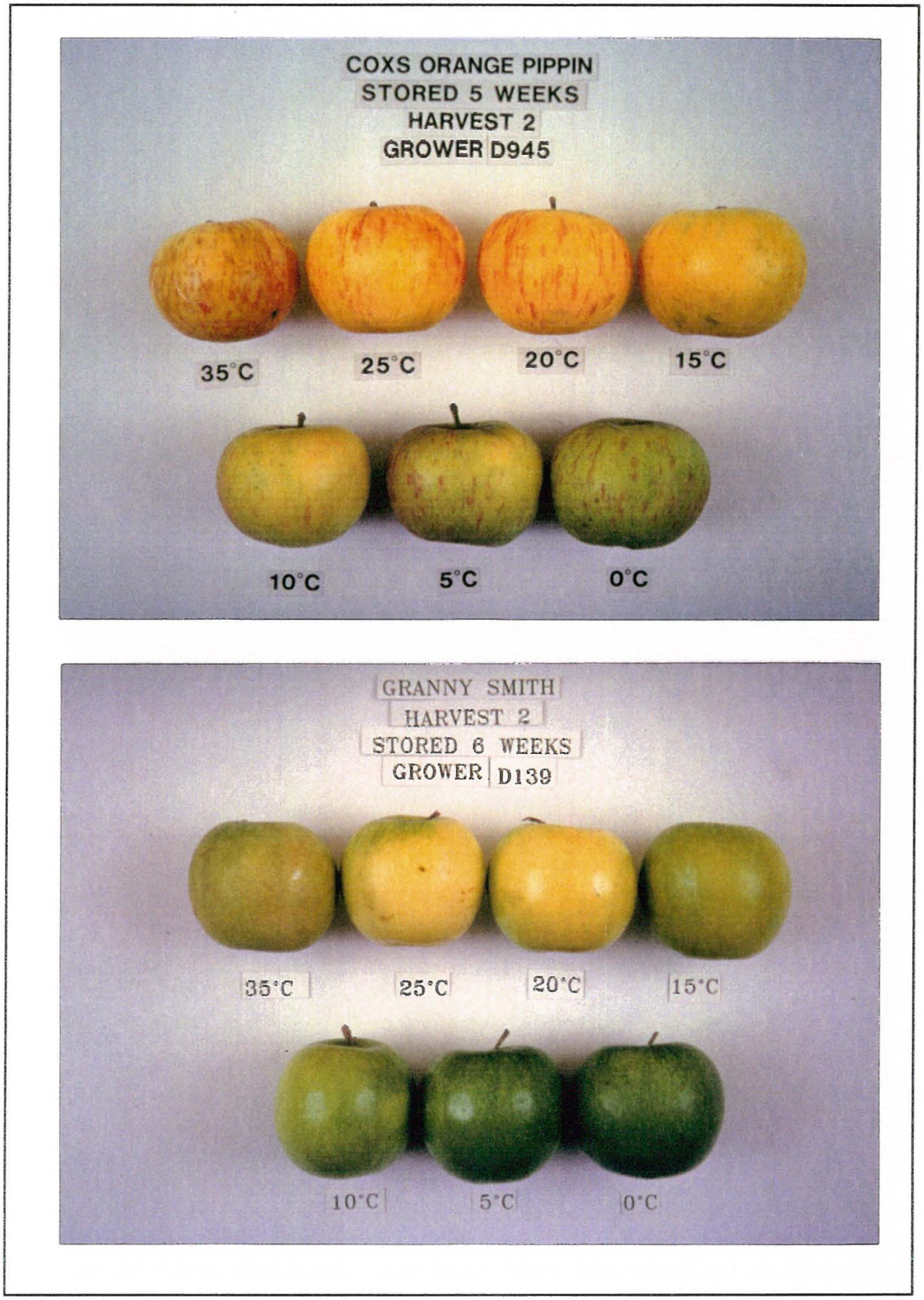


Figure 4.2 Comparison of Cox's Orange pippin and Granny Smith apples on removal from storage at different temperatures.

The value of ***k*** for Cox's Orange Pippin fruit was not significantly different between years; there was some variation between harvests within a year but the most significant difference was between growers (Table 4.1). In contrast for Granny Smith fruit the most significant differences were between years with little difference between harvests and growers. The differences observed are described in more detail in the following pages.

4.3.2 Cultivars

There was a difference in ***k*** between Cox's Orange Pippin and Granny Smith due mainly to the magnitude of change of colour parameters during storage (Figures 4.3 to 4.8). Both cultivars exhibit the same pattern of response to temperature for chlorophyll a, chlorophyll b, total chlorophyll and hue angle.

Table 4.1 Significance levels of ***k*** in quality parameters with temperature when assessed by year, harvest and grower.

	Cox's Orange Pippin			Granny Smith		
Parameter	Year	Harvest (Year)	Grower	Year	Harvest (Year)	Grower
Chl a	NS	NS	**	***	NS	NS
Chl b	NS	*	**	***	*	NS
Total Chl	NS	*	***	***	NS	NS
Hue Angle	NS	***	***	*	*	*
Lightness	*	NS	NS	***	NS	NS
Colour Chart Score				***	***	NS
Firmness	NS	NS	*	NS	NS	*
Total Soluble Solids	NS	*	*	NS	*	*

1 Significance levels: NS not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$

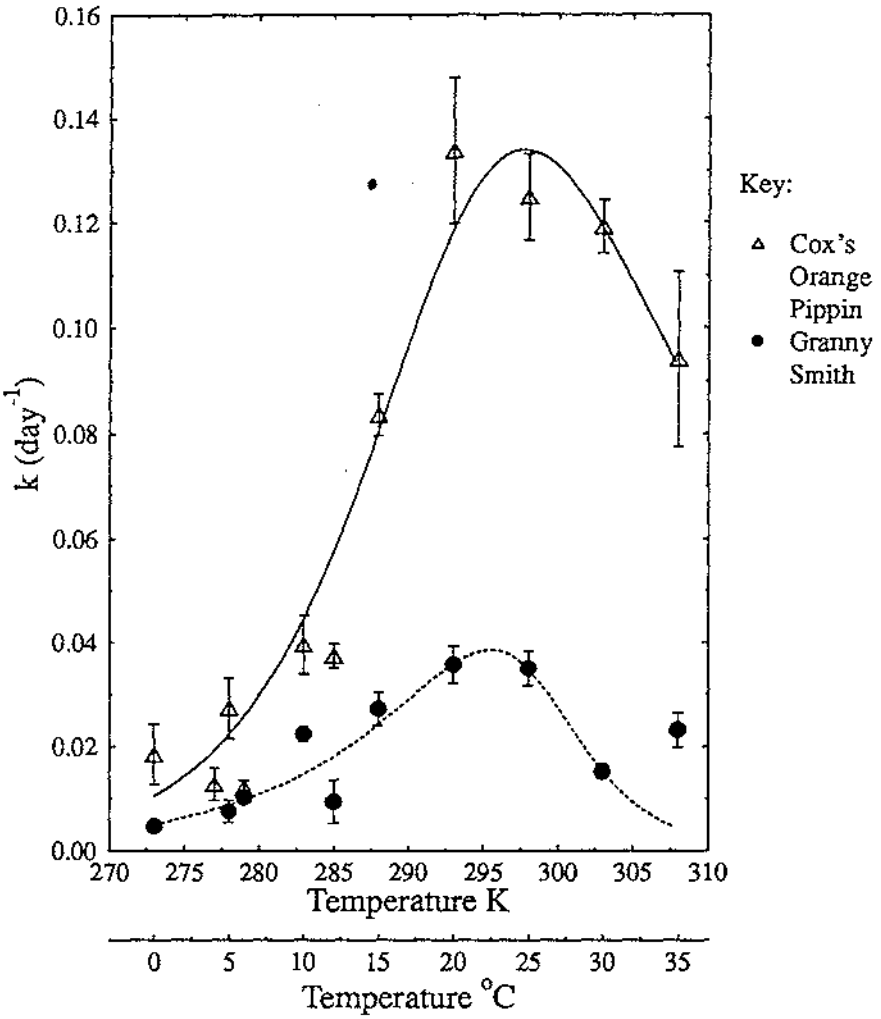


Figure 4.3 Rate constants of chlorophyll a in the skin of Cox's Orange Pippin and Granny Smith apples at various temperatures during 1989 and 1990. Rates were averaged over growers, harvests and years. Lines of best fit were calculated from equation [4.3] the parameters used were: for Cox's Orange Pippin: Ka = 3.51×10^{16}; Ea/R = 11641.70; $\Delta S/R$ = 62.32; $\Delta H/R$ = 18389.42; $r^2 = 0.803$; $p<0.01$; and for Granny Smith: Ka = 6.43×10^{10}; Ea/R = 8234.74; $\Delta S/R$ = 114.90; $\Delta H/R = 34289.68$; $r^2 = 0.715$; $p<0.05$.

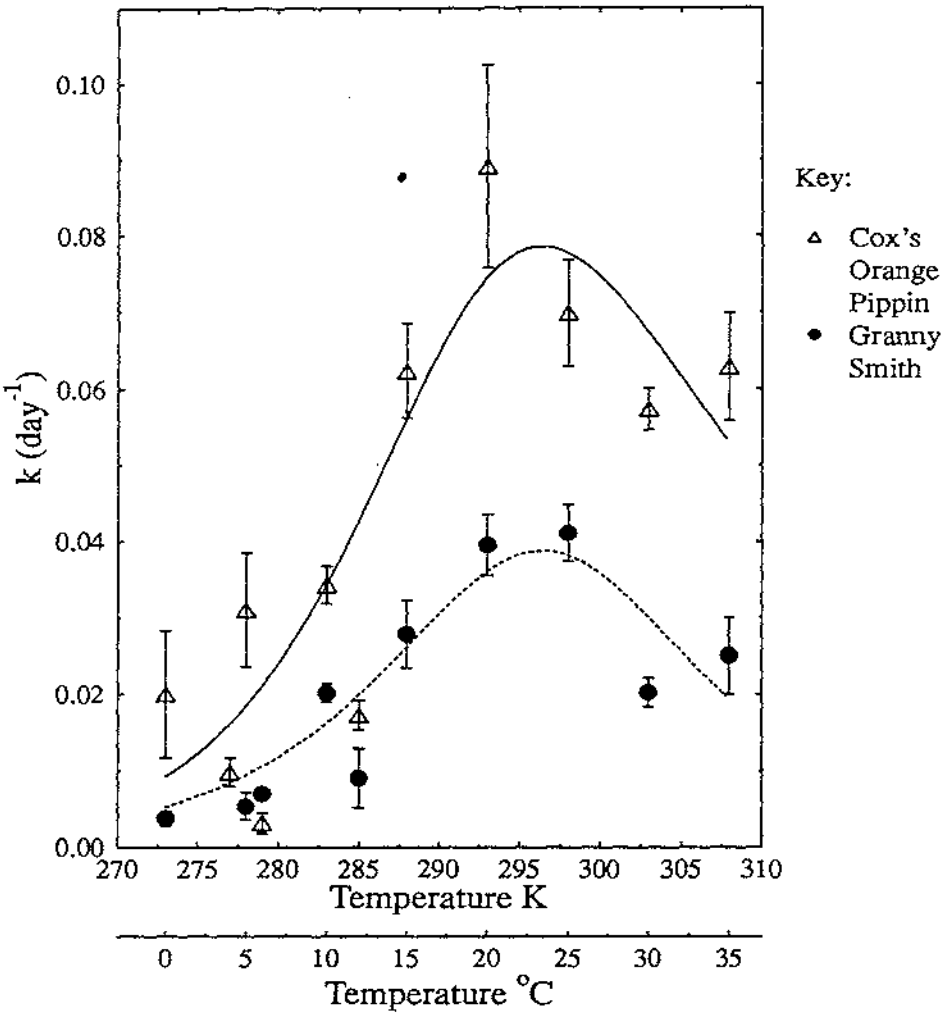


Figure 4.4 Rate constants of chlorophyll b in the skin of Cox's Orange Pippin and Granny Smith apples at various temperatures during 1989 and 1990. Rates were averaged over growers, harvests and years. Lines of best fit were calculated from equation [4.3] the parameters used were: for Cox's Orange Pippin: Ka = 2.13×10^{15}; Ea/R = 10910.71; $\Delta S/R$ = 57.96; $\Delta H/R$ = 17004.75; r^2 = 0.545; NS; and for Granny Smith: Ka = 7.72×10^{11}; Ea/R = 8902.27; $\Delta S/R$ = 67.30; $\Delta H/R$ = 20013.71; r^2 = 0.712; $p<0.05$.

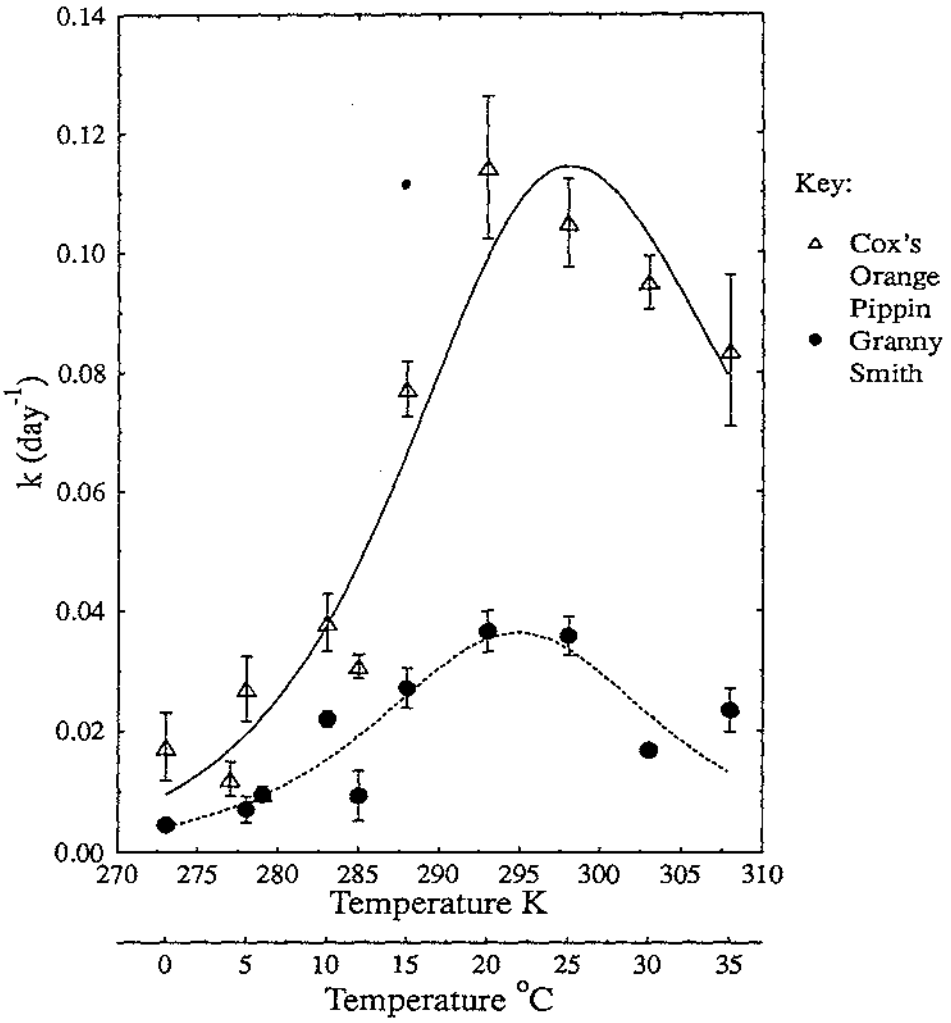


Figure 4.5 Rate constants of total chlorophyll in the skin of Cox's Orange Pippin and Granny Smith apples at various temperatures during 1989 and 1990. Rates were averaged over growers, harvests and years. Lines of best fit were calculated from equation [4.3] the parameters were: for Cox's Orange Pippin: $Ka = 2.45 \times 10^{15}$; $Ea/R = 10941.52$; $\Delta S/R = 62.23$; $\Delta H/R = 18438.70$; $r^2 = 0.775$; $p<0.01$; and for Granny Smith: $Ka = 5.75 \times 10^{13}$; $Ea/R = 10139.60$; $\Delta S/R = 75.50$; $\Delta H/R = 22313.05$; $r^2 = 0.726$; $p<0.05$.

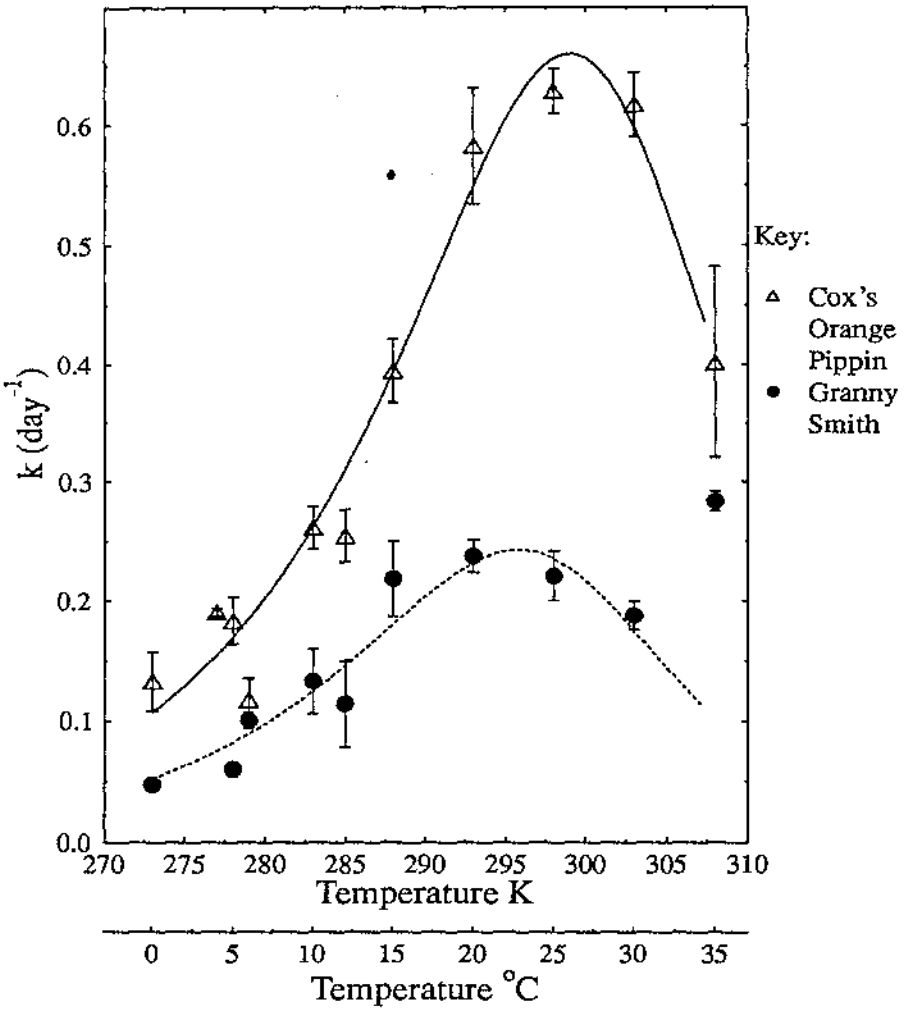


Figure 4.6 Rate constants of hue angle in the skin of Cox's Orange Pippin and Granny Smith apples at different temperatures. Rates were averaged over growers, harvests and years. Line of best fit was calculated from equation [4.3] the parameters were: for Cox's Orange Pippin: Ka = 1.46×10^{10}; Ea/R = 6998.58; $\triangle$S/R = 68.45; $\triangle$H/R = 20667.94; $r^2 = 0.836$; $p<0.01$; and for Granny Smith: Ka = 3.89×10^{9}; Ea/R = 6831.44; $\triangle$S/R = 69.06; $\triangle$H/R = 20636.52; $r^2 = 0.693$; $p<0.05$.

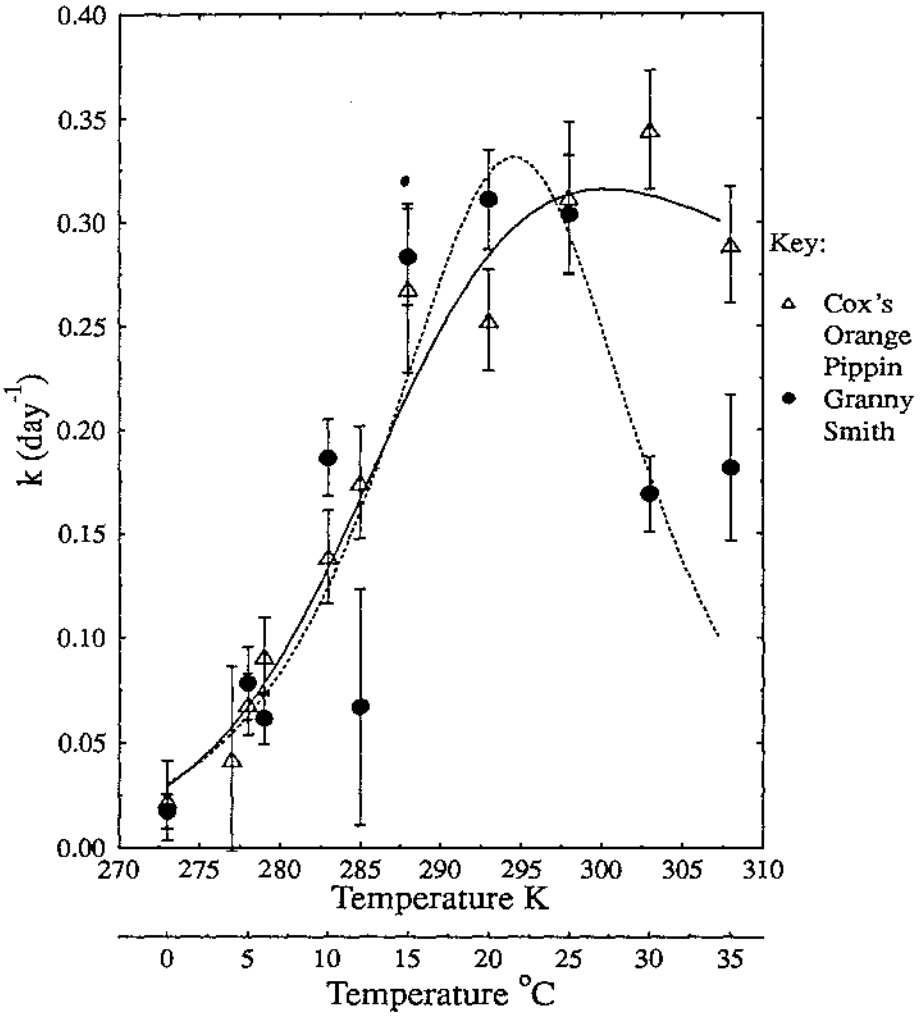


Figure 4.7 Rate constants of lightness of Cox's Orange Pippin and Granny Smith apples at different temperatures. Rates were averaged over growers, harvests and years. Line of best fit was calculated from equation [4.3] the parameters used were: for Cox's Orange Pippin: Ka = 1.73×10^{20}; Ea/R = 13668.98; ΔS/R = 53.13; ΔH/R = 15317.60; r^2 = 0.714; $p<0.01$; and for Granny Smith: Ka = 1.09×10^{16}; Ea/R = 11033.77; ΔS/R = 85.31; ΔH/R = 25204.30; r^2 = 0.734; $p<0.01$.

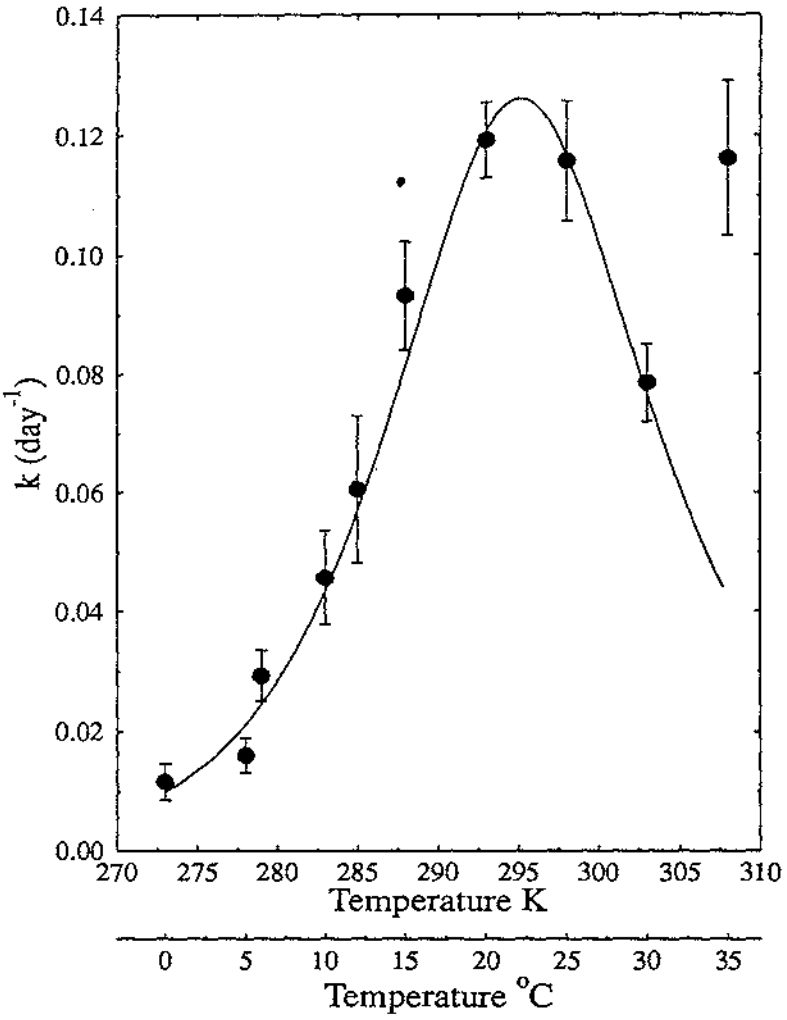


Figure 4.8 Rate constants of colour chart score in the skin of Granny Smith apples at various temperatures. Rates are averaged over growers, harvests and years. Line of best fit was calculated from equation [4.3] the parameters were: Ka = 3.21×10^{16}; Ea/R = 11636.15; ΔS/R = 82.29; ΔH/R = 24311.78; $r^2 = 0.829$; $p<0.01$.

Total chlorophyll loss ***k*** values were higher in Cox's Orange Pippin than in Granny Smith fruit (Figure 4.5). The maximum ***k*** of chlorophyll loss at optimum temperature for Cox's Orange Pippin was about 0.12 day^{-1} at 25°C, and 3 times greater than the 0.04 day^{-1} at 22°C for Granny Smith. It is worth noting that even at 0°C Cox's Orange Pippin lost chlorophyll faster than Granny Smith.

The ***k*** of hue angle was similar to that of chlorophyll with temperature (Figure 4.6). Maximum ***k***'s of 0.66 day^{-1} and 0.25 day^{-1} occurred at 26°C and 23°C for Cox's Orange Pippin and Granny Smith apples respectively. These were similar to those found for chlorophyll loss. Granny Smith fruit had a high ***k*** of hue angle change at 35°C which did not follow the predicted pattern; the reason for this is not known.

The relationship between temperatures and increase in lightness was the same for both cultivars (Figure 4.7). The optimum temperature was higher for Cox's Orange Pippin (27°C) than for Granny Smith (22°C) but very close to the hue angle optimum temperatures. Colour chart score ***k*** for Granny Smith followed the same pattern as for chlorophyll, hue angle and lightness (Figure 4.7). The optimum temperature was 22°C.

Table 4.2 Colour parameters of Cox's Orange Pippin and Granny Smith apples stored during 1989 and 1990.

Cultivar	Chl a (ng/mm^2)	Chl b (ng/mm^2)	Total Chlorophyll (ng/mm^2)	Hue Angle (°)	Lightness (%)	Firmness (N)	Total Soluble Solids (°Brix)
Cox's Orange Pippin	22.8b[1]	6.4b[2]	29.0b	109.1b	70.0a	69.7b	11.0a
Granny Smith	62.1a	17.0a	79.1a	117.5a	60.7b	74.2a	9.9b

1 Average of initial measurements.
2 Differences between letters within columns were significantly different at $p<0.05$ according to Duncans multiple range test.

