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.

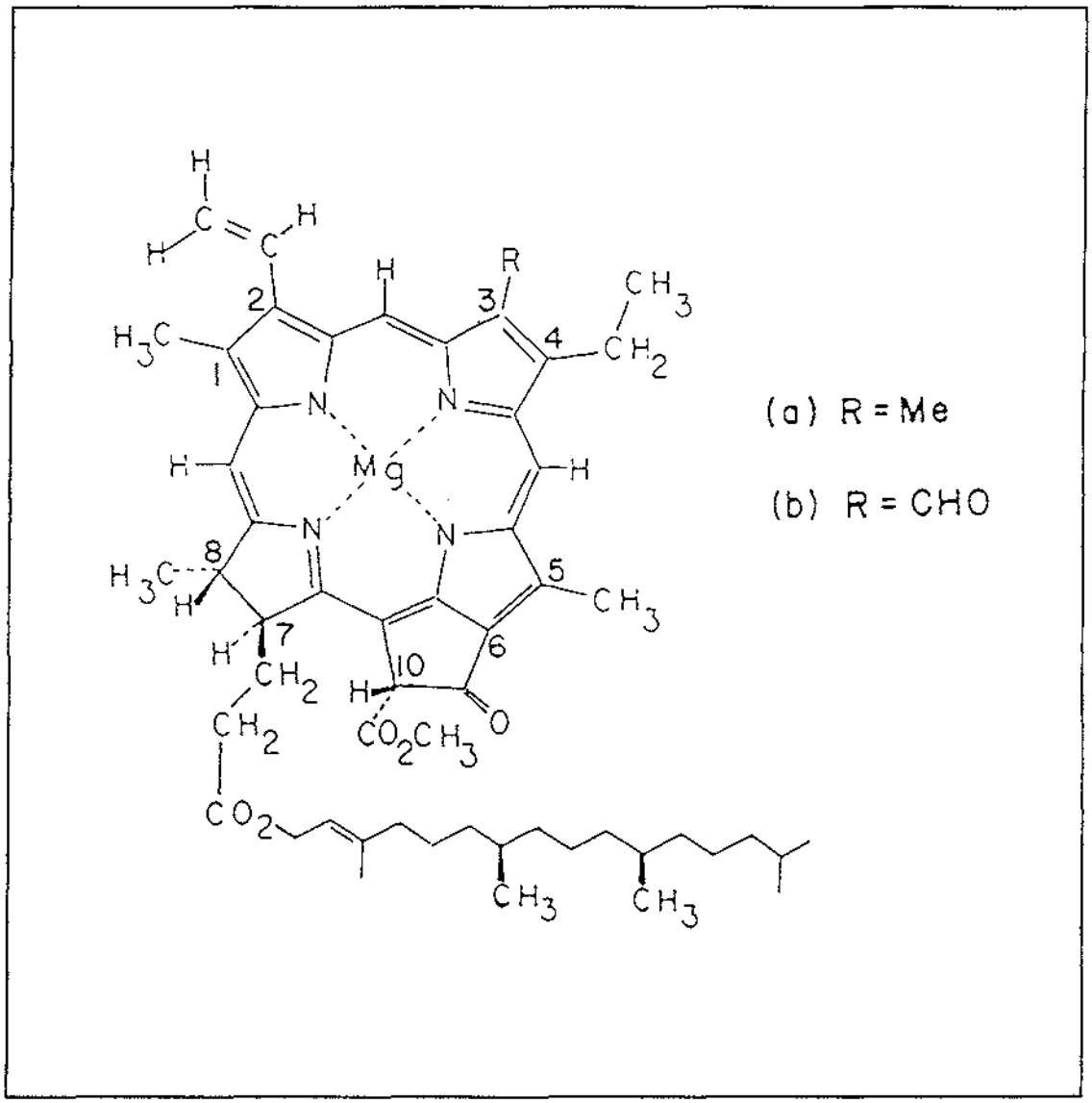
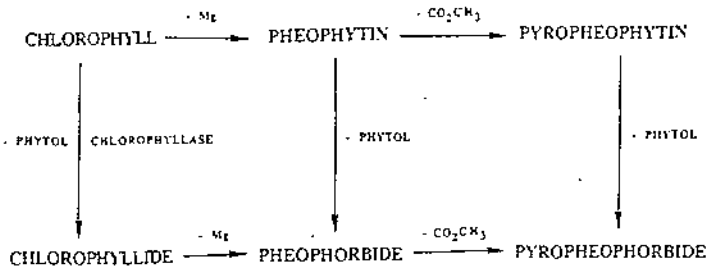


Figure 1.1 Structure of chlorophylls (Schwartz and Lorenzo 1990).



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).

