The Relationship between Respiration Rate and Storage Life of Fresh Produce

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PLEASE NOTE

The greatest amount of care has been taken while scanning this thesis,

and the best possible result has been obtained.
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Thesis Summary

This project examines whether there is a direct link between respiration and the rate of senescence of fresh produce. Treatments that increase the storage life of fresh products, such as cooling, modified atmospheres and semi-permeable coatings, often decrease respiration rates. This suggests that it may be possible to assess the effectiveness of a specific storage treatment in terms of its effect on reducing respiration. If this is so, total respiration during storage should sum to a constant regardless of changes in the storage conditions. Such an equivalence between "respiration life" and storage life has been demonstrated in only a few cases. Respiration data from a wide range of published work was analysed from the viewpoint of testing this hypothesis. In general, the results were positive, with some reservations that were further investigated by experiment.

To enable this study, a new method of measuring respiration was developed. This combined some of the attributes of flow-through and static systems. The "respirometer" consists of a sealable container equipped with sensors for oxygen, carbon dioxide and temperature that are linked to a computer. The atmosphere inside the container is refreshed with air or a special gas mixture once the oxygen level is depleted to a specified level by the respiration of the produce. The rate of change of gas concentration inside the container is then used to compute the respiration rate in terms of oxygen and carbon dioxide flux. The advantages of this system derive from its simplicity - the accuracy of measurements is limited mainly by the precision of the sensors, while the equipment was portable and easily interfaced with a computer. When compared with established methods of measuring respiration, the respirometer gave comparable results.

Many criteria have been published for establishing the end of storage life. Several methods were compared using broccoli. Physical methods correlated well with results from a kit that detects proteases involved in programmed cell death in animal tissues. However colour, measured as hue angle ($H^\theta$) appeared to be the method most sensitive to the early stages of senescence. Subsequent experiments therefore used colour as the primary determinant of broccoli storage life.

The summed respiration over the life of other products was determined by defining the end of life as the stage at which the product was no longer marketable. Products included a fruit-vegetable (capsicums), climacteric and non-climacteric fruit (melons and grapes respectively) and an inflorescence (broccoli). Capsicums were stored at different temperatures or partially coated with
wax. Total respiration at non-chilling temperatures was similar within 3 of the 4 replications, but varied between the replicates. Total respiration also varied between melons stored in different atmospheres. In this case, the composition of the storage atmosphere had little effect on storage life, but respiration rate was changed significantly. In contrast, treatment with sulfur dioxide increased the storage life of grapes, but had little effect on the respiration. Respiration life was also determined for grapes held at a range of temperatures. Total respiration was similar at several of the temperatures used, but was not constant under all conditions. It was concluded that respiration is unlikely to be a reliable indicator of storage life when quality is primarily a function of the onset of rots.

Total respiration during storage was determined for broccoli stored at various temperatures, in different atmospheres, and treated with 1-methylcyclopropene (1-MCP). Storage life was ended primarily by loss of chlorophyll, although at temperatures close to 0°C rots became more significant. Total O₂ consumption over the life of the product was similar at all temperatures above 6°C. However, respiration life was increased by storage at lower temperatures or in an atmosphere containing 10 kPa CO₂, as well as by treatment with 1-MCP. The results suggest that the amount of carbohydrate respired before chlorophyll breakdown occurs can be increased by limiting the effects of ethylene. As a result, there may be an equivalence between respiration and storage life of broccoli under some circumstances, but this equivalence can be changed.

These experiments suggested that respiration and storage life were linked only under a limited range of conditions. The question then arises, if respiration rate is not determined by the metabolic demands of senescence, what factors do control O₂ consumption? To answer this question, other factors affecting respiration rates on and off the plant were examined.

The net respiration rate of developing capsicums was studied. To do this, their internal atmospheres were monitored under field conditions, where photosynthesis can offset respiratory loss of CO₂. The results suggested that the structure of the capsicum fruit is particularly well adapted to retain respiratory CO₂ within the internal cavity, where it can be refixed by photosynthesis. This is likely to reduce the loss of carbohydrates during fruit development. The results obtained suggested further experiments on the storage of capsicums after they had been detached from the plant.

Respiration rates of melons are also likely to be affected by harvest, as fruit ripening while attached to the plant respired less than harvested fruit. In this case, the difference appeared to be due to an inhibition of the respiratory climacteric in attached fruit. This was so even though the ethylene climacteric was unaffected. Using normally ripening Charantais melon fruit, as well as
those in which ACC oxidase had been genetically disabled, it was demonstrated that sensitivity to ethylene, at least in terms of respiration, was reduced by attachment to the plant. It was also found that preventing ethylene synthesis increased disease resistance. These results further demonstrate the pivotal role played by ethylene in regulating plant metabolism.

The respiration rate of many fruit is also likely to be affected by their limited permeability to gases. The permeance of capsicum and melon fruit to $O_2$ was measured by different methods, including the rate of flux of $O_2$ through plant tissue, and simultaneous measurements of $O_2$ consumption and internal $O_2$ concentrations. It was found that gas exchange in detached capsicum fruit occurs primarily through the pedicel tissue, the cuticle having a much lower gas permeance. Previous experiments using capsicum fruit on the plant had supported the hypothesis that this was an adaptation allowing recycling of respired $CO_2$.

Melon cuticles were considerably more permeable to gases than those of the capsicums, although in this case the underlying flesh also represented a significant barrier to gas exchange. Permeance was found to vary between melon varieties, and decreased following ripening. A general result of these experiments was that, despite variations between individual fruit, overall permeance is genetically determined. As permeance influences respiration rate and, potentially, storage life, it could be an important criterion for the selection of cultivars with better storage potential.

In conclusion, respiration rate may be a guide to storage life for products that have clearly defined stages of senescence, and for which rots are not the primary cause of the end of acceptability. The development of the respirometer should make it possible to examine this relationship for many other commodities. However, while respiration may be a function of the rate of senescence under some circumstances, it is also affected by other factors. These include photosynthesis, attachment to the plant, and permeance to gases. Directions for future work therefore include the contribution of photosynthesis to fruit development, the effects of ethylene on respiration rates of non-climacteric fruit while attached to the plant, and interactions between ethylene synthesis and disease resistance.
Declaration

The research reported in this thesis has not been submitted for a higher degree at any other institution.

Publications


Bower, J.H., Patterson, B.D., and Jobling, J.J., 2000, Permeance to oxygen of detached Capsicum annuum fruit, Australian Journal of Experimental Agriculture, 40: 457-463

Bower, J.H., Holford, P., Pech, J.C., Ben-Amor, M., 200-, Environmental conditions affect the development of a respiratory climacteric on the vine, in press

Bower, J.H., and Patterson, B.D., 200-, Does total respiration during storage of fresh produce remain constant under changing conditions?, under internal review


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Abbreviations

1-MCP 1-methylcyclopropene
ACC Aminocyclopropane-1-carboxylic acid
ACP Anaerobic compensation point
ADAM Analog data assimilation module
ADP Adenosine diphosphate
ANOVA Analysis of variance
AOX Alternative oxidase
AS Antisensed (melon)
ATP Adenosine triphosphate
CA Controlled atmosphere
CAM Crassulacean acid metabolism
CPP 32 Cysteine protease 32
DAP Days after pollination (melon)
DM Dry matter
DNA Deoxyribonucleic acid
DW Dry weight
GC Gas chromatograph
H° Hue angle (colour)
ICE Interleukin-1β-converting enzyme
ID Internal diameter (tube)
Kmax Maximum rate of a given reaction
kPa Kilopascals (pressure)
LDPE Low density polyethylene (film)
Ln Natural logarithm
MA Modified atmosphere
N Newtons (force)
NDIR Non-dispersive infra red (CO2 sensor)
NR Normally ripening (melon)
P Permeance
p probability
PCD Programmed cell death
pNA p-nitroanilide
PVC Polyvinylchloride (film)
Q10 Quotient (change in rate resulting from a 10°C change in pressure)
R Resistance
RH Relative humidity
RNA Ribonucleic acid
RQ Respiratory quotient (CO2 prod./O2 cons.)
RQB Respiratory quotient breakpoint
SLR Single lens reflex
STS Silver thiosulfate
TCA Tricarboxylic acid
Introduction and Scope of this Thesis

It could be argued that on a worldwide basis there is no shortage of food. Rather, there is a shortage of methods to store, transport and distribute food (Sherman, 1987). Senescence of fruit and vegetables leads to waste of valuable produce. It also results in a loss of returns to producers and reduces nutritional value and quality (Wills et al., 1998, Paull, 1992). Improving our understanding of senescence could bring about new ways of controlling and improving produce storage life (Sherman, 1987).

Harvested fruit and vegetables are on the road to death. Detaching fruit, leaves, stems or other edible parts of a plant removes them from their source of water, nutrients and hormones. This means that they must rely on their own reserves to maintain their metabolism. Some storage organs, such as potatoes and onions, are 'functionally self-sufficient', in that they contain large energy stores, respire slowly, and have both effective barriers to water loss and active wound repair mechanisms (Morris and Pogson, 200-). However, most produce must rely on more limited carbohydrate reserves. For products which contain few carbohydrates and / or which are developing rapidly at harvest (mushrooms, flowers, asparagus, sprouts etc.) these reserves may be quickly exhausted, hastening the onset of senescence.

Plant senescence, whether of whole plants or detached parts, is a natural and inevitable degenerative process. The speed with which it occurs is under genetic control, although it may also be influenced by external factors (Watada et al., 1984, Nooden, 1988). This is in contrast to aging, which is a consequence of external damage accumulated over time (Leopold, 1975). Senescence is also different to necrosis, which occurs as a result of major, non-correctable plant damage such as severe chilling (Davies and Sigee, 1984). Symptoms of senescence include chlorophyll degradation, water loss, loss of cellular integrity, decreased ability to resist pathogen attack and, in the case of bulbs and tubers, sprouting (Morris and Pogson, 200-). While aging and necrosis can also influence storage life, often the rate of senescence is the most important factor determining produce storage life.

Respiration rate is an indicator of the metabolic activity of produce, and so may also indicate how quickly it is senescing (Blanke, 1991). In general, fruit and vegetables with high respiration rates tend to have short storage lives, while those with low respiration rates tend to have longer storage lives (Phan et al., 1975, Kader, 1985, Blanke, 1991, Wills et al., 1996). Many of the treatments used
to increase the storage life of products, such as cooling, atmosphere modification, or reducing gas permeability, also reduce respiration rates. However, a direct relationship between respiration rate and storage life has been shown for relatively few products. Respiration rate has been demonstrated to be inversely correlated with storage life for asparagus (King et al., 1988, Brash et al., 1995), brussels sprouts (Lyons and Rappaport, 1958) and cherries (Sekse, 1988).

The respiration rate of produce is affected by many factors. A number of these are intrinsic to the produce itself. These include permeance to gases (Wardlaw, 1936, Cameron and Yang, 1982, Banks, 1997), maturity (Peterson et al., 1987, Wills et al., 1998) and genotype (Pratt, 1972, Robbins et al., 1989, Manolopoulou and Papadopoulou, 1997). Photosynthesis by the fruit or vegetable (Blanke and Lenz, 1989, Cipollini and Levey, 1991, Pauli, 1992) and attachment to the plant (Smock, 1972, Biale and Young, 1981, Sheill and Saltveit, 1993) may also influence respiration rate.

External factors that slow respiration are often used to increase storage life. Temperature has a greater effect on respiration rate and storage life than any other factor (Wills et al., 1998). Modifying the storage atmosphere by increasing CO₂ and/or decreasing the O₂ concentration, or by removing ethylene can also significantly decrease produce respiration rates (Kader, 1986a). Furthermore, the effects of modified atmospheres and low storage temperatures are often additive (McGlasson, 1992).

If the respiration rate of fresh produce is linked to its storage life, then total substrates consumed between harvest and a given point during senescence should remain constant. For example, asparagus consumes around 550 mmol O₂. kg⁻¹ between harvest and the end of storage life, regardless of the storage temperature used (Brash et al., 1995). Such a measure of “respiration life” could be a useful tool in predicting storage life under different conditions. For example, cumulative respiration could be used to predict the quality at out-turn after storage or transport, or give a dollar value to the degree to which produce is cooled. Furthermore, the question of what controls produce senescence is significant. While there is considerable evidence that senescence is regulated by plant development, the mechanism by which this occurs is not fully understood (Huber, 1987).

The aim of this thesis was to determine the role of respiration in the mechanisms controlling plant senescence. To do this, both respiration rate and storage life needed to be measured for a number of economically significant horticultural products. As the project developed, it became clear that the rate of respiration was not the sole factor affecting produce senescence. The specific aims of the study were therefore;

- To develop a new method of measuring respiration rates of fresh produce.
Chapter 1 - Introduction

- To examine different methods of assessing senescence and the end of storage life for a particular product, in this case broccoli was used.

- To calculate total respiration during acceptable storage life for a range of different commodities. These included a climacteric and a non-climacteric fruit (melons and grapes respectively), a fruit vegetable (capsicum), and a floral vegetable (broccoli).

- To assess the effects of pre-harvest factors including attachment to the plant and photosynthesis on the nett respiration rates of fruit.

- To examine the influence of permeance to gases on respiration rates, and monitor changes in permeance during fruit development.


2

Introductory Literature Review

2.1. Senescence

Senescence is an important biological phenomenon that occurs in most harvested plant tissues (Romani, 1984). It can be defined as a series of actively controlled degenerative processes. These occur after physiological or horticultural maturity, and lead to death of the tissue (Watada et al., 1984). Senescence has been described by Solomos (1988) as “organised disorganisation”, indicating that it is a process which requires energy to continue. It is also as natural and necessary to plant development and reproduction as growth. Senescence of leaves may assist the development of fruit, and fruit senescence in the form of ripening is often necessary for seed dispersal (Solomos, 1988). While these processes may be influenced by external factors, senescence itself is under internal, genetic control (Watada et al., 1984, Nooden, 1988).

The terms senescence and aging are often used interchangeably by biologists. However, plant scientists have tended to separate their meanings. As a general rule, aging is passive while senescence is an active process. Deterioration occurring as a result of damage accumulated over time may be regarded as aging rather than senescence (Leopold, 1975, Nooden and Leopold, 1978). For example, withering and abscission of flowers after pollination are actively controlled processes and so are associated with senescence. On the other hand, the gradual loss of the ability to sprout in seeds or tubers is considered to be aging (Nooden and Leopold, 1988).

Necrosis is described as the death of cells. It can result from some extreme trauma such as chilling or wounding. It may also occur as a hypersensitive response to pathogen attack (Nooden, 1988). The plant is unable to correct such damage, and so this has a major effect on cell metabolism (Davies and Sigee, 1984). Necrosis is different to senescence in that death is rapid, control is limited, and it can occur at any time during plant development.
2.1.1. Changes During Senescence

As senescence progresses, different genes are sequentially expressed. These have a number of effects on the biochemistry of cells (Smart, 1994). Examples include the disintegration of organelle structures, loss of proteins, increased peroxidation of lipids and permeability of membranes, and the generation of oxidative compounds and enzymes leading to breakdown of cell components (Sacher, 1973, Legge et al., 1986, Deschene et al., 1991, Del-Rio et al., 1998).

Loss of chlorophyll is one obvious symptom of senescence. Degradation of chlorophyll is one of the earliest signs of senescence in leaves (Smart, 1994) as well as other green vegetables (Paull, 1992). Even before the chloroplast structure is lost, the ability of chlorophyll to take part in photosynthesis may be reduced (Nooden and Leopold, 1978, Tolwonen, 1992). Chlorophyll degradation may occur more rapidly if the plant tissue is in darkness, as usually occurs during storage (Lipton, 1987, Paull, 1992). Recent work has shown that the degradation of chlorophyll during senescence occurs as a distinct series of steps. In the initial stages the grana thylakoids lose their tight organisation, degenerating into a series of globules. These globules become larger, and are eventually ejected into the cytosol (Teraı̈ et al., 2000). This further supports the view of senescence as an organised, active process.

However, such processes of cellular and biochemical changes are not important to consumers. To the consumer, senescence is the immediately obvious deterioration that occurs after harvest (Romani, 1987). This includes not only colour changes, but also sprouting, water loss, and a reduced ability to repair wounds and resist pathogens leading to rots (Morris and Pogson, 200-). Once these changes occur, the produce will no longer be attractive to eat. The end of storage life may therefore be considered as a particular stage of senescence, and one which may or may not have biological significance.

2.1.2. Defining Senescence

Senescent cells are characterised by a progressive loss of the ability to maintain a steady state, or homeostasis (Samis, 1966). Homeostasis is characteristic of all dynamic, living tissues (Solomos, 1994a). While plant cells are able to respond to stress, they are able to maintain homeostasis. Sources of stress may include physical injury, invasions by microorganisms, damaging concentrations of CO₂ or O₂, and exposure to ethylene. However, even in the absence of stress, a considerable amount of metabolic activity is involved in maintaining homeostasis.
(Romani, 1987). For example, up to 50% of the proteins in ripening bananas are replaced daily (Brady and O'Connell, 1976).

The terms ripening and senescence are used interchangeably by some researchers (Sacher, 1973, Grierson, 1987). Indeed, Huber (1987) suggested that ripening may be viewed as a "functionally modified, protracted form of senescence". The respiratory climacteric itself was once regarded as the manifestation of senescence (Romani, 1984). Certainly, many of the changes associated with senescence also occur during ripening of fruit. However, consumers would not regard the ripening of fruit as a degradative process. Fruit quality is usually at its best during or immediately after ripening. Subsequently, senescence may be considered to be the phase which follows the attainment of maximum quality (Romani, 1984).

2.1.3. Mechanisms of Senescence

While the symptoms of senescence are well defined, the underlying mechanisms are less well understood (Paull, 1992). Senescence appears to be regulated by a balance of phytohormones. In turn, this balance is influenced by a range of factors including availability of carbohydrates, light, injury, temperature and sources of stress such as injury and water loss (Morris and Pogson, 200-). On the one hand, cytokinins and gibberellins retard senescence, while on the other, abscisic acid and ethylene promote senescence (Nooden and Leopold, 1978). The progress of senescence can therefore be determined by the relative amounts of phytohormones such as these.

Ethylene has been shown to cause the onset of senescence in many different plant tissues (Paull, 1992). One of the most immediate effects of ethylene is to stimulate respiration. In climacteric fruit this is associated with ripening. However, it has been shown that the extra energy generated by increased metabolism during the climacteric is not needed for ripening to continue (Rhodes, 1980). This suggests that ethylene-induced increases in respiration are essentially stress responses (Romani, 1984). Even Biale (1964) remarked that cells react to the warnings of imminent death with increased activity. In this case, the respiratory rise during the climacteric may be regarded as a homeostatic response to the degradative effects of ethylene. Postclimacteric senescence is a consequence of an unsuccessful attempt to maintain homeostasis (Romani, 1984).

There is some evidence to support the suggestion that increased respiration reflects an attempt by cells to maintain homeostasis. Work with potatoes and oranges demonstrated that a recovery time was needed between treatments in order for the respiratory response to ethylene to be
repeated (Reid and Pratt, 1972). Limiting the carbohydrates available to pear cells also diminished the effects of ethylene on respiration (Brady and Romani, 1988). These results suggest that the response to ethylene is a function of cellular fitness. Furthermore, minimum ethylene exposure times are needed to initiate ripening in many products. These may be up to 5 days depending on fruit type and maturity as well as ethylene concentration (Knee et al., 1985). For example, avocados may not respond to ethylene at all in the first 48 hours after harvest, particularly if they are immature (Adato and Gazit, 1974). Minimum exposure times may represent the time needed to overcome the homeostatic response (Romani, 1984).

Polyamines may also have a role in maintaining homeostasis. The polyamines spermidine and spermine have been found to inhibit the activities of several enzymes involved in senescence. Polyamines have also been shown to stabilise and protect cell membranes and scavenge potentially damaging oxygenative radicals (Wang and Kramer, 1989). For example, exposure to 1mM of spermine for 10 minutes significantly inhibited chlorophyll loss in dark stored leaves (Kaur-Sawhney and Gaistone, 1979). High levels of polyamines may regulate cellular pH, preventing acidification due to high CO₂ (Philosoph-Hadas et al., 1993).