Granny Smith fruit differed from Cox's Orange Pippin fruit in being darker, greener, firmer and lower in total soluble solids (Table 4.2). Total chlorophyll content was 2.7 times greater, hue angle 8.34° higher and lightness lower by 9.28%. Granny Smith were 7.27N firmer and 1.02 °Brix lower than Cox's Orange Pippin. The difference in chlorophyll content was in the same proportion for each cultivar with the ratios of chlorophyll a and chlorophyll b being similar, 3.59 and 3.66 for Cox's Orange Pippin and Granny Smith respectively.

4.3.3 Years.

Granny Smith fruit at initial assessment had a significantly higher hue angle and similar lightness in 1990 compared to fruit in 1989 while initial values for Cox's Orange Pippin fruit were the same in both years (Table 4.3). Initial chlorophyll contents were higher in 1989 than in 1990 by 9.76 ng/mm^2 and 4.87 ng/mm^2 for Granny Smith and Cox's Orange Pippin respectively. This was caused by lower chlorophyll b levels being present in fruit in 1989 than in 1990; chlorophyll a levels were similar in both years. Although chlorophyll content was lower in 1990 than in 1989 for both cultivars, the hue angle was lower in Cox's Orange Pippin and higher in Granny Smith. Firmness of Granny Smith fruit was higher in 1989 than in 1990 while Cox's Orange Pippin firmness was the same in both years. Total soluble solids were higher in 1990 for Cox's Orange Pippin than in 1989 but lower in 1990 compared to 1989 for Granny Smith fruit. In both cultivars low firmness was associated with low total soluble solids and high firmness with high total soluble solids.

Table 4.3 Quality parameters of Cox's Orange Pippin and Granny Smith fruit at initial assessment during 1989 and 1990

Cultivar	Year	Chl a (ng/mm^2)	Chl b (ng/mm^2)	Tot Chl (ng/mm^2)	Hue Angle (°)	Lightness (%)	Firmness (N)	Total Soluble Solids (°Brix)
Cox's Orange Pippin	1989	24.5b[1]	7.1c	31.6c	109.8c	70.0a	69.0b	10.6b
	1990	21.1b	5.6d	26.8d	108.4c	69.9a	70.5b	11.4a
Granny Smith	1989	64.1a	19.9a	84.0a	116.8b	60.4b	76.6a	10.2c
	1990	60.2a	14.1b	74.2b	118.1a	61.0b	71.7b	9.7d

1 Differences between letters within columns were significantly different at $p<0.05$ according to Duncans multiple range test.

Colour change parameters, apart from lightness ($p<0.05$), were not significantly different for Cox's Orange Pippin fruit in 1989 and 1990 (Table 4.4). Highly significant differences between years were found in ***k*** of Granny Smith fruit for chlorophyll a, chlorophyll b, total chlorophyll, lightness and colour chart score ($p<0.001$) and hue angle ($p<0.01$). Fruit in 1989 showed a very low ***k*** from 0°C to 6°C after which there was a steep rise until a maximum was reached around 24°C (see Tables A2.1 to A2.11 in Appendix 2). In contrast some fruit in 1990 had higher ***k*** at low temperatures, 0°C to 10°C, than above 10°C which resulted in a higher average ***k*** values of each colour parameter measured.

Table 4.4 Rate constants of change in quality parameters during 1989 and 1990[1].

Cultivar	Year	Chl a (ng/mm^2)	Chl b (ng/mm^2)	Tot Chl (ng/mm^2)	Hue Angle (°)	Lightness (%)	Colour Chart score
Cox's Orange Pippin	1989	0.065NS[2]	0.037NS	0.054NS	0.35NS	0.19 *	
	1990	0.075NS	0.054NS	0.067NS	0.38NS	0.18 *	
Granny Smith	1989	0.017 ***	0.018 ***	0.017 ***	0.16 *	0.14 ***	0.07 ***
	1990	0.024 ***	0.025 ***	0.024 ***	0.17 *	0.21 ***	0.08 ***

1 Comparisons made between years for each individual cultivar not between cultivars.
2 Significance levels: NS not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

4.3.4 Harvests.

In general apples harvested early were greener and darker than those harvested late in both 1989 and 1990 (Table 4.5). While some differences found were significant they were small and unlikely to have been noticeable to an observer, for example Granny Smith 1990 hue angle differed by only 0.2° for early and late harvested fruit. Late harvested Granny Smith fruit in 1989 had the opposite trend. The hue angle in 1989 in early harvested fruit was lower, indicating less green fruit, than late harvested fruit.

Later harvested fruit tended to be softer than earlier harvested fruit. Cox's Orange Pippin 1989 early harvest fruit had lower firmness than late harvest fruit but this was not significant. Total soluble solids tended to be higher in later harvested fruit than early harvested fruit and this was associated with softer fruit.

Table 4.5 Quality parameters from start and end of commercial harvest during 1989 and 1990[1].

	Cox's Orange Pippin				Granny Smith			
	1989		1990		1989		1990	
	Early	Late	Early	Late	Early	Late	Early	Late
Chl a (ng/mm^2)	27.6c[2]	21.4d	22.4b	19.9c	62.1b	66.1a	62.2a	58.1c
Chl b (ng/mm^2)	7.7a	6.5d	6.0b	5.3c	19.5a	20.3a	14.5c	13.7d
Total Chl (ng/mm^2)	35.3a	27.9d	28.4b	25.1c	81.6a	86.4ab	76.7b	71.8c
Hue Angle (°)	109.3c	110.3d	110.1b	106.6a	116.6b	117.0b	118.2a	118.0b
Lightness (%)	68.3c	71.7b	69.9a	70.0ab	61.2b	59.6c	60.5d	61.5a
Colour Chart Score					2.4b	2.0c	1.7d	3.1a
Firmness (N)	63.5b	74.5b	74.2a	66.7b	77.8a	75.4b	72.7c	70.8d
Total Soluble Solids (° Brix)	10.0d	11.2b	11.0c	11.7a	9.8c	10.7a	9.4d	9.9b

1 Average of initial measurements.
2 Differences between letters within a row for each year were significantly different at $p<0.05$ according to Duncans multiple range test.

Late harvested Cox's Orange Pippin fruit tended to have a higher ***k*** value for chlorophyll b, total chlorophyll loss and hue angle change than early harvested fruit (Table 4.6). Granny Smith apples had significant differences in the colour chart score ***k*** in both years for both harvests and for hue angle in 1990.

Table 4.6 Average ***k*** values for quality parameters of Cox's Orange Pippin and Granny Smith apples from the start and end of commercial harvest in 1989 and 1990[1].

	Cox's Orange Pippin				Granny Smith			
	1989		1990		1989		1990	
	Early	Late	Early	Late	Early	Late	Early	Late
Chl a (ng/mm^2)	0.05NS[2]	0.08NS	0.06NS	0.09NS	0.02NS	0.02NS	0.02NS	0.02NS
Chl b (ng/mm^2)	0.03*	0.05*	0.04*	0.07*	0.02NS	0.02NS	0.03NS	0.02NS
Total Chl (ng/mm^2)	0.05*	0.06*	0.06*	0.08*	0.02NS	0.02NS	0.02NS	0.02NS
Hue Angle (°)	0.36NS	0.35NS	0.30***	0.45***	0.16NS	0.16NS	0.18*	0.15*
Lightness (%)	0.18NS	0.20NS	0.16NS	0.20NS	0.14NS	0.14NS	0.23NS	0.20NS
Colour Chart Score					0.06***	0.07***	0.08***	0.07***

1 Comparison made between harvests within a year.
2 Significance levels: NS not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Chlorophyll a loss in Cox's Orange Pippin apples was higher than chlorophyll b loss at all storage temperatures (Figures 4.3 and 4.4, Tables A2.1 to A2.6 in Appendix 2). Differences between late harvest ***k*** values of chlorophyll a and chlorophyll b loss were the same in both 1989 (0.21 day^{-1} compared to 0.15 day^{-1}, a 0.06 day^{-1} difference) and 1990 (0.16 day^{-1} compared to 0.10 day^{-1}, a 0.06 day^{-1} difference). Maximum ***k*** values of chlorophyll a and chlorophyll b loss were 1.85 and 2.4 times higher for late harvested fruit than for early harvested fruit. The differences were smaller in 1990, 1.25 and 1.2 times for chlorophyll a and chlorophyll b loss respectively.

Granny Smith fruit had similar average ***k*** of chlorophyll a and chlorophyll b loss during 1989 and 1990. Maximum ***k*** values of chlorophyll b loss were higher than chlorophyll a loss in fruit from both harvests in 1989 and 1990. The size of the difference was 0.007 and 0.009 day^{-1} in 1989 and 1990 for early harvest fruit. Late harvest fruit had smaller differences of 0.0003 and 0.005 day^{-1} (Tables A2.2 and A2.4, Appendix 2). Late harvest fruit in 1989 had higher ***k*** of chlorophyll a and chlorophyll b loss than early harvest fruit, by 1.4 and 1.5 times. In 1990 the

maximum ***k*** of chlorophyll a and chlorophyll b loss were similar between harvests.

Prior to storage Cox's Orange Pippin apples had higher levels of internal ethylene than Granny Smith apples indicating that the fruit had started to ripen (Table 4.7).

Table 4.7 Internal ethylene levels (ppm) of Cox's Orange Pippin and Granny Smith fruit prior to storage in 1990.

Cultivar	Harvest	Ethylene level
Cox's Orange Pippin	Early	4.9 ± 1.1
	Late	4.2 ± 0.9
Granny Smith	Early	0.5 ± 0.1
	Late	1.9 ± 0.4

4.3.5 Growers.

The largest difference in average chlorophyll content between fruit from each grower was 9.7 ng/mm^2 for Cox's Orange Pippin and 7.5 ng/mm^2 for Granny Smith (Table 4.8). This represents a difference between growers of up to 27.6% in chlorophyll content for Cox's Orange Pippin and 7.8% for Granny Smith. Total chlorophyll differences observed between growers was due to chlorophyll a and chlorophyll b being present in lesser amounts but in similar proportion. Chlorophyll a to chlorophyll b ratios ranged from 2.8 to 3.9 for Cox's Orange Pippin and 3.1 to 4.1 for Granny Smith. The differences observed in chlorophyll content whilst large were not significant (Table 4.8) within cultivars while differences between cultivars were large.

Fruit from Cox's Orange Pippin grower D946 had a significantly lower hue angle and chlorophyll than fruit from other Cox's Orange Pippin growers. Hue angles from Granny Smith fruit from different growers were not significantly different.

Cox's Orange Pippin fruit from grower D265 had a significantly lower lightness than fruit from other Cox's Orange Pippin growers. No significant differences were found with Granny Smith fruit from different growers. Colour chart scores also showed no significant differences between Granny Smith growers fruit.

Both cultivars of fruit from Grower D389 were significantly softer than fruit from other growers. All other growers fruit had similar firmness values. Major differences were found in levels of total soluble solids. In general Cox's Orange Pippin fruit had higher total soluble solids levels than Granny Smith fruit. Significant differences were found between Cox's Orange Pippin growers fruit with D946 having the highest total soluble solids and grower D389 the lowest. Granny Smith growers fruit had no significant differences in total soluble solids.

Grower D946's fruit had the highest total soluble solids, the lowest chlorophyll content, lowest hue angle at the initial measurement and also the highest ***k*** of colour change for chlorophyll a, chlorophyll b, total chlorophyll, hue angle and lightness (Table 4.9). In contrast fruit from grower D389 had the lowest ***k*** of colour change measured by loss of chlorophyll, change in hue angle and lightness (Table 4.9). Fruit from other growers had similar ***k***. The lowest ***k*** of firmness change were found in fruit from growers D265, D389 and D946. Growers D592 and D252 fruit had the lowest ***k*** of total soluble solids change.

No differences in the ***k*** of chlorophyll loss, lightness or change in colour chart score was found between growers of Granny Smith apples.

Table 4.8 Quality parameters of Cox's Orange Pippin and Granny Smith apples obtained from different growers in 1989 and 1990 at initial measurement.

Grower	Chl a (ng/mm^2)	Chl b (ng/mm^2)	Totchl (ng/mm^2)	Hue Angle (°)	Lightness (%)	Colour Chart Score	Firmness (N)	Total Soluble Solids (°Brix)
Cox's Orange Pippin								
D265	27.7b[1]	7.4c	35.1b	109.3b	67.5b		70.9a	10.7bcd
D389	23.4b	7.1c	30.5b	108.7bc	69.7ab		60.2b	10.2cde
D592	22.4b	5.8c	28.1b	109.0bc	71.1a		75.3a	11.3b
D646	20.5b	7.3c	27.8b	111.7b	72.3a		73.7a	11.1b
D252	20.5b	5.4c	25.8b	109.4b	71.0a		68.3a	10.9bc
D946	20.0b	5.4c	25.4b	106.0c	69.7ab		70.2a	12.3a
Granny Smith								
D139	60.3a	16.2b	76.4a	117.0a	61.3c	2.1a	74.38a	9.97de
D341	58.4a	19.1a	77.4a	116.2a	61.4c	2.4a	74.46a	9.78e
D069	64.3a	19.7a	84.0a	116.7a	59.8c	2.1a	74.61a	10.22cde
D075	66.7a	16.2b	82.9a	119.3a	59.5c	2.2a	74.38a	9.83e

1 Letters within a column values not followed by a letter are significantly different at $p<0.05$.

Table 4.9 Rate constants of change in quality parameters per day of Cox's Orange Pippin and Granny Smith apples from different growers during storage at different temperatures in 1989 and 1990.

Grower	Chl a (ng/mm^2)	Chl b (ng/mm^2)	Totchl (ng/mm^2)	Hue Angle (°)	Lightness (%)	Colour Chart Score
Cox's Orange Pippin						
D265	0.058cd[1]	0.038bc	0.051bc	0.33b	0.18ab	
D389	0.050c	0.024c	0.040c	0.36b	0.13b	
D592	0.077ab	0.046b	0.064ab	0.36b	0.17ab	
D646	0.075ab	0.045b	0.062ab	0.33b	0.22a	
D252	0.078ab	0.053ab	0.069a	0.36b	0.19ab	
D946	0.085a	0.067a	0.077a	0.48a	0.21a	
Granny Smith						
D139	0.020a	0.021a	0.020a	0.17ab	0.17a	0.07a
D341	0.017a	0.018a	0.018a	0.18ab	0.18a	0.08a
D069	0.020a	0.022a	0.020a	0.15ab	0.16a	0.07a
D075	0.023a	0.024a	0.023a	0.14b	0.19a	0.08a

1 Letters within a column values not followed by a letter are significantly different at $p<0.05$.

4.4 Discussion

Yellowing of apples is a result of chlorophyll breakdown rather than production of yellow pigments (Knee 1972). Increases in the total carotenoid content of ripening apples has been observed in the varieties Golden Delicious (Workman 1964) and McIntosh (Francis *et al* 1955) but Bramley's Seedlings apples maintained carotenoid levels (Knee 1975). The changes in carotenoids levels are small in comparison to the almost total destruction of chlorophyll. Thus change in colour is due mainly to reduction in chlorophyll content and variation in the ***k*** are primarily due to changes in ***k*** of chlorophyll loss.

4.4.1 Modified Arrenhius Equation

For the first time the relationship between temperature and colour change (change in chlorophyll content, hue angle and lightness) has been characterised using a modified form of the Arrenhius equation [4.3] (Johnson and Thornley, 1985). The Arrenhius equation describes the following observations that ***k*** values rise exponentially as temperature increases reaching a maximum at an optimum temperature after which ***k*** declines (Figures 4.3 to 4.8). This indicates that there are two transition regions for ***k*** in response to temperature; 0°C to 6°C and 20°C to 30°C (Figures 4.3 to 4.8). The modified form of the Arrenhius equation is probably describing an enzyme mediated response to temperature that is once the process of colour change reaches a certain temperature the enzyme activity reduces. This may be due to denaturing of the proteins involved (Bailey and Ollis, 1977). Holden (1961) had a similar response curve for chlorophyllase activity at temperatures between 0°C and 50°C in leaves of several plants but no measurements were made at temperatures between 0°C and 15°C (Figure 4.9).

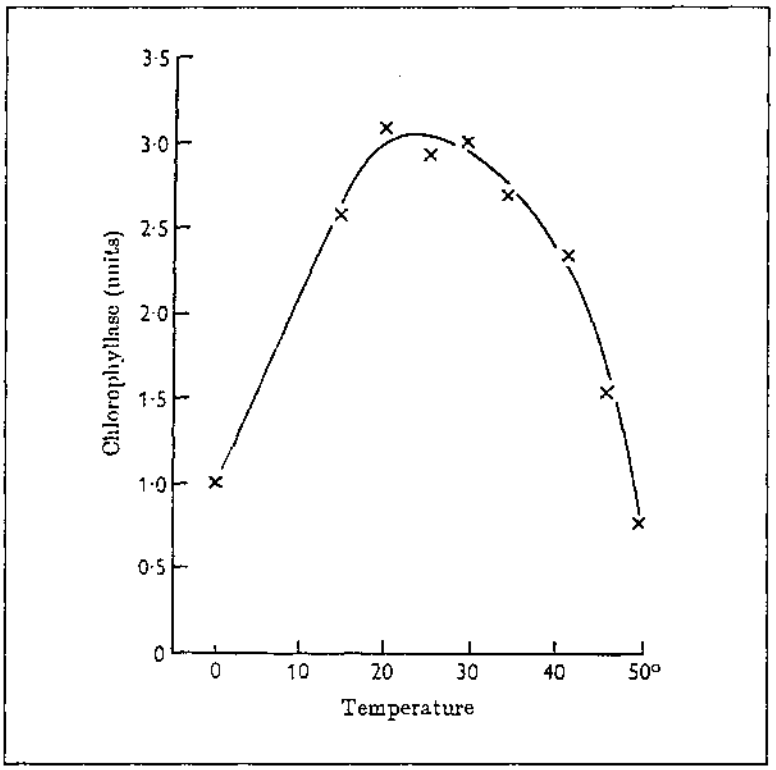


Figure 4.9 Change in chlorophyllase activity with temperature (Holden 1961).

The modified Arrenhius equation describes the relationship between temperature and colour change very well, r^2 values ranged from 0.545 to 0.836 for Cox's Orange Pippin and 0.693 to 0.829 for Granny Smith fruit (Figures 4.3 to 4.8). With the exception of Cox's Orange Pippin chlorophyll b data this represents significance at least at $p<0.05$ for chlorophyll a, chlorophyll b, total chlorophyll, hue angle, lightness and colour chart score. Therefore the Arrenhius equation can be used with confidence to predict values of ***k*** in temperatures commonly found during the postharvest handling of apples.

The modified Arrenhius equation has the following limitations where parameters for equation [4.3] were estimated using the least square procedure in which the degree of error in parameter estimates depends on the distribution of temperatures used in the experiment with error in parameters being greater with fewer observations. Using temperatures over an appropriate range is important to maximise the contribution of each data point in reducing parameter estimate error. This can be illustrated by removal of a data point and comparing the T_{max} (equation [4.5]) values, and the estimate of T_{max} error (Feng *et al* 1990) where r^2 changes little if one data point is removed but the standard error of estimated parameters can be halved. Standard errors in ***k*** illustrate the range over which ***k*** values can occur at any one temperature (Figures 4.3 to 4.8). Statistical analysis cannot determine which type of curve to use to best fit the process of colour change with temperature. Experimenter judgement is required to do this therefore the modified Arrenhius equation [4.3] is only the best guess as to the true nature of the colour change relationship to temperature. The modified Arrenhius equation is based on current understandings of the relationship between temperature and chemical reactions and the nature of enzyme responses to increasing temperature and is therefore based on sound physical and chemical theory. For this reason the modified Arrenhius equation was preferred over any other possible equation for the same shaped response curve.

To determinate more accurately the relationship between colour change and temperature a greater number of measurements of ***k*** in the regions 0°C to 6°C and 20°C to 30°C would be necessary.

4.4.2 Chlorophyll

For the first time chlorophyll loss and colour change ***k*** have been measured over the temperature range of 0°C to 35°C. In addition ***k*** of chlorophyll change have been linked to an objective measure of colour, hue angle, and a subjective measure of colour, colour charts. The units of ***k*** are the inverse of time, for example day^{-1} of chlorophyll a. Other researchers (Holden 1961, Hansen 1956, Looney and Patterson 1967) have used enzyme activity (chlorophyllase; the principle enzyme thought to be involved in chlorophyll degradation) as their unit of change.

The relationship of ***k*** to temperature for colour change is similar to that shown by Holden (1961) for chlorophyllase activity in sugar beet, pea, bean, wheat and barley leaves. The optimum temperature reported by Holden was about 25°C, similar to that for the apples studied (Figure 4.9). Hansen (1956) and Laval-Martin (1969) investigated chlorophyll loss in skin of pears over a range of temperatures. Re-analysis of their data using the modified Arrenhius equation is illustrated in Figure 4.10. Different varieties have different ***k*** values of chlorophyll loss but the same relationship with temperature and different optimum temperatures. A similar relationship of colour change with temperature for different pome fruit implies the mechanism by which chlorophyll is lost may be the same. Further research is required to confirm this. Lyons and Rappaport (1962) presented results on the change in appearance of Brussel Sprouts in which colour was important. Re-analysis of their data is illustrated in Figure 4.10. No optimum temperature was noted as the experiment examined the effect of temperature up to 20°C but the relationship between ***k*** of quality loss and temperature 0°C to 20°C is similar to apples over the same temperature range.

Background colour change of apples has been investigated by: Rhodes and Wooltorton (1967); Knee (1972 and 1980) for Cox's Orange Pippin; Watkins *et al* (1991); Mussini *et al* (1985) for Granny Smith; Johnson and Ertan (1983) for Idared; Workman (1964) for Golden Delicious and Grimes Golden; Gorski and Creasy (1977) for Golden Delicious. But these authors have presented results for only one or two temperatures over a large range with few intermediate points between 0°C and 20°C. Thus little can be concluded on the nature of the relationship between apple colour change and temperature from the literature.

Chlorophyll loss or colour change ***k*** values have been measured for other fruit and vegetables: green beans (Groeschel *et al* 1966); Bearss lemon (Jahn 1976); Shamouti oranges (Apelbaum *et al* 1976); broccoli (Lebermann *et al* 1968); mangoes (Medlicott *et al* 1986); citrus (Amir-Shapira 1987); Hamlin oranges (Jahn *et al* 1973) and oranges (Knee *et al* 1988). All of these reports have studied changes at one to three temperatures; thus it is not possible to characterise a relationship of chlorophyll loss and colour change with temperature from this limited information.

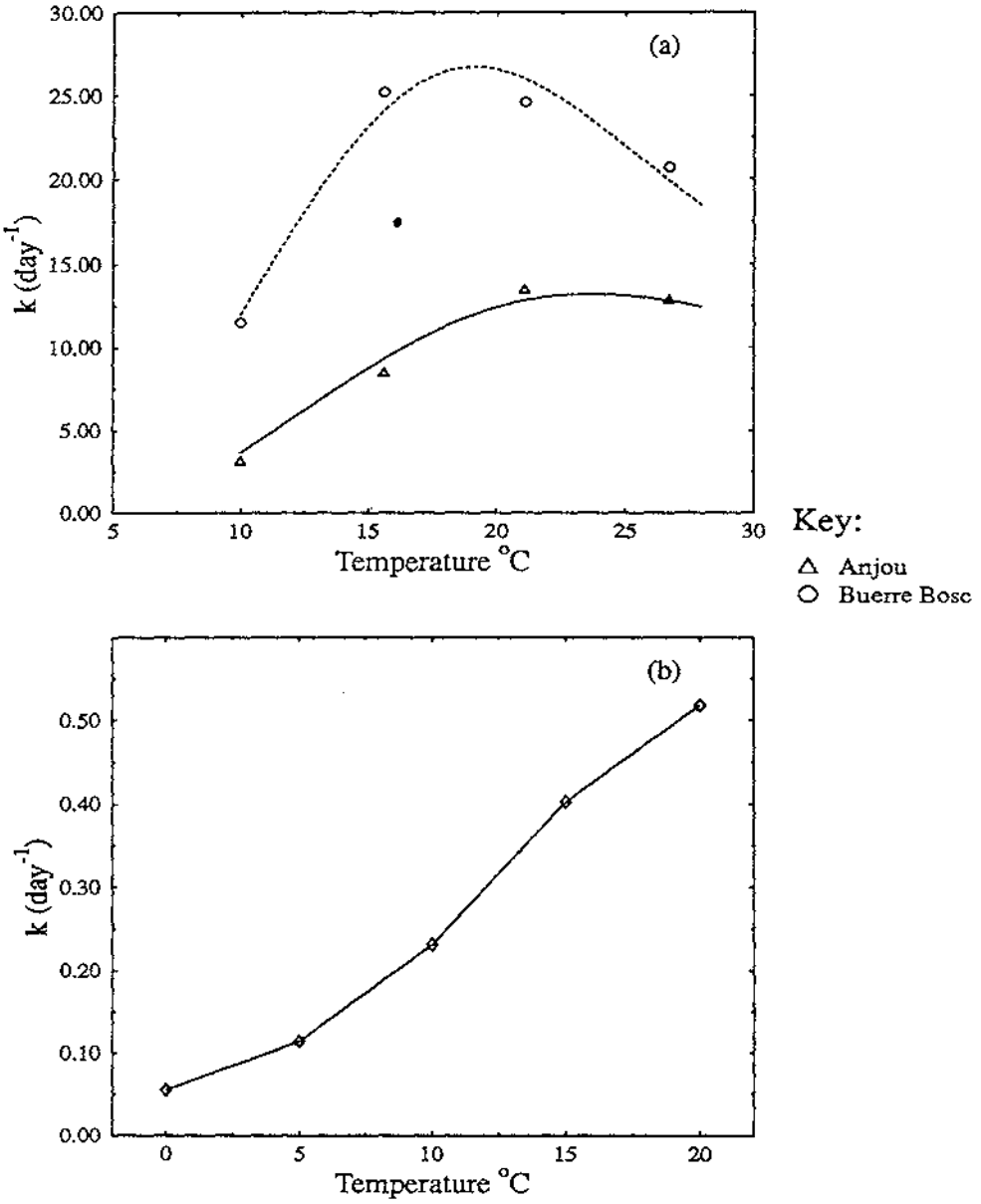


Figure 4.10 (a) Rate constants of chlorophyll loss of Anjou, Buerre Bosc pears (Hansen 1956) and (b) Change in quality rating of Brussel Sprouts (Lyons and Rappaport 1962). Rate constants calculated using equation [2.1] and fitted to the arrenhius equation [4.3].

Chlorophyll a disappeared more rapidly than chlorophyll b (Tables 4.2 to 4.7) which has been found previously (Laval-Martin, 1969). Very similar temperature optima for ***k*** of chlorophyll a and chlorophyll b loss may indicate that a similar process may be occurring to degrade both chlorophylls.

Knee (1972) reported that in Cox's Orange Pippin apples there was no difference in the rate of chlorophyll a and chlorophyll b loss when judged by chlorophyll a to chlorophyll b ratios, in contrast to data presented in this thesis, but he did not report chlorophyll a or chlorophyll b ***k*** values. This result may have been due to the method used where strips of skin from the stem to the calyx were taken for pigment extraction. Areas of red blush contain anthocyanin which interfere with chlorophyll measurement affecting the spectrophometric readings taken (personal observation). Knee used extraction in aqueous acetone and chlorophyll was calculated using the differential equations of Arnon (1949). Porra *et al* (1989) re-examined Arnon's equations and has determined that there were significant errors in the extinction coefficients of chlorophyll a and chlorophyll b. These errors have led to an ever increasing underestimate of the chlorophyll a to chlorophyll b ratio as the ratio calculated according to Arnon increases. Such errors in chlorophyll a to chlorophyll b ratios may have led to erroneous results. Variation in chlorophyll content may also have been due to red speckling in addition to red blush found in Cox's Orange Pippin apple skin. In the results presented here care was taken to use skin which was free from red patches.

The reason for variation in levels of chlorophyll a and chlorophyll b between years is not known but the different ***k*** values of chlorophyll a and chlorophyll b may affect overall ***k*** values when estimated by hue angle or colour chart score. Thus the contribution that each chlorophyll makes to fruit colour varies from year to year as the proportion of chlorophyll a and chlorophyll b varies. This may account for differences found in ***k*** as chlorophyll a is lost faster than chlorophyll b. Therefore if there is little chlorophyll b then the chlorophyll a decreases quickly resulting in an apple which yellows quickly. If the chlorophyll b levels are high yellowing may be slower. For example, Granny Smith fruit had similar

levels of chlorophyll a in 1990 and 1989 but slightly lower levels of chlorophyll b in 1990 than 1989. Total chlorophyll ***k*** values were significantly different between 1990 and 1989 but ***k***'s of chlorophyll a and chlorophyll b were similar from 1989 to 1990. Thus in Granny Smith ***k*** of total chlorophyll differed due to the proportion of chlorophyll a and chlorophyll b changing. There was no difference in ***k*** values between years in total chlorophyll loss for Cox's Orange Pippin fruit (Table 4.4). Differences in ***k*** of chlorophyll a and chlorophyll b may account for some cultivar differences in ***k***.