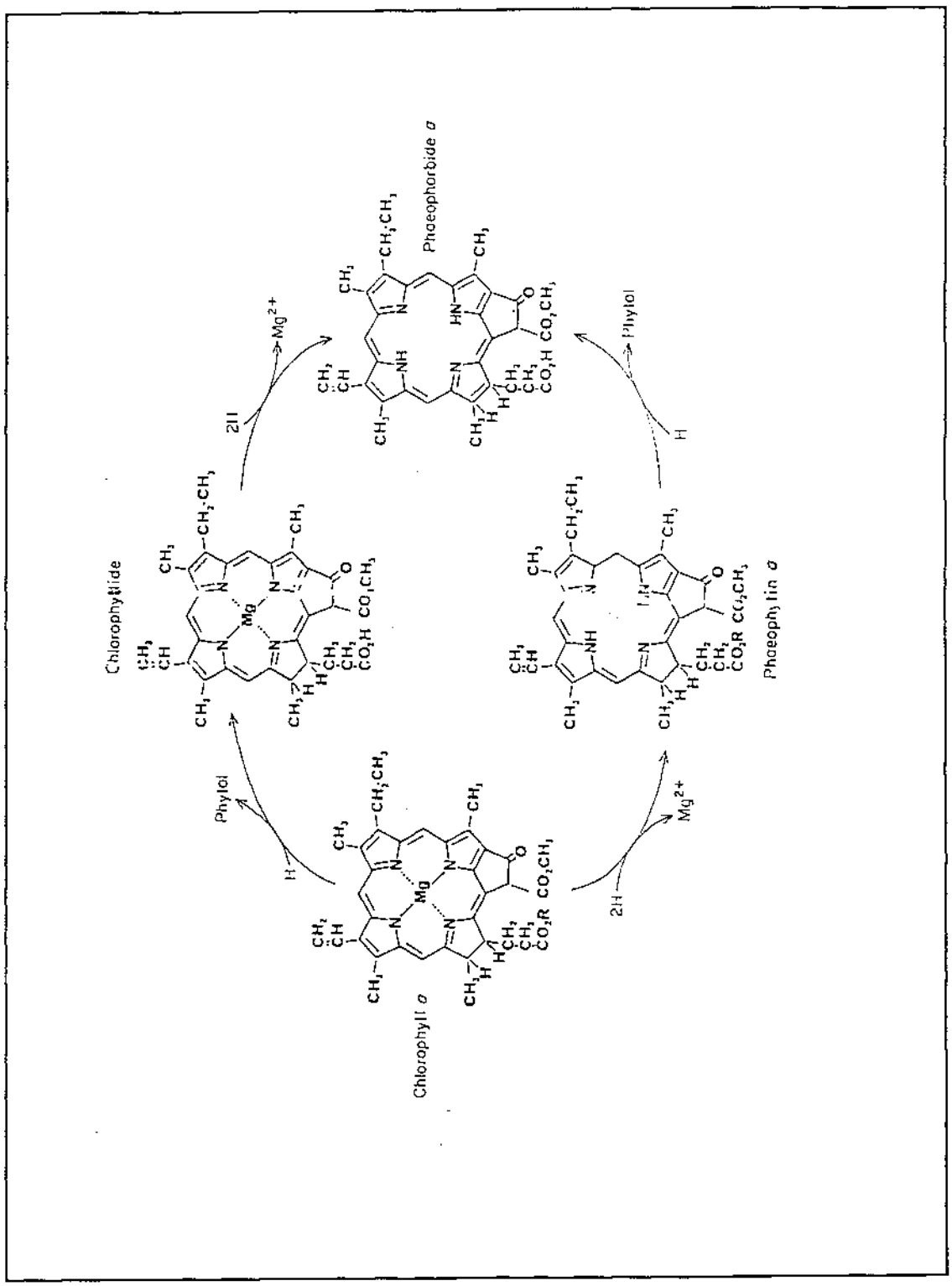


Figure 1.2 Formation of chlorophyll derivatives by demetalation and dephytolation (Hendry *et al* 1987).

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).

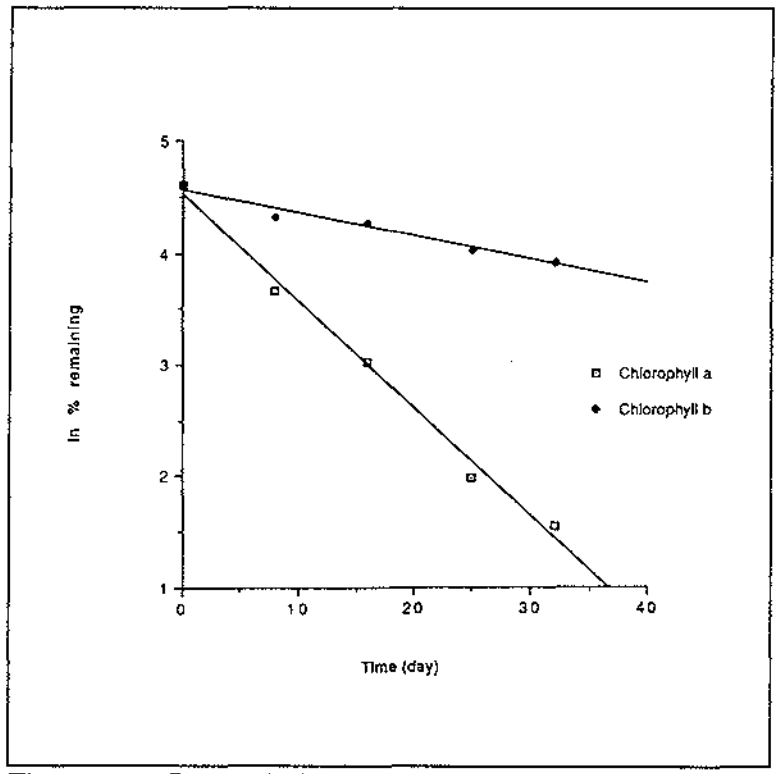


Figure 1.3 Degradation rate plot of chlorophylls a and b during storage of aseptically packaged spinach puree (Schwartz and Lorenzo 1990).