Cytokinins have been shown to delay senescence in many fruit, vegetables and flowers (Nooden and Leopold, 1979). In particular, cytokinins have been shown to be effective in retarding loss of chlorophyll (Rushing, 1990, Downs et al., 1997) as well as protein and ribonucleic acid (RNA) (Nooden and Leopold, 1979). Cytokinins are normally translocated from the plant roots, and the loss of this supply is thought to be one cause of senescence in detached leaves (Morris and Pogson, 200-).

2.1.4. Programmed Cell Death

As an active procedure, senescence elicits the formation of senescence associated genes (SAG). This process requires specific functions by the cell nucleus and changes in gene expression (Greenburg, 1996). Essentially, this means that senescence is a form of programmed cell death (PCD). While PCD certainly occurs in plants, it has been more extensively studied in animal cells. Inappropriate PCD in animals can result in diseases such as Aids (death of helper T cells) or Alzheimer’s disease (death of brain neurons) (Pennell and Lamb, 1997). On the other hand, if PCD is turned off cells can multiply indefinitely, resulting in cancerous tumors (Lane et al., 1995). In plants, PCD is responsible for the formation of hollow xylem vessels, aspects of embryogenesis, and hypersensitive responses to pathogen attack and ozone damage (Greenberg, 1996, Havel and Durzan, 1996, Kettunen et al., 1999). However, PCD may also be associated with the early senescence of harvested products.
PCD is defined as an active process that occurs during development, and in response to environmental cues (Greenburg, 1996). One particular form of PCD is referred to as apoptosis. Originally an ancient Greek word that referred to the natural abscission of plant leaves (Havel and Durzan, 1996), apoptosis is now defined by a series of events that include:

- Contraction of the entire cell
- Blebbing of the plasma membrane
- Disassembly and degradation of the nuclear DNA
- Redistribution of nuclear pores

(Kumar and Lavin, 1996)

Similar events have been found during senescence in plants. Also, many of the genes that control apoptotic pathways in animals are similar to those found in plants (Mittler and Lam, 1995, Havel and Durzan, 1996). Chemicals known to induce apoptosis in animal cells produced similar symptoms, including DNA laddering, in tomato cells (Woltering et al., 1999). Ethylene has also been shown to be involved in PCD in plants, while inhibitors of ethylene biosynthesis or action reduce PCD (Kettunen et al., 1999, Woltering et al., 1999). Perhaps further study of the processes of PCD and apoptosis could contribute to an improved understanding of the physiology underlying senescence in plants.

### 2.1.5. Classifying Different Types of Senescence

Senescence of plant tissues can be categorised according to its primary causes. Pearl and Miner (1935) proposed three types of survival curves that may be generally extrapolated to describe mortality of all organisms (Figure 2.2.1). Curve A represents a population where mortality occurs at a constant rate due to random external forces such as predation. Curve B represents a population where susceptibility to death increases greatly once the organism reaches maturity. This is typical of slowly ripening fruit such as oranges or capsicums, where death may be due to a combination of both senescence and aging. Curve C represents a population where death occurs rapidly once the population reaches a certain point of maturity. Flowers, and climacteric fruit such as bananas, tomatoes and avocados follow this pattern, where senescence is the primary cause of death (Nooden, 1988).
Figure 2.2.1. - Mortality of different populations; Curve A – constant mortality, curve B – mortality increases with maturity, curve C – rapid mortality at a predetermined point, (adapted from Nooden, 1986).

According to Figure 2.2.1., the time taken until 100% mortality of the population remains the same regardless of external impacts on the survival rate. However, reducing the effects of predation, aging and other external factors results in a greater percentage of survivors at intermediate points in time. The model is therefore analogous to the storage life of fruit and vegetables. In this case, postharvest handling and storage methods can greatly increase mean produce life, even though total storage capacity may be finite.
2.2 Respiration

The study of respiration was pioneered in the early 1920's by Franklin Kidd and Charles West. Their research arose in part from the recognition that respiration generates heat during cold storage. However, there was also some interest at the time in the role of respiration in fuelling plant cells, and whether respiration could be used as an index for metabolic activity (Blackman, 1954). It was Kidd and West who first recognised that ripening fruits underwent an increase in respiration and coined the term “climacteric” (Kidd and West, 1930). Frederick Blackman also studied this phenomenon during the 1920's. Blackman recognised that the course of the climacteric differed between individual fruits, and was associated with both a change in colour and the production of ethylene (Blackman, 1954). It is curious to note that although Kidd and West used 4-5 apples for each measurement during their work and drew on a store containing several tonnes of fruit, Blackman measured a total of just 21 individual apple fruit! Nevertheless, the work of both Kidd and West and Blackman has formed the basis of modern postharvest physiology (Laties, 1995)

Just as understanding senescence assists in measuring storage life, understanding respiration is important when determining appropriate methods for measurement and predicting the effects of storage conditions. Respiration is the process which releases the energy stored in carbohydrates in a controlled way, transforming it into a form that can be utilised by plant cells. Respiration is effectively the opposite of photosynthesis, as it breaks down carbon compounds. Energy released by respiration allows the plant to grow, develop, and maintain homeostasis (Romani, 1987). Respiration measures the metabolic activity of cells, and accordingly varies considerably during plant growth, maturation, ripening and senescence.

2.2.1. Biochemistry of Respiration

Under aerobic conditions, respiration is an oxidative reaction. The generalised equation for respiration describes the breakdown of glucose in the presence of oxygen;

\[ C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{energy} \]

In reality, this is considerable simplification of the processes involved. Carbohydrates are stored within plants in many different forms, including starches, sugars (such as sucrose, fructosans, mannitol etc), lipids, organic acids and sometimes proteins (Taiz and Zeiger, 1991). These
compounds must be converted into glucose or fructose before they can be used. Respiration itself occurs in three separate stages, each of which occurs as a series of separate steps.

Glycolysis is the initial stage. Each hexose molecule is phosphorylated, before being split into two molecules of triose phosphate, each of which is subsequently converted into pyruvate. In the process energy is released, and this is trapped as 2 NADH and 2 ATP molecules for each original glucose molecule;

\[ \text{Glucose} + 2\text{NAD}^+ + 2\text{ADP} + 2\text{Pi} \rightarrow 2\text{pyruvate} + 2\text{NADH} + 2\text{ATP} + 2\text{H}^+ + 2\text{H}_2\text{O} \]

This series of reactions is common (with slight variations) to all living cells, from the smallest bacteria to the largest mammal. The process does not require oxygen, suggesting that it is one of the original biochemical pathways needed for life itself (Raven et al., 1992).

Where O$_2$ is not present, plants have to utilise anaerobic respiration to regenerate the NAD$^+$ needed for glycolysis to continue. This is achieved by the reduction of pyruvate to either lactate or ethanol (Taiz and Zeiger, 1991). However, neither process produces any further energy yield, so the total energy produced by each molecule of glucose is only the ATP synthesised during glycolysis.

Under aerobic conditions, the second stage of respiration involves the oxidation of pyruvate to CO$_2$ and H$_2$O. Pyruvate is transported within the cell from the cytosol into the mitochondrial matrix. Here it is oxidised and decarboxylated, transforming it into acetyl-CoA and producing a molecule of NADH (Raven et al., 1992). Acetyl-CoA then enters the tricarboxylic acid (TCA) cycle, also known as the Krebs cycle in honour of its discoverer, Sir Hans Krebs (Taiz and Zeiger, 1991). Here the molecules are further broken down, and the carrier substrates are regenerated via eight different carbon molecules; citrate, cis-aconitate, isocitrate, α-ketoglutarate, succinate, fumarate, malate and oxaloacetate (Raven, et al., 1992). Carbon dioxide is released, and the energy generated is stored in the forms of ATP, NADH and FADH$_2$ (Appendix 1.).

The third stage in respiratory metabolism involves the reoxidation of NADH and FADH$_2$, using this energy to generate more ATP. This process is catalysed by the electron transport chain, this being a complex system of enzymes, electron carriers (e.g. ubiquinones, cytochromes) and cytochrome oxidase. The process occurs in the membranes of the mitochondria, forming 2-3 molecules of ATP by the oxidation of each molecule of NADH (Raven et al., 1992);

\[ \text{NADH} + \text{H}^+ + \frac{1}{2} \text{O}_2 + 2\text{ADP} + \text{Pi} \rightarrow \text{NAD}^+ + \text{H}_2\text{O} + 2\text{ATP} \]
A certain proportion of this last stage occurs by a different pathway. The alternative oxidase (AOX) pathway branches from the electron transport chain at the level of ubiquinone. It is non-phosphorylating after this point, and so is a less efficient converter of oxidative energy to ATP than the primary electron pathway. Excess energy is dispersed as heat. In several flowers, including the voodoo lily (*Sauromatum guttatum*) and the skunk cabbage, this heat is used to produce the strong odours used to attract pollinators (Cruz-Hernandez and Gomez-Lim, 1995). The AOX is estimated to account for around 10-25% of respiration under normal conditions (Taiz and Zeiger, 1991).

The purpose of the AOX pathway is unclear. One possible purpose relates to the increased flux through this pathway during ripening of many climacteric fruits (Duque and Arrabaca, 1999). Hydrogen cyanide is produced as a by-product of ethylene synthesis. As the AOX is cyanide resistant, it has been suggested that this pathway allows respiration to continue despite the accumulation of cyanide. However, cyanide levels do not appear to rise high enough in ripening fruits to account for increased activity of the alternative oxidase (Yip and Yang, 1988).

The AOX may allow plant tissues to cope better with stress. During ripening, increased respiration rates result in amounts of energy being produced that cannot be readily used by the plant tissue. The AOX may provide a way of oxidising the large electron flow without producing excess amounts of ATP (Lange and Kader, 1997, Duque and Arrabaca, 1999). An imbalance between ATP production and ATP usage during ripening will quickly exhaust available supplies of ADP. It can also result in increased production of reactive oxygenative compounds by the cytochrome oxidase pathway, as well as fermentative reactions and the production of ethanol. The AOX is not dependant on the availability of ADP, and so reduces production of these potentially damaging compounds (Purvis, 1997).

### 2.2.2. Aerobic and Anaerobic Respiration

Anaerobic respiration is much less efficient than aerobic respiration. While aerobic respiration conserves approximately 40% of the energy liberated by the oxidation of glucose (Burton, 1982, Taiz and Zeiger, 1991) anaerobic respiration conserves less than 3%. This has implications for the storage life of fresh produce, as either their carbohydrate reserves will be exhausted much more quickly under anaerobic conditions, or insufficient energy will be produced to maintain vital functions.

A shift from aerobic to anaerobic metabolism occurs when the atmospheric O₂ concentration falls below some critical concentration, usually 1-3% O₂. This varies among products according to
their respiration rate and permeance to gases (Kader, 1986a). Determination of the lower O₂ limit for produce is important when selecting optimal storage atmospheres for produce storage. In general, the most benefit will be obtained using atmospheres just above the lower O₂ limit (Yearsley et al., 1996). Below this O₂ concentration off flavours and odours are likely to occur, and the plant tissue will deteriorate rapidly (Banks et al., 1993).

The transition from aerobic to anaerobic respiration does not occur instantly. The change occurs gradually over a range of O₂ concentrations, so both forms of respiration can occur simultaneously. The point at which anaerobic respiration starts to dominate is the critical value when storing produce. Under aerobic conditions CO₂ evolved and O₂ consumed are approximately equal. This ratio, known as the respiratory quotient (RQ), is calculated by dividing CO₂ production by O₂ consumption (Wills et al., 1998). However, as respiratory metabolism shifts from aerobic to anaerobic and O₂ consumption continues to decrease, CO₂ production will decrease more slowly or can even increase (Figure 2.2.2.). Under these conditions RQ becomes greater than 1 and ethanol and acetaldehyde are produced (Kanellis et al., 1988). The point at which RQ starts to increase is described as the respiratory quotient breakpoint (RQB) (Beaudry et al., 1992). A similar way of defining the point at which anaerobic respiration becomes significant is the anaerobic compensation point (ACP) (Figure 2.2.2.). This is defined as the O₂ concentration at which CO₂ production is minimised (Boersig et al., 1988). These methods may be expected to give similar, but not necessarily identical, results.
Figure 2.2.2. - Changes in O₂ consumption (—) and CO₂ production (—) of a plant tissue in response to decreasing O₂ availability. In this example the RQ breakpoint occurs at approximately 1.2-1.3 kPa O₂, while the anaerobic compensation point is at 1 kPa O₂.

The ACP may vary according to temperature (Beaudry et al., 1992, Joles et al., 1994), produce permeance (Banks et al., 1993, Yearsley et al., 1996), produce maturity (Boersig et al., 1988), CO₂ concentration in the storage atmosphere (Beaudry, 1993, Joles et al., 1994) and cultivar (Gran and Beaudry, 1993). Even though the optimal O₂ concentration may be close to the ACP, this variability means that the risk of anaerobiosis is considerable. As a result it may be preferable to use O₂ levels twice the ACP during controlled atmosphere storage (Chervin et al., 1996).

2.2.3. Metabolism of Different Substrates

Although RQ is often assumed to equal 1 under aerobic conditions, RQ actually varies according to the substrate being utilised (Beevers, 1961). While the complete oxidation of glucose involves equal quantities of O₂ and CO₂, oxidation of malic acid yields an RQ of 1.33, triglycerides (such as fats and lipids) give an RQ of 0.7, and utilisation of proteins results in an RQ of approx. 0.8 (Burton, 1982). RQ may change during produce development, particularly during the ripening of climacteric fruits when carbohydrate demand is greatest (Burton, 1982). Measurement of RQ can
therefore provide a guide as to the type of substrate being respired (Wills et al., 1998). For example:

Respiration of malic acid:

\[ \text{C}_4\text{H}_6\text{O}_6 + 3\text{O}_2 \rightarrow 4\text{CO}_2 + 4\text{H}_2\text{O} + \text{ energy} \]

\[ \text{RQ} = 4/3 = 1.333 \]

Respiration of triglycerides:

\[ \text{C}_{16}\text{H}_{32}\text{O}_6 + 21\text{O}_2 \rightarrow 16\text{CO}_2 + 16\text{H}_2\text{O} + \text{ energy} \]

\[ \text{RQ} = 16/21 = 0.76 \]

Variations in RQ also have implications for the measurement of respiration. Ideally both \( \text{O}_2 \) consumption and \( \text{CO}_2 \) production should be used to measure the respiration rate of plant tissues. However, this is not always possible. Unless anaerobic respiration is occurring, measurement of \( \text{O}_2 \) will indicate metabolic activity even when substrates other than glucose are used.

2.2.4. The Respiratory Climacteric and Ethylene

The climacteric in fruits was originally defined as a rapid increase in respiration rate associated with ripening of some fruits (Burg and Burg, 1962, McGlasson et al., 1978). When it became possible to accurately measure low concentrations of ethylene, it was recognised that this rise in respiration was preceded or accompanied by increased ethylene production (Hulme, 1970). Increases in ethylene production due to positive feedback from ethylene exposure then became the defining feature of climacteric fruits. Ethylene stimulates respiration (Figure 3), and promotes other, irreversible changes associated with ripening. These include the hydrolysis and softening of cell wall materials, changes in organic acids, increased aroma, loss of astringency and changes in colour (McGlasson et al., 1978). The respiration rates of some fruit may increase sixfold during this stage (Biale and Young, 1981).

In contrast, exposure of non-climacteric fruit to ethylene results in only a transitory increase in respiration rate. The magnitude of this increase is proportional to the logarithm of the ethylene concentration (McGlasson et al., 1978). The presence of ethylene does not stimulate its own production as it does in climacteric fruit, and respiration returns to its previous levels once the exogenous ethylene is removed (Lelievre et al., 1997). Apart from its role in de-greening citrus, the effect of ethylene on non-climacteric fruit is essentially to accelerate senescence (Wills et al., 1999). The respiratory response to ethylene in non-climacteric as well as climacteric fruit suggests that it acts as a stress factor in plant tissues (Romani, 1984)
It is difficult to define the minimal amount of ethylene required to stimulate respiration. Previous work has suggested that 0.1 ppm of ethylene is the threshold below which no effects on produce can be observed (Knee et al., 1985). However, even levels as low as this have been shown to promote ripening in bananas (Peacock, 1972), and to significantly reduce the storage lives of a number of non-climacteric fruit and vegetables (Wills et al., 1999). Conversely, preventing ethylene from accumulating in the storage atmosphere, when combined with low O₂ levels, delayed ripening of green bananas by over six months (McGlasson and Wills, 1972). Removal of ethylene is therefore an important method of prolonging produce storage life, as well as avoiding increases in respiration rates (Wills et al., 1998).

2.2.5. Effect of Temperature on Respiration

Temperature has a major effect on respiration rate. One way of quantifying these effects is by applying the Q₁₀ rule. This rule, proposed by the Dutch chemist Van’t Hoff, estimates that for every 10°C increase in temperature, the rate of any reaction will double (Burton, 1982, Wills et al., 1998). Q₁₀ values for respiration rates are calculated by:

\[
Q_{10} = \frac{(\text{respiration rate}_2)}{(\text{respiration rate}_1)}^{\frac{10}{\text{temp}_2 - \text{temp}_1}} = \text{constant (approx. 2)}
\]

The effects of temperature on biochemical pathways are complex, and respiration is not a simple chemical reaction (Burton, 1982). Q₁₀ values are often different to 2. This is particularly the case at low temperatures, when small changes in temperature can have large effects on metabolism. For example, Q₁₀ values for broccoli respiration range from 2.5 to 4.5 between 10-20°C, and 6 to 9 between 0-10°C (Klieber and Wills, 1991, Kader et al., 1989), while Q₁₀ values for brussels sprout respiration were 2.2 between 10-20°C, and 4 between 0-10°C ( Lyons and Rappaport, 1958).

Low storage temperatures can delay the onset of ripening, particularly in climacteric fruit (Wills et al., 1998). This is due to the close interactions between temperature, ethylene and enzyme activity (Field, 1985). Low temperatures greatly reduce ethylene production due to low levels and activity of ACC synthase (Wang and Adams, 1982, Field, 1985). Work with apples has shown that reducing the temperature by 10°C can reduce ethylene production by 50% (Knee et al., 1985).

Cooling also reduces the response to ethylene by plant tissues. This can enable fruits and vegetables to tolerate relatively high ethylene concentrations with minimal effects on respiration.
(McGlasson, 1970, Reid and Pratt, 1972). However, ethylene sensitivity may be thought of in terms of the concentration required to produce half the maximum response. It has been shown that although the maximum response by pea seedlings to ethylene is reduced by low temperature, the ethylene concentration at which half the maximum response occurs is lower at 10°C than at 20°C (Knee et al., 1985). This suggests that although the effects of ethylene are reduced at low temperatures, produce sensitivity is not.

Both ethylene production and responsiveness to the gas may be enhanced in cool stored produce after it is warmed. Cold treatments increased ethylene production and respiration of a number of pear cultivars (Wang et al., 1971) and Granny Smith apples (Jobling et al., 1991), on removal to ambient temperatures. Custard apples also ripen more quickly and with a greater accompanying burst of ethylene after several days storage at 8°C (Alique, 1995). In this way, prior chilling can hasten the ripening of such fruits.

Low storage temperatures also increase the subsequent respiration rates of chilling sensitive produce. Chilling injury, as a form of stress, increases ethylene production by plant tissues (McGlasson, 1970). This then has the effect of increasing respiration rate. For example, respiration rates at ambient temperatures increased by over 60% in bitter melons that had been stored for a time at 7.5°C (Zong et al., 1995), and were doubled in capsicums stored for several days at 4°C (Mencarelli et al., 1993) compared to fruits kept continuously at room temperature.