4.4.3 Hue Angle, Lightness and Colour Chart Score

Although there was a large difference in the ***k*** of chlorophyll for Granny Smith fruit between 1989 and 1990 the difference in ***k*** of hue angle form 1989 to 1990 was small although significant. Cox's Orange Pippin fruit hue angle ***k*** and chlorophyll ***k*** was not different between 1989 and 1990. Cox's Orange Pippin chlorophyll ***k*** had the same differences between 1989 and 1990 as Granny Smith chlorophyll ***k***, 0.007 day^{-1}. Hue angle ***k*** had larger differences 0.03 day^{-1} for Cox's Orange Pippin and 0.01 day^{-1} for Granny Smith fruit in 1989 and 1990. In Granny Smith fruit chlorophyll levels are in the region of the curve where small changes in chlorophyll content result in small changes in hue angle whereas Cox's Orange Pippin fruit were in an area of the curve where small changes in chlorophyll content represent large changes in hue angle. Thus the variation in chlorophyll change is smaller for Granny Smith fruit than for Cox's Orange Pippin. Hence differences in ***k*** of hue angle of Granny Smith are detected more readily than ***k*** of hue angle of Cox's Orange Pippin for the same colour change.

4.4.4 Cultivars

Cox's Orange Pippin and Granny Smith fruit had the same pattern of colour change with temperature and similar temperature optima indicating the same underlying mechanism for colour change may be operating. Average optimum temperatures of chlorophyll a, chlorophyll b, total chlorophyll, hue angle, lightness

and colour chart score ***k*** values were not significantly different between cultivars, Cox's Orange Pippin having an average optimum temperature of 25.3°C and Granny Smith fruit an optimum temperature of 23.4°C, a 2°C difference.

Cox's Orange Pippin fruit firmness and total soluble solids as well as chlorophyll content from different growers varied more than Granny Smith fruit indicating that fruit from individual growers may have been riper, i.e. yellower and softer, than other growers fruit. Data collected was averaged over harvests and the same growers fruit was not used for both early and late harvests therefore additional experiments are required to more precisely determine variations in ***k*** due to grower influence.

Clear cultivar differences in quality parameters are found between Cox's Orange Pippin and Granny Smith fruit. Granny Smith fruit had a higher chlorophyll content and a lower ***k*** than Cox's Orange Pippin fruit. Reasons for this are not known but may be due to slower ***k*** of senescence of Granny Smith fruit indicated by a lower respiration rate (Dadzie 1992) and ethylene levels. In addition Granny Smith fruit are slower ripening and have a smaller climacteric peak than Cox's Orange Pippin.

Fruit maturity is thought to be important when considering ***k*** of colour change, firmness and total soluble solids changes (Biale and Young, 1962). Looney and Patterson (1967), Rhodes and Wooltorton (1967) measured chlorophyllase activity in relation to the respiration rate in McIntosh apples, at 23°C, and Cox's Orange Pippin apples, at 12°C, and found that changes in chlorophyllase activity closely mirrored changes in respiratory activity. Rhodes and Wooltorton (1967) reported that the increase in chlorophyllase activity commenced before the climacteric rise in respiration and continued to be high after the climacteric peak was reached. This increase in activity is thought to be associated with the chloroplast transformation to chromoplasts (Bain and Mercer, 1964) as electron microscope studies have shown that chloroplast lamellae disintegrate as the climacteric proceeds (Bain and Mercer, 1964). There is a lag phase after

harvest in the change in colour of Golden Delicious and Grimes Golden apples (Workman 1964). This lag phase corresponds with the development of the respiratory climacteric as later harvested fruit which have entered the climacteric do not show a lag in colour change. Pigment changes in apples due to chlorophyllase activity could provide an objective measure of physiological age (Looney and Patterson 1967). This may be possible in Golden Delicious and Grimes Golden apples as there is a reasonably good association between the time to the climacteric maximum and time to reach 91° hue or complete yellowness for these cultivars (Workman, 1964) (Table 4.10). A more practical use would be predicting the preclimacteric minimum to determine when the apples start to ripen. The ground colour of Jonathan apples has been considered as a maturity index as there was a good correlation with colour storage disorder incidence (Faragher *et al* 1984) but these authors considered an absolute measure of ground colour too variable which could be influenced by other factors.

Table 4.10 Days to reach climacteric maximum compared to days to yellow to 91° Hue at 20°C for Golden Delicious apples. Data reworked from Workman (1964).

Harvest Date	Days to reach climacteric maximum.	Days to reach 91° Hue Angle
7/9	20	26
14/9	13	26
21/9	11	16
28/9	10	15
5/11	7	9
11/11	6	5

Chlorophyll degradation is thought to be ATP and oxygen dependent (Brown et al 1991) therefore if ***k*** is linked to the respiration rate of apples (Looney and Patterson 1967, Rhodes and Wooltorton 1967) then a lower respiration rate may

help to explain differences in ***k*** between Granny Smith and Cox's Orange Pippin fruit. At 0°C ***k*** values are higher in Cox's Orange Pippin compared to Granny Smith fruit, this may be associated with the difference in respiration rates at 0°C (Dadzie 1992) between these cultivars. Workman (1964) investigated in detail two apple cultivars, Golden Delicious and Grimes Golden, in which yellowing is often a desirable feature. Grimes Golden had a higher respiration rate and higher ***k*** at 20°C than Golden Delicious. In this study Cox's Orange Pippin fruit lost chlorophyll and subsequently yellowed, 3 to 5 times faster than Granny Smith fruit. The respiration rate is approximately 2 times faster for Cox's Orange Pippin fruit (16.38 mls CO_2/kg/hr) than for Granny Smith fruit (9.52 mls CO_2/kg/hr) (Dadzie 1992). Granny Smith also have higher chlorophyll levels, 2.7 times that of Cox's Orange Pippin, thus more chlorophyll needs to be lost than Cox's Orange Pippin fruit before yellowing is noticeable.

Internal browning was found in Cox's Orange Pippin and Granny Smith fruit after 1 to 2 weeks storage at 25°C and above. The amount of injury was slight at 25°C but severe at 35°C indicating that it was some form of high temperature injury. Hue angle ***k*** values for Granny Smith were higher than expected at 35°C and did not fit the modified Arrenhius curve (Figures 4.6 to 4.8). This internal browning may have indirectly influenced background colour. Colour of apple flesh under the skin has a large influence on fruit colour as the skin is thin, in pears for example, the chlorophyll layer containing cells is only a few micrometers thick (Bain and Mercer 1964). The light passes through the skin and out again readily by diffuse reflection (Figure 1.5), therefore the flesh colour probably contributes significantly to the fruit colour. Most fruit in this study had a white or very pale green flesh which probably does not contribute greatly to the colour measured by the chromameter. A brown or dark brown flesh colour, such as bruise, has the effect of making the skin appear darker. Thus a browning of the flesh would be expected to alter the colour of an apple. The ***k*** for chlorophyll was considerably less than the ***k*** for hue angle which indicates there may not have been a reduction in the loss of chlorophyll associated with a change in ***k*** of colour change (Figures 4.3 to 4.5).

4.4.5 Preharvest Factors

In order to establish an accurate predictive model of colour change with temperature, variations in ***k*** values due to harvest, year and growers need to be accounted for. Preharvest factors may cause differences between growers fruit ***k*** values; such factors may include differences in location, microclimate and cultural practices, early and late commercial harvests and yearly changes. The relative importance of preharvest factors may differ for Cox's Orange Pippin and Granny Smith (Table 4.1). For Granny Smith fruit most variation in colour change is accounted for by differences between years. This may imply that climactic conditions from year to year have a greater influence on Granny Smith appearance than differences due to grower management. With Cox's Orange Pippin fruit differences from year to year were less important than grower differences. To more clearly establish the causes of variation in ***k*** due to preharvest factors more detailed research is necessary on such things as mineral content, position on the tree, shading (Jackson *et al* 1971, Wilkinson and Sharples 1967).

Fruit used in this study had probably started to ripen when received which may have been due to delays in precooling and coolstorage amounting to 1-2 days for fruit received from Hastings. For Cox's Orange Pippin ripening and consequently yellowing could have been initiated during this time. Granny Smith fruit change colour and ripen more slowly and may have been less affected than Cox's Orange Pippin fruit.

4.4.6 Early and Late Harvests

There was a general trend for Cox's Orange Pippin and Granny Smith fruit from early harvests to have high firmness and low total soluble solids. The hue angle and lightness of early harvest fruit was similar to late harvest fruit although chlorophyll levels were lower (Table 4.5). Colour change ***k*** values were different

between harvests but not as much as the between year differences of Granny Smith or between grower differences of Cox's Orange Pippin fruit. Late harvest Granny Smith fruit may have been more mature (Fidler and North, 1971) than early harvest fruit indicated by higher levels of internal ethylene (Table 4.7). Cox's Orange Pippin fruit had the same internal ethylene levels in both harvests which appeared to be high and indicated that fruit were undergoing ripening when received. Applications of ethephon have resulted in accelerated yellowing of Jonathan apples (Brohier and Faragher 1984). The consequences of this are that yellowing had started before the fruit were placed in the temperature treatments and some fruit may have been more advanced in terms of ripening than other fruit. This may have led to more variation in ***k*** values and colour than would otherwise be the case.

Using average ***k*** values of quality parameters does not indicate the magnitude of differences in ***k*** at different temperatures between harvests, years or growers, for example at low temperatures late harvested fruit may have higher ***k*** values than early harvested fruit but ***k*** values at optimum temperatures are lower (Tables A2.1 to A2.11, Appendix 2 and Table 4.6). Generally average ***k*** values tended to be higher for late harvested than early harvested fruit. The results presented here demonstrate that maintenance of apple fruit colour requires good temperature control of both early and late harvested fruit but in particular late harvested fruit as ***k*** values are higher than early harvested fruit. Therefore as late harvested fruit yellow more quickly, the time in storage before reaching unacceptable yellowness is less than for early harvested fruit.

4.4.7 Growers

Considerable differences in apple colour, firmness and total soluble solids were found between fruit from different growers (Table 4.8). As a result fruit from some growers were greener, softer and had lower total soluble solids than other growers fruit. Comparing fruit from different growers visually was not possible as fruit arrived at different times (Chapter 2). Some fruit had hue angle differences

of greater than 3° which can be considered to be an easily distinguished colour difference (Personal observation, Figure 3.13, Chapter 3). The human eye is very sensitive seeing green colours and is more sensitive to green when mixed with yellow pigments; in mixes of yellow and green pigments containing as little as 9% green are considered to be more green than yellow (Gorski and Creasy 1977).

The largest differences found between growers fruit was in firmness and total soluble solids indicating that lines were probably at different stages of maturity (Lott, 1964). For example fruit from Grower D389 had high chlorophyll levels and low total soluble solids and was probably more immature than fruit from grower D946 which had low chlorophyll levels and high total soluble solids. Colour change ***k*** values were similar between growers fruit for Granny Smith but varied greatly for Cox's Orange Pippin fruit. For example, Grower D389's fruit had almost half the ***k*** of chlorophyll loss compared with fruit from grower D946. This would imply that a predictive model of colour change for Cox's Orange Pippin may need to be based on individual growers fruit whereas a model for Granny Smith does not.

4.4.8 Methods of Measuring Colour

Chlorophyll content is correlated with hue angle, lightness and colour chart measurements (Chapter 3). Hue angle varies with the same chlorophyll content. It is possible for fruit with low chlorophyll to have a higher hue angle than another fruit with more chlorophyll. This is opposite to what may be expected. The reason for this may be that hue angle is a measure of the total pigment mix, indicating green, yellow and red pigments. Changing levels of yellow pigments as well as green pigments may be the cause of variation in hue angle. Fruit lightness also does not always correlate well with fruit chlorophyll content. In some fruit the hue angle was similar to other fruit but the lightness higher or lower than the other fruit. This indicates that lightness is a measure independent of hue angle. Examples of this can be seen in Tables 4.2 and 4.7 where Cox's

Orange Pippin fruit from grower D946 had low chlorophyll levels, the most green fruit according to hue angle but were light in colour. In contrast fruit from grower D389 had high levels of chlorophyll but were yellower according to the hue angle and darker. Granny Smith fruit had similar results where grower D075's fruit had the lowest chlorophyll level but the highest hue angle and were darker. Thus average hue angle and lightness results are not always reliable indicators of colour. Therefore average values should always be used with caution.

4.4.9 Conclusions

The ***k*** at which colour changes and chlorophyll is lost depends on storage temperature and fits well the modified form of the Arrenhius equation. Colour change and chlorophyll loss is slowest between 0°C and 6°C and most rapid at about 25°C, above this temperature ***k*** is reduced. This relationship may be related to respiration rate and follow the climacteric rise in respiration. To minimise colour change and chlorophyll loss, storage at a temperature as low as practicable is recommended. Variation in ***k*** was found with year, harvest and grower. There may be an association between colour and fruit maturity, as fruit with low total soluble solids have high chlorophyll contents but the association is not found with ***k***.

Granny Smith fruit change colour more slowly than Cox's Orange Pippin apples. The probable reasons for the difference may be higher chlorophyll levels and a lower respiration rate for Granny Smith compared to Cox's Orange Pippin apples. Year to year differences were more significant for Granny Smith and grower to grower differences were most significant for Cox's Orange Pippin fruit. The reasons for these differences could not be determined from this study, but are worthy of further research. Such work could help explain problems such as mixed maturity.

Chapter 5

Effect of Atmosphere Composition on Colour Change of Apples

5.1 Introduction

Controlled (CA) or modified atmosphere (MA) storage conditions are widely used to supplement low temperatures and further extend apple storage life. Modified atmospheres or controlled atmosphere is where oxygen levels are decreased and carbon dioxide levels are increased in comparison to levels normally found in air (Kader *et al* 1989). Modified atmospheres are generated by respiratory action of apples when enclosed in polymer films. This is in contrast to the externally generated CA storage system which requires specialised equipment which can accurately control atmosphere composition. In New Zealand CA conditions of 2% CO_2 and 2% O_2 are used for commercial storage of selected apple cultivars (Hamish Tough NZAPMB pers. comm.).

The mode of action of CA and MA storage of apples is not fully understood (Kader *et al* 1989). In general lower levels of O_2 and elevated levels of CO_2 are assumed to reduce respiration rate thereby reducing apple deterioration. But postharvest deterioration can be a result of other factors in addition to high respiration rates. Such factors include; ethylene biosynthesis and action, compositional changes, physical injuries, water loss, physiological and pathological disorders.

Low O_2 and high CO_2 levels in the atmosphere are known to affect the rate of yellowing in apples but the relationship between colour change and atmosphere composition is poorly characterised. A large number of studies on horticultural

crops mention colour changes when considering CA or MA effects but only as incidental factors these include: red and black currants (Agar *et al*, 1991), Bartlett pears (Allen and Claypool, 1949), brussel sprouts (Lyons and Rappaport, 1962), green beans (Groeschel et al, 1966), Idared apples (Johnson and Ertan, 1983), Cox's Orange Pippin apples (Knee, 1980; Smith *et al*, 1987; Stow and Genge, 1990; Stow, 1986, 1989;), Bramley's Seedling apples (Knee, 1975), Golden Delicious and Delicious apples (Lau, 1985), Granny Smith apples (Little *et al*, 1982; Watkins *et al*, 1991), broccoli (Lipton and Harris, 1974; Lieberman and Hardenburg, 1954; Lebermann *et al*, 1968; Wang, 1979), bananas (Liu, 1970), asparagus (Wang *et al*, 1971). In general fruit remain greener as the oxygen level is reduced and carbon dioxide levels are increased. Hewett *et al* (1989) indicated that green colour was proportional to oxygen concentration as a quadratic function.

In order to develop a model of colour change in response to the composition of the external atmosphere more detailed data is required than is presently available. Experiments were conducted in order to characterise the relationship between oxygen, carbon dioxide and yellowing by measuring the colour change in various controlled atmospheres.

5.2 Methods

A sample of ten fruit was taken seven times during storage from each of the controlled atmospheres containing different mixes of oxygen and carbon dioxide at 20°C. Chlorophyll content, hue angle and lightness and in the case of Granny Smith apples colour chart score were measured according to the techniques described in Chapter 2. The experiment was conducted using fruit from several growers of one harvest. The experiment was repeated over two seasons, 1989 and 1990 and with two cultivars. This represents a database of readings from 2240 fruit. The values obtained at each sample time were averaged and used to calculate k, equation [2.1], for each storage temperature. Values of k were graphed (Figures 5.2 to 5.20), using the GLE graphics package (a general

purpose graphics package produced by the DSIR Gracefield), and regression equations determined, using the SAS statistical program. Differences between parameters, cultivars, seasons, and growers were determined using analysis of variance and multiple analysis of variance procedures of the SAS statistical program, for chlorophyll levels, hue angle, lightness, colour chart score, firmness and total soluble solids. The declining exponential curve function [2.1] was used to calculate the rate constant of chlorophyll loss. Values of ***k*** were compared to study the effects of atmosphere composition on colour change.

5.3 Results

Cox's Orange Pippin and Granny Smith apples for all parameters of colour change ***k*** calculated from equation [2.1] showed good fits and exhibited no 'lack of fit' (the standard errors of parameters were not large being in general 10-15% of ***k***).

5.3.1 Oxygen

Cox's Orange Pippin and Granny Smith apples which were stored in low oxygen atmospheres were greener than fruit stored in high oxygen atmospheres when compared on removal from storage (Figure 5.1). When the ***k*** values of total chlorophyll for Cox's Orange Pippin are compared to percent oxygen in the external atmosphere there is clearly a trend of increase in ***k*** with increase in oxygen level (Figure 5.2). Whilst the relationship is linear over the range of O_2 concentrations of 5% to 17% this doesn't describe well ***k*** values in the range 0 - 5% oxygen or above 17% O_2. It was therefore concluded that the relationship approximated a sigmoid curve more closely than a straight line. There are several sigmoidal functions which have been presented in the literature to define plant growth rates (France and Thornley, 1984) but none have been used for ***k*** of colour change and O_2 levels. One equation which defines a sigmoidal relationship well is the Gompertz equation (France and Thornley 1984). This

fitted the ***k*** values observed best at low oxygen levels compared to other equations. The Gompertz equation consists of the following :

$$k = A_o \frac{e^{Y_o(1-e^{-D_e O_2})}}{D_e} \qquad [5.1]$$

where k = rate constant of the reaction

A_o = rate constant at zero concentration

Y_o = mean value at zero concentration

D_e = decay in specific rate constant of change

O_2 = percent oxygen

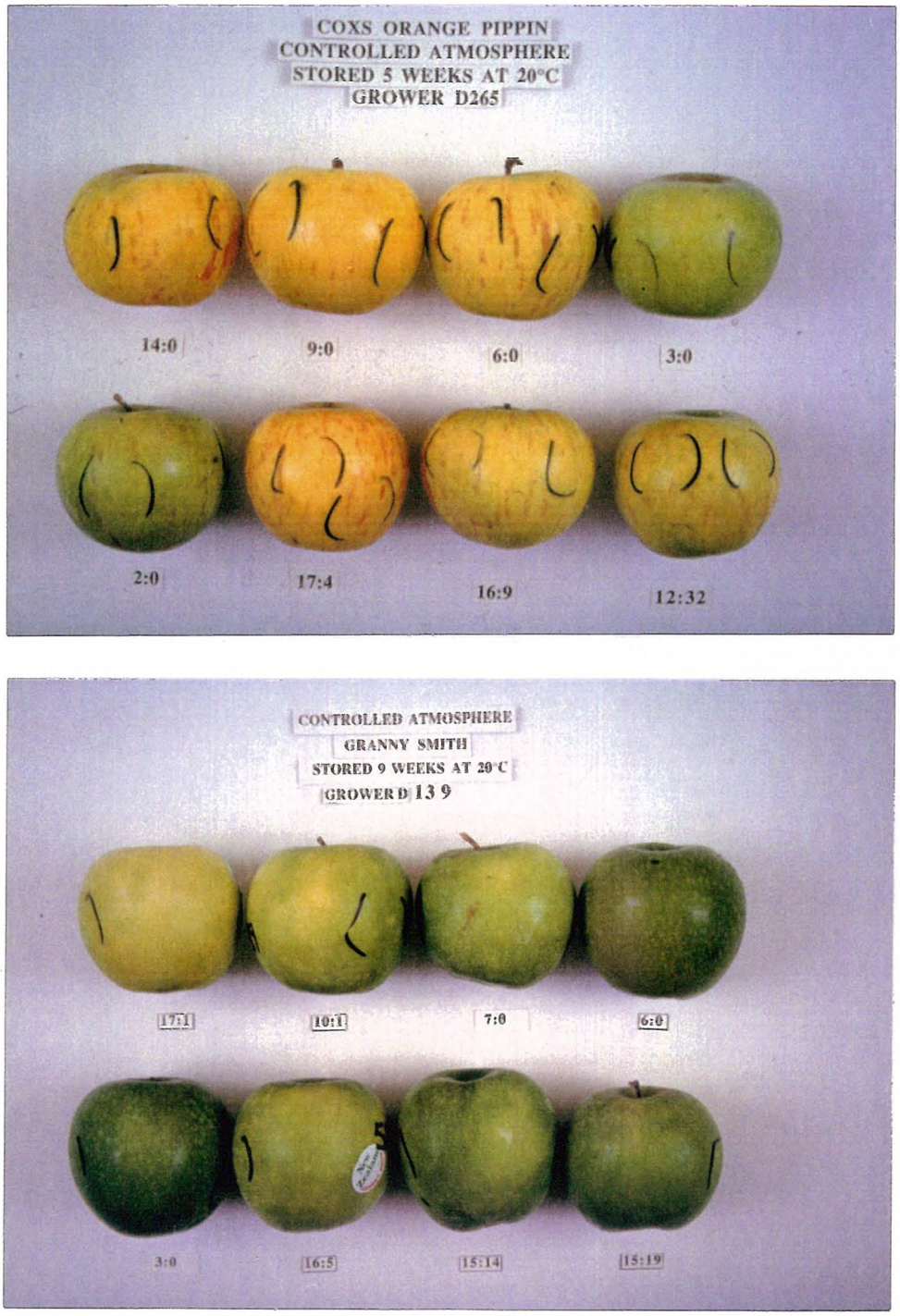


Figure 5.1 Comparison of Cox's Orange Pippin and Granny Smith apples upon removal from storage in different controlled atmospheres.

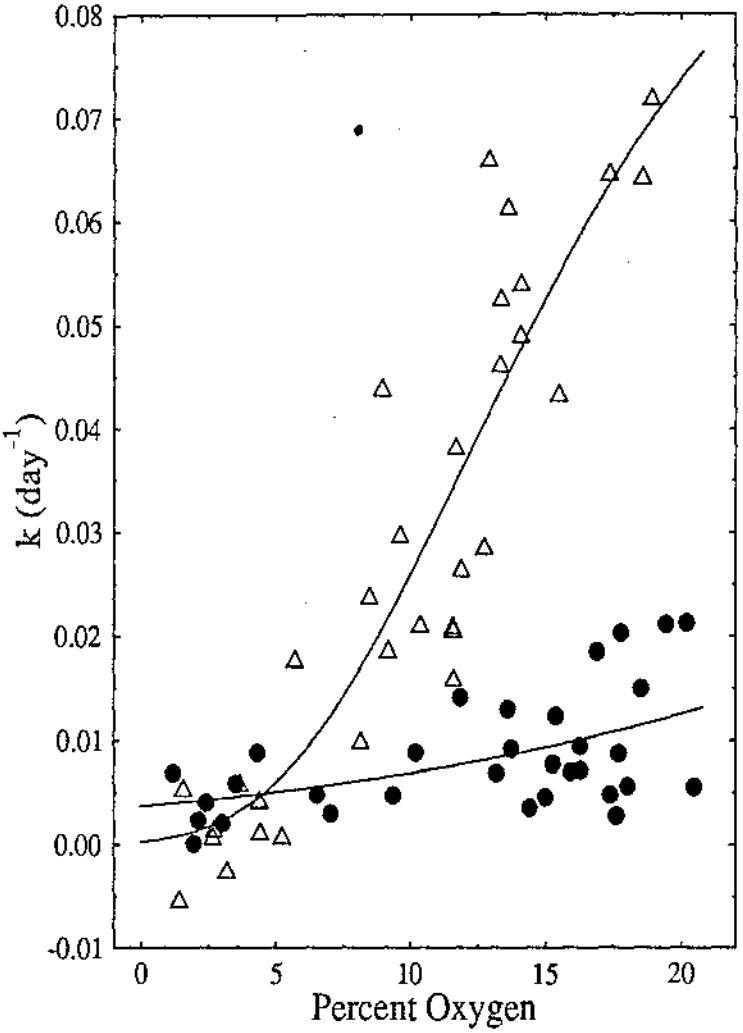


Figure 5.2 Rate constants of total chlorophyll over different oxygen levels in the storage atmosphere of Cox's Orange Pippin (△) and Granny Smith (●) apples stored at 20°C for eight or sixteen weeks. Rates are for each oxygen concentration. Line of best fit was calculated from equation [5.1] the parameters were: for Cox's Orange Pippin: A_o = $4.19x10^{-5}$; Y_o = 5.86; D_e = $1.46x10^{-1}$; r^2=0.824; $p<0.001$; and for Granny Smith; A_o = $1.11x10^{-5}$; Y_o = $2.12x10^{1}$; D_e = $2.97x10^{-3}$; r^2=0.278; $p<0.01$.

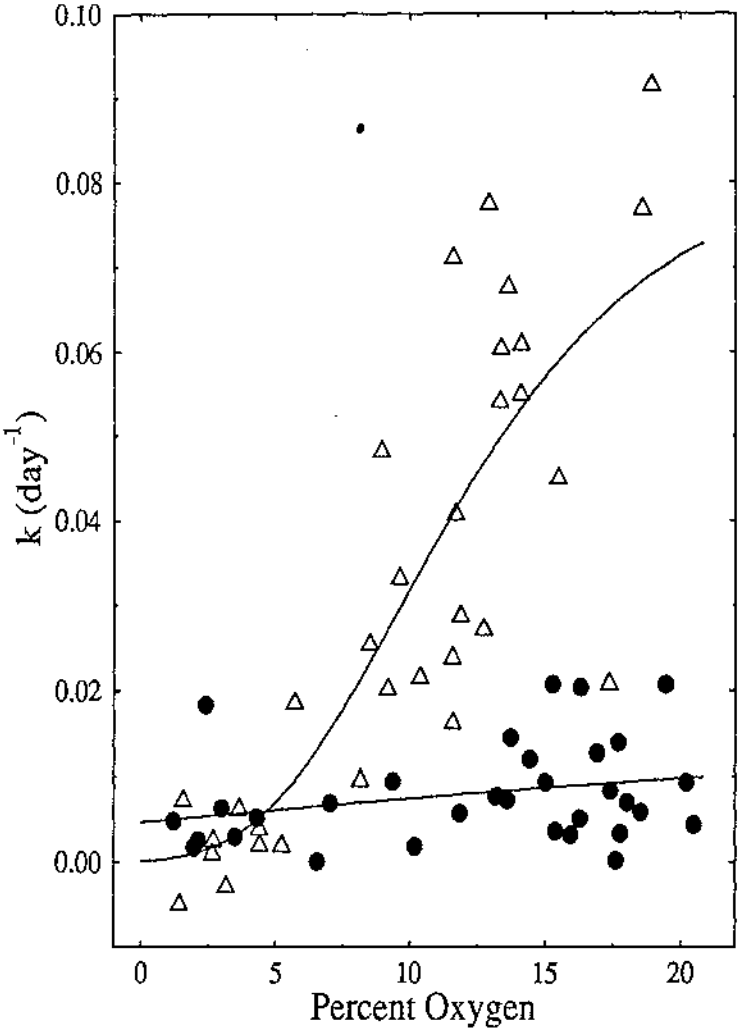


Figure 5.3 Rate constants of chlorophyll a over different oxygen levels in the storage atmosphere of Cox's Orange Pippin (△) and Granny Smith (◯) apples at 20°C for eight or sixteen weeks. Line of best fit was calculated from equation [5.1] the parameters were: for Cox's Orange Pippin: A_o = $2.56x10^{-5}$; Y_o = 6.42; D_e = $1.92x10^{-1}$; r^2=0.676; p<0.001; and for Granny Smith: A_o = $2.44x10^{-4}$; Y_o = 1.14; D_e = $5.24x10^{-2}$; r^2=0.073; p<0.1.