Increasing the temperature does not always increase respiration, but may reduce it in some circumstances. High temperatures, particularly those over 35°C, have been reported to inhibit ripening of many fruits (McGlasson, 1970, Klein and Lurie, 1990). This effect is partly due to inactivation of the ethylene synthesising system at temperatures over 35-36°C (Field, 1985, Lelievre et al., 1997). Heat treatments are now used for both reducing chilling injury and disinfection of produce. Short exposure to high temperature has been shown to significantly reduce respiration rates in apples (Klein and Lurie, 1990).
2.2.6. Effects of $O_2$ and $CO_2$ Concentrations on Respiration

Reducing the $O_2$ concentration in the storage atmosphere can have the effect of reducing produce respiration rates. The benefits of controlled atmosphere (CA) and modified atmosphere (MA) storage methods have been recognised for more than 60 years (Kader et al., 1989), and are used commercially to increase produce storage life. They are most effective when combined with low temperatures, the effects of both treatments being additive (Kader, 1985b, Wills et al., 1998). As well as slowing down respiration and the rate of senescence, CA and MA treatments can reduce ethylene production and sensitivity, lessen some physiological disorders, inhibit pathogen growth, and be used for produce disinfestation (Kader, 1985).

Elevated $CO_2$ may act as either a suppresser or a stimulator of respiration, depending upon its concentration and the tissue involved (Mathooko, 1996). $CO_2$ is known to inhibit several enzymes involved in the TCA cycle, as well as phosphofructokinase, an enzyme involved in glycolysis (Kerbèl et al., 1988). Exposure to high $CO_2$ may also lower the cytoplasmic pH, to the point where normal metabolism is interrupted (Mathooko, 1996).

The effect of $CO_2$ may be largely due to its effects on the production and sensitivity to ethylene (Mathooko, 1996). Some $CO_2$ is required during ethylene biosynthesis, with an optimum concentration of around 2 kPa in vitro (Lelievre et al., 1997). However, higher levels reduce this process, possibly due to inhibition of the activity of ACC oxidase (Gorny and Kader, 1994). Elevated $CO_2$ has long been recognised to reduce ethylene sensitivity, with a 10 kPa concentration sufficient to eliminate the effects of 0.1Pa (1ppm) of ethylene (Burg and Burg, 1967). This inhibition of ethylene action is primarily through competition for the binding site (Burg and Burg, 1967, Kubo et al., 1990, Gorny and Kader, 1994).

High $CO_2$ levels do not depress respiration in all cases, however, and may even have the opposite effect. Heightened $CO_2$ has been shown to increase the proportion of total respiration that occurs via the alternative oxidase pathway (Lange and Kader, 1997). As this pathway is less efficient than that via cytochrome oxidase, respiration may have to increase simply to meet energy demands. Increases in respiration due to high $CO_2$ stress must be balanced against decreases due to feedback inhibition of $CO_2$ production.

The effects of $CO_2$ on respiration and quality are highly specific to particular produce types. Of 18 different fruits and vegetables exposed to high concentrations of $CO_2$ (60 kPa) for 24 hours, the
O₂ consumption rate decreased in only four (Kubo et al., 1990), with most remaining unaffected by the treatment. However, 60 kPa CO₂ has been shown to stimulate respiration in products such as cucumbers and carrots (Kubo et al., 1990; Mathooko, 1996), while only as little as 5 kPa CO₂ increased respiration rates and browning of mushrooms (Briones et al., 1992). Some apples are also damaged by this concentration of CO₂, although other varieties will tolerate up to 10 kPa CO₂ with little effect (Burton, 1974). Maturity may be another source of variation, with immature fruit less tolerant of high CO₂ than ripe fruit (Kader et al., 1989).

In contrast, the effects of low O₂ are generally beneficial as long as anaerobic metabolism is avoided. However, O₂ concentrations must sometimes be taken to levels close to the anaerobic compensation point for there to be a substantial benefit in terms of reduced respiration or increased storage life (Beaudry, 2000). Raised CO₂ concentrations (Kader et al., 1989), and increases in temperature (Beaudry, 2000) can increase the O₂ concentration at which anaerobic respiration commences. This means that a beneficial atmosphere can become damaging with only a small change in concentrations.

Recommended O₂ concentrations for increasing storage life range from 1-5 kPa (Kader, 1980). O₂ is required for the re-oxidation of NADH and FADH₂ by the electron transport chain. This reaction is catalysed by cytochrome oxidase. However, this enzyme has a high affinity for O₂, and its activity is not limited by these concentrations (Burton, 1978). The maximum rate (Kₘ) occurs with only 0.1-0.15% O₂ in the atmosphere around the cell (Beaudry, 2000). The alternative oxidase has a slightly lower affinity, being saturated at around 1-3% O₂ (Beaudry, 2000). Reductions in O₂ consumption by 1-5% O₂ are therefore not directly due to restriction of the activity of these enzymes. Instead, the effect could be due to another regulatory enzyme or enzymes that respond to low levels of O₂ by inhibiting the initial stages of glycolysis (Solomos, 1994a). In other words, low O₂ may limit the activities of other oxidative enzymes with lower affinities for O₂ (Solomos, 1982, Kanellis et al., 1991, Rahman et al., 1993).

Low O₂ may also reduce respiration rate by changing gene expression. This would not inhibit respiration directly, but could result in reduced respiration due to a general decrease in metabolic rate (Solomos, 1994b). This is supported by work with capsicums in which it was found that exposure to 1.5% O₂ for 24 hours significantly reduced respiration by whole fruits, but not that by isolated mitochondria (Rahman et al., 1993, Rahman et al., 1994). However, Beaudry (2000) has suggested that these results may be due to greater diffusive resistance in whole plant tissues relative to that of individual cells.
The inhibition of respiration by relatively high \( \text{O}_2 \) concentrations is likely to be related to the gas permeance of produce. Fruit, as bulky plant tissues, have limited permeance to gases. The epidermal layer in particular can be a significant barrier to gas exchange (Ben-Yehoshua \textit{et al.}, 1963). The magnitude of the gradient in gas concentration between the internal tissues and the external atmosphere is likely to be a function of produce respiration, permeance, and the external gas concentration (Burg and Burg, 1965, Cameron and Reid, 1982). Variability in fruit permeance may be one reason for the different responses of many products to modified atmosphere storage, an issue discussed in greater detail in Chapter 7 of this thesis.

Low \( \text{O}_2 \), like high \( \text{CO}_2 \), reduces the effects of ethylene on plant metabolism. This is likely to be due to inhibition of binding of ethylene to the receptor site by \( \text{O}_2 \) concentrations below 8 kPa (Burg and Burg, 1967). Induction of ACC synthase may also be suppressed, ethylene production being halved by reducing \( \text{O}_2 \) concentration to around 2.5 kPa (Kader, 1980). Low \( \text{O}_2 \) concentrations have been shown to delay ripening in various fruit (Solomos, 1982) including several apple varieties (Sfakiotakis and Dilley, 1973, Lidster \textit{et al.}, 1983). \( \text{O}_2 \) reductions affect a variety of metabolic processes associated with ripening, including softening, colour changes and volatile production (Beaudry, 2000).

The decrease in respiration under CA and MA conditions might not be the cause of increased storage life, but rather a response to a general decrease in metabolic activity (Solomos, 1994a). It is hoped that the subsequent parts of this thesis will help to clarify the validity of this statement, not just in relation to the effects of CA and MA, but also to other factors which affect the respiration rates of produce.
2.3 Respiration Life

2.3.1. What is meant by Respiration Life?

The rate of senescence of harvested produce often appears to be related to its respiration rate (Burton, 1978, Ryall and Pentzer, 1981). For example, cool temperatures, modified atmospheres, semi-permeable coatings, and applications of novel compounds reduce respiration rate and also prolong storage life. Conversely, treatments that speed up respiration such as increased temperature, exposure to ethylene, stress and wounding, decrease storage life. It has often been assumed that the effects of such treatments on storage life are due to their influence on respiration (Kader, 1986a).

It has been suggested that respiration rate may be used as a guide to the potential storage life of different products (Wills et al., 1998). Products that have high rates of respiration, such as mushrooms, asparagus, and bananas, often have short storage lives. Products with low respiration rates, such as potatoes, apples, and grapes, generally have a longer storage lives. This is illustrated in Figures 2.3.1 and 2.3.2, in which mean respiration rates during storage are compared with approximate storage lives for a number of commodities.

However, the trends depicted in Figures 2.3.1 and 2.3.2 might not hold true in all cases. If respiration is very rapid (>100 mg CO₂.kg⁻¹.h⁻¹) then further increases in respiration may have minimal effects on storage life (Ryall and Lipton, 1980). Senescence could differ between climacteric fruit and non-climacteric fruit, and data on storage life as recorded in the literature is often variable. For example, the storage life of strawberries kept at 0°C has been reported to be 6 days (AUF Manual, 1980) or 14 days (Thompson, 1996). Likewise, broccoli kept at 0°C is described as having a storage life of 12 days (AUF Manual, 1980), 24 days (UC Davis Fact Sheet, 2000), 35 days (Pogson and Morris, 1997) or 56 days (Klieber and Wills, 1991).

Storage life can vary because of many factors including cultivar, maturity and vigour at harvest, humidity in the storage atmosphere, presence of micro-organisms and exposure to ethylene. However, one of the most important causes of the reported variability could be the different criteria used to estimate the end of life. For example, even though most studies record the end of broccoli storage according to yellowing, this may be evaluated in several ways. These include a visual grading scale (Ku and Wills, 1999), hue angle (H°) (Irving and Joyce, 1995), chlorophyll content (Bastrash et al., 1993) and chlorophyll fluorescence (Tolvonen, 1992).
Chapter 2 - Literature Review, Respiration Life

Figure 2.3.1. - Relationship between respiration rate and storage life of fruit; data compiled from Thompson (1996), AUF Product Manual (1980), UC Davis Fact Sheets (2000), and Ryall and Pentzer (1981). Full data set with sources presented in Appendix 2.

Figure 2.3.2. - Relationship between respiration rate and storage life of vegetables; data compiled from Thompson (1996), AUF Product Manual (1980) and UC Davis Fact Sheets (2000). Full data set with sources presented in Appendix 2.

The rate of senescence has been found to be a function of temperature for many fruit and vegetables (Thorne and Alvarez, 1982). Accumulated heat units can be used to predict storage life of asparagus (King et al., 1988), yellowing of broccoli (Brockwell, 1999), softening and colour loss
of apples (Burmeister et al., 1999) and ripening of tomatoes (Thorne and Alvarez, 1982). Respiration rates also vary as a function of temperature, increasing 2 - 6 times for a rise in temperature of 10°C (Ryall and Pentzer, 1981). This raises the question of whether the effects of temperature on storage life are due mainly to changes in respiration rate.

Senescence is a controlled process regulated by gene expression. It therefore requires energy to proceed (Morris and Pogson, 200-). As respiration indicates the metabolic activity of the plant tissue, it could be expected that the two processes are closely coupled. This means that if respiration rate is halved, storage life should approximately double. If this is true, then the total amount of O₂ consumed during storage life will remain constant regardless of the storage conditions. This measurement of cumulative respiration during storage may be referred to as the "respiration life" of the product.

Total respiration during storage has been calculated for a few crops. These include asparagus (Brash et al., 1995), brussels sprouts (Lyons and Rappaport, 1958) and cherries (Sekse, 1988). However, measurements of respiration rates and quality changes have been published for many others. Using this published data, I have estimated total respiration during storage life for a variety of fruit and vegetables. The results of this survey of the literature are summarised in Table 2.3.1.

2.3.2. Respiration Life of Leafy Vegetables

The factors controlling senescence of leafy vegetable crops have been little studied despite their economic and nutritional importance (Lipton, 1987, Paull, 1992). Yellowning, due to loss of chlorophyll, is usually the primary symptom of senescence in these products (Morris and Pogson, 200-). Many leafy vegetables have a high surface area relative to their volume. This increases water loss, a source of stress that multiplies the rate of chlorophyll degradation (Lipton, 1987, Paull, 1992). Leafy vegetables often have low levels of carbohydrate reserves, another factor that could lead to early senescence signalled by yellowning. Both dehydration and carbohydrate depletion are involved in the senescence of seedlings and single leaves (Nooden and Leopold, 1988). However, most vegetables are more complex than these plant parts, suggesting that the processes in leafy vegetables may be different to those previously studied (Morris and Pogson, 200-).

Leafy vegetables often consist of both mature and immature leaves. They may also be composed of more than one type of tissue, and vary widely in their structural arrangement (Morris and Pogson, 200-). Interactions among different tissues can complicate both the mechanisms and the measurement of senescence. For example, untrimmed lettuces lost quality more rapidly than those from which the outer leaves were removed. It was proposed that this effect was due to the greater
susceptibility to damage and microbial infection of mature leaves (Pratt et al., 1954). The outer leaves of Chinese cabbage have very low carbohydrate reserves (Wang, 1983) and contain few polyamines compared to the inner leaves (Wang and Kramer, 1989). This may be the reason why mature leaves are less able to maintain homeostasis than young leaves (Romani, 1987).

The rate of chlorophyll degradation is often determined by storage temperature. One way of expressing this is as the time taken until one-third of chlorophyll is lost (Paull, 1992). Another method is to count the number of leaves with substantial yellowing. This technique was used to calculate the storage life of brussels sprouts stored at various temperatures (Lyons and Rappaport, 1958). Using this information, cumulative CO$_2$ production during acceptable storage life could be calculated. The results were similar irrespective of the storage temperature, ranging from 420-511 mmol.kg$^{-1}$ (Table 2.3.1).

However, total respiration during storage may not be constant for other leafy vegetables. Data published on cabbages (Guffy and Hicks, 1984) suggested that the variety that deteriorated most quickly respired faster than other, similar varieties. Although it was not possible to calculate storage life from the presented data, differences in the rate of senescence were greater than differences in CO$_2$ production. This suggests that total CO$_2$ production during storage was not the same for the cultivars tested. The time taken until lettuce quality fell to “fair” (Grade 5) was estimated from published data on lettuces presented by Pratt et al., (1954). This was combined with the accompanying data on respiration rates to estimate the total CO$_2$ production during storage life. In this case, my calculations showed that total CO$_2$ production during storage was not constant, being approximately 200 mmol.kg$^{-1}$ at 20°C compared to 93 mmol.kg$^{-1}$ at 0°C.

### 2.3.3. Respiration Life of Shoots and Inflorescences

Immature plant parts, such as shoots and floral tissues, are still growing at harvest. These vegetables have high respiration rates and often senesce rapidly (Huber, 1987). For example, at 20°C asparagus becomes unacceptable within 2-5 days (Irving and Hurst, 1993), while yellowing of broccoli may occur in only 2-3 days (Morris and Pogson, 200-). The rates of senescence of both asparagus and broccoli are strongly related to storage temperature (King et al., 1988, Klieber and Wills, 1991). As with leafy vegetables, the primary symptom of senescence is chlorophyll loss.

After asparagus is harvested, carbohydrate and protein levels fall, respiration rate declines, and ammonium ions accumulate (King et al., 1990). Changes in protein and ammonium levels can be symptoms of carbohydrate starvation, as has been observed in other plant tissues (Kader, 1987, Irving and Hurst, 1993). To test the relationship between storage life and respiration, asparagus
spears were held at a range of temperatures between 0-20°C for approximately 4 days. They were then transferred to 20°C to measure their subsequent storage life. Total CO₂ evolved between harvest and senescence was the same at all storage temperatures used (Brash et al., 1995). If storage of asparagus is limited by the supply of carbohydrates, then a close relationship between respiration and life is entirely explicable. However, Irving and Hurst (1993) found that endogenous reserves of glucose, sucrose and fructose were sufficient to maintain respiration well past the end of storage life. Despite this, respiration rate remains an excellent predictor of asparagus storage life (Brash et al., 1993).

Broccoli is another perishable product, and senescence can occur rapidly under warm, drying or excessively wet conditions (Wang, 1979). At harvest broccoli florets consume almost 1% of their dry weight every hour (King and Morris, 1994a). As with asparagus, respiration rates, carbohydrate reserves, and organic acids decrease significantly soon after harvest (King and Morris, 1994b). The causes of the end of broccoli storage life vary. At temperatures below 5°C, the main factor limiting storage is the onset of rots, while at higher temperatures yellowing is more significant (Klieber and Wills, 1991).

I estimated the total CO₂ production of broccoli during storage using data from a number of publications. Excepting where storage life was stated (Klieber and Wills, 1991), broccoli was assumed to be unacceptable once hue angle \( (H^a) = 115 \), this value representing colour after \( \approx 2 \) days at 20°C (King and Morris, 1994a, Tian et al., 1994, Irving and Baird, 1996). Total respiration was estimated from the associated graphs of O₂ uptake / CO₂ production. The results varied considerably between the different publications, ranging from 208-224 mmol kg⁻¹ for broccoli held at 10-20°C (Klieber and Wills, 1991), to 2.7 mol kg⁻¹ for broccoli treated with cytokinins (Irving and Baird, 1996) (Table 2.3.1). Most of this variation may be attributed to differences in measurements of respiration rates. For example, 'Shogun' broccoli heads held at 20°C in air were reported to have mean respiration rates of 4.7 mmol CO₂ kg⁻¹ h⁻¹ (Klieber and Wills, 1991), 4.5 mmol CO₂ kg⁻¹ h⁻¹ (King and Morris, 1994a), 16.6 mmol CO₂ kg⁻¹ h⁻¹ (Tian et al., 1994) and 24 mmol O₂ kg⁻¹ h⁻¹ (Irving and Baird, 1996). While conditions at the time of measurement are likely to vary, such large discrepancies are difficult to understand.

To overcome the large variations in recorded respiration rates, the data were normalised by multiplying the days of storage life from each study by standard values for respiration rate. This resulted in a similar figure for cumulative respiration at 20°C in all cases. The results calculated from the data of Klieber and Wills (1991) suggested that storage life was increased by a greater factor than CO₂ production at low temperatures. As a result, cumulative respiration during storage was increased. When the storage temperature was varied, total CO₂ production increased with the amount of time spent below 2°C. Similar results were calculated for broccoli treated with the
cytokinin BAP (6-benzylaminopurine) at 20°C (Irving and Baird, 1996). These results support the observation that senescence occurs in a different way when storage conditions are used which inhibit chlorophyll degradation.

2.3.4. Respiration Life of Other Vegetables

Unlike the vegetables discussed so far, many fruit vegetables and storage organs have substantial energy reserves at harvest. In this context, storage organs include tubers, bulbs, and roots. These are essentially nutrient stores as well as reproductive structures (Wills et al., 1998). They are therefore "functionally self sufficient", containing large reserves of starch, fructans, and other carbohydrates. These reserves are further conserved by low respiration rates (Morris and Pogson, 200-). Root vegetables and swollen hypocotyls such as carrots and radishes do not have the same barriers to moisture loss as tubers and bulbs. However, all these plant parts can be stored for extended periods under suitable conditions (Morris and Pogson, 200-).

Potato respiration rates vary as the tubers develop. Mature potatoes respire more slowly than young tubers (Kader, 1987), this decrease being up to 90% compared to initial rates (Morris and Pogson, 200-). For example, potatoes that were harvested in July had a respiration rate of 8.6 mg CO₂ kg⁻¹ h⁻¹. Potatoes from the same planting that were harvested two months later respired more slowly, at around 2.9 mg CO₂ kg⁻¹ h⁻¹ (Peterson et al., 1981). Respiration rate also increased in response to injury. It was concluded that measurements of respiration were a good indicator of potato maturity and injury levels, and that these were important determinants of storability (Peterson et al., 1981).