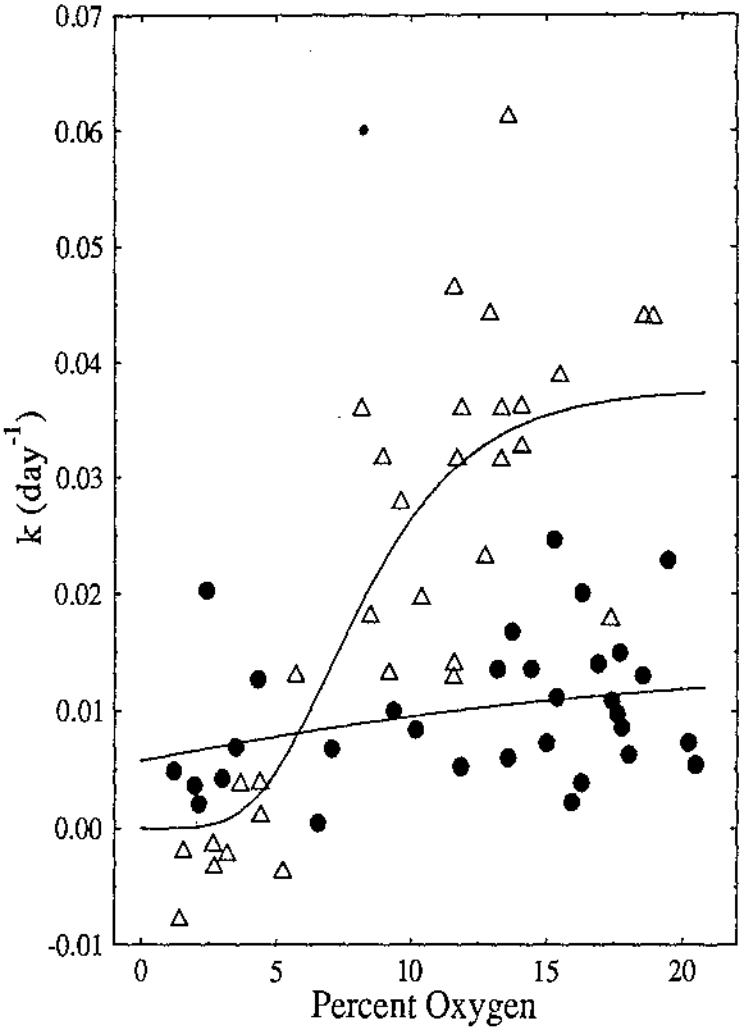


Figure 5.4 Rate constants of chlorophyll b over different oxygen levels in the storage atmosphere of Cox's Orange Pippin (△) and Granny Smith (◯) apples at 20°C for eight or sixteen weeks. Line of best fit was calculated from equation [5.1] the parameters were: for Cox's Orange Pippin: A_o = $1.10x10^{-7}$; Y_o = 11.69; D_e = $3.50x10^{-1}$; r^2=0.708; p<0.001; and for Granny Smith; A_o = $4.86x10^{-4}$; Y_o = $8.82x10^{-1}$; D_e = $8.43x10^{-2}$; r^2=0.088; p<0.1.

Cox's Orange Pippin chlorophyll ***k*** values fitted to external oxygen concentration are well described by the Gompertz equation (Figures 5.2 to 5.4). Chlorophyll ***k*** values increased as the oxygen level rose. Fruit ***k*** of chlorophyll stored in 1% to 4% oxygen changed little and represented a lag phase before ***k*** values rose. Total chlorophyll ***k*** values at oxygen levels above 4% increased linearly up to about 18% oxygen before very slowly tapering off. The increase in ***k*** was eight fold from low oxygen levels, around 1% to 6% to normal atmospheric levels of oxygen of 21% (Figure 5.2). The asymptote of chlorophyll ***k*** was more pronounced for chlorophyll b and chlorophyll a (Figures 5.2 and 5.3) than total chlorophyll. Data for chlorophyll b was more variable data for chlorophyll a.

The pattern of chlorophyll loss with O_2 was different for Granny Smith fruit (Figures 5.2 to 5.7). Chlorophyll ***k*** for Granny Smith changed little over the range of oxygen levels used in this experiment (Figure 5.2 to 5.4). For levels of O_2 in the range of 1% to 6%, chlorophyll ***k*** was similar for both Granny Smith and Cox's Orange Pippin but Cox's Orange Pippin ***k*** was considerably higher than Granny Smith ***k*** from 6% to 21% oxygen. Total chlorophyll ***k*** increased slowly as oxygen increased from 1% to 13%. Chlorophyll a ***k*** values rose slightly over the range of oxygen levels present while ***k*** of chlorophyll b showed only a very slight rise in ***k*** with increased O_2 level. These changes in ***k*** fitted equation [5.1] poorly for chlorophyll's a and b.

The same pattern to that found for chlorophyll loss was observed for change in hue angle (Figure 5.5). The ***k*** values for hue angle were approximately 5 times greater (0 to 0.45 $day^{-1)}$ than ***k*** for chlorophyll (-0.02 to 0.08 day^{-1}) in the same conditions thus changes in colour are larger when expressed as ***k*** of hue angle. There was no lag phase at low O_2 levels, 1% to 6%, in contrast to ***k*** values for chlorophyll. Hue angle ***k*** values of Cox's Orange Pippin were higher at low O_2 levels and had a larger linear component in the sigmoidal curve than chlorophyll loss. Hue angle ***k*** values for Granny Smith fruit have a rising trend with increase in oxygen level (Figure 5.5) but this was not significant. The trend was similar to

total chlorophyll ***k*** although more pronounced. Values of ***k*** for hue angle of Granny Smith were between 0.03 and 0.10 day^{-1}, compared to chlorophyll loss, 0.01 to 0.04 day^{-1}. While not as great as Cox's Orange Pippin this is a further indication that hue angle ***k*** values are larger than chlorophyll ***k*** values for the same colour change.

Lightness had a more pronounced sigmoidal pattern of ***k*** than hue angle for Cox's Orange Pippin fruit (Figure 5.6). Lightness ***k*** at oxygen levels below 3% changed little and reached an asymptote at levels above 16% oxygen for Cox's Orange Pippin fruit. Granny Smith fruit lightness ***k*** values rose slowly from 0% O_2 to 21% O_2 (Figure 5.6). This is similar to the pattern found with hue angle and chlorophyll. The ***k*** values of lightness for Granny Smith were lower than ***k*** values for Cox's Orange Pippin but the difference in values was less than hue angle ***k*** or chlorophyll ***k***. Variation in ***k*** was high for Granny Smith fruit so that the relationship of lightness ***k*** to O_2 was poorly defined.

Colour chart score ***k*** values for Granny Smith had a rising trend with colour chart score increase and fitted only the exponential phase of the sigmoidal curve. There was a large variation in the data at oxygen levels greater than 15% resulting in poor definition of the relationship with oxygen.

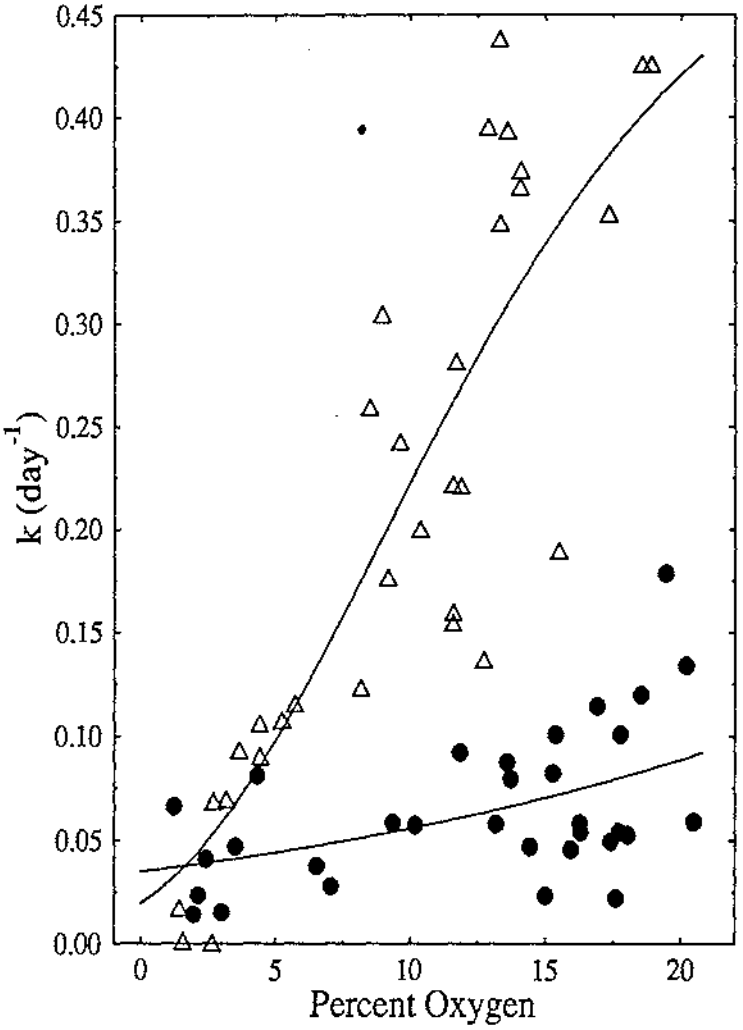


Figure 5.5 Rate constants of hue angle over different oxygen levels in the storage atmosphere of Cox's Orange Pippin (△) and Granny Smith (○) apples at 20°C for eight or sixteen weeks. Line of best fit was calculated from equation [5.1] the parameters were: for Cox's Orange Pippin: $A_o = 2.65 \times 10^{-3}$; $Y_o = 3.28$; $D_e = 1.33 \times 10^{-1}$; $r^2 = 0.731$; $p < 0.001$; and for Granny Smith: $A_o = 1.58 \times 10^{-5}$; $Y_o = 103.33$; $D_e = 4.50 \times 10^{-4}$; $r^2 = 0.262$; $p < 0.1$.

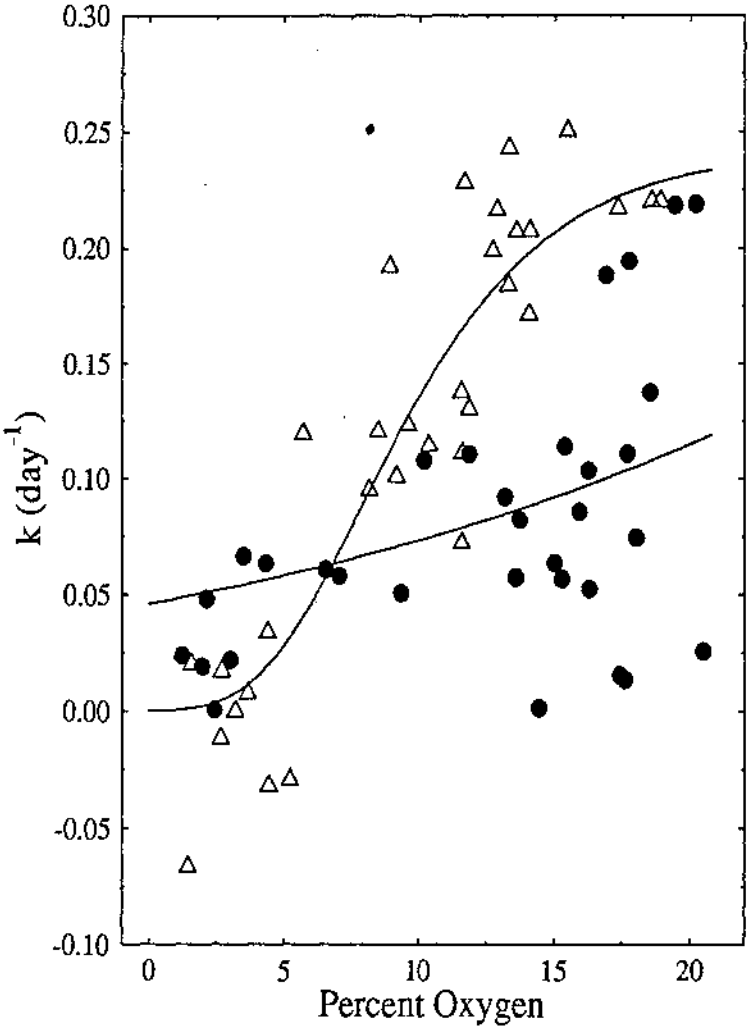


Figure 5.6 Rate constants of lightness over different oxygen levels in the storage atmosphere of Cox's Orange Pippin (△) and Granny Smith (◯) apples at 20°C for eight or sixteen weeks. Line of best fit was calculated from equation [5.1] the parameters were: for Cox's Orange Pippin: $A_o = 2.30 \times 10^{-5}$; $Y_o = 9.18$; $D_e = 2.61 \times 10^{-1}$; $r^2 = 0.815$; $p < 0.001$; and for Granny Smith: $A_o = 1.34 \times 10^{-4}$; $Y_o = 16.15$; $D_e = 2.90 \times 10^{-3}$; $r^2 = 0.231$; $p < 0.1$.

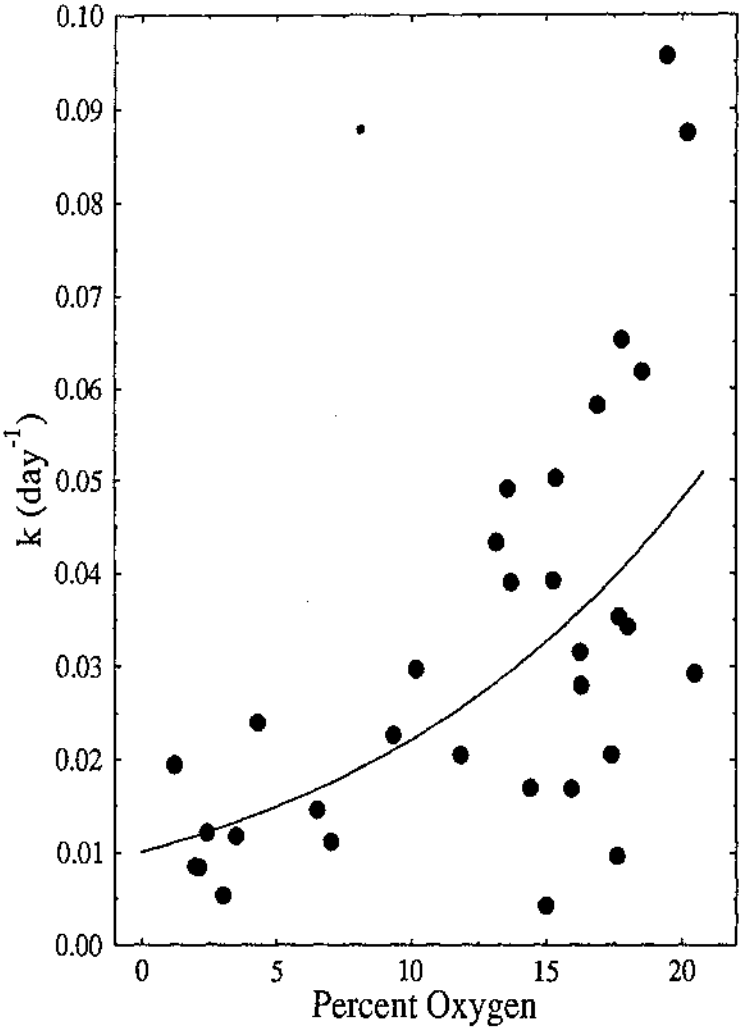


Figure 5.7 Rate constants of colour chart score over different oxygen levels in the storage atmosphere of Granny Smith (◯) apples at 20°C for sixteen weeks. Line of best fit was calculated from equation [5.1] the parameters were: for Granny Smith: $A_o = 2.00 \times 10^{-5}$; $Y_o = 44.01$; $D_e = 1.80 \times 10^{-3}$; $r^2 = 0.407$; $p < 0.05$.

5.3.2 Carbon Dioxide

Total chlorophyll ***k*** values when compared to levels of CO_2 in the atmosphere have a different pattern of ***k*** to that with O_2 (Figure 5.2 and 5.8). A different function to that used for O_2 is required to accurately describe the relationship of ***k*** with percent CO_2. In addition the relationship is more complex than is the case for O_2 with a large amount of scatter in ***k*** values at CO2 levels below 1% (Figures 5.8 to 5.10). Above 1% CO_2 the pattern of ***k*** with CO_2 appears to decline reaching a base asymptote. Therefore relationship between ***k*** for chlorophyll and atmospheres containing greater than 1% carbon dioxide can be described by a declining exponential function [5.2] with an asymptote above zero (Figures 5.8 to 5.9) for both Cox's Orange Pippin and Granny Smith apples.

$$k = A_r + A_o e^{-K_{CO_2} CO_2} \qquad [5.2]$$

where K_{CO2} = rate constant for carbon dioxide
A_o = rate constant at zero concentration
A_r = rate constant of the asymptote
CO_2 = percent carbon dioxide

Thus atmospheres containing more than 1% CO_2 may inhibit chlorophyll loss, reaching maximum inhibition between 10% to 15% CO_2 for Granny Smith apples. Carbon dioxide at high levels, 26% to 32%, did not appear to further reduce ***k*** of chlorophyll. The pattern of chlorophyll ***k*** with CO_2 was different for Cox's Orange Pippin and Granny Smith fruit. Granny Smith fruit ***k*** values fitted a declining exponential curve well for total chlorophyll and chlorophyll a but poorly for chlorophyll b. Cox's Orange Pippin fruit had a large degree of scatter at different CO_2 levels making defining the relationship of ***k*** with CO_2 difficult to establish.

Hue angle ***k*** values decline as levels of CO_2 rise for Granny Smith and Cox's Orange Pippin fruit (Figure 5.11). The curve for Granny Smith is similar to chlorophyll ***k*** but the data has less variation and is described well by a declining exponential function. Cox's Orange Pippin fruit ***k*** values had a poor fit to CO_2 which may be due to four very low ***k*** values between 2% and 5% CO_2 (Figure 5.11). Removal of the four very low values from the analysis is likely to give a better fit to equation [5.2].

Lightness ***k*** values had a high degree of scatter for Cox's Orange Pippin apples when compared to CO_2 level (Figure 5.12) indicating no clear relationship is present. Granny Smith fruit ***k*** values are variable but fit a declining exponential function well with the same pattern noted with hue angle and chlorophyll.

Colour chart score ***k*** values had the same pattern of response to increasing CO_2 as chlorophyll, hue angle and lightness (Figure 5.13).

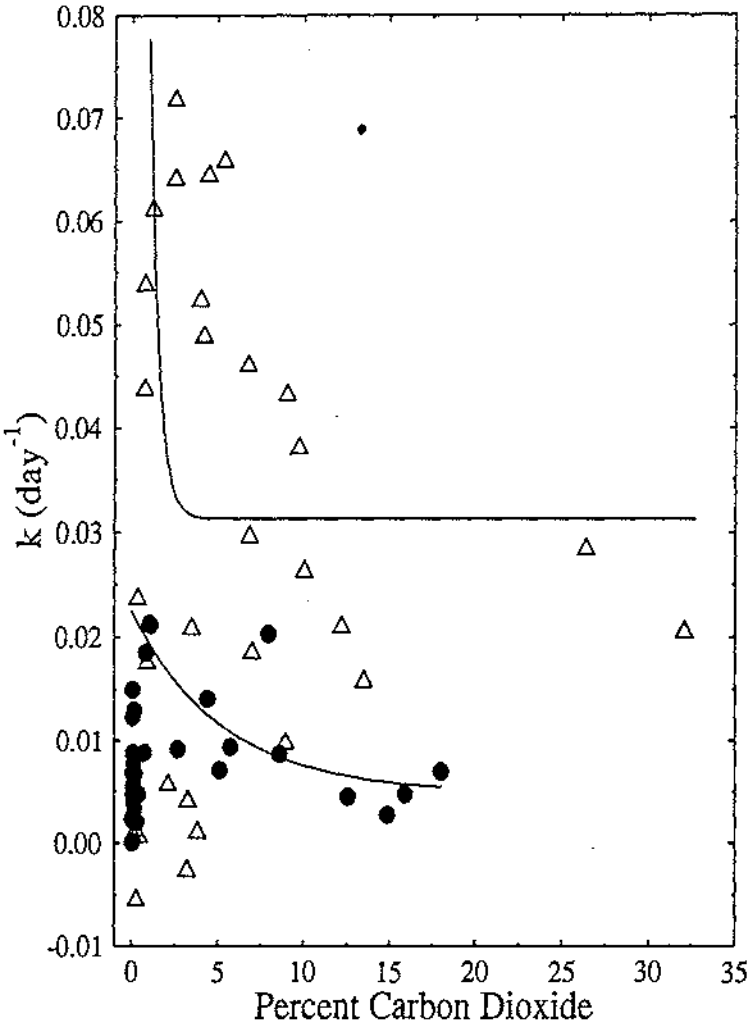


Figure 5.8 Rate constants of total chlorophyll over different carbon dioxide levels in the storage atmosphere of Cox's Orange Pippin (△) and Granny Smith (○) apples at 20°C for eight or sixteen weeks. Rates are for each carbon dioxide concentration. Line of best fit was calculated from equation [5.2] the parameters were: for Cox's Orange Pippin: A_r = 0.031; A_o = 0.341; K_{CO_2} = 1.996; r^2 = 0.070; NS; and for Granny Smith: A_r = 0.005; A_o = 0.018; K_{CO_2} = 0.187; r^2 = 0.555; $p<0.01$.

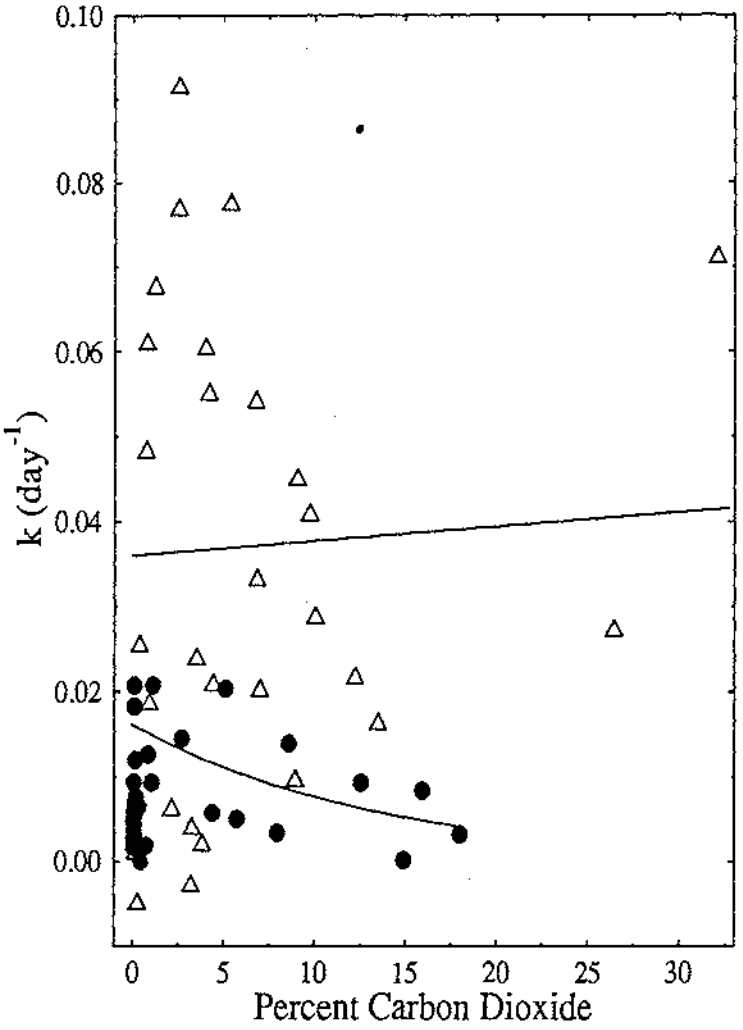


Figure 5.9 Rate constants of chlorophyll a over different carbon dioxide levels in the storage atmosphere of Cox's Orange Pippin (△) and Granny Smith (◯) apples at 20°C for eight or sixteen weeks. Rates are for each carbon dioxide concentration. Line of best fit was calculated from equation [5.2] the parameters were: for Cox's Orange Pippin: A_r = 0.321; A_o = -0.285; K_{CO_2} = 0.001; r^2 = 0.002; NS; and for Granny Smith: A_r = -0.001; A_o = 0.017; K_{CO_2} = 0.072; r^2 = 0.337; $p<0.01$.

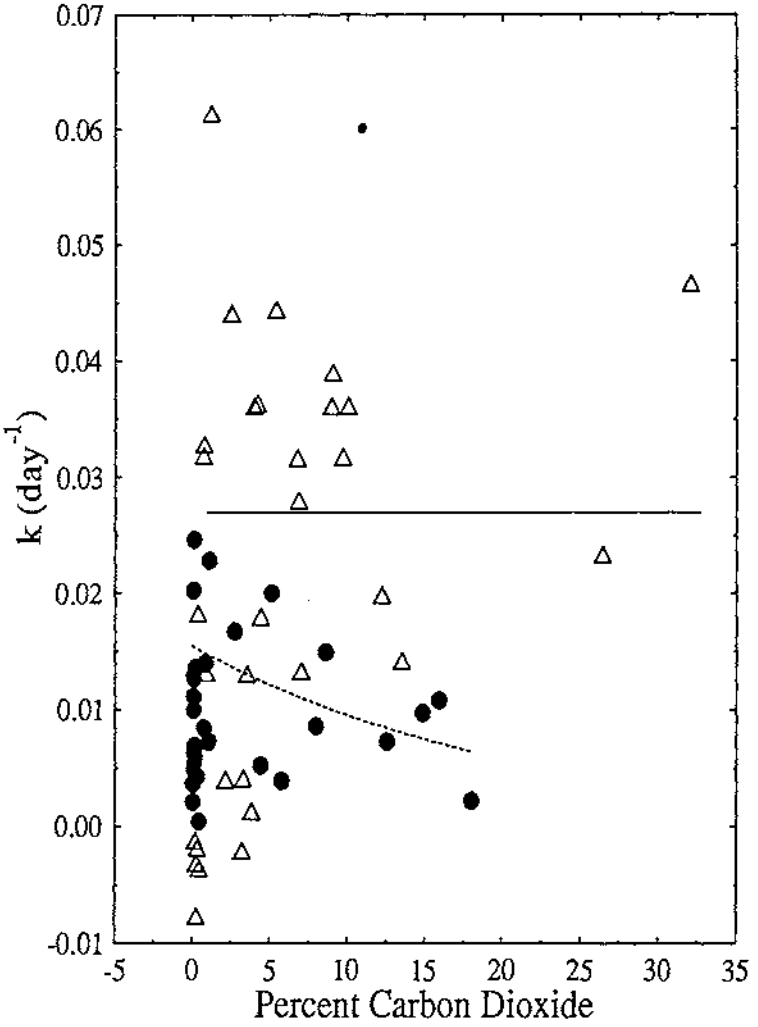


Figure 5.10 Rate constants of chlorophyll b over different carbon dioxide levels in the storage atmosphere of Cox's Orange Pippin (△) and Granny Smith (○) apples at 20°C for eight or sixteen weeks. Rates are for each carbon dioxide concentration. Line of best fit was calculated from equation [5.2] the parameters were: for Cox's Orange Pippin: A_r = 0.027; $A_o = -2.2 \times 10^6$; $K_{CO_2} = 1.36 \times 10^6$; $r^2 = 0.000$; NS; and for Granny Smith: $A_r = -0.0001$; $A_o = 0.016$; $K_{CO_2} = 0.048$; r^2 = 0.191; NS.

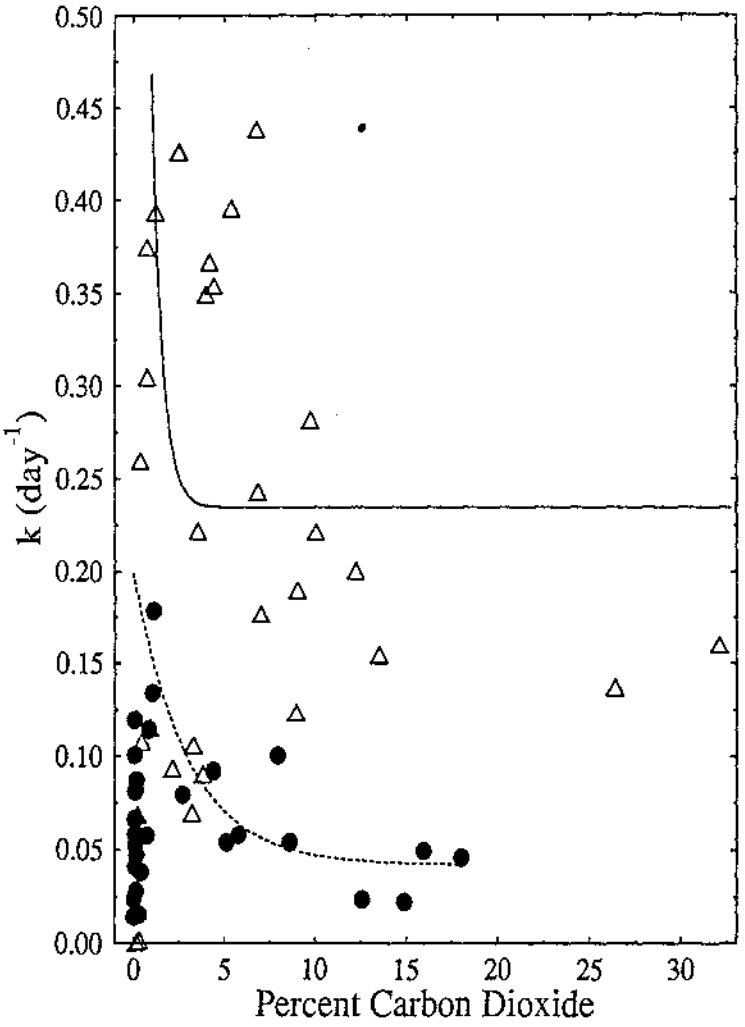


Figure 5.11 Rate constants of hue angle over different carbon dioxide levels in the storage atmosphere of Cox's Orange Pippin (△) and Granny Smith (○) apples at 20°C for eight or sixteen weeks. Rates are for each carbon dioxide concentration. Line of best fit was calculated from equation [5.2] the parameters were: for Cox's Orange Pippin: A_r = 0.234; A_o = 1.413; K_{CO_2} = 1.805; r^2 = 0.067; NS; and for Granny Smith: A_r = 0.042; A_o = 0.157; K_{CO_2} = 0.338; r^2 = 0.762; $p<0.001$.

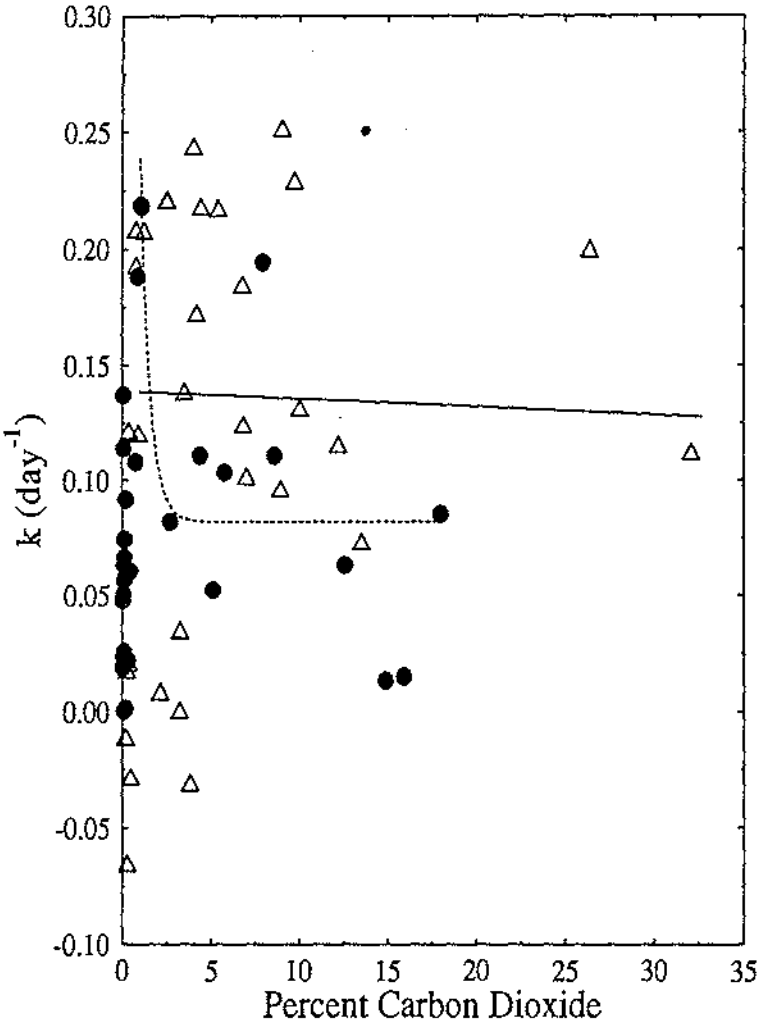


Figure 5.12 Rate constants of lightness over different carbon dioxide levels in the storage atmosphere of Cox's Orange Pippin (△) and Granny Smith (◯) apples for 20°C for eight or sixteen weeks. Rates are for each carbon dioxide concentration. Line of best fit was calculated from equation [5.2] the parameters were: for Cox's Orange Pippin: A_r = -19.33; A_o = 19.469; K_{CO_2} = 0.000; r^2 = 0.001; NS; and for Granny Smith: A_r = 0.082; A_o = 0.972; K_{CO_2} = 1.826; r^2 = 0.546; $p<0.01$.

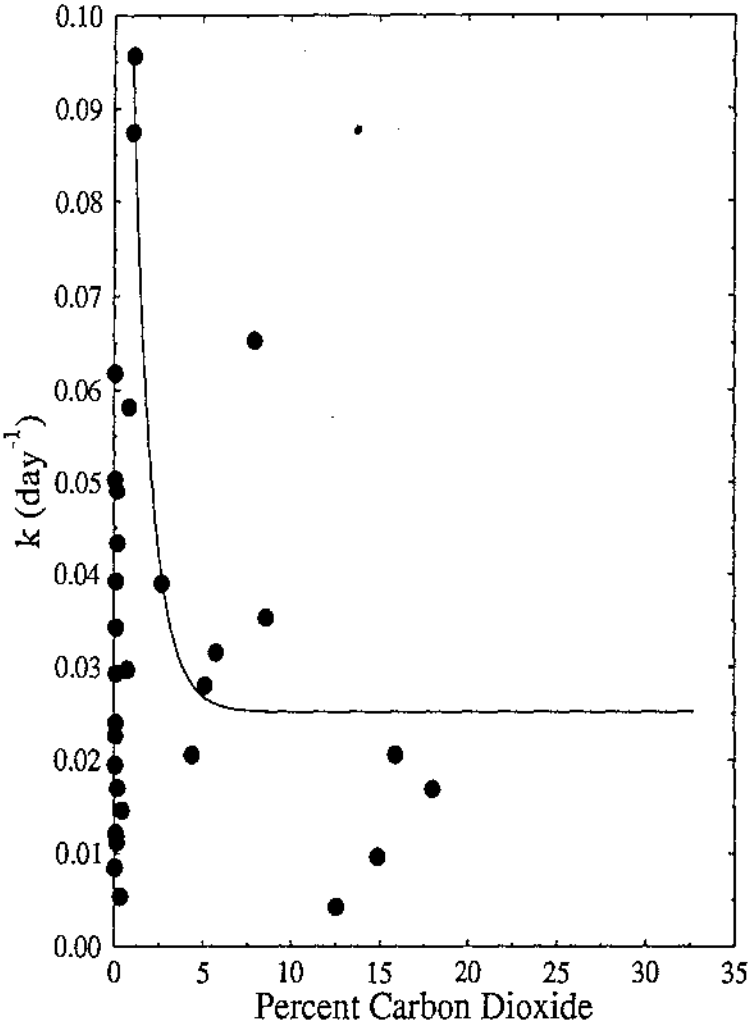


Figure 5.13 Rate constants of colour chart score over different carbon dioxide levels in the storage atmosphere of Cox's Orange Pippin (△) and Granny Smith (○) apples at 20°C for sixteen weeks. Rates are for each carbon dioxide concentration. Line of best fit was calculated from equation [5.2] the parameters were: for Granny Smith: $A_r = 0.025$; $A_o = 0.182$; $K_{CO_2} = 0.938$; $r^2 = 0.728$; $p<0.001$.

5.3.3 Interaction between oxygen and carbon dioxide

In an attempt to increase the precision of the predicted ***k*** values for a given O_2 and CO_2 atmosphere the Gompertz equation for O_2 and the declining exponential function for CO_2 were combined [5.3] and used to estimate ***k***.

$$k = A_o \frac{e^{Y_o(1-e^{-D_e O_2})}}{D_e} e^{-K_{CO_2} CO_2} \qquad [5.3]$$

The equation was derived using the assumption that a change in oxygen level has a greater influence on ***k*** than a change in CO_2, i.e. carbon dioxide effects are supplemental to oxygen effects as the same decrease in O_2 has a greater inhibitory effect on ***k*** than the same increase in CO_2. For Cox's Orange Pippin fruit both O_2 and CO_2 have an influence on ***k*** of chlorophyll (Figures 5.14 and 5.15). Reduction in O_2 levels results in a rapid reduction in ***k*** compared to increasing CO_2. Carbon dioxide inhibits ***k*** more as O_2 increases. When O_2 levels are low high CO_2 levels increase the lag phase and lower the maximum ***k*** values.

Estimates of ***k*** using equation [5.3] are compared to estimates of ***k*** using [5.1] and [5.2] in Figure 5.15 for Cox's Orange Pippin apples. The r^2 values for Cox's Orange Pippin fruit increased when the combined equation [5.3] was fitted to ***k*** values (Table 5.1). The greatest increase in fit was for hue angle which had 14.5% more variation accounted for taking into account both O_2 and CO_2 rather than with O_2 alone. Values of ***k*** estimated by equation [5.3] are closer to the actual values illustrated in Figure 5.15 than estimates of ***k*** by equations [5.1] or [5.2].

Table 5.1 Values of r^2 for fits to equations [5.1], [5.2] and [5.3] for Cox's Orange Pippin and Granny Smith apples stored in different atmospheres at 20°C during 1989 and 1990.

Parameter	Oxygen [5.1]	Carbon Dioxide [5.2]	Combined Equation [5.3]
Cox's Orange Pippin			
Chlorophyll a	0.676	0.002	0.682
Chlorophyll b	0.708	0.000	0.709
Total Chlorophyll	0.823	0.000	0.890
Hue Angle	0.737	0.000	0.882
Lightness	0.815	0.000	0.827
Granny Smith			
Chlorophyll a	0.072	0.327	0.118
Chlorophyll b	0.089	0.199	0.142
Total Chlorophyll	0.294	0.556	0.375
Hue Angle	0.263	0.762	0.458
Lightness	0.232	0.546	0.320
Colour Chart Score	0.407	0.728	0.594

Total chlorophyll ***k*** response surface to CO_2 and O_2 levels in the atmosphere for Granny Smith is different to Cox's Orange Pippin apples (Figure 5.16). Unlike Cox's Orange Pippin the rise in ***k*** values is exponential with oxygen increase whereas carbon dioxide increase has an inhibitory effect on ***k***. The net effect of high CO_2 levels at low O_2 levels is to increase the lag phase of the curve which remains exponential. Increases of the same magnitude in CO_2 or O_2 levels have a similar promotive or inhibitory effect on ***k*** for Granny Smith, unlike Cox's Orange Pippin, where O_2 has more influence on ***k*** than CO_2. Estimates of ***k*** using equation [5.3] are compared to estimates of ***k*** using equations [5.1] and [5.2] in Figure 5.17. The combined equation [5.3] did not account for more

variation in the fits of k to both O_2 and CO_2. But r^2 values for the the combined equation were better than the r^2 values for O_2 and worse than r^2 values for CO_2. This indicates there is a significant cultivar difference of yellowing in response to O_2 or CO_2 in the atmosphere.

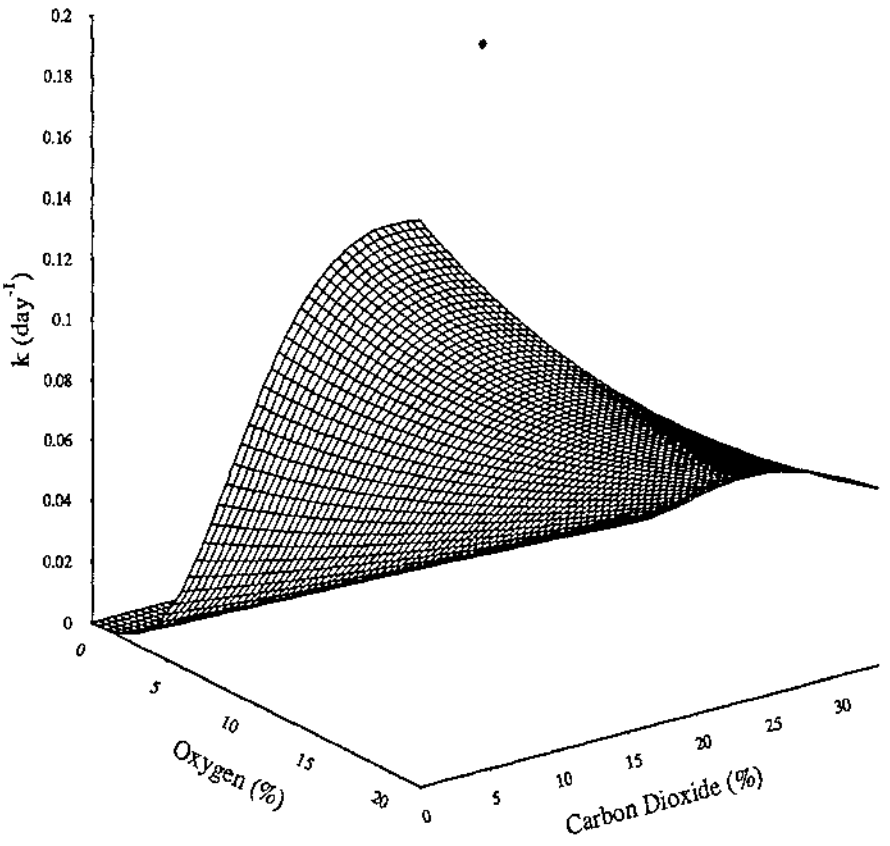


Figure 5.14 Three dimensional surface plot of k for total chlorophyll of Cox's Orange Pippin apples versus O_2 and CO_2 levels calculated from equation [5.3]. Parameters of the equation were: $A_o = 8.90 \times 10^{-6}$; $Y_o = 7.65$; De = 0.22; $K_{CO2} = 0.035$; $r^2 = 0.890$ $p<0.001$.

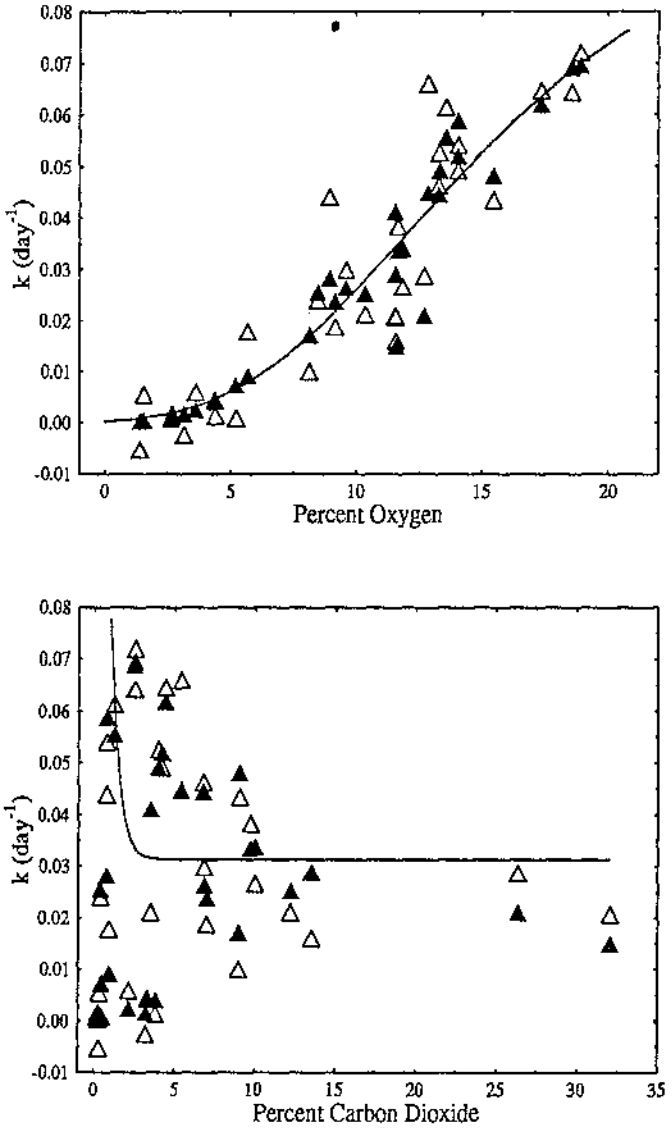


Figure 5.15 Estimates of rate constants of total chlorophyll by the combined Gompertz/declining exponential equation [5.3] for Cox's Orange Pippin apples, fits (△) and actual data (△). Lines are fits for the individual equations taken from figures 5.1 and 5.7. Parameters for the combined equation were: A_o = 8.90×10^{-6}; Y_o = 7.65; D_e = 0.22; K_{CO_2} = 0.035; r^2 = 0.890; $p<0.001$.

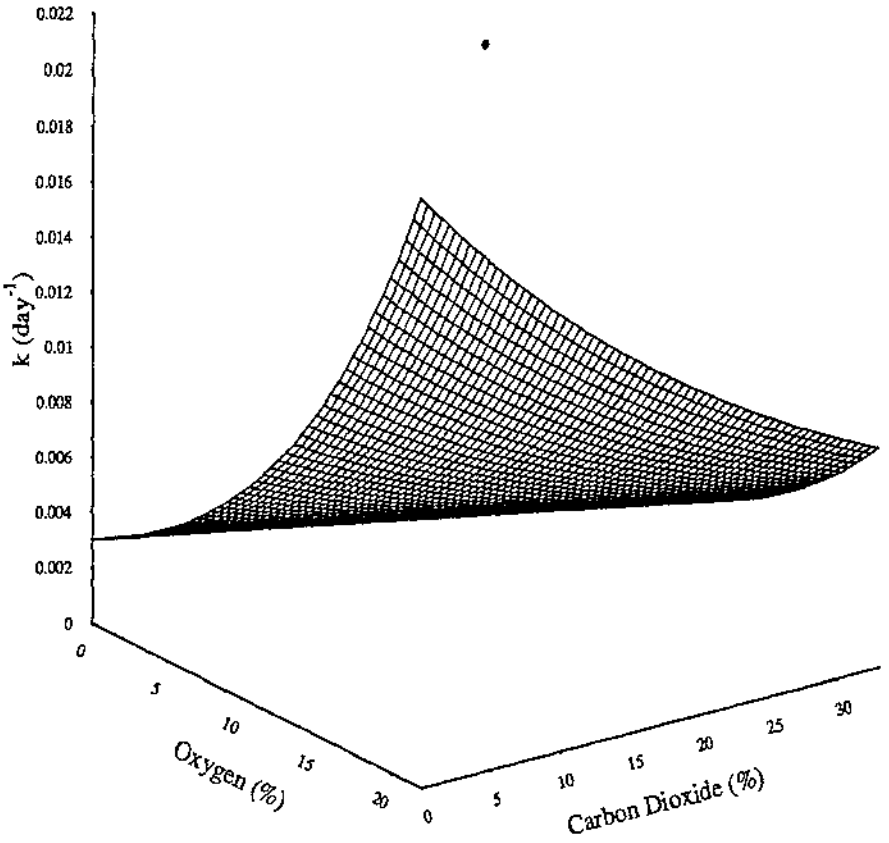


Figure 5.16 Three dimensional surface plot of k for total chlorophyll of Granny Smith apples versus O_2 and CO_2 levels calculated from equation [5.3]. Parameters of the equation were: $A_o = 6.0x10^{-6}$; $Y_o = 45.50$; De = 0.002; $K_{CO_2} = 0.03$; $r^2 = 0.375$ $p<0.05$.

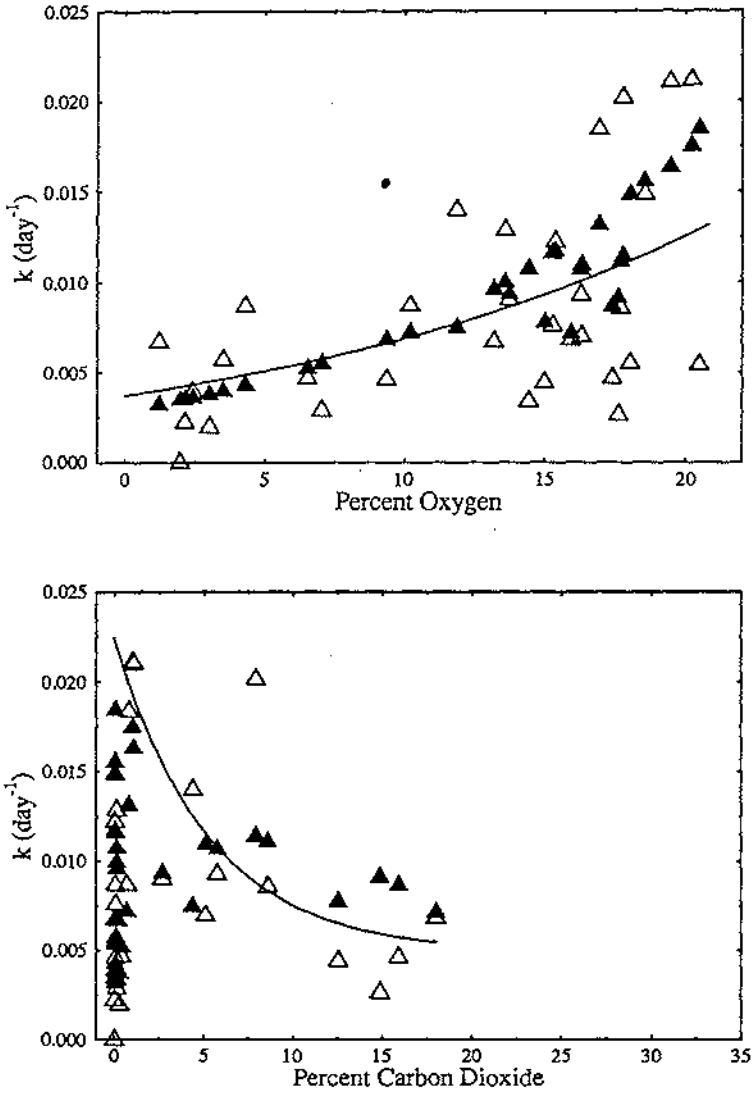


Figure 5.17 Estimates of rate constants of total chlorophyll by the combined Gompertz/declining exponential equation [5.3] for Granny Smith apples, fits (△) and actual data (△). Lines are fits for the individual equations taken from figures 5.1 and 5.7. Parameters for the combined equation were: A_o = 6.00x10^{-}6; Y_o = 45.50; D_e = 0.002; K_{CO_2} = 0.03; r^2 = 0.375; $p<0.05$.

5.3.4 Ethylene

There was no identifiable pattern of colour change with ethylene (Figure 5.18). Ethylene was scrubbed from the atmospheres tested therefore ethylene levels should have been close to zero. Apple fruit ripen during storage at temperatures above 10°C, during which time large amounts of ethylene are produced. These levels of ethylene are difficult to remove from the storage atmosphere adequately. Therefore some atmospheres tested may have higher levels of ethylene than expected in spite of an ethylene scrubber being used. This may be the reason for there being a wide scatter in ***k*** values with ethylene.

5.3.5 Cultivars

Similar differences between Granny Smith and Cox's Orange Pippin fruit were found to those in the temperature experiment (Tables 4.2 and 5.2). Granny Smith fruit total chlorophyll content was 2.6 times greater (2.7 times in the temperature experiment), hue angle 7.3° (8.3°) higher and lightness lower by 9.9% (9.3%), 11.1N (7.3N) firmer and 1.6°Brix (1°Brix) lower than Cox's Orange Pippin fruit. Levels of chlorophyll a and chlorophyll b were in the same proportion for each cultivar despite the large absolute difference between cultivars. Ratios of chlorophyll a and chlorophyll b were similar, 3.5 and 3.3 for Cox's Orange Pippin and Granny Smith respectively.

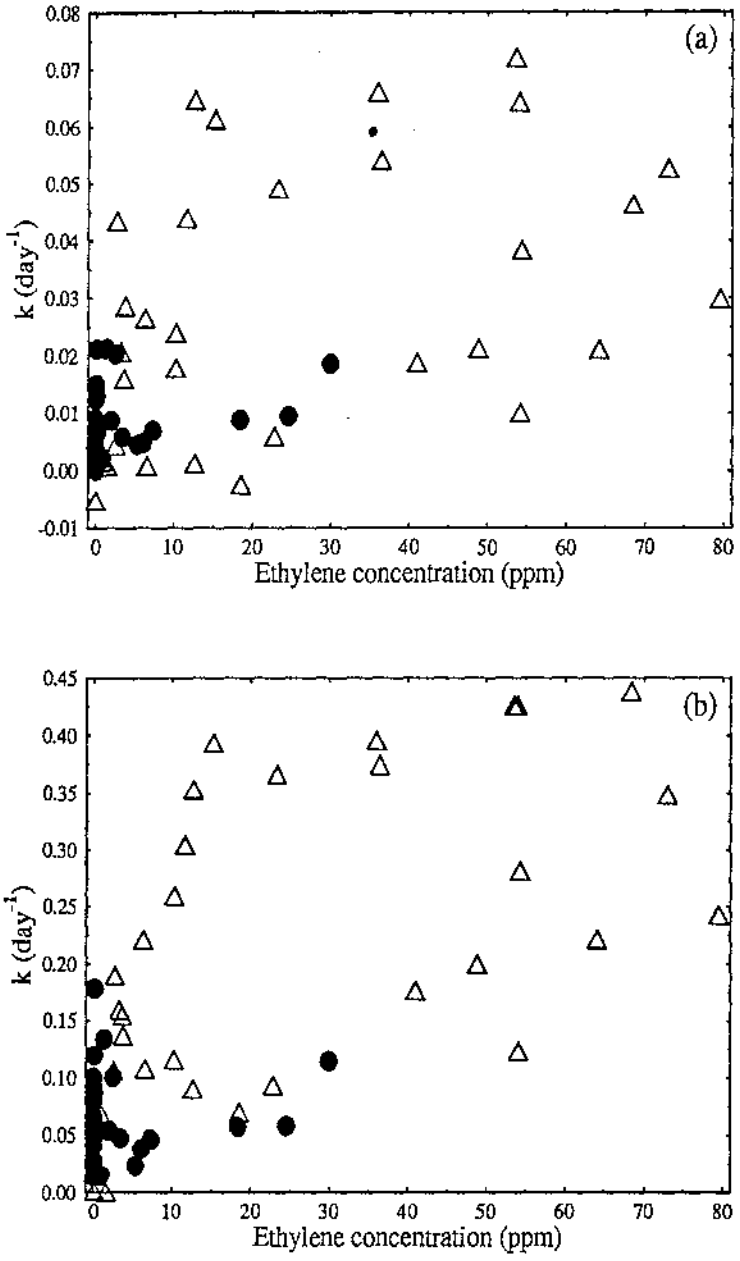


Figure 5.18 Rate constants of (a) total chlorophyll and (b) hue angle in relation to ethylene level for Cox's Orange Pippin (△) and Granny Smith (◯) apples.

Table 5.2 Colour parameters of Cox's Orange Pippin and Granny Smith apples stored during 1989 and 1990.

Cultivar	Chl a (ng/mm^2)	Chl b (ng/mm^2)	Total Chlorophyll (ng/mm^2)	Hue Angle (°)	Lightness (%)	Firmness (N)	Total Soluble Solids (°Brix)
Cox's Orange Pippin	25.5b[1]	7.3b[2]	32.8b	110.8b	69.5b	83.75b	10.52a
Granny Smith	65.8a	20.0a	85.8a	118.1a	59.6a	94.89a	8.97b

1 Average of initial measurements.
2 Different letters within columns are significantly different at $p<0.05$ according to Duncans multiple range test.

Chlorophyll a, chlorophyll b and total chlorophyll ***k*** values were 3.5, 2.5 and 3.5 times higher for Cox's Orange Pippin fruit than for Granny Smith fruit (Table 5.3).

Table 5.3 Rate constants of quality parameters during 1989 and 1990.