Many fruit vegetables are harvested at an immature stage, and have relatively short storage lives. A study on Asian vegetables found that those that respired most rapidly also lost quality most rapidly (Zong et al., 1992). Using the data of Zong et al., 1992, I estimated the days taken until quality fell to poor (grade 3) for four Asian vegetables at different temperatures. Multiplying this figure by the relevant mean respiration rate gave the approximate CO₂ production during storage (Table 2.3.1.). Total CO₂ production varied between products, and was reduced at temperatures that caused chilling injury. For example, total respiration by yard long beans during storage was 803-820 mmol kg⁻¹ at 12.5 - 20°C, but 589 mmol kg⁻¹ at 10°C. This suggests that calculation of total respiration may show whether storage life has been ended prematurely by chilling damage.

Respiration was not a good indicator of storage life for capsicums coated with a range of emulsions. Dipping capsicums in a mineral oil or cellulose based coating reduced textural changes and retarded weight loss. However, respiration was not significantly affected by the treatments
Total respiration must therefore have been higher in the coated fruit. Part of the variability in both this study and that of Zong et al. (1992) may be due to the use of subjective, visual scales, leading to variability in measurements of storage life.

2.3.5. Respiration Life of Climacteric Fruit

Climacteric fruit are defined by the autocatalytic induction of ethylene that is associated with ripening (Burg and Burg, 1962). Ethylene stimulates respiration, so the ethylene climacteric is accompanied by a respiratory climacteric. Fruit contain large amounts of carbohydrates as sugars as well as starch and sometimes lipids (Wills et al., 1998). Respiration is therefore unlikely to be limited by insufficient energy. Depending on the magnitude and duration of the climacteric, respiration stimulated by ethylene may account for a substantial portion of the total postharvest respiration. Given the variability of the climacteric and a non-limiting carbohydrate supply, it might be expected that total respiration is less likely to remain constant in climacteric fruit than in the commodities already examined.

Respiration rates of different apple cultivars can reflect their storability. Long-lived cultivars such as "Granny Smith" and "Red Delicious" have lower respiration rates than shorter storing cultivars such as "Anna" and "Gala" (UC Davis Product Fact Sheets, 2000). Heat treatments (Klein and Lurie, 1990), surface coatings (Saftner, 1999) and diphenylamine (DPA) dipping (Lurie et al., 1989) reduce respiration rates of apples and enhance retention of firmness and color. I analysed the measurements presented by Saftner (1999) for "Gala" and "Golden Delicious" apples by linear regression so as to calculate the number of days until firmness decreased by 15 N from the values at harvest. These figures were combined with respiration data to calculate total CO₂ production during storage. The results ranged from 0.8 – 1.1 mol.kg⁻¹ for "Gala" apples that were untreated, coated with shellac, or coated with a fruit wax. This was different to results for "Golden Delicious" in which total CO₂ production was tripled in the shellac treated fruit compared to the other two treatments (Table 2.3.1.). Such differences between cultivars are intriguing.

Unlike apples, European pears undergo distinct changes in colour and firmness during ripening. These changes can be significantly reduced by treatments that also restrict respiration. For example, 12 kPa CO₂ significantly inhibited pear softening and reduced respiration rates during ripening (Wang and Mellenthin, 1975), as did reducing O₂ to 1-0.5 kPa (Ke et al., 1990). Similar treatments have been studied using cultured pear cells (Brady and Romani, 1988). Respiration rate was monitored while reducing the O₂ concentration, adding ethylene, or changing the availability of carbohydrates in the growing media. An excellent correlation was found between respiration rate and cell viability (Brady and Romani, 1988).
Like apples and pears, kiwifruits can be stored for extended periods if ripening is inhibited. A good correlation between the rate of softening and CO$_2$ production has been shown for kiwifruits pre-treated with ethylene (Ritenour et al., 1999) as well as for different varieties (Dexing et al., 1991, Manolopoulou and Papadopoulou, 1997). As described for apples, I was able to use the data of Manolopoulou and Papadopoulou, (1997) to estimate the storage lives of four kiwifruit cultivars. Polynomial equations were fitted to firmness measurements to estimate the days taken until the fruit fully softened (5 lb pressure). Cumulative respiration was calculated from the area under the CO$_2$ production curve up to this point. Storage life ranged from 54 to 120 days. However, total CO$_2$ production during storage was relatively constant, being between 220 to 270 mmol.kg$^{-1}$ for the varieties tested.

Total pre-climacteric O$_2$ consumption was reported to vary among bananas held in different atmospheres at 20°C (McGlasson and Wills, 1971). However, when I recalculated this figure to include O$_2$ consumption during the climacteric, the total respiration varied only slightly between the treatments. A study of banana ripening by Esguerra et al., (1992) showed that ‘Senora’ bananas took between 4 and 11 days to ripen to peel colour 4 at different temperatures. Results from my calculations of cumulative respiration from this data were consistent between the treatments, and similar to those extrapolated from McGlasson and Wills (1971). Even though the time to ripeness varied from 4 days to 140 days in these two studies, all the bananas produced between 400 - 500 mmol.kg$^{-1}$ of CO$_2$ during storage and ripening.

Like bananas, tomatoes undergo substantial changes during ripening. Chlorophyll is degraded and β-carotene and lycopene are synthesised, changing the skin colour from green to red. Cell walls are partly broken down, resulting in softening (Grierson, 1987). Using the data of Ghaouth et al. (1992) it can be estimated that tomatoes dipped in water, 1% chitosan or 2% chitosan took 16, 21 and 24 days respectively to reach grade 4 (two-thirds pink, U.S. Dept of Ag. Visual Standards) at 20°C. By summing the area under the CO$_2$ evolution graph for the same time interval, I calculated the total respiration for this degree of ripening. The results were similar between treatments, being 212-241 mmol.kg$^{-1}$ (Table 2.3.1.). Storage atmospheres containing low O$_2$ concentrations can also delay ripening and reduce tomato respiration rates (Louie and Lee, 1989). Furthermore, ripening of tomato fruit can be predicted from storage temperature (Thorne and Alvarez, 1982). These results suggest that respiration rate may be a good indicator of the storage life of tomatoes.

My analysis of other studies indicated that cumulative respiration during storage remains approximately constant for a number of other climacteric fruit. For example one study found that guava fruit were affected by their orientation during storage. Guavas placed with the pedicel upwards retained quality longer and had lower respiration rates than fruit placed upside-down or
sideways (Siddiqui et al., 1991). This study recommended that an organoleptic rating of 5 was unacceptable. Fitting polynomial equations to the data allowed calculation of the number of days until quality reached grade 5. As previously, this was combined with the relevant respiration data to calculate total CO₂ production. Again, cumulative respiration was similar between the treatments, being 233 - 243 mmol kg⁻¹.

Avocado fruit gave a similar result. Total respiration was calculated from data presented by Wills and Tirmazi (1982), who had extended storage life by around 50% using calcium chloride dips. The area under the O₂ consumption graph was summed up to the point indicating that the fruit were ripe. Total O₂ consumption between harvest and full ripeness was similar between the treatments, being 591 - 626 mmol kg⁻¹.

Custard apples provided a third example. The storage life of the fruit was calculated from the data of Alique and Oliveira (1994) by estimating the time taken until firmness decreased from 185 N to 60 N. Total CO₂ production during storage under various controlled atmospheres was calculated as previously and found to be 177 - 227 mmol kg⁻¹.

From the studies assessed, it seems possible that cumulative respiration is constant for climacteric fruit under different storage conditions. This is an unexpected result, given the respiratory upsurge in response to ethylene production during the climacteric. Although the significance of these similarities cannot be assessed without a measure of variability, the results suggest that further research on climacteric fruit could be worthwhile.

2.3.6. Respiration Life of Non-Climacteric Fruit

In general, the respiration rates of non-climacteric fruit gradually decline during maturation and ripening (Wills et al., 1998). Modifications associated with ripening such as changes in colour, soluble solids, texture and aroma are often more gradual in non-climacteric than climacteric fruit. In climacteric fruit, the end of marketable life is often determined by excessive softening or colour changes associated with ripening (Grierson, 1987, Sumnu and Bayindirli, 1995). For non-climacteric fruit, storage life is more likely to be terminated by rots (Swanson and Weckel, 1975, Robbins and Fellman, 1993, Yahia et al., 1993, Sekse, 1996, Baka et al., 1999) or by moisture loss (Nagar, 1994, Bayindirli et al., 1995). This may make the end of storage life more variable and difficult to determine than in climacteric fruit.

The susceptibility of fruit to decay is influenced by many factors. On the one hand, the loss of the ability to maintain homeostasis and resist pathogen growth can be a sign of senescence (Romani,
1987). However, interactions between fruit, the environment, and micro-organisms are complex, and decay may not be explicable in terms of metabolic activity alone. Other factors that will increase the growth of pathogens include:

- heavy initial microbial loads due to growing or packing conditions,
- fruit maturity at harvest,
- free moisture or high humidity during storage,
- poor temperature control,
- damage during harvest and transport,
- delay in application of fungicides
- the pH of the fruit tissue.

Low temperatures restrict the growth of some organisms more than others. For example, *Rhizopus* is unable to grow below 4.5°C, whereas *Penicillium* and *Botrytis* will continue to grow slowly at temperatures as low as 0°C.

As a result of fungal growth, storage life of some fruit is likely to be strongly influenced by factors other than the metabolism of the fruit itself. For example, the storage life of grapes in different controlled atmospheres can depend on the fungicide used, respiration rate being less important (Yahia et al., 1983). Cranberries provide another example of this behavior, the early onset of rots meaning that the storage life of these fruit is unrelated to their respiration rate (Swanson and Weckel, 1975). This could be expected when dealing with an aggressive pathogen, a susceptible product, or storage conditions that favor growth of the pathogen over metabolic activity by the fruit.

Published work suggests that a relationship between storage life and respiration may exist for some non-climacteric fruit. Raspberry cultivars that respired rapidly at harvest were softer and developed rots sooner than cultivars that had a slower respiration rate (Robbins et al., 1989). Total CO₂ production was used to estimate storage potential in sweet cherries (Sekse, 1988, Sekse, 1996). In this case, total CO₂ evolved before 5% of the fruit developed rots was found to be constant at temperatures between 0-12°C, although it was higher at 20°C. Respiration rate was found to be correlated with decay of strawberries exposed to UV light and stored at 4°C or 13°C (Baka et al., 1999). Using the presented data, I estimated the time taken until 10% of fruit had fungal decay and used this to calculate cumulative CO₂ production. Total respiration was 38 – 56 mmol.kg⁻¹, a much lower range than that calculated for other non-climacteric fruit.

However, the respiration rate of some other non-climacteric fruit may be unrelated to storage life. Using the data of Bayindirli et al. (1995), I calculated total respiration during storage for mandarins dipped in different coatings and stored at 20°C. In this case, the end of storage life was estimated as the time until 15% of initial weight was lost. Although skin coatings reduced both water loss and
respiration rate, total respiration during storage varied from 697 mmol.kg\(^{-1}\) to 359 mmol.kg\(^{-1}\). However, weight loss is more strongly affected by the partial pressure difference in water vapour than by metabolic activity, so this may not have been a valid method of measuring senescence.

Storage life of loquats was estimated from published results of Ding et al., (1998), the end of life being when 10% of fruit showed evidence of rots (as for strawberries). Total respiration was estimated from the area under the CO\(_2\) production curve. Cumulative respiration increased from 229 – 602 mmol.kg\(^{-1}\) as the storage temperature rose from 1°C to 20°C. This suggests that increases in temperature had a greater effect on respiration rate than on the time until onset of decay.

Sekse (1996) used his recorded data on respiration and storage life to estimate total CO\(_2\) production by cherries during different storage and transport procedures. It was concluded that cherries transported by the ordinary transport route would have less than one day of shelf life remaining when they reached the point of sale (Sekse, 1996). The accuracy of this prediction is likely to be affected by other storage conditions such as humidity, condensation, microbial load and the maturity of the fruit at harvest. Nevertheless, this study demonstrates that information about total respiration during storage can be used to assess the effectiveness of different storage conditions.
Table 2.3.1. - Total respiration (mmol.kg⁻¹) between harvest and the end of storage life for various commodities; data extrapolated from published papers. Total respiration calculated from the area under the curve on graphs or recorded mean rate, storage life estimated from changes in a given quality parameter.

<table>
<thead>
<tr>
<th>Reference</th>
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<th>Treatment</th>
<th>Total Resp. mmol.kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vegetables</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brash et al., 1995</td>
<td>Asparagus</td>
<td>0°C then 20°C</td>
<td>564</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5°C then 20°C</td>
<td>564</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10°C then 20°C</td>
<td>562</td>
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<td></td>
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<td>15°C then 20°C</td>
<td>549</td>
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<tr>
<td></td>
<td></td>
<td>20°C then 20°C</td>
<td>582</td>
</tr>
<tr>
<td>Klieber and Willis, 1991</td>
<td>Broccoli</td>
<td>continuously at 20°C</td>
<td>303</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 weeks at 0°C, then 20°C</td>
<td>461</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 weeks at 0°C, then 20°C</td>
<td>641</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 weeks at 0°C, then 20°C</td>
<td>738</td>
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<td>8 weeks at 0°C, then 20°C</td>
<td>834</td>
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<td></td>
<td>2 weeks at 1.5°C, then 20°C</td>
<td>405</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 weeks at 1.5°C, then 20°C</td>
<td>587</td>
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<tr>
<td></td>
<td></td>
<td>6 weeks at 1.5°C, then 20°C</td>
<td>598</td>
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<td>8 weeks at 1.5°C, then 20°C</td>
<td>778</td>
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<tr>
<td>Klieber and Willis, 1991</td>
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<td></td>
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<td></td>
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<td>20°C</td>
<td>224</td>
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<td>Broccoli</td>
<td>cv green beauty at 20°C</td>
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<td>cv shogun at 20°C</td>
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<td></td>
<td></td>
<td>with propylene at 20°C</td>
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<td></td>
<td></td>
<td>with BAP at 20°C</td>
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</tr>
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<td>5°C</td>
<td>500</td>
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<td></td>
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<td>10°C</td>
<td>459</td>
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<tr>
<td></td>
<td></td>
<td>15°C</td>
<td>420</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20°C</td>
<td>507</td>
</tr>
<tr>
<td>Pratt et al., 1954</td>
<td>Lettuce</td>
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<td>93</td>
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<tr>
<td></td>
<td></td>
<td>5°C</td>
<td>148</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10°C</td>
<td>190</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15°C</td>
<td>145</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20°C</td>
<td>168</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25°C</td>
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</tr>
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</tr>
<tr>
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<td>------------------</td>
<td>-----------</td>
<td>-------------</td>
</tr>
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<td>Zong et al., 1992</td>
<td>Bitter Melon</td>
<td>10°C</td>
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<tr>
<td></td>
<td></td>
<td>12.5°C</td>
<td>369</td>
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<tr>
<td></td>
<td></td>
<td>15°C</td>
<td>384</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20°C</td>
<td>305</td>
</tr>
<tr>
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<td>Fuzzy Melon</td>
<td>10°C</td>
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<td>164</td>
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<td></td>
<td></td>
<td>15°C</td>
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<tr>
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<td>20°C</td>
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<tr>
<td></td>
<td>Luffa</td>
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<tr>
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<td>20°C</td>
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<tr>
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<td>Yard Long Bean</td>
<td>10°C</td>
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<td>12.5°C</td>
<td>872</td>
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<td>15°C</td>
<td>923</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20°C</td>
<td>803</td>
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**Climacteric Fruit**

<table>
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<th>Reference</th>
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<th>Treatment</th>
<th>Total Resp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saffner, 1999</td>
<td>Apple - Golden</td>
<td>control at 0°C</td>
<td>640</td>
</tr>
<tr>
<td></td>
<td>Delicious</td>
<td>shellac at 0°C</td>
<td>2,087</td>
</tr>
<tr>
<td></td>
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<td>waxed at 0°C</td>
<td>824</td>
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<td></td>
<td>Apple - Gala</td>
<td>control at 0°C</td>
<td>1,001</td>
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<tr>
<td></td>
<td></td>
<td>shellac at 0°C</td>
<td>1,096</td>
</tr>
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<td></td>
<td></td>
<td>waxed at 0°C</td>
<td>851</td>
</tr>
<tr>
<td>Willis and Tirmazi, 1982</td>
<td>Avocado</td>
<td>control at 20°C</td>
<td>591</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2% CaCl₂ at 20°C</td>
<td>612</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4% CaCl₂ at 20°C</td>
<td>626</td>
</tr>
<tr>
<td>McGlasson and Willis, 1971</td>
<td>Banana</td>
<td>air at 20°C</td>
<td>360</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5% CO₂, 20% O₂ at 20°C</td>
<td>400</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0% CO₂, 3% O₂ at 20°C</td>
<td>520</td>
</tr>
<tr>
<td>Eguerra et al., 1992</td>
<td>Banana</td>
<td>15°C</td>
<td>430</td>
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<tr>
<td></td>
<td></td>
<td>20°C</td>
<td>496</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25°C</td>
<td>404</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30°C</td>
<td>412</td>
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<td>Alique and Oliveira, 1994</td>
<td>Custard Apple</td>
<td>air at 9°C</td>
<td>177</td>
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<td></td>
<td></td>
<td>3% O₂ at 9°C</td>
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<td>3% O₂, 3% CO₂ at 9°C</td>
<td>227</td>
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<td>3% O₂, 6% CO₂ at 9°C</td>
<td>193</td>
</tr>
<tr>
<td>Siddiqui et al., 1991</td>
<td>Guava</td>
<td>Upright position at 25°C</td>
<td>234</td>
</tr>
<tr>
<td></td>
<td></td>
<td>upside-down position at 25°C</td>
<td>233</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sideways position at 25°C</td>
<td>243</td>
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<tr>
<td>Manolopoulou and Papadopoulou, 1997</td>
<td>Kiwfruit</td>
<td>cv Hayward at 0°C</td>
<td>254</td>
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<tr>
<td></td>
<td></td>
<td>cv Allison at 0°C</td>
<td>230</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cv Monty at 0°C</td>
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<tr>
<td></td>
<td></td>
<td>cv Bruno at 0°C</td>
<td>269</td>
</tr>
<tr>
<td>E Ghaouche et al., 1992</td>
<td>Tomato</td>
<td>control at 20°C</td>
<td>212</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1% chitosan at 20°C</td>
<td>239</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2% chitosan at 20°C</td>
<td>241</td>
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</table>
In addition to the points already discussed, the results presented in Table 2.3.1. give rise to a number of general observations. I had expected that total respiration might be increased at low temperatures due to greatly reduced rates of decay and water loss as well as reduced sensitivity to ethylene. This effect was indeed observed with broccoli. However, a number of other products including lettuce, strawberries and loquats gave the opposite result. For these products, total respiration was greatest at the highest storage temperature. This suggests that the effect of low temperature storage on respiration was greater than the accompanying effect on storage life for these highly perishable products.

In general, the data suggested that climacteric fruit are more likely to have a constant "respiration life" than are non-climacteric fruit. This was unexpected, as the respiration climacteric occurs in response to ethylene rather than being a function of metabolic activity (Romani, 1984). It might therefore be expected that cumulative respiration during the climacteric would not be related to the rate of senescence. However, adding CO₂ production during the climacteric to pre-climacteric banana respiration more or less eliminated the differences in total respiration reported by McGlasson and Wills (1971). Other climacteric fruit gave similar results. In contrast, total respiration during storage was not constant for the non-climacteric fruit for which I analysed data. One reason for this may be that the end of storage life of these fruit was determined using subjective scales or the onset of disease. Both of these methods may increase the variability of the results.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Product</th>
<th>Treatment</th>
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<tbody>
<tr>
<td>Non-Climacteric Fruit</td>
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<tr>
<td>Sakse, 1988</td>
<td>Sweet Cherry</td>
<td>0°C</td>
<td>170</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4°C</td>
<td>181</td>
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<td>190</td>
</tr>
<tr>
<td></td>
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<td>12°C</td>
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<td></td>
<td></td>
<td>20°C</td>
<td>248</td>
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<td>Ding, et al., 1995</td>
<td>Loquat</td>
<td>1°C</td>
<td>229</td>
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<td></td>
<td></td>
<td>5°C</td>
<td>281</td>
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<td></td>
<td></td>
<td>10°C</td>
<td>358</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20°C</td>
<td>602</td>
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<tr>
<td>Bayindirli et al., 1995</td>
<td>Mandarin</td>
<td>Control at 20°C</td>
<td>697</td>
</tr>
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<td></td>
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<td>Semperfresh at 20°C</td>
<td>359</td>
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<td></td>
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<td>Jonfresh at 20°C</td>
<td>516</td>
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<tr>
<td>Baka, et al., 1999</td>
<td>Strawberry</td>
<td>4°C</td>
<td>46</td>
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<td>4°C + 0.25 kJ/m² UV-C</td>
<td>40</td>
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<td>4°C + 1 kJ/m² UV-C</td>
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<td>13°C</td>
<td>54</td>
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<td>13°C + 0.25 kJ/m² UV-C</td>
<td>56</td>
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<td></td>
<td></td>
<td>13°C + 1 kJ/m² UV-C</td>
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</table>
Chapter 2 - Literature Review, Respiration Life

It might have been expected that total respiration by vegetables with smaller carbohydrate reserves would be less than fruit that have greater reserves of sugars at harvest. However, this was not the case. For example, the total respiration of asparagus was approximately 560 mmol kg\(^{-1}\), compared to around 400 mmol kg\(^{-1}\) for bananas and 600 mmol kg\(^{-1}\) for avocados. In other words, the initial content of carbohydrate reserves seems not to correlate with the respirable content of carbohydrates or lipids.

A final observation is that total respiration was between 200 – 500 mmol kg\(^{-1}\) for the majority of the products examined. The published data used to compile this table is likely to be affected by factors such as different produce maturities and growing conditions, the criteria used to assess storage life, and the gap between harvest and the start of measurements. Given these sources of variation, the data would appear to be remarkably consistent.

In general, the results from this examination of the literature suggest that the relationship between respiration and storage life is poorly defined for most products. The large variations in results from different sources and the differences between climacteric and non-climacteric fruit are intriguing. The next chapters of this thesis therefore examine methods of measuring respiration life, and calculate total \(O_2\) consumption for a range of different horticultural products.
3
Development and Application of the Respirometer

3.1. Methods of measuring respiration

Methods used to measure respiration rates of fresh produce have changed greatly in the last 70 years. Even today, methods vary considerably among researchers. Gas exchange may be measured indirectly from chemical reactions or pressure changes, or directly using one of the many different types of gas sensor that are commercially available. Systems may be manual, automated, or a combination of both, with measurements at intervals of seconds, hours or even weeks. Devices to measure respiration have been developed to measure all types of plant materials from pollen grains to whole trees. Given the abundance of techniques already available, the development of another method would appear unnecessary.

Each system of measuring respiration has a number of advantages and disadvantages. These are described in the following sections and are summarised in Appendix 4. For example, the main advantage of a static system is that it is easy, reliable and accurate. However, the flow-through system has the advantage of being more easily automated. In considering the best method to use to measure respiration, I began to consider whether there could be some way of combining these systems. The aim was to create a device that could monitor changes in respiration continuously over long periods of time. However, the system also needed to be simple, reliable and reasonably inexpensive. How this was achieved is described in this chapter.

3.1.1. Early Methods of Determining Respiration

One of the first methods used was the Claypool-Keefer method. The sample being measured was placed in a sealed vessel. Air was flushed through this vessel at a constant, known velocity, then passed through a dilute solution of sodium bicarbonate. Ionisation of the H₂O and CO₂ to HCO₃⁻ and H⁺ changed the pH of the solution. By adding a suitable indicator, such as bromphenol blue, changes in pH could be determined colourimetrically. The amount of CO₂ that had been reacted with the solution was calculated by comparison with a calibration curve prepared with known CO₂
concentrations (Claypool and Keefer, 1942, Watada and Pratt, 1960). Using a flow board, this method could be adapted to measure multiple samples at the same time.

Another way of measuring CO₂ production was with a Warburg manometer. The sample was placed in a small vessel next to a vial or a central well containing a CO₂ absorbant, usually a solution of NaOH. To maintain constant temperature, the container was immersed in a water bath. The main opening of the vessel was sealed, and a smaller outlet pipe connected to a narrow column of fluid. As CO₂ produced by the plant sample was absorbed, the pressure inside the vessel fell, and a corresponding volume of indicator fluid was drawn up the column.

Respiration rate was therefore indicated by changes in the height of the fluid over time (Umbreit et al., 1964). Manometers may still be used for analysing respiration rates of small samples, being simple to operate and accurate over limited time periods. However, both manometry and the Claypool Keefer method are limited by the need for an operator to be present, and cannot easily be used to indicate changes in respiration rate.

3.1.2. Flow-through systems

The most commonly used method of measuring respiration of fruit and vegetables has been a flow-through system. A gas stream is passed through a sealed container of fruit or vegetables at a constant rate. As the initial O₂ and/or CO₂ concentrations in the gas supply are known, these can be compared to those in the gas stream leaving the container. The changes in O₂ and CO₂ indicate the respiration rate of the produce enclosed (Dilley and Dewey, 1969, Lee et al., 1991, Peppelenbos et al., 1996).

There are a number of advantages and disadvantages involved with the use of flow-through systems. As it is easy to connect many containers to a gas stream, a large number of samples may be measured at once. Also, any combination of gases may be used, allowing an infinite range of possible atmospheres (Smith et al., 1997). Leshuk and Saltveit (1990) designed a system which gradually reduced the O₂ concentration in the gas stream. This allowed accurate determination of the anaerobic compensation point for some products. Flow-through systems may be designed which are relatively cheap and easy to use (Piergiovanni et al., 1999). More complex systems may include automatic sampling of the O₂ and CO₂ concentrations in the gas efflux, allowing measurements at regular intervals (Dilley and Dewey, 1969, Bohling and Bauer, 1993, Smith et al., 1997).

However, there are a number of systematic problems that are associated with the use of flow-through systems. Flow rate must be measured accurately as even small changes can lead to
relatively large changes in apparent respiration rate. These errors are systematic, and are not easily estimated. The rate of flow is also critical in terms of allowing a measurable change in the gas mixture leaving the container. If the flow rate is too fast, the changes in composition in the gas leaving the container will not be detectable. However, too slow a flow rate may result in changes to the atmosphere inside the container that significantly affect respiration rate. In some cases it may be necessary to alter the flow rate used during storage of some products. For example, the respiration rate of bananas may increase sixfold during ripening (Brady et al., 1970). This could make it necessary to increase in the ventilation rate to avoid excess CO₂ within the container.

Another difficulty may occur because the small changes occurring in O₂ are difficult to measure against the large background concentration in air. For this reason it is often easier to measure CO₂ production, as the concentration of CO₂ is naturally low. However, CO₂ is a by-product of oxidative metabolism. For CO₂ production to be a true indicator of respiration, it must be assumed that the respiratory quotient (RQ) = 1. As substrates other than glucose are respired by many fruits and vegetables (Talz and Zeiger, 1991), O₂ consumption may be a better indicator of metabolic activity than CO₂ production.

Additionally, the flow of gas around the produce sweeps away ethylene and other volatiles that could influence the produce respiration rate. This is different to the conditions generally used during storage, when rates of ventilation used may be lower.

### 3.1.3. Static systems

In static systems the produce is sealed inside a container. Respiration depletes O₂ from inside the vessel while increasing the concentration of CO₂. After a suitable time interval the changes in these gases may be measured by gas chromatography (Shiomi et al., 1996). Sealable containers can be used as a quick, easy way to gain a single rather than a continuous measurement of respiration rate. This method may be useful for measurements taken at intervals during storage, or where a large number of individual fruits or vegetables need to be measured at the same time. However, the time interval used is critical. As with flow-through systems, too short a time interval may not allow a measurable modification of the atmosphere, while too long a time interval may result in conditions which affect respiration. Furthermore, this method is not suitable for measuring respiration rates in atmospheres other than air.

Automatic systems have been devised by a number of researchers. Either the O₂ (Bosset et al., 1982) or the CO₂ (Forcier et al., 1987, Andrich et al., 1993, Varoquaux et al., 1999).
concentrations inside the container are monitored by gas chromatography. When the gas concentrations exceed set limits or a set time limit elapses, the container air is flushed through a reactant such as sodium hydroxide to absorb the evolved CO₂. Pure O₂ is then injected to equalise the container pressure. Respiration may be calculated from the rate of change inside the container (Varoquaux et al., 1999), the change in pressure after CO₂ removal (Forcier et al., 1987) or the amount of additional O₂ required (Bosset et al., 1982, Andrich et al., 1993). These systems are generally more complex than flow-through systems. They can be difficult to use, rely on highly accurate measuring equipment, and may be affected by cumulative small leaks from the many valves required (Varoquaux, pers. comm.).

Permeable containers may also be used for respiration rate measurements as long as their permeance to O₂ and CO₂ are known. Sealed plastic bags have been used to measure respiration rate under different gas concentrations. Once the package reaches a steady state the rate of permeation through the film will be equal to the rate of respiration of the produce. As long as the area and permeability of the film are known, respiration rate can be calculated by measuring the partial pressure difference between the package and the air. This method also allows easy measurement of the respiratory quotient. A range of atmospheres can be generated by varying either the size of the packages or the volume of produce inside (Beaudry et al., 1992, McGlasson, 1992, Chau and Talasila, 1995, Christie et al., 1995).

Using permeable packages to measure respiration rate is simple, cheap, and reflects the conditions experienced in a modified atmosphere package. When ample time (of the order of a week) can be allowed for the packages to reach a steady state, it can provide useful information. In particular, information on respiration rate may be gathered for a wide range of different atmospheres (Beaudry et al., 1992, Cameron et al., 1995).

However, information on respiratory behavior is often required more urgently, and results may be affected by a number of different factors. Changes in produce respiration rate cannot be readily measured, and are likely to confuse the results. Condensation inside the packages may not only increase the incidence of disease, but also affect gas permeation through the film. Film permeability may also vary between, and even within, batches, potentially leading to systematic errors. Leaks in packages can also confuse the results. Using permeable packages to measure respiration rate therefore has limited applications.

The remainder of this chapter describes how a new respirometer system was designed and built, illustrates its practical use, and discusses the advantages and disadvantages of this method.
3.2. Development of a Respirometer

3.2.1. Introduction and Aims

The ideal respirometer for measuring the respiration rates of fresh produce would be reliable, accurate, and able to measure a wide range of produce types, whether large or small, and respiring fast or slow. It also needed to be reliable, simple to set up and use, and be repairable without specialised skills, equipment or materials. An ideal respirometer should also be sufficiently inexpensive that at least six vessels containing samples could be measured simultaneously. If possible, it should measure both O₂ consumption and CO₂ production. Other attractive features might be portability, particularly if the device could be taken into the field, and that recorded should be easily transferable into graphing or statistical analysis programs.

The initial design of the respirometer involved using a sealed container equipped with sensors so that the atmosphere inside could be measured automatically. Initially, I tried using systems that calculated how much air need to be added in order to maintain the atmosphere inside the vessel. These required very accurate monitoring of the air input, as well as rapid mixing of the internal atmosphere. One option used a mass flow meter, another involved peristaltic pumps. However, this method proved complex and unreliable, and setting up multiple systems would have been expensive.

To overcome this difficulty, I decided to allow the atmosphere to fluctuate inside each container. It could be refreshed either after a set time interval, or when the gas concentrations reached a predetermined concentration. By eliminating the need to regulate flow rate, this method proved much simpler and more reliable than the first option. Initially, I planned to refresh the containers using compressed air or a gas mixture. While this method worked reasonably well, there was considerable risk of leaks from the solenoid valves allowing air from the cylinder into the respirometer containers. Also, it was difficult to maintain pressure and avoid leaks from the gas cylinder itself. Using separate pumps for each container eliminated these problems. This method of refreshing the atmosphere proved most effective, and is the basis of the method described in the remainder of this chapter.
3.2.2. Materials and Methods

3.2.2.1. Respirometer Construction

The main part of the respirometer can be constructed from any sealable container. The initial design used glass jars. While these could be made gas tight, it was difficult to fit the lids without using large amounts of sealant. I decided it would be better to use a container with a lip at the top so as to allow the lid to be clamped on. Plastic ( polymethylacrylate) goldfish bowls with a wide lip around the rim were therefore used. They are inexpensive, readily available, and a lid can be easily made from a flat acrylic sheet. Holes can be drilled in the lid to allow insertion of the sensors as well as the air inlet and outlet tubing (Figure 3.01).

*Figure 3.01 – Configuration of a respirometer used for measuring the rates of O₂ consumption and CO₂ production by ripening bananas.*
Other static systems have used a gas chromatograph (GC) to periodically check the gas concentrations inside sealed containers. This can introduce potential errors. For example, a significant volume of air may be contained in the tubing, and pressure will be reduced if the sample is not completely returned to the container. Using a GC also means that all the containers cannot be checked simultaneously, as at least 5 minutes is commonly required to analyse each sample. If eight respirometers are in use, this will increase the sampling interval to 40 minutes. Such a long time interval between readings could result in significant modification of the internal atmosphere. For the respirometer to work, the atmosphere inside each container needs to be continually monitored.

Various gas sensors could be used without changing the general principle of the method. In this case I used KE-25 model O₂ sensors manufactured by GS Japan Storage Battery Co. Ltd., Osaka, Japan. These sensors combine a lead anode with a gold coated cathode. Oxygen molecules diffuse through a membrane and are reduced at the gold electrode (Figaro Engineering Inc., Japan, product specifications). The small current created by this reaction is proportional to the O₂ concentration, reaching approximately 12mV in 21kPa O₂. To test whether O₂ consumption by the sensor would affect readings, one of the units was sealed with foil and monitored for several days. However, the decrease in internal O₂ was less than fluctuations due to electronic noise. O₂ consumption by the sensor was therefore concluded to be insignificant within the experimental context.

KE-25 O₂ sensors operate by diffusion, so can be mounted directly onto each respirometer without the need for a pump. They also do not require an external power supply, do not give off heat and have internal temperature compensation. Their response time is stated to be around 12 seconds and, although it may take longer than this for a stable reading when large changes in O₂ occur, this is fast enough for the current purpose. The sensors are also inexpensive, costing around A$80 each. Their primary disadvantages are that the output is low and affected by background noise, and that the calibration can change significantly over time. As they measure the partial pressure of O₂ rather than the concentration, the sensors are affected by changes in atmospheric pressure (Figure 3.02). This means that regular recalibration is required, particularly if there is a cool change in the weather. However, overall they proved an affordable and reliable method for measuring O₂.
Figure 3.02 - Effect of changes in atmospheric pressure (— ) on the output of a KE-25 O₂ sensor (----- )

CO₂ can be effectively measured by non-dispersive infrared (NDIR) detection. This is possible because CO₂ molecules absorb light at the specific wavelength of 4.26 µm. NDIR sensors consist of an infrared source, a sampling space for gas, an optical filter and a detector unit (Figure 3.03). As the CO₂ concentration within the sampling space increases, more of the light emitted by the source is absorbed before reaching the detector. The voltage output is therefore \( V = k (v_s - v_o) \), where \( v_s \) is the detector signal with the sample, \( v_o \) is the detector signal when the cell is filled with N₂ and \( k \) is a constant. This output voltage is a non-linear function of the gas concentration (http://www.eas.ualberta.ca/profs/jwilson/eas327/eas327u4.html). In some sensors, therefore, the signal is linearized according to Beer's Law

\[
V_s = V_o e^{-kP}
\]

Where \( P \) is the CO₂ concentration (www.tsi.com/his/homepage/appnote/ndir_co2.htm).

Figure 3.03 - Diagram of NDIR sensor (Adapted from Vaisala single beam NDIR CO₂ sensor specifications).
Initially I used a CO₂ sensor for enclosures model WA 456-B, manufactured by the Analytical Development Co., Ltd., England. This device was calibrated against standard gases (0, 5.6 and 9.8 kPa CO₂) and was found to be linear. However, experiments monitoring gas concentrations inside sealed containers of mushrooms found fluctuations in the apparent rate of CO₂ production (Figure 3.04). These occurred at the same O₂ concentrations regardless of the temperature. As O₂ consumption did not display these fluctuations it seemed likely that the irregularities were due to the linearising function built into the CO₂ sensor. To test this theory the CO₂ sensor was placed inside an incompletely sealed container flushed with 9.6 kPa CO₂. The rate of leakage had the same irregularities as found previously, demonstrating that the effect was due to equipment error.

As previously stated, absorbance at the 4.26 µm wavelength is a non-linear function of CO₂ concentration. While this relationship can be approximately linearised by a Beer’s Law Plot, it may vary significantly from linear over the relatively large range of 0-10 kPa. It seems probable that the voltage output had been internally converted into a series of linear regression lines. The variation between the actual CO₂ concentration and that output by the sensor may not have been significant under other circumstances. However, differentiating the output to calculate the rate of change lead to small irregularities being expressed as significant peaks and troughs.

![Figure 3.04](image)

*Figure 3.04 - Fluctuations in the apparent rate of CO₂ production (○○○) compared to O₂ consumption (●●●) by mushrooms inside a sealed vessel. Fluctuations in CO₂ production were due to imprecise linearisation of the sensor.*

I decided it would be better to use a non-linearised CO₂ sensor. The GMM 12C non-linear sensor manufactured by Vaisala, Helsinki, Finland was suitable. This sensor has a maximum output of
V at 10KPa CO\(_2\). The Vaisala sensor was compact and reliable, and did not require the complicated wiring of the ADC sensor. At just under A$1,000, the sensor was considerably more expensive than the O\(_2\) sensors, a factor which prohibited mounting one on every respirometer. However, two units were purchased for use in checking RQ.

Unlike the ADC sensor, which relies on diffusion, the Vaisala sensor requires air to be pumped through the cell. The device was externally mounted, and air from the container was circulated through the sensor using a small pump. It was extremely important to avoid leaks in the recirculating system as it contained a positive pressure. I tested several diaphragm pumps, but was unable to completely seal the units. Instead, I used small peristaltic pumps with a flow rate of around 100ml/min. The pumps were connected to the respirometer vessels and the sensors using Tygon\(^6\) tubing (Fuel and Lubricant, F-4040-A, Cole Palmer Laboratory Supplies). Unlike many other types of tubing, this material is both flexible and has extremely low permeability to CO\(_2\).

A variety of temperature sensors can be employed including thermocouples, theristors and solid state temperature sensors. Thermocouples were used initially. They were not suitable due to their low output, which was difficult to read accurately against background noise. They were also somewhat fragile for use in respirometers. Solid state sensors are more robust than thermocouples, and are more accurate over the temperature range used than theristors. Initial measurements were made using model LM 335s (manufactured by National Semiconductor). These sensors have a linear output that changes by 10mV for each degree Kelvin. They are highly sensitive and accurate when their output is measured against a voltage standard (approx. 2.5 volts, from an LM336). A circuit diagram is shown in Figure 3.05. The voltage adjust line was not connected as the sensors were calibrated and corrected using the computer software. These sensors required recalibration if the storage temperature changed by greater than 10°C as the resistance varied with temperature. As moisture causes current leakage, each sensor was encased in silicone.
More recently, LM 35 sensors have become available. These are similar to LM 335s, but are preset to produce close to 0 volts at 0°C. This eliminates the need for an offset voltage. For comparison, a circuit diagram of this system is also shown (Figure 3.06). Both of these sensors require a 8-15V power supply, and are accurate to within 0.1°C when correctly calibrated.

To ensure the airtightness of the container a 1cm wide, 1-2mm thick gasket was formed onto the rim of each bowl. Black RTV silicone rubber (window sealant) was found to be flexible enough for this purpose. It adhered well to the acrylic if the bowl rim was roughened with coarse sandpaper first. A thick, even bead of silicone was then piped around the edge. The bowl was placed upside-down on a sheet of polyethylene cling film that had been thoroughly wetted and smoothed flat onto a perspex or glass sheet. The bowl was weighted down with some heavy objects, and left to
cure. Cling film is one of the few plastics that does not adhere to silicone, so the mould could be easily removed the following day. Initially, the gasket was coated with a thin layer of silicone grease before securing the lid. However, I found water to be just as effective, the liquid making it easy to see any areas not fully in contact. The lid was secured with six to eight bull-dog clips to give a hermetic seal.