Cultivar	Chl a (ng/mm^2)	Chl b (ng/mm^2)	Total Chl (ng/mm^2)	Hue Angle (°)	Lightness (%)	Colour Chart Score
Cox's Orange Pippin	0.07 ***[1]	0.05 ***	0.07 ***	0.22 ***	0.12 *	
Granny Smith	0.02 ***	0.02 ***	0.02 ***	0.07 ***	0.08 *	

1 Significance levels within a column: NS not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

5.3.6 Years

Cox's Orange Pippin fruit at initial assessment had higher total chlorophyll and chlorophyll b but not chlorophyll a in 1989 compared to fruit in 1990 (Table 5.4), the differences were 3.4 ng/mm^2 and 1.6 ng/mm^2 respectively. Lightness, hue angle, chlorophyll a and firmness were the same for fruit from 1989 and 1990. Total soluble solids were higher in 1990 fruit indicating 1990 fruit may have been

slightly more mature than 1989 fruit. Granny Smith fruit showed the same trends as Cox's Orange Pippin fruit. Chlorophyll a, chlorophyll b, total chlorophyll, firmness and total soluble solids were higher in 1989 fruit than in 1990 fruit, the difference in total chlorophyll between 1989 and 1990 being 12.6 ng/mm^2. There was no difference in fruit lightness between years but hue angle was lower in 1989 than in 1990.

Table 5.4 Quality parameters of Cox's Orange Pippin and Granny Smith apples at initial assessment during 1989 and 1990.

Cultivar	Year	Chl a (ng/mm^2)	Chl b (ng/mm^2)	Total Chl (ng/mm^2)	Hue Angle (°)	Lightness (%)	Firmness (N)	Total Soluble Solids (°Brix)
Cox's Orange Pippin	1989	15.8a[1]	7.5a	23.3a	109.5a	69.2a	74.4a	10.1a
	1990	14.0a	5.9b	19.9b	110.0a	70.6a	72.5a	11.0b
Granny Smith	1989	59.4a	27.0a	86.4a	114.2b	62.6a	72.3a	10.8a
	1990	52.4b	21.4b	73.8b	115.4a	65.6a	67.5b	10.1b

1 Different letters within a column are significant at $p<0.05$ according to Duncans multiple range test.

There were no differences in ***k*** between Cox's Orange Pippin and Granny Smith fruit in 1989 and 1990 (Table 5.5).

Table 5.5 Rate constants of colour change per day in quality parameters during 1989 and 1990.

Cultivar	Year	Chl a (ng/mm^2)	Chl b (ng/mm^2)	Total Chl (ng/mm^2)	Hue Angle (°)	Lightness (%)	Colour Chart Score
Cox's Orange Pippin	1989	0.08NS	0.06NS	0.07NS	0.25NS	0.13NS	
	1990	0.06NS	0.05NS	0.06NS	0.18NS	0.11NS	
Granny Smith	1989	0.02NS	0.03NS	0.02NS	0.02NS	0.06NS	
	1990	0.01NS	0.02NS	0.02NS	0.05NS	0.05NS	

1 Significance levels within a column: NS not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

5.3.7 Growers

The difference between average chlorophyll content of fruit from each grower is as high as 29.8 ng/mm^2 for Granny Smith and 5.0 ng/mm^2 for Cox's Orange Pippin (Table 5.6). This represents a difference between growers of 20.5% in chlorophyll content for Cox's Orange Pippin and 31.4% for Granny Smith. Total chlorophyll differences observed between growers was due to lower levels of chlorophyll a and chlorophyll b. Chlorophyll a to chlorophyll b ratios ranged from 3.3 to 2.3 for Cox's Orange Pippin and 2.1 to 2.3 for Granny Smith. Despite the similar percentage differences between growers of Cox's Orange Pippin and Granny Smith fruit only fruit from Granny Smith grower D139 was different in chlorophyll content to fruit from other Granny Smith growers. Cox's Orange Pippin fruit showed no significant differences in chlorophyll levels between growers (Table 5.6).

Granny Smith grower D341 fruit had a lower average hue angle indicating the fruit was less green compared to the other Granny Smith growers fruit. Grower D075's fruit had the lowest chlorophyll content but a hue angle similar to grower D139's fruit. Hue angle and lightness values of Cox's Orange Pippin fruit from different growers were not significantly different. Lightness was significantly different between each Granny Smith growers fruit tested but grower D341's fruit

had a lower hue angle than other growers fruit. Grower D139's fruit were the darkest, had highest hue angle and highest chlorophyll content.

Cox's Orange Pippin grower D389's and Granny Smith grower D139's fruit were significantly softer than other growers fruit. There was no significant difference in Cox's Orange Pippin fruit in total soluble solids which were similar to levels in Granny Smith fruit. Total soluble solid levels of Granny Smith fruit which were different for each growers fruit although they had the same range of values as Cox's Orange Pippin.

Growers D265 and D075 had the highest total soluble solids and lowest chlorophyll content, for Cox's Orange Pippin and Granny Smith respectively, but similar ***k*** values to fruit with lower soluble solids and higher chlorophyll contents (Table 5.7). There were no differences in ***k*** between different growers fruit for Cox's Orange Pippin and Granny Smith fruit.

Table 5.6 Quality parameters of Cox's Orange Pippin and Granny Smith apples of each grower prior to storage in controlled atmospheres.

Grower	Chl a (ng/mm^2)	Chl b (ng/mm^2)	Total Chl (ng/mm^2)	Hue Angle (°)	Lightness (%)	Firmness (N)	Total Soluble Solids (°Brix)
Cox's Orange Pippin							
D252	14.5a[1]	6.4a	20.9a	110.8a	71.2a	71.2a	10.5a
D265	14.9a	4.5a	19.4a	109.3a	69.1a	84.2a	10.8a
D389	16.9a	7.5a	24.4a	109.7a	70.2a	64.9b	10.1a
Granny Smith							
D075	46.2b	18.8b	65.0b	115.0a	67.5a	72.4a	10.8a
D139	66.4a	28.4a	94.8a	115.5a	62.3c	67.2b	10.2c
D341	44.4b	21.4b	65.8b	113.1b	64.7b	72.9a	10.5b

1 Different letters within a column and cultivar are significantly different at $p<0.05$ according to Duncans multiple range test.

Table 5.7 Rate constants of colour change per day of quality parameters during 1989 and 1990.

Grower	Chl a (ng/mm^2)	Chl b (ng/mm^2)	Total Chl (ng/mm^2)	Hue Angle (°)	Lightness (%)	Colour Chart Score
Cox's Orange Pippin						
D252	0.046 NS[1]	0.032 NS	0.042 NS	0.168 NS	0.083 NS	
D265	0.079 NS	0.058 NS	0.071 NS	0.221 NS	0.142 NS	
D389	0.089 NS	0.055 NS	0.076 NS	0.249 NS	0.116 NS	
Granny Smith						
D075	0.015 NS	0.018 NS	0.020 NS	0.058 NS	0.102 NS	
D139	0.022 NS	0.024 NS	0.015 NS	0.053 NS	0.061 NS	
D341	0.018 NS	0.025 NS	0.028 NS	0.097 NS	0.093 NS	

1 Significance levels within a column: NS Not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

5.4 Discussion

5.4.1 Oxygen and Carbon Dioxide

Cox's Orange Pippin apples ***k*** values for colour changes at varying O_2 concentrations are well described by the Gompertz equation. Regression coefficients ranged from 0.824 to 0.676 which are all significant to least at $p<0.05$. This finding further refines the quadratic relationship of hue angle as a function of oxygen level (Hewett *et al* 1989). The Gompertz function defined the change in hue angle well at low oxygen levels in comparison to the poor definition of the quadratic function. The relationship of oxygen to colour is similar to that reported by Scott *et al* (1964) where Jonathan and Delicious apples had a higher ground colour score at low oxygen levels, 3-6% oxygen, compared to 18%-21% oxygen. Linear relationship for each cultivar of oxygen to ground colour score was highly significant. Granny Smith fruit had a poorly defined relationship of k with O_2 and the relationship did not did have a sigmoidal pattern. This represents a fundamental difference in each cultivars colour change

response to O_2. The model used is likely to be correct for Cox's Orange Pippin apples and indicates that O_2 has a large influence on the rates of colour change. This would imply that yellowing could be retarded well by the use of lower than normal atmospheric levels of O_2 and even that small changes in levels of O_2 will reduce yellowing rates. Granny Smith apples colour change appears to be relatively insensitive to changes in oxygen level in the atmosphere. Thus Granny Smith fruit would be expected to receive relatively little benefit in terms of reduced yellowing rates from a lowering of the O_2 level in the atmosphere.

Chlorophyll ***k*** values have a sigmoidal relationship with external oxygen at 20°C which is in contrast to the asymptotic relationship for days to lose 1µg/cm^2 chlorophyll reported by Knee (1980a) at 3.5°C. Chlorophyll a and chlorophyll b ***k*** have the same relationship with external O_2 but differ in magnitude, chlorophyll b ***k*** being less than chlorophyll a ***k***. This is consistent with results from the temperature experiment. This may be due to chlorophyll loss at 20°C being four times that at 3.5°C (Figures 4.3 to 4.5, Chapter 4). If oxygen is a requirement for chlorophyll breakdown whether for ATP production via respiration (Brown *et al* 1991) or directly in the breakdown process (Thomas and Matile 1985) then oxygen demand would be expected to be greater at 20°C than 3.5°C. Additionally the skin of apples represents a significant barrier through which O_2 has to diffuse (Dadzie 1992). Restricting oxygen supply by a barrier to oxygen diffusion would result in a sigmoidal relationship with ***k***, as is found with respiration rate (Dadzie 1992). Results from this experiment do not indicate whether there is limited O_2 supply for respiration or chlorophyll breakdown. At 3.5°C demand for O_2 is very much less than at 20°C as respiration is slower and the apple skin resistance to O_2 diffusion may not limit the O_2 supply sufficiently to inhibit chlorophyll breakdown. Without a limit to O_2 the expected relationship with ***k*** would be similar to a typical Michaelis-Menton curve of enzyme activity. Apples also ripen more rapidly at 20°C than 3.5°C and the effect of temperature on ***k*** of physiological changes associated with ripening, e.g. cell wall breakdown and the climacteric rise in respiration rate, may also have an influence.

Some fruit at O_2 levels below 5% have an apparent increase in chlorophyll content instead of a loss in chlorophyll (Figures 5.2 to 5.7 and Appendix 3) which has been noted by Knee (1980a) for 1% O_2. This may be due to difficulties of estimating chlorophyll loss when chlorophyll content is relatively constant, i.e. the regression line is essentially horizontal. The method used to estimate ***k*** for chlorophyll therefore estimates ***k*** poorly at low O_2 levels. At the high end of the O_2 curve, above 17% O_2, the reaction would be limited by availability of enzyme and substrate and not O_2 thus the rate of increase in chlorophyll ***k*** values may slow and eventually plateau.

The relationship of hue angle ***k*** with O_2 is similar to total chlorophyll relationship with ***k*** for both Cox's Orange Pippin and Granny Smith. The curve rises steeply without a lag phase, for Cox's Orange Pippin fruit, unlike total chlorophyll ***k***. Granny Smith has the same curve as for chlorophyll loss. Lightness ***k*** values had a more pronounced sigmoidal relationship than for chlorophyll or hue angle but was very similar to chlorophyll b. The Granny Smith curve was the same as the curve for other colour parameters. The lightness and hue angle relationships appear to measure different aspects of colour changes. Hue angle is measuring the colour change and must therefore depend on the mix of pigments in the skin providing the colour. For Cox's Orange Pippin the steepness of the curve for hue angle in Figure 5.5 would indicate that there is a high degree of inhibition of ***k*** when the O_2 level is reduced. Lightness ***k*** values are not inhibited as much at levels of O_2 above 15% than at levels below 15% O_2 where there is a high degree of inhibition of ***k***. This may be an indication that while the colour change is inhibited, fruit lightening changes reach a maximum ***k*** more rapidly but have a greater initial lag phase. This may be an indication of changes in other pigments occurring at the same time as chlorophyll is degraded. Storage in low O_2 atmospheres for Cox's Orange Pippin is therefore a good method of minimising ***k*** of colour change. Granny Smith fruit are relatively insensitive to changes in O_2 in comparison to Cox's Orange Pippin. The hue angle and lightness relationship with ***k*** are very similar. However, storage in low O_2 atmospheres minimises ***k*** for

this cultivar also. The colour chart score ***k*** relationship to O_2 is similar to the other colour parameters measured and follows the same trends.

The respiration process in plants uses O_2 and produces CO_2. Therefore in any experiment in which the relationship with O_2 is examined, care must be taken to remove the effect of CO_2. This is to prevent possible interactions on the ***k*** of colour change of O_2 and CO_2. In this study levels of CO_2 were not closely controlled in 1989, the flow rate of the gas mix was assumed to be sufficient to remove CO_2 produced by the apples during respiration. The experiment was conducted at 20°C during which time the fruit ripened. Apples undergo a large increase in CO_2 production when ripening due to the respiratory climacteric (Wills *et al* 1981). This additional CO_2 increased the levels of CO_2 in the atmospheres studied. In 1990 a CO_2 scrubber kept levels of CO_2 low in the chambers used for storing apples for CA. The relationship of ***k*** had the same pattern in 1990 as in 1989 when the CO_2 levels were uncontrolled (Figure 5.19). Thus removing possible CO_2 interactions by lowering levels of CO_2 in the atmosphere had no effect on the pattern of ***k*** with O_2. This implies that the relationships of O_2 and CO_2 with ***k*** are the result of separate processes and that O_2 affects the rate of col;our change differently to CO_2.

Levels of CO_2 above 1% inhibit colour change, in agreement with Burton (1982) and Apelbaum *et al* (1976), while levels below 1% have little effect on ***k***. The same relationship to CO_2 was seen with all calculations of ***k*** with differences between methods being one of magnitude. Reworking of data from Wang *et al* (1971) on asparagus shows the same relationship of CO_2 to ***k*** (Figure 5.20). Carbon dioxide levels above 1% and ***k*** appear to described by a declining exponential function. However, fits for Cox's Orange Pippin were poor whereas Granny Smith fits were good thus highlighting a significant cultivar difference in the relationship of ***k*** to CO_2. Such a difference between cultivars has been noted for Jonathan and Delicious apples where more variation is accounted for in a linear regression by O_2 for Jonathan apples but CO_2 accounts for more variation in Delicious apples skin ground colour (Scott *et al* 1964). Further work is

required to fully assess the effect of CO_2 on ***k*** at low and high levels. The lack of inhibition of ***k*** at CO_2 levels below 1% may be ascribed to the effect of CO_2 on general metabolism, most probably suppression of respiration. As yet there is little evidence in the literature for this idea. Additionally high CO_2 levels do not reduce ***k*** to very low levels but to an asymptote which is cultivar dependent. Therefore CO_2 may not inhibit ***k*** directly. Granny Smith fruit ***k*** are inhibited more and have a clear declining exponential relationship with CO_2 compared to Cox's Orange Pippin apples. The reason for this is not known but is an example of a fundamental cultivar difference between Cox's Orange Pippin and Granny Smith fruit.

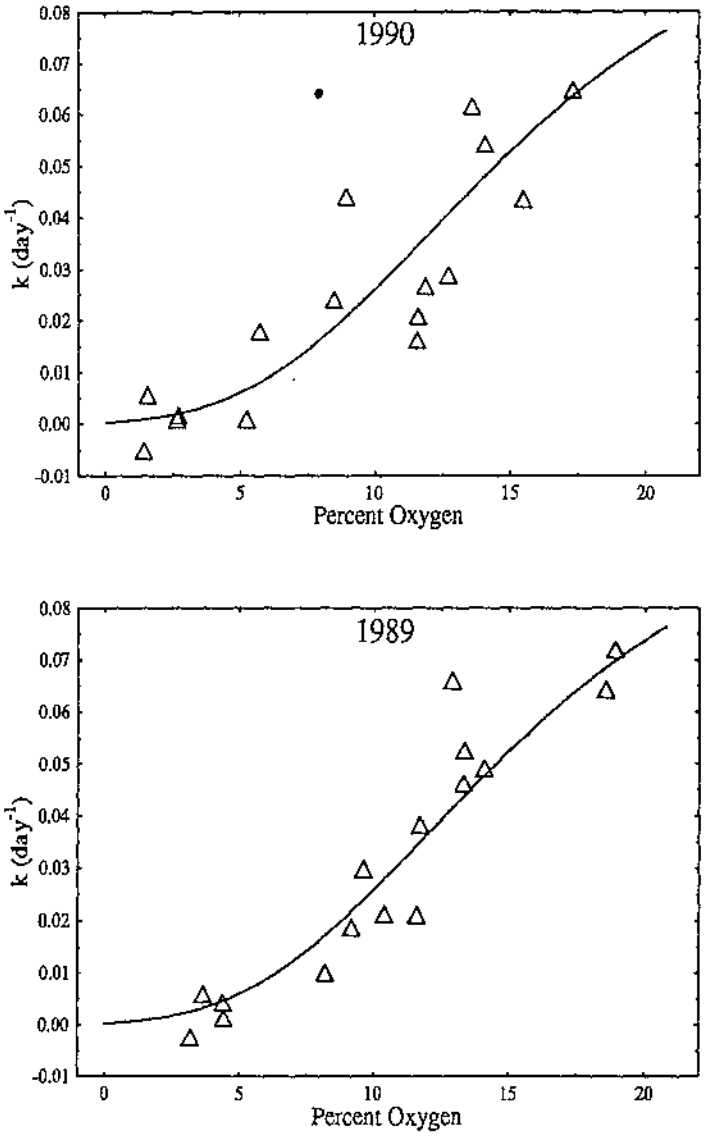


Figure 5.19 Rate constants of total chlorophyll over different oxygen levels in the storage atmosphere of Cox's Orange Pippin apples during 1989 and 1990 at 20°C for eight weeks. Rates are for each oxygen concentration. Line of best fit was calculated from equation [5.1] the parameters were: A_o = $4.19x10^{-5}$; Y_o = 5.86; D_e = $1.46x10^{-1}$; r^2=0.824; p<0.001;

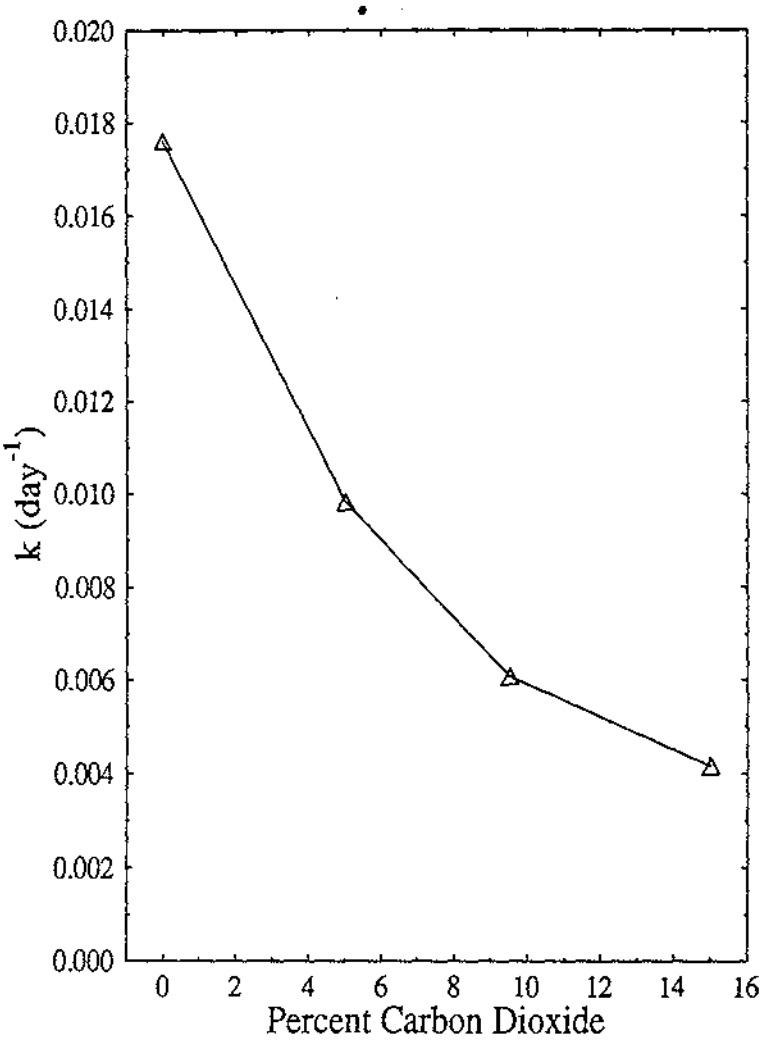


Figure 5.20 Rate constants of total chlorophyll over different carbon dioxide levels in the storage atmosphere of fresh asparagus at 1.7°C for eleven days. Oxygen levels were 21% for 0% carbon dioxide and 3% for other carbon dioxide levels. There was no 3% oxygen 0% carbon dioxide treatment. Data reworked from Wang *et al* (1971).

Oxygen and CO_2 together in the atmosphere may interact to reduce ***k*** of colour change more than for each gas alone, even though each gas may be influencing ***k*** values of colour change independently. When ***k*** is plotted three dimensionally using equations [5.1] and [5.2] the surface generated allows possible interactions to be interpreted. The effect of increased CO_2 is to increase the lag phase and to reduce the magnitude of the curve with respect to O_2 (Figure 5.14 and Figure 5.16). To improve prediction of ***k*** to O_2 and CO_2 levels in the atmosphere the Gompertz equation [5.1] for O_2 and declining exponential function [5.2] for CO_2 were combined additively and is presented as equation [5.3]. The improvement in predicted values for each O_2 and CO_2 level for Cox's Orange Pippin are presented in (Figures 5.14 to 5.16). Predicted values from equation [5.3] are closer to actual values than when calculated by equations [5.1] and [5.2]. The r^2 values of the combined equation for Cox's Orange Pippin fruit are greater than O_2 or CO_2 alone (Table 5.1). The combined CO_2 and O_2 equation [5.3] for Granny Smith fruit gives fits to ***k*** that are lower than CO_2 indicating that equation [5.3] is not an improvement over equation [5.2]. Other measurements of ***k***, hue angle, lightness and colour chart score, had the same relationship with O_2 or CO_2 as chlorophyll content and the combined equation could be expected to be similar to that for chlorophyll. Equation [5.3] is useful therefore in estimating the influence of O_2 and CO_2 on yellowing for Cox's Orange Pippin apples but not Granny Smith apples.

To accurately determine the relationship between CO_2 and colour change, other factors which may influence colour change should be removed or maintained at a constant level during the experiment. In the case of CO_2 there may be considerable interaction of colour change with O_2. If O_2 has an overriding effect on colour change then when ***k*** values are plotted against CO_2 levels the pattern seen may be more influenced by levels of O_2 than levels of CO_2. A closer examination of Cox's Orange Pippin total chlorophyll ***k*** values in relation to CO_2 concentration was made for 6 storage conditions where O_2 was maintained in the range of 11.6 to 11.9% and CO_2 ranged from 3.5 to 32% (Table 5.8). No

significant pattern was apparent indicating that conclusions drawn about CO_2 and rates of colour change must be made with caution. Although the pattern described in section 5.3.2 is found with asparagus (Wang *et al* 1971), the pattern for apples may be different when oxygen levels are held constant. Therefore the CO_2 relationship for Cox's Orange Pippin presented in this thesis is a best guess. Granny Smith fruit, however, have a clear relationship between values of ***k*** and CO_2 but a poorly defined relationship with O_2. The same comment on the Cox's Orange Pippin experiment can be applied to the relationship between O_2 and colour change ***k*** values for Granny Smith fruit. Further experiments are required in which the levels of oxygen the same for a range of CO_2 concentrations in order to determine if these two gases interact with one another with respect to colour change.

Table 5.8 Values of ***k*** for total chlorophyll of Cox's Orange Pippin apples stored in controlled atmospheres with O_2 concentrations ranging between 11.6 and 11.9%.

CO_2 (%)	O_2 (%)	Tot Chl ***k***
3.5	11.6	0.048
4.4	11.9	0.014
9.7	11.7	0.088
10.0	11.9	0.061
13.5	11.6	0.037
32.1	11.6	0.048

5.4.2 Ethylene

Values of ***k*** varied greatly for similar levels of ethylene in the external atmosphere (Figure 5.18). While there was no clear relationship between ethylene and ***k*** ethylene levels may trigger colour change and thereby increase ***k*** by stimulating the respiratory climacteric (Knee 1980a, Apelbaum *et al* 1976). Fruit which were kept at 20°C or above would have ripened and passed the climacteric during which high levels of ethylene were produced. Ethylene

stimulation of respiration may not have a large influence on chlorophyll loss as Grimes Golden apples exposed to 1000ppm ethylene at 15°C had an abrupt rise in respiration but not an increase in chlorophyll loss (Workman 1964). Further research would be required to clarify ethylene's role in the rate of colour change.

5.4.3 Cultivar

The relationship of ***k*** with O_2, CO_2 or a combination of O_2 and CO_2 for Cox's Orange Pippin was not the same for Granny Smith fruit. The reason for this is not known. Differences in ***k*** values noted between Cox's Orange Pippin and Granny Smith apples may be due to their respective respiration rates and skin resistances. The O_2 demand of Granny Smith fruit is less than Cox's Orange Pippin which may be due to a lower respiration rate at 20°C (9 $cm^3/kg/hr$ compared to 17 $cm^3/kg/hr$, (Dadzie 1992)) but similar skin resistance. If respiration is important in regulating the speed of chlorophyll loss then low O_2 and low respiration rates could result in a slow rate of chlorophyll loss. Extending the range of CO_2 and O_2 combinations by including atmospheres with more than 20% O_2 may allow Granny Smith fruit's response to O_2 to be characterised. Chlorophyll loss is thought to be faster at 100% O_2 than at 21% O_2 (Burton 1982), though 60% O_2 did not affect the rate of yellowing of Grimes Golden and Golden Delicious apples (Workman 1964). Thus there may be a level of O_2 above which no increase in ***k*** occurs, i.e. the process is O_2 saturated.

Skin resistance is known to affect the internal atmosphere of an apple fruit (Dadzie 1992). Reductions in external levels of O_2 correspond to reductions in internal levels by a similar amount. Even in normal atmospheric levels of O_2 apple fruit have reduced levels of internal O_2. According to Dadzie (1992) Cox's Orange Pippin have on average 15% internal O_2 and 6% internal CO_2 while Granny Smith have on average 18% internal O_2 and 4% internal CO_2 when in air. Cox's Orange pippin apples lose chlorophyll approximately seven times faster at 18% O_2 than Granny Smith fruit. These differences in atmosphere levels are

relatively small when compared to the difference in ***k*** values between cultivars and therefore may not be important influences on colour change.

5.4.4 Preharvest factors

Differences in colour parameters of growers fruit were small for Cox's Orange Pippin fruit which had similar hue angles, lightness and chlorophyll contents. There may have been maturity differences between different growers fruit. As an example grower D389 Cox's Orange Pippin fruit were softer and had lower total soluble solids than fruit from growers D252 and D265. Granny Smith fruit from grower D139 were different to other growers fruit being darker with more chlorophyll but the same hue angle, softer with lower total soluble solids than other growers fruit. As noted in the fruit from the temperature experiment the largest difference between growers was in firmness and total soluble solid content which may have been related to fruit maturity. There were no differences in ***k*** between years and the initial colour of fruit. The influence of maturity differences on colour change are difficult to quantify in the controlled atmosphere experiments as only one harvest was used.

5.5 Conclusions

Considerable differences in ***k*** values of chlorophyll, hue angle and lightness exist in the response of Cox's Orange Pippin and Granny Smith apples to O_2 level in the external atmosphere. Cox's Orange Pippin ***k*** values fit a sigmoidal relationship with O_2, but ***k*** values for Granny Smith fruit have a different pattern of a rising trend with increased O_2 level. The relationship between CO_2 and ***k*** follows a declining exponential relationship for chlorophyll levels above 1% with the level of inhibition reaching a minimum asymptote. Reduced O_2 levels inhibit ***k*** of chlorophyll, hue angle, lightness and colour chart score more than similar increases in carbon dioxide levels.

Oxygen and carbon dioxide relationships with ***k*** can be added for Cox's Orange Pippin fruit but is no improvement over CO_2 for Granny Smith. This would imply that CO_2 has the major influence in Granny Smith fruit. A function which combines O_2 and carbon dioxide effects improves the prediction of ***k*** for hue angle and chlorophyll for Cox's Orange Pippin fruit. Granny Smith ***k*** values were influenced more by CO_2 than O_2 and the same combined equation [5.3] used for Cox's Orange Pippin may not be appropriate for Granny Smith.