3.2.2.2. Atmosphere Refreshment

As previously stated, the internal atmosphere was refreshed using an air pump. I found that diaphragm air pumps used for fish tanks were suitable. They have a flow rate of 1-2L minute\(^{-1}\), are cheap and readily available from aquarium shops (Figure 3.01). These pumps are not necessarily air tight, and so cannot be reliably used with special gas mixtures. In this case, a peristaltic pump may be more suitable. Alternatively, the pumps can be placed into a sealed container (such as a lidded aquarium), which is then supplied with the gas mixture.

The pumps were plugged into switching boxes. These were turned on and off by the computer as required. I found that it was necessary to use a mechanical rather than a solid state relay. The solid state relays tended not to switch off cleanly with the inductive load of a diaphragm pump. This meant that the pump continued to run when turned off, albeit at a much slower speed. Mechanical relays are readily available, cheap, simple to connect and easily mounted into standard electrical boxes. They switch on or off in response to a 12V signal that can be taken directly from the ADAM data modules. However, most workplaces require a licensed electrician to construct such devices as they use mains voltage (240V).

When the pumps were turned off, the air outlet of each respirometer was sealed by a one way valve. This was initially constructed using a container of water and a float made of polystyrene foam. The outlet tube was inserted through the polystyrene and glued so as to leave the end flush with the bottom of the float (Figure 3.07). This resulted in a very small back pressure of less than 2 mm of water. Later versions of the respirometer replaced this device with commercially-produced one way valve (Cole Palmer miniature Check Valve with Viton diaphragm). This was more robust than the water + float system and gave negligible back pressure. Respiration rates of mushrooms were tested using both systems, and no differences were apparent. This showed that the check valve was just as effective as the water + float under experimental conditions.
In some circumstances it may be necessary to improve the mixing of the air inside the respirometer. Insufficient gas mixing was mainly a problem when using bulky produce, or under rapidly changing conditions. As an electric motor generates heat, I constructed a magnetic fan. This used a magnetic flea equipped with blades and glued onto a central pivot (Figure 3.08). When the respirometer was placed on a magnetic stirrer the fan span, circulating the internal atmosphere. As the stirrer also gave off heat, it was insulated from the respirometer using a polystyrene sheet wrapped in aluminium foil.
Chapter 3 - Development of a Respirometer

Figure 3.08 - Magnetic stirrer used to circulate the atmosphere inside a closed vessel.

3.2.2.3. Calibration of Sensors

The O₂ sensors were calibrated before use by recording the mV reading in air (20.95%). These sensors reliably read ± 0.1 mV when exposed to high purity nitrogen, so this was not repeated after an initial calibration. Recalibration was necessary every few days, or whenever there was a significant change in atmospheric pressure.

Calibration of the CO₂ sensor was complicated by the relative humidity (RH) of the air. Water vapour absorbs energy at a similar wavelength to CO₂ (2.6 μm), and is likely to affect readings from the Vaisala sensor. Either water vapour needed to be compensated for when calibrating the sensor, or calibration had to take place under the same approximate RH that would occur during use. Therefore, the CO₂ sensor was not calibrated using known gas mixtures, but by monitoring O₂ and CO₂ changes inside a sealed container of carrots. As the RQ is close to unity, [CO₂] should equal 20.95 - [O₂]. This method of calibration was checked by periodically analysing samples from the container by gas chromatography (Gow Mac series 580 TCD). A power equation was then fitted to the data so that the CO₂ concentration could be calculated from the voltage output (Figure 3.09).

![Graph](image)

Figure 3.09 - Calibration curve for non-linear CO₂ sensor; showing readings made using a gas chromatograph (▲), the difference between O₂ sensor reading and air (---), and the calculated curve that best fits the data (----); CO₂ concentration = 0.6 * 22^{(voltage * 0.11) * 0.89} - 0.72.
3.2.2.4. **The Computer**

In order to automate the respirometers, I needed to develop a controlling system. The simplest way to do this was to link the CO₂, O₂ and temperature sensors to a computer. Many different interfaces could be used, but I decided to standardise the system by using ADAM 4000 series data loggers (Advantech, USA). These are small, addressable data gatherers that can be daisy chained together. Each module has different features. For example, a 4018 has 8 input channels that can monitor voltage ranges as low as 0-20mV. A 4050 has 8 output channels that can switch on or off. This allowed me to design an efficient interface system linking the respirometers and the computer. An RS-232 link between the ADAMs and the computer communications port (comport) converted voltage readings (analogue signals) into a digital form that could be processed by the computer.

A number of commercial interface programs are available which are compatible with Microsoft Windows and which use this type of input (eg. ADAM DLL Driver package, Genie). However, Windows is known to interfere with the function of comports. Older style, DOS computers are plentiful, and can often be obtained free of charge. Also, I decided that a simple but purpose built software system would give better control over the equipment than a generic program. The simplest way to communicate with the ADAM modules was to write a program in GW Basic. Later, I wrote more complex versions of the software using Quick Basic. An example of one of these is included in Appendix 5.

After initiating the program, the operator is prompted to enter the weight of produce in each container, the number of readings required, and the time intervals between readings. The calibration points for the sensors may be either read from file, or re-entered as required. Minimum and maximum set points for O₂ concentration are also entered. These may be close together if it is necessary to hold O₂ at a particular percentage, although a gap of 2kPa is suitable for most purposes. All of the initial setpoints for each respirometer are recorded onto individual files.

Raw data from the sensors, along with the calculated respiration rates and the time of measurement are recorded as well as displayed on the computer monitor. When the minimum O₂ concentration is reached, a signal is sent to the switching box, turning the pump on. The pump flushes the container with air until the O₂ concentration reaches the set minimum percentage. This system is similar to that used in devices such as ovens and airconditioners, which maintain temperature between upper and lower limits. In this case, once the pump turns off calculations of respiration can recommence. The pump status is printed to screen and file, as respiration rates are not calculated during and immediately after flushing cycles.
At the end of the experiment, the file is downloaded onto a diskette. It can then be imported into a spreadsheet program as a delimited text file. The data can be processed, mean values or statistics calculated, and the results interpreted graphically.

3.2.2.5. **Plant Materials**

To test the effectiveness of the method, six respirometers were used to monitor the changes in respiration rate during the ripening of bananas. The results recorded in this way could then be compared with those gathered by other methods. Mature green bananas (*Musa* sp. cv. Cavendish Williams) which had not been treated with ethylene were therefore obtained from a commercial supplier. The fruit density was found by weighing a number of fruits then measuring their volume by displacement of water. A dozen bananas were randomly selected from a single hand, and groups of two were weighed and placed in each of the six respirometers equipped with O\textsubscript{2} sensors. The volume of the bananas (weight x density) was subtracted from the bowl volume to calculate the liters of air inside each vessel.

After sealing, the respirometers were covered with blackout cloth to eliminate photosynthesis by the green bananas. The temperature was maintained between 20-22°C for the duration of the experiment. The control program was set so as to allow the O\textsubscript{2} to fall to 18 kPa. Once this was reached, the container was flushed with air until the internal O\textsubscript{2} concentration increased to 20 kPa. Readings were taken every six minutes.

Respiration was monitored for one day after sealing. Sufficient ethylene was then injected into each of the respirometers to give an initial concentration of at least 16 μL/L. This is enough to initiate ripening in bananas. The resultant changes in respiration were measured over the following 10 days. After this period all the bananas had fully ripened, and the containers were opened.

As a separate experiment, pairs of bananas were placed into two respirometers equipped with both O\textsubscript{2} and CO\textsubscript{2} sensors. The same procedure was followed as already described. As both CO\textsubscript{2} production and O\textsubscript{2} demand were measured the RQ could be calculated. RQ values were output to screen as well as recorded on file.

3.2.2.6. **Calculations**

The concentrations of O\textsubscript{2} and CO\textsubscript{2} were calculated using the predetermined calibration points. The data was then used to estimate O\textsubscript{2} demand and CO\textsubscript{2} production as shown;
\[
\frac{\text{change in gas concentration (\%)} \times \text{container volume (ml)}}{100 \times \text{weight (kg)} \times \text{reading interval (h)}} = \text{respiration rate (ml.kg}^{-1}.\text{h}^{-1})
\]

Respiration rates were converted from ml.kg\(^{-1}.\text{h}^{-1}\) to mmol.kg\(^{-1}.\text{h}^{-1}\). This was done using the formula;

\[
\frac{P \times V}{R \times T} = n
\]

where \(P\) = pressure (kPa), \(R = 8.314\) (the ideal gas constant), \(T = \text{temperature (Kelvin)}\), \(V = \text{volume (L)}\), and \(n = \text{number of moles}\). Data on barometric pressure during the experimental period were provided by the nearby School of Earth Sciences, Macquarie University, North Ryde, NSW (http://atmos.es.mq.edu.au/~aws2/).

Readings taken immediately before and after atmosphere refreshment were not used as the new air would have taken time to mix and be rehumidified once the pump cycle was completed. It may also be interesting to consider the time taken for the new atmosphere to diffuse through the fruit. This is demonstrated by the non-linear changes in O\(_2\) immediately following each atmosphere refreshment. It is generally more accurate to discard a number of readings after the flush cycle so that those remaining relate to the steady state atmosphere.

The remaining data from each flushing cycle were combined to calculate the mean rate of respiration during the relevant time period. This allowed each cycle to be plotted as a single point. The standard deviation around each mean was calculated using Quattro Pro statistical software (Corel Suite 7, Corel Co., Ltd.). Data from the CO\(_2\) sensor were treated in the same way as those from the O\(_2\) sensors. The respiratory quotient (RQ) was calculated by dividing the resultant mean rates of CO\(_2\) production by O\(_2\) demand.

### 3.2.3. Results

#### 3.2.3.1. Oxygen Measurements

As shown in Figure 3.10, the respirometer successfully controlled O\(_2\) concentrations to within the specified range. Where the data points do not exactly coincide with the maximum and minimum setpoints, the pumps turned on or off in between readings. Figure 3.10 shows how the cycles become more closely spaced as the fruit start to ripen. The slopes of the lines indicate the rate of oxygen consumption by the bananas.
Chapter 3 - Development of a Respirometer

Allowing $O_2$ to fall to 18 kPa from 20 kPa would be expected to result in $CO_2$ increasing to around 3 kPa. To check whether this modification affected respiration rate I examined a number of cycles to see whether $O_2$ demand varied over this range. Cycles were analysed that occurred during the banana’s climacteric increase in respiration (days 1-3). As shown in Figure 3.11, the rate of $O_2$ consumption did not decrease between the start and the end of each cycle.

Figure 3.10 - Fluctuations in $O_2$ concentration inside a respirometer containing ripening bananas.

Figure 3.11 - Effect of changes in $O_2$ concentration between 18-20 kPa on the rate of $O_2$ consumption by ripening bananas; each point represents one measurement, with measurements taken at a 6 minute intervals; data are from cycles at four different times during the respiratory climacteric.
The climacteric was initiated simultaneously in five of the six containers of bananas, with one container delayed by half a day. Data from this container was shifted in time to allow comparison with the remainder of the fruit. To allow statistical analysis of the results, a mean value was calculated for each respirometer container over every 12 hours of measurements. The values could then be compared, and an overall mean calculated. The confidence interval for each time interval ranged from 0.06 to 0.52 mmol.kg\(^{-1}\).h\(^{-1}\) (Figure 3.12). It was concluded that differences in ripening behavior among the replicates were not significant.

![Graph showing O\(_2\) consumption over time.](image)

*Figure 3.12 - \(O_2\) consumption rate of ripening bananas; points represent mean values calculated at 12 hour intervals; error bars represent the standard deviation of each mean (n=6).*

### 3.2.3.2. Carbon Dioxide Measurements

Although this method of measuring respiration directly controls \(O_2\), \(CO_2\) is also indirectly controlled. The results from the \(CO_2\) sensor showed that \(CO_2\) rose to a maximum of around 3.5% during the experiment. These data were processed in the same way as for the \(O_2\) sensor. \(CO_2\) production was shown to closely follow \(O_2\) demand (Figure 3.13).

By dividing \(CO_2\) production by \(O_2\) demand, the respiratory quotient (RQ) could be calculated for each cycle. RQ fluctuated between 0.9 and 1.1 over 9 days of measurements, and the mean value was found to be not significantly different to 1 (Figure 3.13). This is consistent with previously published results (Brady *et al.*, 1970, Marcellin and Dick, 1983).
3.2.3.3. **Comparison with Published Data**

The results from this experiment were compared to those published in the literature. Rates of O₂ demand resembled those recorded under similar conditions by Brady *et al.* (1970) and McGlasson and Wills (1971). The rates of CO₂ production recorded by Marcellin and Dick (1983), Kanellis *et al.* (1989), Hall *et al.* (1991), Aceto and Bautista (1993) and Domínguez and Vendrell (1994) are also comparable to these results. Figure 3.14 shows the comparison between those of these sources and the results gained using the respirometer. Given that some variation will occur due to differing growing conditions, cultivars and experimental methods, the results found with the respirometer are consistent with those recorded by established methods.
Figure 3.14 - Comparison of mean experimental results found using respirometers (----) (n=6) with previously published data by Brady et al., 1970 (●), Bai et al., 1991 (●), Acedo and Bautista, 1993 (●) and Dominguez and Vendrell, 1994 (▲).

3.2.4. Discussion

Although the system allowed oxygen to fluctuate between 18kPa and 20kPa, respiration rates did not change significantly between these points. This meant that measurements conducted in this way were unlikely to be different to readings made in air. It seems probable that many plant products would behave in a similar manner, and could be analysed using the method described.

It may also be important to consider the time taken for the atmosphere to diffuse through the fruit after each pump cycle. The initial readings after a flushing cycle may not relate to a steady state atmosphere, particularly given the observed variability of O₂ and CO₂. This means it is often necessary to discard more than one reading after each flushing cycle. The calculation of respiration rate may be made more accurate by using a first derivative calculation on the slope of the line rather than the difference between two points.

As the system is automated, large amounts of data can be collected simultaneously on a number of different replicates. Mean values calculated using many samples taken from one container are likely to be more accurate than single readings made by injection into a gas chromatograph. This observation is supported by the small standard deviations calculated for our experimental data when compared to the total respiration rate. The other advantage of an automated system is that data can be collected continuously over a long period.
In future studies, the carbon dioxide concentration could be controlled by the same method that oxygen is currently replenished. That is, once a maximum set point is reached, a pump circulates the container air through a scrubber until the CO₂ concentration returns to a minimum concentration. Compensation for pressure changes would be necessary, and respiration rate calculations would need to take into account the resulting changes in container volume. Diffusion between the scrubber and the container must be minimised using a system of one way valves. If oxygen is simultaneously allowed to fall to a low level this method could effectively generate a controlled atmosphere while still measuring changes in respiration rate.

The method was found to be an effective method for measuring the RQ of bananas. This could prove useful when examining different metabolic processes in harvested fresh produce. However, monitoring CO₂ production requires care. As gas density changes with temperature and absolute humidity, the sensor has to be calibrated for each change of temperature. The oxygen sensors contain internal temperature compensating thermistors, so are less affected by changes in temperature. Accurate calibration of sensors is essential if RQ calculations are to be accurate.

Although the respirometer was an effective tool for measuring oxygen demand, the current lack of control over carbon dioxide levels is one of its disadvantages. Although CO₂ did not rise over 3.5 kPa, this concentration could still be enough to influence the respiration rate of some plant tissues. For example, it has been recommended that CO₂ should not rise over 1 kPa during ripening of bananas (McGlasson, pers. com.). A possible solution would be to allow oxygen to fluctuate between 20 kPa and 20.5 kPa, effectively preventing CO₂ concentration from increasing past this concentration.

Within the limitations described, the respirometer should adaptable to measure the respiration rate of produce of any size or shape. It can be used to give a rapid determination of respiration rate over an hour, or used to monitor changes occurring over several weeks. It is particularly inexpensive to build if only information about O₂ demand is required. It is simple to build, portable, and would appear to have considerable potential as a scientific tool.
3.3 Key Points

- A respirometer was developed which combined some of the features of flow-through and static methods of measuring respiration.
- To operate, produce is sealed into a vessel equipped with O$_2$, CO$_2$ (optional) and temperature sensors that are connected to a computer. When O$_2$ falls below a preset concentration, a pump turns on and flushes the container with air or a gas mixture until the O$_2$ reaches a set maximum level. Data on gas concentrations and respiration rate are recorded at predetermined intervals.
- To test the method, respirometers were used to monitor changes in respiration rate during the ripening of bananas.
- Mean O$_2$ consumption was calculated from the six respirometers used, and was found to be consistent with previously published data.
- The method is compared to existing techniques for determining respiration, and advantages and disadvantages of different methods are discussed.
4

Measuring Changes in Quality and Senescence

Any treatment that retains quality is, in simple terms, a method of delaying senescence. The biochemical processes that underlie senescence are certainly of considerable interest to the plant physiologist. However, the ultimate aim of all postharvest practices is to meet the needs of consumers (Shewfelt, 1999). Understanding what constitutes quality, and how it can be measured, is therefore essential in any study of senescence.

4.1. What is Quality?

Quality is a term that is frequently used, but difficult to define (Shewfelt, 1999). Quality is subjective, and means different things to different people. For example, to transporters good quality fruit is that which is robust enough to transport easily. Retailers are mainly interested in appearance, while to the consumer flavour and nutritional value are also likely to be important (Wills et al., 1998). Quality can be defined as “fitness for a purpose” (Wills et al., 1998, Abbott, 1999). This means that quality essentially depends on the perspective of the viewer (Shewfelt, 1999).

Quality attributes of a product can include its sensory appeal, nutritional characteristics, biochemistry, mechanical properties and defects (Abbott, 1999). Many quality attributes are difficult to analyse subjectively. Appearance is often used as the measure of postharvest quality because it is relatively easy to evaluate (Shewfelt, 1999). However, using one particular attribute is often not a good indication of overall product quality (Dull and Huime, 1971). For example, an apple may be well coloured and glossy, but if the internal flesh is mealy then the fruit could not be considered a good quality.

Shewfelt (2000) has suggested that quality should relate to consumer acceptability. The critical elements that determine quality may therefore vary both between geographical areas and between market segments. Unlike manufactured items, the management of fruit and vegetables cannot always be tailored to meet exact consumer requirements (Shewfelt, 2000). That is, it is not yet possible to make a blue tomato or a square apple. However, consistency in meeting consumer expectations may be a large component of overall product quality.
Postharvest quality is affected by preharvest treatments. These include cultural practices, maturity, and harvesting methods (Dull and Hulme, 1971, Kader, 1986b). For example, high levels of nitrogenous fertilisers are associated with improved resistance to bruising in potatoes (Barielle et al., 2000) and good quality and flavour in tomatoes (Variyam et al., 1989), but reduced colour development and keeping quality in apples (Neilson et al., 1999). Storage potential of apples is also strongly influenced by harvest date (Jobling et al., 1993, Drake and Eisele, 1994, Song and Bangerth, 1996). Choosing the optimum date to harvest apples is a balance between loss of firmness and greater susceptibility to injury and disease as the fruits ripen, and the increases in size, flavour and colour development associated with greater maturity (Beaudry et al., 1993, DeLong et al., 1999). In effect, an improvement in one quality parameter is at the expense of another. Position on the tree is another factor that can influence postharvest quality of not only apples, but also other fruits. Greater exposure to light and heat during growth reduces chilling injury of avocados, but increases its occurrence in citrus and persimmons (Woof and Ferguson, 2000). These examples illustrate that preharvest practices can have strong effects postharvest, but that these effects differ between commodities.
4.2. Methods of Measuring Broccoli Quality

There are many ways of measuring produce quality. Methods may be subjective or objective (Abbott, 1999), destructive or non-destructive (Watada, 1989), product based or consumer based (Shewfelt, 1999). As there are too many methods to cover in a short review, the remainder of this chapter examines some of the procedures used for evaluating changes in broccoli quality.

4.2.1. Colour

Colour is the most commonly used criterion when assessing broccoli. This is often done using a colorimeter, such as the Gardner XL-845 colour meter or the Minolta Chroma Meter. These devices describe colour as a point on a three dimensional grid. The CIE "L, a, b" system is the most commonly used as it was devised to register different colours in a similar way to the human eye (Abbott, 1999). The a value grades from green (-a) to red (+a), the b value grades from blue (-b) to yellow (+b), and the L value indicates reflectivity or brightness on a scale from 0 (black) to 100 (white). Colorimeters have the advantage of being easily standardised against a standard tile, and so allow remote comparisons of different colours. Their main disadvantages are that only a small area of the product (approx 1cm²) is actually measured, and that readings can be affected by uneven surface textures.

As the chlorophyll is lost from broccoli and it becomes yellow, the values of all three parameters change. Tomkins et al. (1993) found that broccoli had become unacceptable when the b+ value increased from 20 to 22. However, yellowness is described not only by an increase in yellow tone but also by a decrease in other tones (Shewfelt, 1993). A better discrimination can be therefore be gained by combining the a and b values to calculate the hue angle: \( H^\phi = \tan^{-1} \frac{b}{a} \). This parameter was found to show the best discrimination between different broccoli cultivars (Shewfelt et al, 1984). \( H^\phi \) decreases from around 130 to less than 100 during broccoli senescence (King and Morris, 1994a, Tian et al., 1994, Irving and Joyce, 1995, Irving and Baird, 1996). Surprisingly, no references were found which correlated the \( H^\phi \) value with visual appearance or acceptability.

An alternative method of evaluating colour is simply to compare the heads to a colour grading system. For example, the subjective rating scale developed by Wang (1979) ascribes values between 10 and 0, 10 being dark green, and 0 completely yellow. Klieber and Willis (1991) considered that broccoli quality was unacceptable once it fell below 8 on this scale. Rating scales
such as this can be used more reliably when combined with a series of photographs, such as have been developed for bananas and tomatoes (McGlasson et al., 1986, Wills et al., 1998).

4.2.2. Chlorophyll content / activity

As previously stated, loss of chlorophyll is one of the earliest signs of product senescence. One way of determining total chlorophyll is by extraction with N-dimethylformamide and then colorimetric measurement (Pogson and Morris, 1997). Chlorophyll content of broccoli declines from around 300-450 mg kg\(^{-1}\) at harvest to less than 100 mg kg\(^{-1}\) after protracted storage (Perrin and Gaye, 1986, Makhlouf et al., 1989a, Makhlouf et al., 1991, Bastrash et al, 1993, Pogson and Morris, 1997). Shewfelt et al. (1984) found that changes in total chlorophyll concentration between broccoli cultivars were not significant despite obvious differences in colour. However, other studies have found a close correlation between changes in chlorophyll content during storage and H\(^{\theta}\) values (\(R^{2} = 0.86\)) (Perrin and Gaye, 1986, Pogson and Morris, 1997).

Chlorophyll can also be monitored non-destructively by measuring fluorescence. This method measures light re-emitted by excited chlorophyll, as opposed to reflected light (Abbott, 1999). In this case it is chlorophyll function that is measured, rather than chlorophyll content. Disruption of the chlorophyll membranes during the early stages of senescence reduces the capacity of chlorophyll to respond to light. This method may therefore be an early indicator of deterioration or stress (Schapendonk et al., 1992). For example, changes in chlorophyll fluorescence indicated the beginnings of ethanol accumulation and off odours in broccoli stored in modified atmospheres, even though the heads remained green (DeEll and Toivonen, 2000). It is also fast, enabling a large number of samples to be measured (Song et al., 1997). Changes in fluorescence are well correlated with changes in total chlorophyll, and are independent of physiological maturity (Toivonen and Dell, 1998). Damage to apples from bruising and superficial scald as well as general senescence can be measured quickly and accurately by chlorophyll fluorescence (Song et al., 1997, Mir et al., 1998). Measurements of chlorophyll fluorescence also indicate senescence of broccoli, declining by nearly 70% during extended storage at 1°C (Toivonen, 1992).

Unlike methods that extract chlorophyll, measurement of chlorophyll fluorescence is a comparative rather than absolute. By measuring fluorescence at different wavelengths, it is possible to compare the activity of chlorophylls a and b. However, the ratio between these two types does not usually change during storage (Mir et al., 1998). One way of expressing total fluorescence is as F\(\text{var}\). This represents the maximum amplitude of the variable component of fluorescence (F\(\text{m}\)) relative to the non-variable component (F\(\text{o}\)) (Shewfelt et al., 1984, Toivonen, 1992). More commonly, fluorescence is expressed as quantum yield or "non-photochemical quenching" (\(F\text{v} / F\text{m}\)). This is calculated by dividing the change in fluorescence (F\(\text{m} - F\text{o}\)) by F\(\text{m}\) (Song et al., 1997).
4.2.3. Physical changes

Weight loss is a quality factor that is very important to consumers. It is also easily, quickly and cheaply measured. Changes in weight and shrivelling are a direct function of the storage conditions. For example, after 10 weeks storage at 0°C and 100% relative humidity (RH), weight loss of broccoli was less than 4%. Decreasing the humidity to 94% RH more than tripled weight loss after the same time period (Klieber and Wills, 1991). Other ways of reducing weight loss involve wrapping the heads in film (Forney et al., 1989, Toivonen, 1997), or placing the cut stump of the broccoli in water (Irving and Joyce, 1995).

Maintaining high RH around broccoli in order to prevent dehydration can easily lead to the damp conditions that encourage rots. Decay is the main factor limiting broccoli storage life at 0°C (Klieber and Wills, 1991). Rots are also likely to be severe if condensation occurs on the florets at temperatures over 5°C (Perrin and Gaye, 1986, Klieber and Wills, 1991). Rot severity is necessarily assessed on a subjective scale. For example, 1 = no rots, 5 = severe rotting (Tomkins et al., 1993).

Changes in texture are often related to weight loss. One way of measuring texture is to use shear resistance (SR). This measures the force required to cut through the broccoli stem (Batal et al., 1982). SR has been found to be correlated with changes in other broccoli quality attributes, including pH and colour (Lipton and Harris, 1974). However, the texture of the product includes factors such as stringiness, crispness, and waxiness (Wills et al., 1998, Abbott, 1999). Measurements of shear resistance can give some information, but measure stem firmness rather than texture.

4.2.4. Sensory evaluation

Quality is often graded according to subjective rating scales. As these evaluations use human senses, they should indicate consumer perceptions and acceptability. Attributes evaluated may include colour, odour, decay, taste, texture, turgidity, floret opening and compactness (Lebermann et al., 1968a, Lebermann et al., 1968b, Kasmire et al., 1974, Wang, 1979, Perrin and Gaye, 1986, Forney, 1995). Quality factors may be graded separately, or the broccoli given an overall rating that includes a number of attributes (Tomkins et al., 1993, Ku and Wills, 1999). For example, on a scale of 1-5, grade 3 could stand for 30% yellowing of florets, slight rotting and no longer saleable (Ku and Wills, 1999).

Off-odours are a common problem encountered during broccoli storage, particularly in the presence of >10% CO₂ (Ballantyne et al., 1988, Makhlouf, 1989, Izumi et al., 1996). This can result in losses,
even though the odours normally disappear during subsequent holding in air (Kasmire et al., 1974). Off odours in broccoli are the result of production of sulfur compounds such as dimethyl sulfide, dimethyl disulfide, and hydrogen sulfide as well as methanethiol (Derba, et al., 1998). Ammonia may be another source of off-odours. As ammonia is formed by the breakdown of chlorophyll, proteins and acids in senescent tissues, levels increase simultaneously with yellowing (King and Morris, 1994b, Downs et al., 1997). Off-odours/volatiles can be detected using gas chromatography, but are more commonly assessed using subjective scales. Wang (1979) proposed a scale from 0 – normal to 10 – nauseating. Other workers have recorded only whether odours are present or not (Ballantyne et al., 1988) or devised their own scales (Kasmire et al., 1974, Makhlouf et al., 1989b).

Flavour is even more difficult to analyse than odour. Flavour is a combination of taste and aroma, and is likely to be due to a large number of different compounds. For example, gas chromatography has revealed over 225 volatile compounds in grapes (Shewfelt, 1993). As a result, flavour is normally measured by presenting lightly cooked broccoli florets to panels of tasters. The different components of taste – bitter, sweet, sour, salt and umami – need to be analysed separately if possible. The tasters grade each of these attributes according to a hedonic scale. This may be from 1-9, where 9 is excellent, 5 barely acceptable, and 1 repulsive (Lebermann et al., 1968a, Batal et al., 1982). No taste-test results could be found in the published work surveyed. This is likely to be due to the subjective nature of the tests, which can fail to produce statistically significant results.

4.2.5. Compositional Changes

Compositional changes can be measured objectively for some products, and these changes correlated with sensory evaluation. For example, eating quality of pineapples was found to be highly correlated with soluble solids concentration (SSC%) (Smith, 1988), while the consumer acceptability of lychees could be estimated from the ratio of SSC% to total acids (Underhill and Wong, 1990). Carbohydrate levels in broccoli do not significantly affect flavour, but may be a general indicator of senescence. Other constituents, such as protein and ascorbic acid, affect nutritional value.

As an inflorescence, broccoli contains relatively small carbohydrate reserves at harvest. Sucrose has been estimated to comprise 10% (Pogson and Morris, 1997) to 50% (Tian et al., 1997) of total sugars, with glucose and fructose making up the remainder. Florets initially contain around 50 µmol sucrose·g⁻¹ dry weight (DW) (Irving and Joyce, 1995). Broccoli florets also contain low levels of starch. However 50-95% of starch reserves are utilised within 24 hours at 20°C (King and Morris, 1994b, Tian et al., 1997). Sugar concentrations also decline greatly during storage at 20°C. This has led to the suggestion that lack of carbohydrates may be a factor limiting broccoli storage life (Irving and Joyce, 1995), or alternatively, that sugar status regulates changes in gene expression associated with senescence (Downs et al., 1997).

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The nutritional value of broccoli changes during storage. Ascorbic acid – vitamin C – is particularly easily lost, especially if RH is low or the broccoli has been bruised or otherwise injured during harvest (Barth et al., 1993, Lee and Kader, 2000). Citric and malic acids decrease rapidly after harvest (King and Morris, 1994b), as do levels of protein, which fall from 120-150 mg.g\(^{-1}\) DW to around 40 mg.g\(^{-1}\) DW (Pogson and Morris, 1997, Downs et al., 1997).

### 4.2.6. Programmed Cell Death

The study of programmed cell death (PCD) in plants is still in its infancy. Markers for PCD in animal cells are distinctive, and have been studied extensively, and it is possible that similar events may occur during the senescence of many plant tissues (Kreeger, 1996). Research into animal tissues has implicated a role for cysteine proteases in PCD. In particular, proteases of the interleukin-1β converting enzyme (ICE) family have been associated with many of the early changes characteristic of apoptosis (Kumar and Lavin, 1996) (See Section 2.1.4.). It has been shown that genes coding for similar compounds are activated during the early stages of plant senescence (Greenberg, 1996). For example, the cDNA encoding cysteine proteinase SmCP was up-regulated during senescence of *Solanum melongena* leaves and fruit (FangXiu et al., 1999). It is not known whether the ICE family of proteases occurs in plant cells. However, such markers of PCD may be present in different plant tissues. They could therefore provide a measurement of senescence that would allow comparison not just among different cultivars, but even among different species.
4.3. Measurement of Broccoli Quality and Senescence

4.3.1. Aims

I decided to test for the presence of ICE family of proteases in senescent broccoli. If these enzymes were present in broccoli cells, they could be used as an objective measure of the onset of senescence. The results for broccoli were therefore compared with a number of other methods of measuring senescence, including colour, weight loss, and chlorophyll fluorescence.

4.3.2. Materials and Methods

4.3.2.1. Plant Materials

Three separately packed boxes of fresh broccoli were obtained from the Sydney markets. Ten heads from each box were selected for uniformity of size and quality, and each group designated as A, B or C. The heads were surface sterilised by being dipped in a 200 ppm solution of sodium hypochlorite for 1 minute and then dried with paper towels. Each group of ten heads was stored in a lidded plastic container at 20°C. The heads were weighed daily, and a Minolta CR-300 colorimeter was used to record the L, a and b values of the central florets. Hue angle was calculated by the formula;

\[ H^o = \tan^{-1} \left( \frac{b}{a} \right) \times \frac{180}{\pi} + 180 \]

Changes in colour were also monitored separately by photography. Two additional broccoli heads were selected and placed under a light-proofed plastic cover at 20°C. This was to create similar conditions to those inside the storage containers while allowing the heads to be photographed. Photographs were taken at approximately the same time each day for five days using a Canon Eos 1000F SLR camera.

4.3.2.2. Chlorophyll Fluorescence

At each sampling time one head was removed from each of the three replicates. The heads were dark conditioned for 15 minutes by enclosing in a black, light proof bag. While still in the dark, six central branchlets were broken off each head so that they could be measured separately. Fluorescence was analysed using a WALZ KL1500 chlorophyll fluorometer (Heinz Waiz GmbH,
Germany) with the excitation level set at 650 W.m⁻². Floret fluorescence before the light source was
turned on (F₀) and the maximal fluorescence (Fm) were recorded and used to calculate Fv/Fm. The
florets were then excised, divided into two sub-samples, and immediately frozen in liquid nitrogen for
further analysis.

4.3.2.3. Detection of Programmed Cell Death

The ApoAlert™ CPP32 Assay Kit (Clontech Laboratories Inc., USA) was used to test for the ICE type
protease CPP32. CPP32 is a cysteine protease that breaks down some of the cellular proteins
involved in the early stages of apoptosis in animal cells (Clontech Technical Service, 1997). The kit
contains a labelled substrate that is added to the sample solution. The substrate is cleaved by any
CPP32 in the solution, producing a compound that can be detected by either fluorescence emission
or colorimetry (Figure 4.3.1). Different substrates are used according to the method of measurement.
If detection is by fluorescence emission, the substrate used produces 7-amino-4-
trifluoromethylcoumarin (AFC) on cleavage. The substrate used for colorimetric detection produces
p-nitroaniline (pNA) on cleavage.

![Diagram of detection process]

Figure 4.3.1. - Mode of detection of CPP32 enzyme by ApoAlert CPP32 Assay Kit (Clontech
Laboratories Inc., USA). Different substrates are used according to whether detection is by
fluorometry or colorimetry. Adapted from ApoAlert Product Protocol booklet, Clontech, USA.

As the ApoAlert kit had not previously been trialled with plant materials, I conducted some initial tests
to determine whether the method would work with horticultural products. The fluorescence detection
kit is usually recommended, and was trialled first. However, the samples prepared using the kit were
of the order of 200 µl volume, while the available detector required approximately 2ml for accurate
analysis. This made it necessary to use the colorimetric test instead. I then conducted a trial using
different plant tissues at varying stages of senescence. These included carnation flowers (Dianthus
sp.) at four developmental stages (bud, open paintbrush, fully open, and petal drop), broccoli at three
developmental stages (immature buds, mature, and yellowing), young and senescent leaves of lettuce (*Lactuca sativa*) and young and senescent leaves of brush box (*Lophostomen confertus*).

The assays were conducted according to the kit instructions. 15mg of frozen, ground tissue was used for each test. Samples were centrifuged for several minutes after addition of the lysis buffer, and again following incubation with the DEVD-pNA substrate. Control solutions consisted of a sample without added substrate, another without added plant tissue, and a third that was kept on ice rather than incubated at 37°C. The absorbance at 405nm was measured by placing supernatant from each solution in a plate reader (*Bio-Rad* microplate reader, model 3550-UV). A calibration curve for absorbance was developed using the pNA solution supplied in the kit (Figure 4.3.2). This included control solutions of plant material without added substrate, and substrate without plant material.

![Calibration Curve](image)

**Figure 4.3.2. - Calibration of the spectrophotometer to pNA concentration.**

Initial results using the different plant materials were promising, so I prepared solutions for CPP32 testing from the frozen broccoli samples. Enzyme analyses were repeated twice for each of the 3 replicate tissue samples. If there was no significant difference between the boxes, this would give six readings for each date during storage.

I decided that it would be more appropriate to express CPP32 activity in terms of total protein in the sample rather than sample weight. This would eliminate differences due to dehydration, and would be a better indicator of what was occurring at the cellular level. Protein content of each sample was determined using 15mg of frozen tissue analysed by the Folin-Lowry method (*Lowry et al.*, 1951).
4.3.3. Results and Discussion

4.3.3.1. Physical Changes

Analysis of the data using CoStat statistical software found that box C lost weight and changed colour significantly faster than box A (α = 0.01). However, the other parameters measured did not differ between the replicates. Furthermore, the actual numerical differences were small. Therefore, the data were combined. Differences over time are summarised in Appendix 6.

Rapid physical changes were observed in the broccoli. After the first 24 hours the broccoli lost around 2% of its initial weight each day (Figure 4.3.3). However, it seems likely that a disproportionate amount of moisture was lost from the florets compared to the stem, as shrivelling and wilting was clearly visible (Appendix 7).

![Graph showing weight loss of broccoli over time](image)

**Figure 4.3.3.** Weight loss of broccoli stored at 20°C; error bars indicate the standard deviation of each mean value (n=30).

Colour changed significantly during storage, with the greatest yellowing during days 3 and 4 (Figure 4.3.4). The results were consistent with previously published data (King and Morris, 1994a, Tian et al., 1994, Tian et al., 1995).
Figure 4.3.4. - Changes in hue of broccoli stored at 20°C; error bars indicate the standard error of each mean value (n=30).

4.3.3.2. Changes in Chlorophyll Fluorescence

Chlorophyll fluorescence decreased markedly after day 3 (Figure 4.3.5.). It had been expected that chlorophyll fluorescence would decrease before or at the same time as apparent changes in colour. However, the results showed that changes in chlorophyll fluorescence lagged at least a day behind decreases in hue angle. This suggests that measurements of colour are a more sensitive way of measuring chlorophyll degradation than are changes in chlorophyll function measured as $F_v/F_m$.

Figure 4.3.5. - Changes in chlorophyll fluorescence of broccoli stored in the dark at 20°C; error bars indicate the standard error of each mean value (n=18).
4.3.3.3. Detection of Cysteine Protease CPP32

In the preliminary trials there was a consistent increase in absorbance at 405nm in older plant tissues relative to young tissues (Figure 4.3.6.). This suggests that the added substrate was cleaved to form pNA, thereby indirectly indicating the presence of the ICE protease CPP32. The exception to this was the carnation petals, for which absorbance decreased during the later stages of maturity and senescence. However, this may have been due to the role that CPP32 plays in the very early stages of PCD (Clontech Technical Services, 1997). Similar results have been reported for Solanum melongena flowers. In these flowers, the senescence associated cysteine protease SmCP was highly expressed during development, but was less active during seed development (FangXiu et al., 1999). It is possible that by the time the carnation flowers had fully opened, the enzyme CPP32 was no longer necessary for the continuation of PCD.

![Graph showing protease activity at different stages of development and senescence.](image)

Figure 4.3.6. - Preliminary results from ApoAlert CPP32 Assay Kit showing changes in pNA absorbance of carnation petals (--- ▲ ---), brush box leaves (--- ■ ---), lettuce leaves (--- ● ---) and broccoli florets (--- ● ---) at different stages of development and senescence. Points are mean values of two replicate tissue samples.