There was a poor association of ethylene concentration in the atmosphere with ***k***.

There was no difference in initial colour or ***k*** values between apples from each year and differences between fruit from growers was small.

Chapter 6

General Discussion

6.1 Introduction

The role of chlorophyll in photosynthesis and it's location and structure in the chloroplast of plant cells has been well established for a number of years and chlorophyll synthesis has been elucidated in great detail in that time (Hendry *et al* 1987). The same can not be said for chlorophyll destruction. Much emphasis has been placed on such questions as why plants are green? but little attention has been placed on an equally important question of why do plants yellow as they senesce? Much of the answer to this question in a postharvest sense can be related back to senescence and maintenance of commodity quality. In the biochemical area work by Matile *et al* (1987, 1988) and others using meadow fescue (*F. pratensis*) as a model is serving to elucidate the biochemical breakdown pathway in leaves. In fruit however there is almost no corresponding effort at present being put into understanding chlorophyll breakdown. In the past when maturity indices based on fruit colour were established a great deal of research was carried out measuring fruit colour changes over the growth and maturation phase of apple fruit development. Unfortunately there is a lack of a review for fruit chlorophyll breakdown.

Chlorophyll breakdown appears to be a well controlled event in the life of fruit and leaves. Fruit colour has an obvious role to play in the life cycle of a plant as a sign that fruit are ripe and thus ready to eat. Hence many fruit are brightly coloured to advertise their presence. The evolution of coloured fruit appears to be by a mechanism of chlorophyll breakdown similar to that occurring in senescing leaves, to unmask pigments already present in addition to some additional pigment synthesis. Therefore chlorophyll breakdown in fruit is probably similar to the breakdown process in senescing leaves. This would allow

extrapolation to be made between chlorophyll breakdown reported for leaves and fruit.

Catabolism of leaf chloroplasts and their contents, as well as other cell organelles requires O_2 (Thomas and Matile 1989). In the absence of O_2 yellowing proceeds very slowly or not at all. This catabolism appears to be controlled enzymatically as protein inhibitors are also chlorophyll breakdown inhibitors (Schwartz and Lorenzo 1990). Postharvest technologists use low temperature and low O_2 conditions to greatly slow yellowing (Kader *et al* 1985). This effect on yellowing is often considered secondary to the primary aim of postharvest technology to retard respiration rate in order to prolong fruit storage life (Kays 1991). Temperature dependence of yellowing in a characteristic manner may be further evidence that chlorophyll breakdown is enzymatically mediated.

In some cases carbon dioxide and ethylene have been used to retard or promote colour change respectively. The amount of inhibition of chlorophyll loss by high levels of carbon dioxide varies for different commodities, while in general low O_2 levels appear to always inhibit chlorophyll loss. This is indicated by the results in chapter 5 where levels of CO_2 below 1% had no identifiable relationship with ***k*** values of colour change. Similar results have been found for Shamouti oranges (Apelbaum *et al* 1976). A large amount of research has been done on citrus fruit using ethylene to promote degreening. However, the same use of ethylene on apples has not shown similar results and the use of low O_2 or CO_2 levels can negate the promotive effect of ethylene on degreening.

The nature of the relationship between yellowing and temperature or atmosphere composition is not well understood although the general effect of changes in storage environment have been recorded. Increasingly storage technologists are being required to provide conditions in which apple quality is maintained in better condition than has been previously considered possible. More attention is also being paid to the influence of differing storage conditions during the entire

handling process from harvest to retail on apple fruit quality. In order to estimate the beneficial or detrimental effects of a change in the handling system of apples physiological information is required for several important quality attributes of which colour of the fruit is one. To do this the relationship of colour to temperature and atmosphere composition requires characterisation. To be confident that any relationships observed are valid the same relationships should be found using different methods of colour assessment. The following general discussion attempts to examine the success of the approach taken in this thesis.

6.2 Methods of Measuring Colour

Objective (chromameter and pigment analysis) and subjective (colour chart) methods of measuring colour correlated well with each other but the relationships were mostly non-linear. Chlorophyll, hue angle and lightness changes had the same relationship to each other irrespective of cultivar and appear to depend on pigment concentration (Figures 3.3, 3.5, 3.7, 3.8, 3.10, 3.11). Knee (1980b) found similar results for chlorophyll content, %reflectance and Munsell hue values. The relationships between chlorophyll content, hue angle, lightness and colour chart score are summarised by Figure 6.1. This nomogram could be used to estimate chlorophyll content from hue angles, lightness or the NZAPMB Granny Smith apple maturity colour chart. Figure 6.1 indicates clearly that the same change in chlorophyll concentration is perceived differently, by eye, according to the initial chlorophyll concentration. The differences between hue angles, for example, is the same (5°) but the spacing between the lines is different as the chlorophyll content declines. Therefore changes in chlorophyll when the levels of chlorophyll are high have much less influence on the perceived colour changes than when the chlorophyll content is low. Without a nomogram or knowledge of the relationship between methods of colour assessment making comparisons between methods of measuring colour need to be taken with care. Therefore colours reported in the literature assessed using other methods can only be compared in a very general way as their relationship to methods used here is not known.

All the methods used were interrelated with one another therefore the nature of relationships found will tend to be similar. As such it was not surprising that the relationships with temperature, O_2 and CO_2 had the same pattern irrespective of the method of colour measurement. As a relationship between chlorophyll and hue angle or lightness has been established it is now possible to calculate chlorophyll content using such data. This indirect measure of chlorophyll allows non-destructive assessment of the same position on the same fruit to be measured repeatedly. This may give a more accurate estimates of colour change than is possible using the average measurements of a number of fruit. A disadvantage of using a sample of fruit at each sampling time is the variation in fruit chlorophyll content. Using the same fruit each time would allow a researcher to identify individual fruit variation with respect to colour change more accurately. It is suggested that using hue angle or lightness as an indirect measure of chlorophyll content could be used in future experiments looking at colour change on different locations on a fruit or to examine different fruit maturities to better identify sources of variation between individual fruit.

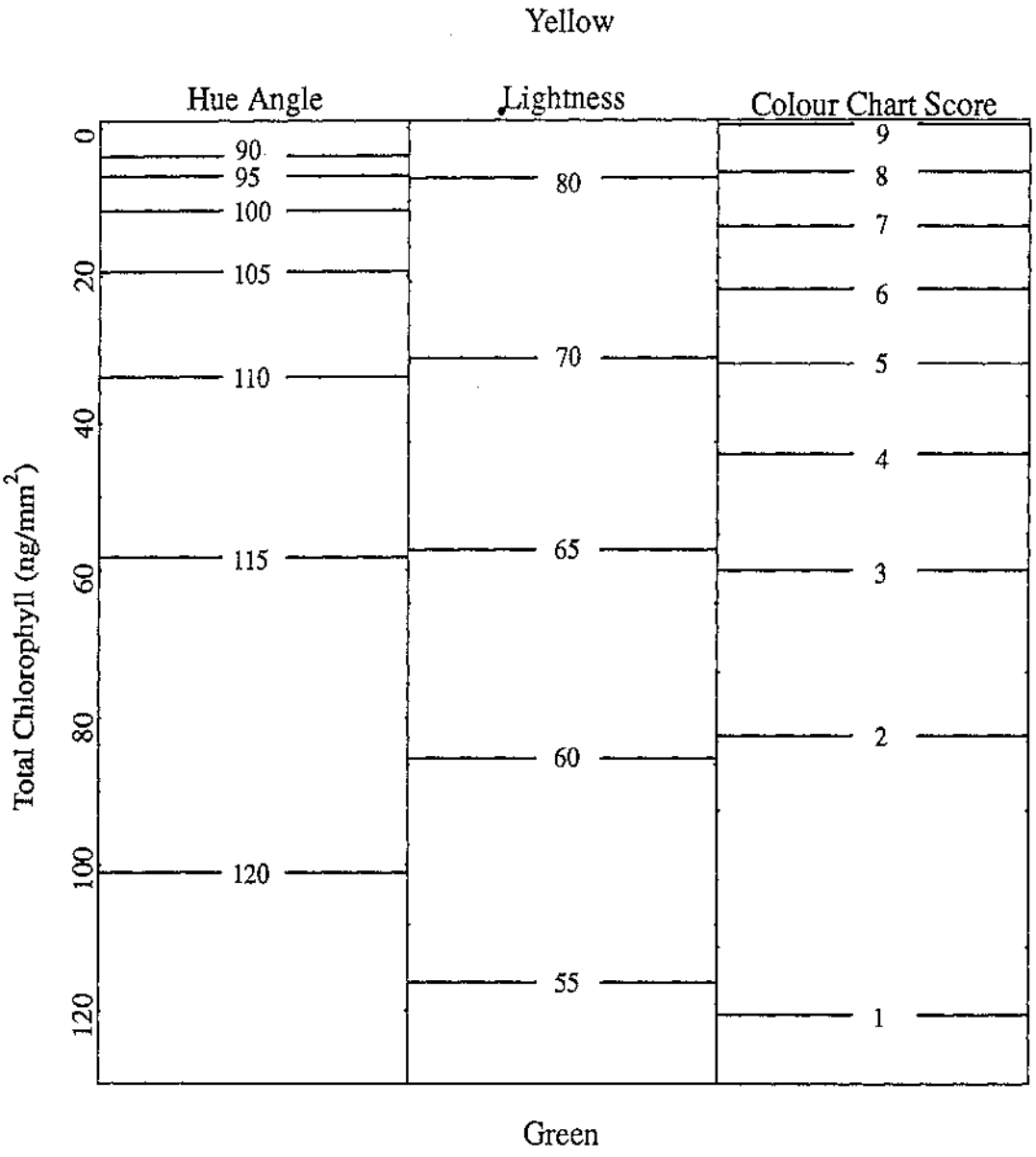


Figure 6.1 Relationship of total chlorophyll content to hue angle, lightness and colour chart score expressed on a common axis.

For measuring colour and colour change the most appropriate method was found to be lightness, as it was the most sensitive and least variable method used for assessing colour changes which is similar to that found by Hirst *et al* (1990). This may be due to lightness being an indication of total pigment concentration rather than a measure of a specific pigments contribution to colour. Chlorophyll levels may possibly vary as the rate of decomposition differs according to chlorophyll type and the state of the senescence process within the plant cell. Levels of other pigments, e.g. carotenoids, which may increase or decrease at the same time as chlorophyll are included in the lightness measurement. The red speckling in Cox's Orange Pippin skin causes variation in colour to be higher than if the fruit were uniformly green. But as the Granny Smith results indicate even apparently dark green apples have levels of yellowness which vary considerably from fruit to fruit. For this reason lightness values are a good reflection of overall pigment changes during the senescence of apple fruit.

Lightness may not be the best measure of apple skin colour with other apple cultivars and fruits as it does not describe colour change or give information as to which pigments are changing. The assessments of effect of temperature, O_2 and CO_2 that used changes in chlorophyll content are examining actual pigment change and reflects something of the metabolic processes occurring. Hue angle or colour charts supply additional information to the experimenter of the change in colour which is important in order to judge when the fruit are overripe or to yellow and unacceptable to consumers. While any one method of colour assessment used in this thesis are equally suitable to determine the nature of the colour change relationship with different environmental conditions each method of colour assessment can contribute additional specific information about fruit colour.

Of interest is the finding that measurements of colour by the chromameter are more sensitive to changes in green colour when chlorophyll levels are low than when chlorophyll levels are high. The chromameter is designed to assess colour

in the same manner as the human eye and so has important practical implications. The eye detects changes in green colour or yellowing poorly when the concentration of chlorophyll is high but can detect small changes in green when the chlorophyll content is low. In dark green apple cultivars, such as Granny Smith, chlorophyll changes may be occurring that are not detectable by eye but which may leave the fruit in a state where only a small amount of additional chlorophyll degradation would result in apparent rapid yellowing. In cultivars with lower chlorophyll concentrations, such as Cox's Orange Pippin, yellowing appears to be more rapid even though it may be at the same rate of chlorophyll decomposition. Therefore during the handling of a cultivar, such as Granny Smith, warmer temperatures and higher O_2 or CO_2 levels may not appear to have as pronounced effect on yellowing as for Cox's Orange Pippin apples. This may hold for the retail period during the handling of the apples with Granny Smith fruit maintaining their appearance longer than Cox's Orange Pippin fruit. It is important therefore for high quality apples to keep the storage temperature as low as practicable as chlorophyll loss can occur which is not apparent but leaves the apple in a state in which the apple appears to yellow rapidly.

6.2 Model of colour change in apples

Chlorophyll degradation is an enzymatic process and the pattern of chlorophyll loss over time would be expected to follow the pattern of decline observed generally for substrates degraded by enzymes (Hendry *et al* 1987, Schwartz and Lorenzo 1990). This pattern is non linear as the rate at which a substrate in a biochemical reaction is used is proportional to the amount of substrate remaining (Bailey and Ollis 1973). The proportional decrease is constant, i.e. as the levels of substrate decline so does the rate at which the process occurs. This results in a pattern known as a declining exponential which is linearised by converting the substrate (chlorophyll) values to their natural log equivalents. This technique has been used only for pears by Laval-Martin (1969) at three storage temperatures. This is the first time that this technique has been used to examine chlorophyll

and associated colour changes in detail for temperature and the atmosphere composition.

In order to characterise the relationship of colour change to temperature and atmosphere composition large number of data need to be collected. Processing the data using the method above has allowed considerable data condensation which in turn has aided interpretation of the overall process of colour change as distinct to changes at individual treatments. However, even once the loss in chlorophyll is transformed to a linear function, comparing across treatments is still difficult as the changes in the rate of chlorophyll loss are represented by many lines. A large number of lines presented together allow the effect of treatments to be assessed but establishing a function for all treatments is not possible unless each treatment's effect can be quantified. In order to model the pattern of change over a range of treatments each treatment needs to be represented by one number. These can then be plotted against treatments and the pattern assessed. The slope of the transformed data for each treatment allows the rate constant of change (k) or the constant proportional change in chlorophyll content to be compared over several temperatures, O_2 or CO_2 levels. This takes a graph or table with many numbers or lines and reduces it to a simpler function. For this method to give a sensible and reliable assessment of a function according to treatment the methods of determining k need also to be reliable.

The method used to obtain k is dependant on the chlorophyll loss function being a declining exponential. It is possible that the function of chlorophyll loss over time is not completely a declining exponential but may have a lag phase before chlorophyll declines. This may be the case as indicated by the poor association of initial chlorophyll levels with predicted chlorophyll levels (Figure 4.2). But the declining exponential function does describe well the majority of points once the degradation process has started. As the effect of the treatment applied becomes more inhibitory the curve of chlorophyll loss over time becomes more horizontal and linear. In such circumstances a non linear function may not be appropriate.

For some treatments there was an apparent increase in chlorophyll level which may have been due to there being almost no change in pigment concentration over the time course of the experiment. As a result the slope of the chlorophyll loss curve may have a large degree of error. It is also possible that highly inhibitory conditions to chlorophyll loss may induce an increase in chlorophyll. This remains a question for future research. Perhaps this indicates that the chlorophyll breakdown process, at least initially, is a balance of synthetic and degradative processes with degradative processes occurring at much faster rates than synthetic processes once senescence starts. Despite these problems the error in values of ***k*** was not sufficient to prevent the relationships with temperature, O_2 or CO_2 being elucidated.

Once the values of ***k*** were obtained and compared across treatments determining the character of the relationship was the next step in formulating a model for yellowing. The equations used in chapters 4 and 5 were derived empirically after examination of a plot of ***k*** with temperature, O_2 or CO_2. The equations are discussed in chapters 4 and 5. The O_2 and CO_2 relationships appear to be cultivar dependant as they were considerably different for Cox's Orange Pippin and Granny Smith fruit. The temperature relationship was the same for each cultivar differing only in magnitude. Combining these two functions would be a requirement of an overall model of the effects of storage conditions changes on the rate of yellowing. Results obtained in this thesis allows a tentative model, equation [6.1], of colour change or chlorophyll breakdown to be proposed.

$$k = K_{O_2 CO_2}^{20^\circ C} * \frac{K_{temp}^{n}}{K_{temp}^{20^\circ C}} \qquad [6.1]$$

where: k = rate constant of the reaction

$K_{o_2co_2}^{20^oC}$ = rate constant at 20°C for a controlled atmosphere

$K_{temp}^{20^oC}$ = rate constant at 20°C in air

K_{temp}^{n} = rate constant at a temperature of *n* in air

Figures 6.2 and 6.3 illustrate different curves of k values calculated by equation [6.1] for different levels of O_2 and CO_2 at a range of temperatures with Cox's Orange Pippin and Granny Smith fruit. As the oxygen level is lowered the k values are reduced more than the same increase in CO_2 level as distances between the curves for part (a), representing CO_2, of Figures 6.2 and 6.3 are relatively equal but are unequal for part (b), representing O_2. This implies that low oxygen conditions effect yellowing more than raised CO_2 levels and this effect is present at all temperatures. The temperature optima does not change in different O_2 or CO_2, levels according to this model. It is possible that the temperature optima may change at different O_2 and CO_2 concentrations, further research would be required to determine this. The curves presented in Figures 6.2 and 6.3 serve as generalisations of yellowing as k may be under or over estimated when related to experimental data. Therefore equation [6.1] should only be used as a guide to the likely value of k.

Equation [6.1] could be used to estimate the length of time before which Cox's Orange Pippin or Granny Smith apples may yellow under certain storage conditions. Using the assumption that previous storage conditions do not influence yellowing equation [6.1] could also be used to estimate differences in the rate of yellowing if storage conditions are changed. For example, Cox's Orange Pippin apples are harvested and left for two days at 15°C before storage

at 3°C for six weeks and start with an average chlorophyll content of 40 ng/mm^2. These fruit will have, on average, 48 percent chlorophyll on removal from storage. Apples which are harvested and stored immediately will have 53 percent chlorophyll on removal from storage. The effect of less than the recommended ideal CA storage atmospheres, such as those from MA packaging, on the rate of yellowing could also be evaluated. Zero degrees and 0% oxygen would be the optimal storage conditions for minimal colour change according to equation [6.1]. These conditions are clearly not practical as 0% O_2 will induce the fruit to become anaerobic. Therefore equation [6.1] is probably not useful in defining optimum storage conditions for a specific apple cultivar unless other quality parameters are included in its formulation. Although the data is not presented in this thesis values for total soluble solids and firmness were recorded at each sample time. Further analysis of these parameters using a similar technique to obtain rates of change has merit and remains a possibility for further analysis.

Granny Smith fruit yellow more slowly than Cox's Orange Pippin fruit over all storage conditions studied. Granny Smith and Cox's Orange Pippin apples exhibit a fundamental difference in their response to O_2 and CO_2. The response of yellowing to O_2 changes for Cox's Orange Pippin is greater than for Granny Smith in which yellowing is more sensitive to changes in CO_2. Cox's Orange Pippin has the same pattern of response to CO_2 as Granny Smith but the Granny Smith response to O_2 is different to Cox's Orange Pippin (Figure 5.1). Thus high CO_2 levels will maintain chlorophyll levels in Granny Smith apples in comparison to low O_2 but be of less benefit for Cox's Orange Pippin apples. The reason for this is not known but may be due to differences in the morphological and physiological characteristics of each cultivar. Granny Smith fruit have a low respiration rate and have a very flat climacteric curve whereas Cox's Orange Pippin fruit ripen rapidly at warm temperatures and have a distinctive climacteric curve. Granny Smith fruit also have much higher levels of chlorophyll in their skin compared to Cox's Orange Pippin thus more chlorophyll needs to be lost before the same differences in colour can discerned by eye. This highlights the

need to have good temperature management for Cox's Orange Pippin as small increases in temperature can have a large influence on increasing the rate of yellowing at temperatures below the temperature optimum.

If respiration provides energy for chlorophyll breakdown and/or pigment synthesis then differences in respiration rate as large as those noted between Granny Smith and Cox's Orange Pippin fruit would account for some of the differences in the rate of yellowing. Physical differences between the fruit like differences in skin resistance to gas diffusion may also be a factor in the difference in the rate of yellowing. Further research is required to investigate these differences between Cox's Orange Pippin and Granny Smith fruit before any definite conclusions can be drawn.

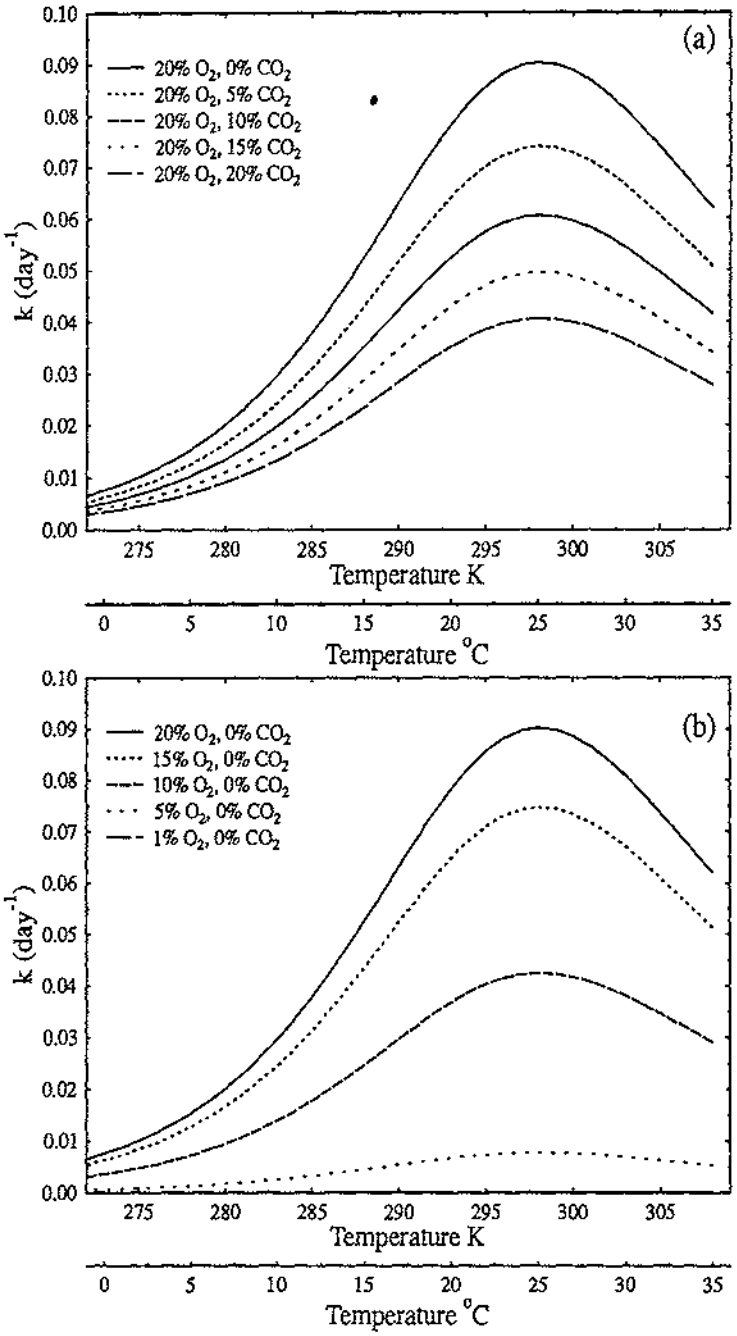


Figure 6.2 Cox's Orange Pippin theoretical rate constants of total chlorophyll with temperature at: (a) constant oxygen levels of 20% and varying carbon dioxide levels; (b) constant carbon dioxide levels of 0% and varying oxygen levels, using equation [6.1].

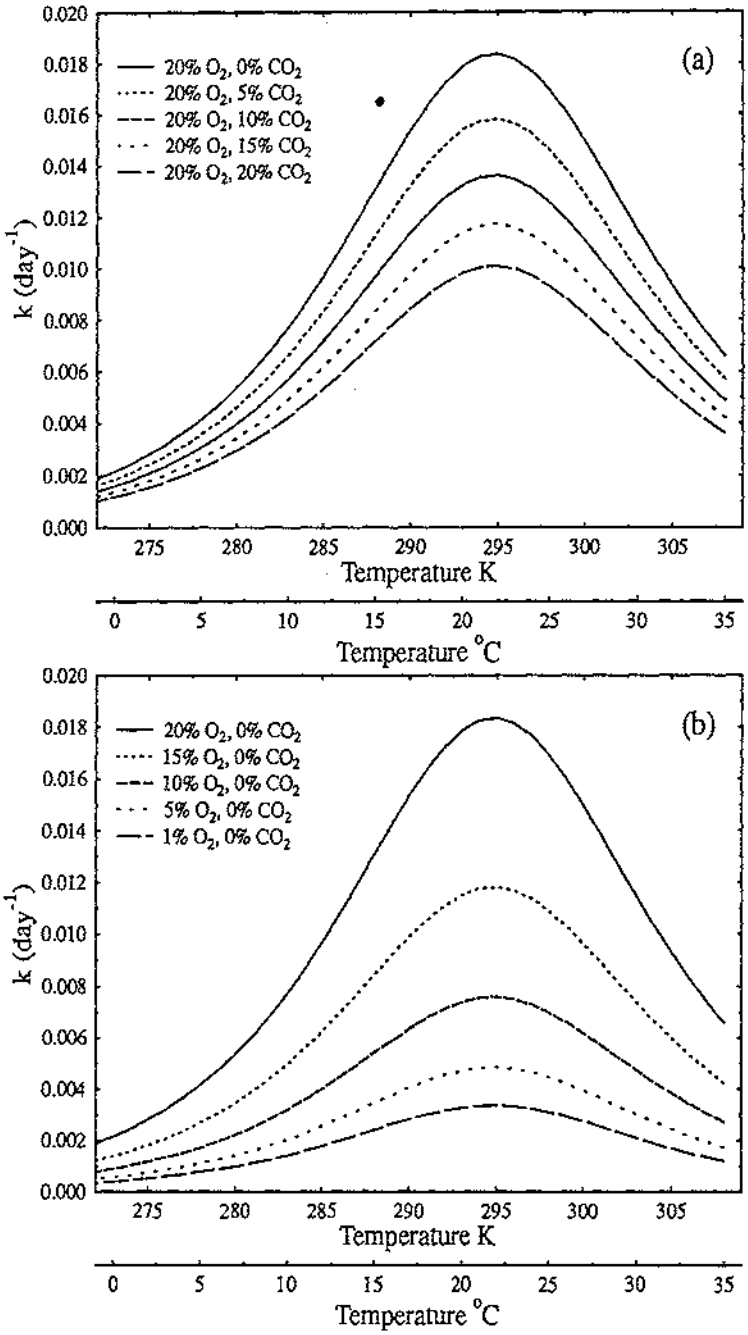


Figure 6.3 Granny Smith theoretical rate constants of total chlorophyll at different temperatures with: (a) constant oxygen levels of 20% and varying levels of carbon dioxide; (b) constant carbon dioxide levels of 0% and varying levels of oxygen, using equation [6.1].

Oxygen appears to be the gas which contibutes the most to yellowing of Cox's Orange Pippin apples. This is due to yellowing being reduced at an increasing rate as oxygen levels fall (Figure 6.2). In contrast the same increase in CO_2 level inhibits yellowing a similar amount (Figure 6.2). Previous research (Fidler and North 1971, Kidd and West 1930, 1936, Leblond 1961, Stow and Genge 1990) has assumed that the inhibitory effect on ***k*** of O_2 and CO_2 is the same. Data presented in this thesis indicates that O_2 and CO_2 have differing influences on the rate of yellowing. While atmospheres containing both O_2 and CO_2 inhibit yellowing more than atmospheres with no CO_2 and the same level of O_2 (Fidler and North 1971) the results presented here indicate this is not always the case. Certain levels of CO_2 (<1%) and O_2 (1%-5%) can have effect on yellowing when changed in these ranges. This has been reported by Drake *et al* (1992) for Delicious apples also. Therefore changes in O_2 levels are more effective in retarding yellowing than CO_2 increases. The practical implications of this in MA applications are that small reductions in O_2 will be more effective in reducing yellowing than the same increase in CO_2. Therefore MA packages will be effective in maintaining the green colour of apples although the atmosphere composition and temperature may not be 'optimum' according to CA research based on other quality attributes (Smith *et al* 1987).

For Granny Smith increased levels of CO_2 retard ***k*** values more than reduced levels of O_2. This is the converse of Cox's Orange Pippin yellowing response and highlights a major cultivar difference in the yellowing response to MA or CA. The reason for this is not known and may be a topic worthy of further research.

High CO_2 levels inhibit enzymes in the tricarboxylic acid cycle in the conversion of succinate to malate and malate to pyruvate in apple fruit tissue (Kays 1991). There is little information on the level of CO_2 which inhibits other metabolic processes, such as respiration. For yellowing the indication is that there is a threshhold level (>1%) at which CO_2 becomes inhibiting to yellowing. To further refine the model of yellowing with CO_2 additional research is required.

6.4 Implications

This thesis did not investigate what the effect storage at one temperature and then removal to another temperature may be on final colour of the fruit. Raising or lowering storage temperature will have a large effect on ***k*** of colour change. For example at 20°C the average ***k*** of total chlorophyll for Cox's Orange Pippin is 0.11 day^{-1} and at 15°C the average ***k*** of total chlorophyll is 0.08 day^{-1}, a 38% reduction. For an average apple with 40 ng/mm^2 chlorophyll it takes 6 days to lose 50% chlorophyll at 20°C and 9 days at 15°C, a 3 day difference (Table 6.1). Substantial improvement may be possible in maintaining colour during retail marketing by judicious use of lower temperatures. At temperatures between 0°C and 6°C there is a larger amount of variation in ***k*** than for temperatures above 6°C (Appendix 2). Small changes in ***k*** at low temperatures result in a large variation in the time at which apple colour is maintained. For example fruit at 5°C take 36 days to lose 50% chlorophyll and at 0°C take 72 days but fruit at 4°C take 41 days (Table 6.1).

Rate constants of colour change at different temperatures may have been influenced by the different rates of ripening at each temperature studied. Ripening is rapid at high temperatures and slow at low temperatures. This may have resulted in ***k*** being over or under estimated when compared to other temperatures over a wide range. For example fruit at 0°C will ripen little during storage and would have reached the same physiological stage as fruit stored at higher temperatures some time long after the experiment concluded. A measurement of respiration rate may have allowed the physiological stage of the fruit to be accounted for in the analysis and thereby relate changes back to the same physiological stage. Fruit removed from one temperature and placed at another may be expected to yellow at the new rate similar to changes observed for respiratory activity when the temperature is changed. Further research is required to establish if there is any carryover effect of storage in other conditions.

Table 6.1 Days to reach certain percentage chlorophyll loss at different temperatures of Cox's Orange Pippin and Granny Smith apples during 1989 and 1990. Days are calculated using equation [4.3].

	Cox's Orange Pippin			Granny Smith		
	10%	50%	90%	10%	50%	90%
Temperature (°C)	4 ng lost	20 ng lost	36 ng lost	8 ng lost	40 ng lost	72 ng lost
0	11.0	72.4	240.5	24.8	163.0	541.6
4	6.2	40.9	135.9	14.6	95.8	318.2
5	5.4	35.6	118.3	12.8	84.1	279.5
6	4.7	31.1	103.2	11.3	74.0	245.9
10	2.8	18.4	61.2	6.9	45.3	150.6
12	2.2	14.5	48.0	5.5	36.2	120.1
15	1.6	10.4	34.6	4.1	26.8	88.9
20	1.1	7	23.2	3.0	19.6	65.1
25	0.9	6.1	20.5	3.1	20.7	68.7
30	1.0	6.8	22.4	4.6	30.5	101.4
35	1.3	8.8	29.3	8.1	53.1	176.4

A practical use of knowledge of the association between yellowing and respiration rate are that ***k*** could be used to determine if stored fruit were ripening or in the climacteric phase. While the relationship of yellowing to respiration needs to be established the idea has merit as colour measurement is less technically demanding than respiration measurements. Small changes in colour are easy to detect using an instrument like the Minolta chromameter. Further respiration changes are difficult to measure at 0°C, the ***k*** may offer an indirect alternative. This would allow coolstore operators to know which fruit are ripening and should be removed. The rate of colour change could also potentially be used to accurately predict fruit maturity as a harvest indicator. Before any of the above could be considered for implementation further research is required.

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Appendix 1

Equations for determining chlorophyll concentration in N,N-dimethylformamide (Porra *et al* 1989).

Chlorophyll a (µg/ml). = $12.00*(A_{665}-A_{700}). - 3.11*(A_{647}-A_{700})..$

Chlorophyll b (µg/ml). = $20.78*(A_{647}-A_{700}). - 4.88*(A_{665}-A_{700})..$

Total Chlorophyll = chlorophyll a + chlorophyll b.

Results are presented in ng/mm^2 which was calculated according to the following:

$$\frac{\text{Chlorophyll a or b (µg/ml). * 3 mls of DMF}}{\text{pi * (diameter of skin disk / 2).}^2}$$

Values obtained were expressed as ng/mm^2 as this gave whole numbers to analyze.

Appendix 2

Rate constants of quality parameter changes at different temperatures.

Table A2.1 Rate constants of chlorophyll a (day^{-1}). for Cox's Orange Pippin apples stored at different temperatures during 1989 and 1990.

Year	1989				1990			
Harvest	Start		End		Start		End	
Grower	D265	D389	D592	D646	D265	D252	D946	D252
Temperature °C								
0	0.008	0.014	0.004	0.007	0.019	0.005	0.044	0.047
4	0.013	0.004	0.013	0.021				
5					0.017	0.015	0.043	0.034
6	0.014	0.008	0.012	0.016				
10					0.057	0.043	0.031	0.028
12	0.038	0.030	0.041	0.041				
15					0.078	0.074	0.090	0.092
20	0.112	0.105	0.196	0.205	0.106	0.103	0.135	0.110
25	0.102	0.084	0.140	0.122	0.126	0.119	0.143	0.164
30	0.124	0.107	0.133	0.113				
35					0.076	0.049	0.129	0.122

Table A2.2 Rate constant of colour change of chlorophyll a (day^{-1}). for Granny Smith apples stored at different temperatures during 1989 and 1990.

Year	1989				1990			
Harvest	Start		End		Start		End	
Grower	D139	D341	D139	D069	D075	D139	D075	D139
Temperature °C								
0	0.007	0.009	0.003	0.002	0.006	0.004	0.005	0.003
5					0.003	0.004	0.001	0.013
6	0.010	0.013	0.008	0.010				
10					0.023	0.018	0.023	0.025
12	0.011	0.022	0.003	0.002				
15					0.032	0.034	0.025	0.018
20	0.029	0.019	0.045	0.027	0.029	0.042	0.047	0.047
25	0.017	0.030	0.043	0.033	0.033	0.051	0.037	0.036
30	0.014	0.011	0.018	0.017				
35					0.030	0.029	0.015	0.019

Table A2.3 Rate constants of chlorophyll b (day^{-1}). for Cox's Orange Pippin apples stored at different temperatures during 1989 and 1990.

Year	1989				1990			
Harvest	Start		End		Start		End	
Grower	D265	D389	D592	D646	D265	D252	D946	D252
Temperature °C								
0	0.002	0.012	0.002	0.000	0.022	0.009	0.056	0.061
4	0.011	0.005	0.009	0.015				
5					0.015	0.019	0.051	0.039
6	0.007	0.002	0.000	0.004				
10					0.042	0.035	0.029	0.031
12	0.021	0.011	0.020	0.016				
15					0.047	0.055	0.069	0.079
20	0.069	0.055	0.148	0.152	0.054	0.056	0.095	0.082
25	0.047	0.032	0.083	0.076	0.070	0.078	0.073	0.100
30	0.061	0.052	0.064	0.052				
35					0.043	0.056	0.079	0.073

Table A2.4 Rate constants of chlorophyll b (day^{-1}). for Granny Smith apples stored at different temperatures during 1989 and 1990.

Year	1989				1990			
Harvest	Start		End		Start		End	
Grower	D139	D341	D139	D069	D075	D139	D075	D139
Temperature °C								
0	0.005	0.006	0.007	0.001	0.006	0.003	0.005	0.001
5					0.003	0.001	0.008	0.010
6	0.006	0.009	0.007	0.007				
10					0.022	0.016	0.022	0.021
12	0.012	0.020	0.005	0.001				
15					0.031	0.040	0.025	0.016
20	0.032	0.020	0.045	0.030	0.035	0.049	0.054	0.052
25	0.021	0.037	0.049	0.037	0.039	0.061	0.045	0.040
30	0.026	0.017	0.017	0.020				
35					0.034	0.035	0.011	0.020

Table A2.5 Rate constant of total chlorophyll (day^{-1}). for Cox's Orange Pippin apples stored at different temperatures during 1989 and 1990.

Year	1989				1990			
Harvest	Start		End		Start		End	
Grower	D265	D389	D592	D646	D265	D252	D946	D252
Temperature °C								
0	0.006	0.013	0.002	0.005	0.020	0.006	0.042	0.045
4	0.012	0.004	0.012	0.020				
5					0.016	0.016	0.041	0.034
6	0.012	0.006	0.008	0.012				
10					0.052	0.041	0.031	0.031
12	0.032	0.024	0.034	0.033				
15					0.069	0.067	0.084	0.088
20	0.096	0.084	0.167	0.171	0.088	0.084	0.122	0.102
25	0.084	0.066	0.118	0.105	0.106	0.105	0.116	0.140
30	0.101	0.084	0.106	0.088				
35					0.067	0.050	0.110	0.106

Table A2.6 Rate constants of total chlorophyll (day^{-1}). for Granny Smith apples stored at different temperatures during 1989 and 1990.

Year	1989				1990			
Harvest	Start		End		Start		End	
Grower	D139	D341	D139	D069	D075	D139	D075	D139
Temperature °C								
0	0.006	0.008	0.004	0.001	0.006	0.004	0.005	0.002
5					0.003	0.003	0.009	0.013
6	0.009	0.012	0.008	0.010				
10					0.023	0.017	0.023	0.024
12	0.011	0.022	0.004	0.001				
15					0.031	0.035	0.025	0.017
20	0.030	0.021	0.045	0.028	0.030	0.043	0.048	0.048
25	0.018	0.031	0.043	0.033	0.034	0.053	0.038	0.037
30	0.017	0.015	0.017	0.018				
35					0.031	0.030	0.014	0.019

Table A2.7 Rate constants of hue angle (day^{-1}). for Cox's Orange Pippin apples stored at different temperatures during 1989 and 1990.

Year	1989				1990			
Harvest	Start		End		Start		End	
Grower	D265	D389	D592*	D646	D265	D252	D946	D252
Temperature °C								
0	0.074	0.090	0.096	0.078	0.103	0.161	0.168	0.296
4	0.191	0.193	0.199	0.181				
5					0.123	0.176	0.207	0.228
6	0.167	0.134	0.083	0.087				
10					0.247	0.210	0.297	0.291
12	0.291	0.287	0.258	0.183				
15					0.315	0.375	0.447	0.441
20	0.547	0.563	0.653	0.535	0.421	0.435	0.621	0.887
25	0.610	0.615	0.575	0.714	0.540	0.629	0.679	0.667
30	0.614	0.633	0.687	0.535				
35					0.331	0.168	0.557	0.547

Table A2.8 Rate constants of hue angle (day^{-1}). for Granny Smith apples stored at different temperatures during 1989 and 1990.

Year	1989				1990			
Harvest	Start		End		Start		End	
Grower	D139	D341	D139	D069	D075	D139	D075	D139
Temperature °C								
0	0.066	0.057	0.046	0.046	0.047	0.024	0.064	0.029
5					0.049	0.080	0.061	0.052
6	0.090	0.119	0.085	0.109				
10					0.139	0.209	0.058	0.127
12	0.151	0.213	0.046	0.046				
15					0.202	0.314	0.137	0.224
20	0.251	0.259	0.309	0.238	0.187	0.257	0.180	0.222
25	0.136	0.232	0.294	0.269	0.189	0.297	0.147	0.206
30	0.149	0.203	0.212	0.189				
35					0.275	0.302	0.260	0.297

Table A2.9 Rate constants of lightness (day^{-1}). for Cox's Orange Pippin apples stored at different temperatures during 1989 and 1990.

Year	1989				1990			
Harvest	Start		End		Start		End	
Grower	D265	D389	D592	D646	D265	D252	D946	D252
Temperature °C								
0	0.081	0.041	0.027	0.064	0.083	-0.051	0.003	-0.067
4	0.076	-0.015	-0.060	0.169				
5					0.095	0.034	0.044	0.099
6	0.088	0.044	0.084	0.148				
10					0.177	0.187	0.109	0.082
12	0.176	0.094	0.180	0.247				
15					0.179	0.199	0.320	0.374
20	0.284	0.183	0.326	0.336	0.173	0.228	0.326	0.164
25	0.455	0.275	0.320	0.265	0.300	0.129	0.278	0.473
30	0.439	0.302	0.297	0.338				
35					0.307	0.253	0.225	0.371

Table A2.10 Rate constants of lightness (day^{-1}). for Granny Smith apples stored at different temperatures during 1989 and 1990.

Year	1989				1990			
Harvest	Start		End		Start		End	
Grower	D139	D341	D139*	D069	D075	D139	D075	D139
Temperature °C								
0	0.022	0.022	-0.019	-0.013	0.052	0.039	0.026	0.010
5					0.032	0.058	0.119	0.103
6	0.027	0.081	0.051	0.088				
10					0.196	0.229	0.128	0.193
12	0.010	0.204	-0.019	-0.013				
15					0.269	0.353	0.223	0.287
20	0.250	0.280	0.314	0.196	0.283	0.398	0.377	0.389
25	0.145	0.288	0.317	0.271	0.298	0.456	0.296	0.357
30	0.144	0.231	0.160	0.140				
35					0.271	0.224	0.142	0.089

Table A2.11 Rate constants of colour chart score (day^{-1}). for Granny Smith apples stored at different temperatures during 1989 and 1990.

Year	1989				1990			
Harvest	Start		End		Start		End	
Grower	D139	D341	D139	D069	D075	D139	D075	D139
Temperature °C								
0	0.015	0.014	0.007	0.006	0.031	0.013	0.008	-0.001
5					0.022	0.007	0.020	0.015
6	0.017	0.030	0.028	0.041				
10					0.035	0.026	0.065	0.057
12	0.018	0.070	0.080	0.074				
15					0.118	0.102	0.069	0.084
20	0.087	0.145	0.125	0.096	0.118	0.132	0.125	0.125
25	0.050	0.122	0.119	0.115	0.128	0.159	0.113	0.120
30	0.059	0.094	0.075	0.086				
35					0.147	0.136	0.094	0.087

Appendix 3

Rate constants of quality parameters in different controlled atmospheres.

Table A3.1 Rate constants of quality parameters for Cox's Orange Pippin apples stored at 20°C.

Year	Grower	CO_2 (%)	O_2 (%)	C_2H_4 (ppm)	Tot Chl	Chl a	Chl b	Hue Angle	Lightness
1989	D389	2.55	18.91	53.57	0.165	0.211	0.101	0.426	0.221
		4.20	14.07	23.28	0.113	0.127	0.083	0.366	0.173
		7.01	9.17	40.97	0.043	0.048	0.031	0.176	0.102
		2.15	3.65	22.83	0.014	0.015	0.009	0.093	0.009
		3.83	4.42	12.63	0.003	0.005	0.003	0.090	-0.030
		12.21	10.37	48.87	0.049	0.050	0.046	0.199	0.115
		5.40	12.89	35.99	0.152	0.179	0.102	0.395	0.212
		6.84	9.61	79.51	0.069	0.077	0.065	0.243	0.124
	D265	2.51	18.57	54.01	0.148	0.177	0.101	0.426	0.221
		4.00	13.34	73.01	0.121	0.139	0.083	0.348	0.244
		8.96	8.16	54.12	0.023	0.022	0.083	0.123	0.096
		3.23	3.18	18.53	-0.006	-0.006	-0.005	0.069	0.001
		3.28	4.39	2.57	0.010	0.010	0.010	0.106	0.035
		9.71	11.69	54.31	0.088	0.094	0.073	0.281	0.229
		6.78	13.31	68.43	0.106	0.125	0.073	0.438	0.185
		3.52	11.57	64.17	0.048	0.056	0.030	0.222	0.139

Table A3.2 Rate constants of quality parameters for Cox's Orange Pippin apples stored at 20°C.

Year	Grower	CO_2 (%)	O_2 (%)	C_2H_4 (ppm)	Tot Chl	Chl a	Chl b	Hue Angle	Lightness
1990	D265	1.23	13.6	15.22	0.141	0.156	0.141	0.394	0.208
		0.74	8.94	11.57	0.101	0.111	0.073	0.305	0.193
		0.91	5.71	10.20	0.041	0.043	0.030	0.116	0.120
		0.17	2.66	1.55	0.002	0.003	-0.003	0.000	-0.011
		0.33	1.57	0.05	0.013	0.017	-0.004	0.001	0.021
		4.43	17.35	12.64	0.149	0.049	0.041	0.353	0.218
		9.04	15.48	2.70	0.100	0.104	0.090	0.190	0.251
		32.08	11.59	3.26	0.048	0.164	0.107	0.160	0.112
	D252	0.76	14.09	36.47	0.124	0.141	0.075	0.375	0.209
		0.37	8.49	10.20	0.055	0.059	0.042	0.259	0.122
		0.45	5.23	6.58	0.002	0.005	-0.008	0.108	-0.028
		0.23	2.70	0.89	0.004	0.006	-0.007	0.069	0.018
		0.27	1.43	0.03	-0.012	-0.01	-0.018	0.017	-0.065
		10.02	11.87	6.32	0.061	0.067	0.083	0.221	0.131
		13.5	11.58	3.62	0.037	0.038	0.033	0.154	0.073
		26.39	12.73	3.75	0.066	0.063	0.054	0.137	0.200

Table A3.3 Rate constants of quality parameters for Granny Smith apples stored at 20°C.

Year	Grower	CO_2 (%)	O_2 (%)	C_2H_4 (ppm)	Tot Chl	Chl a	Chl b	Hue Angle	Lightness
1989	D139	0.10	20.48	0.00	0.006	0.004	0.005	0.059	0.026
		0.11	15.27	0.02	0.008	0.021	0.024	0.082	0.057
		0.17	14.42	0.00	0.004	0.012	0.014	0.0478	0.001
		0.08	9.35	0.02	0.005	0.009	0.010	0.058	0.051
		0.09	2.42	0.00	0.004	0.018	0.020	0.041	0.001
		14.88	17.6	0.01	0.003	0.0002	0.010	0.022	0.013
		5.13	16.3	0.04	0.007	0.020	0.020	0.054	0.052
		2.71	13.72	0.01	0.009	0.014	0.017	0.079	0.082
	D341	0.07	18.53	0.06	0.015	0.006	0.013	0.120	0.137
		0.07	15.37	0.00	0.012	0.004	0.011	0.101	0.114
		0.16	13.58	0.07	0.013	0.007	0.006	0.087	0.057
		0.09	4.31	0.01	0.009	0.005	0.012	0.081	0.063
		0.06	1.21	0.00	0.007	0.005	0.005	0.066	0.024
		15.91	17.4	0.01	0.005	0.008	0.011	0.049	0.015
		4.4	11.85	0.02	0.014	0.006	0.005	0.092	0.111
		1.11	19.45	0.11	0.021	0.021	0.023	0.178	0.218

Table A3.4 Rate constants of quality parameters for Granny Smith apples stored at 20°C.

Year	Grower	CO_2 (%)	O_2 (%)	C_2H_4 (ppm)	Tot Chl	Chl a	Chl b	Hue Angle	Lightness
1990	D139	0.12	18.02	0.01	0.006	0.007	0.006	0.053	0.075
		0.20	13.18	0.29	0.007	0.008	0.014	0.058	0.092
		0.15	7.04	0.03	0.003	0.007	0.007	0.028	0.058
		0.03	2.13	0.02	0.002	0.003	0.002	0.024	0.048
		0.03	1.97	0.01	0.0001	0.002	0.004	0.014	0.019
		1.05	20.21	1.34	0.021	0.009	0.007	0.134	0.219
		8.61	17.69	1.98	0.009	0.014	0.015	0.054	0.111
		7.95	17.77	2.51	0.020	0.003	0.009	0.101	0.194
	D075	0.85	16.91	29.94	0.019	0.012	0.014	0.114	0.188
		0.74	10.19	18.38	0.009	0.002	0.008	0.057	0.108
		0.43	6.53	6.06	0.005	0.000	0.000	0.038	0.061
		0.13	3.5	3.43	0.006	0.003	0.007	0.047	0.067
		0.32	3.01	0.89	0.002	0.006	0.004	0.015	0.022
		5.77	16.27	24.57	0.009	0.005	0.004	0.058	0.103
		12.56	14.99	5.35	0.005	0.009	0.007	0.023	0.063
		18.00	15.93	7.29	0.007	0.003	0.002	0.046	0.085

Appendix 4

Parameter estimates of regression equations of Cox's Orange Pippin and Granny Smith apples stored at different temperatures.

Table A4.1 Parameter estimates and standard errors of the modified form of the Arrenhius equation.

Parameter	Cultivar	A	B	C	D	r^2	T_{max}
Chl a	Cox's Orange Pippin	$3.51x10^{16} \pm 1.41x10^{19}$	11641.7 ± 508816.9	62.32 ± 3.89	18389.4 ± 1378.0	0.803 **[1]	24.73
	Granny Smith	$6.43x10^{10} \pm 1.37x10^{12}$	8234.7 ± 17801.1	114.90 ± 22.39	34289.7 ± 201.8	0.715 *	22.48
Chl b	Cox's Orange Pippin	$2.13x10^{15} \pm 3.16x10^{17}$	10910.7 ± 163344.4	57.96 ± 27.02	17004.8 ± 345.4	0.545 NS	23.37
	Granny Smith	$7.72x10^{11} \pm 7.92x10^{13}$	8902.3 ± 90959.3	67.30 ± 25.44	20013.7 ± 0.00	0.712 *	23.40
TotChl	Cox's Orange Pippin	$2.45x10^{15} \pm 2.46x10^{16}$	10941.5 ± 2598.2	62.23 ± 0.00	18438.7 ± 1021.4	0.775 **	25.11
	Granny Smith	$5.75x10^{13} \pm 2.73x10^{15}$	10139.6 ± 47276.5	75.50 ± 27.39	22313.1 ± 116.2	0.726 *	21.82
Hue Angle	Cox's Orange Pippin	$1.46x10^{10} \pm 3.74x10^{11}$	6009.6 ± 19152	68.45 ± 6.00	20667.9 ± 48.00	0.836 **	26.02
	Granny Smith	$3.89x10^{9} \pm 1.69x10^{10}$	6831.4 ± 0.0	69.06 ± 1.00	20636.5 ± 201.0	0.693 **	22.81
Lightness	Cox's Orange Pippin	$1.73x10^{20} \pm 3.56x10^{22}$	13669.0 ± 297632.9	53.13 ± 58.29	15317.6 ± 175.53	0.714 **	27.26
	Granny Smith	$1.09x10^{16} \pm 1.67x10^{18}$	11033.8 ± 166555.3	85.31 ± 53.54	25204.3 ± 0.0	0.734 **	21.58
Colour Chart	Granny Smith	$3.21x10^{16} \pm 5.90x10^{18}$	11636.2 ± 214587.8	82.29 ± 43.57	24311.8 ± 2.0	0.829 **	22.13

1 NS Not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

NB: Parameters A and B have little effect on the value of ***k*** except to establish a starting point, therefore large standard errors of these values do not greatly affect the curves drawn using these parameters.

Appendix 5

Parameter estimates of fits for colour change and oxygen or carbon dioxide levels in the external atmosphere.

Table A5.1 Parameter estimates and standard errors of the Gompertz growth equation for the relationship between oxygen and colour change ***k***.

Colour Parameter	Cultivar	A_o ($x10^{-4}$).	Y_o	De ($x10^{-2}$).	r^2
Chlorophyll a	Cox's Orange Pippin	0.26 ± 1.05	6.42 ± 0.02	19.16 ± 11.34	0.676 ***[1]
	Granny Smith	2.44 ± 12.69	1.14 ± 3.23	5.24 ± 29.97	0.072 NS
Chlorophyll b	Cox's Orange Pippin	0.01 ± 0.00	11.69 ± 5.44	34.99 ± 6.93	0.708 ***
	Granny Smith	4.86 ± 14.31	0.88 ± 0.92	8.43 ± 28.86	0.089 NS
Total Chlorophyll	Cox's Orange Pippin	0.42 ± 0.00	5.86 ± 0.00	14.64 ± 0.02	0.823 ***
	Granny Smith	0.11 ± 0.00	21.16 ± 448.67	0.30 ± 6.59	0.294 NS
Hue Angle	Cox's Orange Pippin	26.40 ± 16.00	3.28 ± 0.76	13.40 ± 5.74	0.737 ***
	Granny Smith	0.16 ± 50.25	103.34 ± 33074	0.05 ± 14.36	0.263 NS
Lightness	Cox's Orange Pippin	0.23 ± 1.15	7.92 ± 5.23	26.14 ± 9.86	0.815 ***
	Granny Smith	1.34 ± 77.90	16.15 ± 0.00	0.29 ± 17.17	0.232 NS
Colour Chart	Granny Smith	0.20 ± 10.29	44.01 ± 8199.15	0.18 ± 9.42	0.407 *

1 NS Not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Table A5.2 Parameter estimates and standard errors of the declining exponential function for the relationship between colour change ***k*** and carbon dioxide.

Colour Parameter	Cultivar	Ar	A_o	K_{CO2}	r^2
Chlorophyll a	Cox's Orange Pippin	0.321 ± 0.000	-0.285 ± 0.009	0.001 ± 0.003	0.002 NS[1]
	Granny Smith	-0.000 ± 0.027	0.017 ± 0.024	0.072 ± 0.215	0.327 **
Chlorophyll b	Cox's Orange Pippin	0.027 ± 0.004	-2232244 ± 0.000	1369999 ± 0.000	0.000 NS
	Granny Smith	-0.000 ± 0.063	0.016 ± 0.059	0.048 ± 0.296	0.199 NS
Total Chlorophyll	Cox's Orange Pippin	0.031 ± 0.006	0.340 ± 1.897	1.996 ± 4.367	0.000 NS
	Granny Smith	0.005 ± 0.004	0.018 ± 0.005	0.187 ± 0.164	0.556 ***
Hue Angle	Cox's Orange Pippin	0.240 ± 0.000	-11981538 ± 0	20750020 ± 0	0.000 NS
	Granny Smith	0.042 ± 0.013	0.157 ± 0.035	0.338 ± 0.159	0.762 ***
Lightness	Cox's Orange Pippin	-19.329 ± 0.00	19.469 ± 0.026	0.000 ± 0.000	0.000 NS
	Granny Smith	0.082 ± 0.018	0.972 ± 4.593	1.826 ± 4.407	0.546 ***
Colour Chart	Granny Smith	0.025 ± 0.006	0.182 ± 0.147	0.938 ± 0.744	0.728 ***

1 NS Not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$

Table A5.3 Parameter estimates and standard errors of the combined Gompertz growth equation and declining exponential function for the relationship between oxygen and carbon dioxide and colour change ***k***.

Colour Parameter	Cultivar	A_o ($x10^{-4}$)	Y_o	De	K_{co2}	r^2
Chlorophyll a	Cox's Orange Pippin	0.10 ± 0.01	7.52 ± 0.18	0.22 ± 0.04	0.01 ± 0.03	0.682 ***[1]
	Granny Smith	2.84 ± 8.20	1.30 ± 1.70	0.07 ± 0.23	0.03 ± 0.03	0.118 NS
Chlorophyll b	Cox's Orange Pippin	0.00 ± 0.00	12.62 ± 1.00	0.36 ± 0.06	0.00 ± 0.01	0.709 ***
	Granny Smith	5.11 ± 9.45	1.08 ± 0.68	0.10 ± 0.22	0.03 ± 0.02	0.142 NS
Total Chlorophyll	Cox's Orange Pippin	0.09 ± 0.27	7.65 ± 3.34	0.22 ± 0.06	0.04 ± 0.01	0.890 ***
	Granny Smith	0.06 ± 2.80	45.50 ± 2197.62	0.002 ± 0.088	0.03 ± 0.02	0.375 *
Hue Angle	Cox's Orange Pippin	11.65 ± 11.15	4.58 ± 1.08	0.23 ± 0.05	0.04 ± 0.01	0.882 ***
	Granny Smith	0.21 ± 0.00	96.75 ± 6019.82	0.001 ± 0.086	0.04 ± 0.02	0.458 **
Lightness	Cox's Orange Pippin	0.10 ± 0.43	8.94 ± 4.58	0.30 ± 0.07	0.02 ± 0.01	0.827 ***
	Granny Smith	3.18 ± 30.58	8.09 ± 72.43	0.01 ± 0.01	0.03 ± 0.03	0.320 NS
Colour Chart	Granny Smith	1.34 ± 0.01	7.64 ± 17.89	0.02 ± 0.08	0.06 ± 0.02	0.594 ***

1 NS Not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$