The results from more extensive testing of broccoli were consistent with those found in the preliminary examination. Absorbance remained low during the first 3 days of storage at 20°C, then increased significantly during days 4 and 5. Protein content also changed significantly after 3 days of storage (Figure 4.3.7.). When the amount of pNA present was compared to the protein content of the broccoli tissue, the apparent increase in enzyme activity was even greater (Figure 4.3.7.). Changes in absorbance mirrored changes in chlorophyll fluorescence. As with fluorescence, this method did appear to indicate that senescence was occurring. However, it was less sensitive than measurements of hue angle.
Figure 4.3.7. - Changes in protein content of broccoli during storage at 20°C; error bars indicate the standard deviation of each mean value (n=3).

Figure 4.3.8. - Changes in apparent pNA concentration relative to protein content of broccoli tissue. Error bars indicate the standard deviation of each mean value (n=6).

Despite these promising early results, more work is required to determine whether ICE proteases are involved in PCD of plant tissues. Using the Clontech kit gave indirect evidence that the protease CPP32 was active during broccoli senescence, and possibly during the senescence of other plant tissues. However, the kit was not designed for use with plants, and measurements of absorbance may have been affected by other senescence related compounds. The results would also be more convincing if a peak in absorbance could be shown at the 405 nm wavelength. Detection of cysteine proteases using a different method is therefore necessary to confirm these results.
Changes in chlorophyll fluorescence and apparent protease activity occurred at the same time as observed changes in colour and weight loss. However, measurement of hue angle was a more sensitive measure of the early stages of senescence. It was also easy, reliable, and could be used to rapidly process many samples. Unlike the other methods, this test does not provide information about the mechanisms of senescence. However, the results are likely to be in agreement with consumer perceptions. This therefore appears to be the best test to use when evaluating broccoli freshness.
4.4. Key Points

- Quality can be defined as "fitness for a purpose". Criteria used to evaluate quality will vary according to not only the product itself, but also the priorities of the examiner and the end use of the product.

- Measurements of quality can be subjective or objective. Correlations of subjective and objective measurements are useful for evaluating quality, but appear to be rarely documented.

- Broccoli quality can be evaluated in many different ways including colour, chlorophyll content or fluorescence, physical changes such as weight loss, sensory evaluation and biochemical changes. Detection of programmed cell death (PCD) may be another method used in the future.

- A commercial kit designed for detecting PCD in animal cells (Clontech Laboratories Inc., USA) was trialled with a number of plant products including broccoli. The results were compared with measurements of senescence made by conventional techniques including chlorophyll fluorescence, colour, weight loss, and subjective grading.

- The kit appeared to indicate that PCD was occurring in senescent broccoli as well as other deteriorating plant products. However, changes in hue angle (colour) were a more sensitive method of determining the early stages of broccoli senescence.

- While the early results were promising, more work is required to determine whether ICE proteases are involved in PCD of plant tissues.
Chapter 5 - Respiration Life

Measuring the ‘Respiration Life’ of different fruit and vegetables

The previous two sections have examined methods of measuring respiration rate and determining quality and storage life of fresh products. By combining these two measurements, it is possible to calculate the total respiration that occurs during storage life. This may be referred to as the ‘respiration life’ of the product. If respiration rate is directly linked with the rate of senescence, respiration life should be constant under a range of different storage conditions, as was discussed in section 2.3. In this chapter, respiration life is determined for a climacteric and a non-climacteric fruit, a fruit vegetable, and an inflorescence. Products were selected which had a range of respiration rates and storage requirements, which were economically significant and which were readily obtainable from local suppliers. The results are discussed in terms of whether respiration rate may be used as an indicator of senescence in the products examined.
5.1. Relationship between respiration rate and storage life of capsicums (*Capsicum annuum* L.)

5.1.1. Introduction and Aims

Capsicums, or bell peppers as they are sometimes called, are not an easy fruit to store. They are chilling sensitive, and should not be kept at temperatures less than around 7°C. While they lose water and soften rapidly in a dry environment, moist conditions can lead to growth of rots such as *Botrytis cinerea* and *Alternaria alternata* (Meir et al., 1995). Both of these diseases can grow at 7°C, and are a major cause of postharvest loss in capsicums (Otma, 1989). Modifying the storage atmosphere by increasing CO₂ and/or decreasing O₂ has been shown to reduce decay in capsicums (Polderdijk et al., 1993, Luo and Mikitzel, 1996), as have hot water treatments (Gonzalez-Aguilar et al., 1997, Fallik et al., 1999). However, such treatments have had mixed results on quality and storage life (Hughes et al., 1981, Otma, 1989, Luo and Mikitzel, 1996).

Although they are a non-climacteric fruit, bell peppers change in colour during development (Saltveit, 1977). Reddening involves both the degradation of chlorophyll and increased synthesis of carotenoids. Coloured capsicums are often referred to as ripe even though other changes normally associated with ripening, such as softening and increased levels of sugars, do not occur to a major extent. However, other biochemical components do change, and red capsicums often have a different flavour to green fruit (Gross et al., 1986). Colour development is stimulated by injury and disease (Saltveit, 1977), as well as by maintaining a high relative humidity around the fruit with polyethylene bags (Meir et al., 1995). Even though changes in colour are associated with increased ethylene production (Saltveit, 1977), colouring was not accelerated by treatment with propylene, or reduced by injecting silver thiosulfate (STS) into the fruit cavity (Pretel et al., 1995). Red colour development in capsicums is therefore not mediated by ethylene, and remains an incompletely understood process which may or may not be associated with fruit senescence.

As capsicums are non-climacteric and do not undergo a distinct ripening process, it is expected that they will respond to changes in storage conditions in a predictable way (Chen et al., 2000). Storage life is generally ended by rots or water loss (Lerthanangkul and Krochta, 1996), both of which might be expected to respond to changes in temperature and fruit permeance. Capsicums...
should therefore be a suitable fruit to use to study the effects of changes in respiration on maturation and storage life. I have measured respiration as well as various quality parameters of capsicums in order to clarify the causes of senescence in this fruit. Total O₂ consumed during storage was determined for a range of temperatures to determine whether capsicums have a constant “respiration life”.

5.1.2. Materials and Methods

5.1.2.1. Plant Material

Mature capsicum fruit were obtained from the Sydney Markets, and harvested from commercial growers at Ebenezer, Sudbery, and Camden NSW. These will be referred to as lots A, B, C and D respectively. Harvesting took place at different dates during the growing season. Lots A, B and D consisted entirely of green fruit. However, approximately one third of fruit from lot C were showing some red coloration. Only small numbers of capsicums were involved in Lot A, as this had been intended as a preliminary trial. Data from this initial trial have been included for reasons that will be discussed in the overall results. The fruit were immediately transported to the North Ryde laboratories, where the stems were retrimmed to approximately 10-20 mm in length. Fruit were selected that were undamaged, showed no sign of rots, and were not misshapen or unusually large or small.

The capsicums were sorted according to size and, in the case of lot C, colour. Fruit were graded so as to include similar numbers of fruit from each size and colour category in each treatment. They were then weighed and numbered. Capsicums have a relatively thin skin and flesh that encloses a large internal air space. As the gas composition inside this space closely follows that of the external atmosphere (Banks and Nicholson, 2000), the external and internal atmospheres were assumed to be continuous. The volume of each fruit was therefore calculated from its weight, rather than by its displacement in water.

Capsicums from each lot were stored at various temperatures (Table 5.1.1.). Storage was in cardboard trays lined and overwrapped with plastic. The capsicums were placed inside the liners, and the ends turned over to maintain a high relative humidity around the fruit. The conditions in the tray were intended to reflect conditions inside the respirometers, so that the fruit would be in similar conditions at all times.
Table 5.1.1 - Number of fruit stored at each temperature from the four different harvest dates and locations used.

As a separate experiment, extra capsicums were harvested from Sudbery (C) and Camden (D) to test the effects of waxing on storage life. Thirty-six and sixteen fruit were used from harvest lots C and D respectively. Previous work with other Solanaceous fruit including eggplants and tomatoes, as well as capsicums, has shown that applying wax to the pedicel reduces both transpiration and gas exchange (Burg and Burg, 1965, Díaz-Pérez, 1997). Paraffin wax was therefore applied to the pedicel area of half of the capsicum fruit. The remaining fruit were left untreated as a control. All fruit were stored at 20°C in cardboard trays lined and covered with plastic.

5.1.2.2. Measurements of O₂ Consumption

O₂ demand was monitored over the duration of each experiment using respirometers, as were described in Chapter 3. The O₂ concentration inside the bowls was maintained between 19-20kPa, and data were recorded at 6 minute intervals. One respirometer was used at each storage temperature when monitoring capsicums from lots A and D. This was increased to two respirometers during measurements of lots B and C. Measurements of waxed and unwaxed fruit followed the same procedures.

For lot A and B capsicums, individual fruit were placed in the respirometers at each of the storage temperatures. O₂ demand was measured for up to a week, after which each capsicum was replaced by another from the same group. This process was repeated several times so as to calculate the mean rate of O₂ demand by the fruit at each temperature.

An issue confronted during respiration measurements of lots A and B was that the rate of O₂ depletion inside respirometer bowls containing single capsicums was relatively slow. This meant that the O₂ sensors were operating at the limit of their sensitivity. As a result, the experimental
design was altered for lots C and D, with three capsicums used for each determination of respiration rate. This ensured that sampling errors were minimised. Also, this meant that all the fruit were used when calculating the overall mean rate of O₂ consumption, rather than the limited numbers used in the first two trials.

5.1.2.3. **Quality Assessment**

The capsicum fruit were assessed at least weekly during storage. At each examination the fruit were weighed to within 0.01g, and their colour and condition were graded subjectively according to the following rating scales.

<table>
<thead>
<tr>
<th>Colour</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 solid green, no orange</td>
<td>1 firm, glossy, rot free</td>
</tr>
<tr>
<td>2 green, up to 20% orange</td>
<td>2 fairly firm, slightly glossy, small spots appearing on pedicel</td>
</tr>
<tr>
<td>3 green, with 20-50% orange</td>
<td>3 softening, fairly dull, spots appearing over fruit body, significant rots in pedicel</td>
</tr>
<tr>
<td>4 orange / red, 50-20% green</td>
<td>4 soft, dull, rots appearing over fruit body</td>
</tr>
<tr>
<td>5 orange / red, &lt;20% green</td>
<td>5 advanced decay, free liquid from ruptured cells present, odorous</td>
</tr>
</tbody>
</table>

Photographs taken at different colour and quality stages were used to assist in standardisation of grading (Appendix 8).

5.1.2.4. **Calculations**

The rate of O₂ demand was calculated from the changes in O₂ partial pressure inside each respirometer (Chapter 3). As the rate of O₂ demand remained fairly constant over measurement periods of up to a week, the average respiration rate was calculated for each fruit or group of three fruit. The mean respiration rate at each temperature was then calculated on the basis that \( n \) = number of replications at each temperature. Rates of O₂ consumption were graphed against temperature, and a logarithmic equation was fitted to each data set. Using this equation, it was possible to estimate the respiration rate at any temperature.

Decreases in quality grade were plotted against time for individual capsicum fruit. A polynomial equation was calculated for each fruit that described changes over time. The number of days until the capsicum reached the end of acceptability (quality grade = 3) could be estimated from this equation. An example is shown in Figure 5.1.1.
Figure 5.1.1. - Example of how capsicum fruit quality changes over time; each set of points represents evaluations of an individual fruit stored at 10°C, polynomial regression equations calculated for each data set so as to estimate the number of days until quality became unacceptable (grade 3). In this example, storage life ranges from 14 days to 31.5 days.

The value derived from the log relationship between respiration and temperature was multiplied by the days of life to calculate total O₂ consumed during storage. Exceptions to this were made when the measured rate of respiration differed greatly from that derived from the equation. In this case, the measured rate was used, as other factors (such as the early stages of decay) were likely to be influencing fruit metabolism.

Differences in weight loss, development of red colour, O₂ consumption and storage life were analysed using CoStat statistical software. Where possible, randomised complete block analysis was used, but if numbers of replicates were uneven then completely randomised analysis was used. Differences between the means were determined using Duncan's Multiple Range Test. The standard deviation and standard error was also calculated for each mean value.
5.1.3. Results and Discussion

As previously noted, limited numbers of fruit were used in Lot A as this had been intended as a preliminary trial. However, the variability in the results from the other 3 replicates was considerable. I therefore decided to include the preliminary results as these further demonstrated the variability of the response of this product to different storage conditions.

5.1.3.1. Effects of Temperature and Waxing on Weight Loss

After two weeks, weight loss differed significantly among the replicates as well as between the storage temperatures used (α = 0.05). Capsicums from Lot A lost the least weight during storage, followed by Lot C and Lot B, with those from Lot D being the most rapidly dehydrated (Figure 5.1.2.). Weight loss was generally linear over time, as has previously been reported for bell pepper fruit (Miller et al., 1986). Lot D was the exception to this, as the rate of weight loss increased during storage.

![Graph showing weight loss over time for different lots.](image)

Figure 5.1.2. - Weight loss of capsicums from different harvest dates and locations; data from 10°C, other temperatures being similar, each point represents a sampling time, linear trendlines plotted for each data set. Capsicums from Lot A (●) (n=4), Lot B (●) (n=12), Lot C (▲) (n=18) and Lot D (■) (n=16); error bars represent the standard deviation of each mean value.

The greater weight loss from Lot D fruit compared to those from Lots A - C is likely to be due to the storage environment. These fruit were stored in controlled temperature (CT) rooms at the University of Western Sydney, Hawkesbury whereas the other replications were stored at the
laboratories of CSIRO at North Ryde. The CT rooms at CSIRO operate at close to saturated RH (approx. 95%) whereas the rooms at the University have a higher rate of airflow and operate at approximately 90% RH. Even though all fruit were overwrapped in plastic film, these differences could have affected the fruit during extended storage. However, differences between Lots A, B and C are more difficult to explain, as the same rooms were used on each occasion. Janse (1988) found that electrical conductivity of the soil strongly influenced firmness and keeping qualities of capsicums. Possibly the differences in weight loss observed in this study may be related to the cultural conditions during growth, which in turn led to differences in the thickness or waxiness of the cuticle.

Weight loss was generally temperature dependent. That is, loss was most rapid at 20°C, and slowest at 5°C (Figure 5.1.3.). However, when the symptoms of chilling injury or rots began to occur, the rate of weight loss increased markedly. Fruit from Lot D stored at 16°C also lost more weight than expected, dehydrating significantly more than capsicums from the same group stored at 20°C (α = 0.05). In this case the fruit had been stored in a Thermoline controlled temperature cabinet located in a 12°C temperature room. As the cabinet heated air from the surrounding room, relative humidity inside the unit was reduced relative to the external conditions. The plastic overwrap around the fruit gave insufficient protection against the relatively large humidity gradient between the capsicum flesh and the air inside the cabinet. A similar percentage weight loss was reported for capsicums stored at 17°C and 85% RH (Ben-Yehoshua et al., 1983). Weight loss can be effectively prevented by sealing the fruit inside polyethylene bags (Ben-Yehoshua et al., 1983, Lownds et al., 1994), a technique that may have been preferable in the current study.
5.1.3.2. Effects of Temperature and Waxing on Quality

Significant differences were observed between the replications, as well as between the storage temperatures used ($\alpha = 0.05$). As expected, quality deteriorated more quickly at the higher temperatures (Figure 5.1.4). This effect was particularly obvious in Lot B fruit, which rotted within less than a week at 20°C. These fruit were affected by calcium deficiency during growth, resulting in blossom end rot developing soon after harvest at temperatures >10°C. The storage life of this fruit was significantly reduced at 20°C compared to the other replicates ($\alpha = 0.05$).
Chapter 5 - Respiration Life of Capsicums

Figure 5.1.4. - Time of quality retention of capsicums stored at temperatures between 5 – 20°C. Capsicums from Lot A (●)(n=4), Lot B (●)(n=12), Lot C (▲)(n=18) and Lot D (■)(n=16); error bars represent the standard deviation of each mean value.

Storage life was shortened at the other end of the temperature scale due to chilling injury. Skin pitting and rots become obvious after 2-3 weeks of storage at 5°C for capsicums from Lots A and D. Slight chilling damage was also evident in some capsicums from Lot C held at 7°C. It is interesting to note that the replicates appeared to differ in their sensitivity to chilling injury. Fruit from Lot B did not show evidence of chilling injury at 5°C for approximately a month after harvest. In contrast, some fruit from Lot C showed slight chilling injury symptoms after 4 weeks at 7°C, a temperature not usually damaging to capsicums.

One possible explanation for differences in chilling susceptibility relates to capsicum variety. For example, capsicums cv. “Jupiter” and “Capistrano” were not found to be damaged by storage at 5°C, whereas cv. “Galaxy” had symptoms of chilling injury after two weeks (Mercado et al., 1995). In the current trial, varietal information was not recorded.

Another explanation is that the differences in chilling injury may be due to differences in the exposure of each replicate to stress. Stress leads to alterations in gene expression and the formation of protective proteins (Fiorissen et al., 1996; Paull and Chen, 2000). One form of stress, heat treatment, has been shown to reduce chilling injury in many fruit, including capsicums (Mencarelli et al., 1993; Gonzalez-Aguilar, 2000). Such treatments can even occur naturally in the field, as demonstrated for sun-exposed avocados (Woolf et al., 1999). Mean temperatures during growth of Lot C capsicums were around 8°C lower than those experienced by the other replicates. Possibly, this could have resulted in a reduced tolerance for chilling temperatures. On
the other hand, Lot B fruit were stressed by calcium deficiency during growth. Either by inducing the formation of defensive compounds or by changing the water status inside the flesh, this nutrient deficiency may have influenced the fruit resistance to chilling temperatures.

A number of the fruit used in Lot C had a degree of red colouration at harvest, ranging from colour grade 1 (fully green) to 3 (up to 50% orange/red). Storage life at 7°C was significantly shorter for these fruit than for other replicates stored at the same temperature (α = 0.05). This was because reducing the temperature from 20°C to 7°C increased the mean storage life by only 12 days, a smaller increment than observed with the other groups. Closer examination of the data showed that fruit that were colour grade 1.5 – 3 at harvest had significantly shorter storage lives than fully green fruit (α = 0.05). The greater average maturity of these fruit may therefore have reduced their potential storage life at low temperatures, even though differences between Lots A, C and D were not significant at 15-20°C (α = 0.05).

5.1.3.3. Effect of Temperature and Waxing on Colour Development

All but 4 capsicums from Lots A and B remained green throughout storage. However, capsicums from Lots C and D developed red colour at the higher storage temperatures (Figure 5.1.5.). After 9 days, Lot C capsicums held at 15°C and 20°C had developed significantly more colour than those stored at 7°C and 10°C (α = 0.05). Even after over 3 weeks storage, there was still no significant difference between the 7°C and 10°C fruit (α = 0.05). Similarly, colour development was the same at 12°C, 16°C and 20°C for Lot D capsicums (α = 0.05). It was not until day 30 that capsicums stored at 7°C coloured significantly compared to those at 5°C, whereas red colour was significant after 7 days storage at all other temperatures (α = 0.05).
Figure 5.1.5. - Changes in colour of capsicums from Lots C (a) and D (b). Fruit stored at; (graph a) 7°C (●), 10°C (▲), 15°C (●), and 20°C (■) (n=18); or (graph b) 5°C (▲), 7°C (●), 12°C (▲), 16°C (●), and 20°C (■) (n=16); error bars indicate the standard error of each mean value.

There was no correlation between colour development and either quality grade or weight loss for capsicums held at any temperature (α = 0.05). This is at variance with the suggestion by Lownds et al. (1994), that colour development was reduced in packaged bell peppers due to the alleviation of water stress. On the other hand, Meir et al. (1995) found that polyethylene packaging increased colour development, and suggested that this could be due to the increased humidity around the fruit. In the current study, fruit from Lots C and D coloured at similar rates despite a nearly four fold difference in rate of weight loss. As a further test of the possible relationship between colour and quality, the colour grade at the end of storage life was calculated for each capsicum. Colour ranged from 1 or 1.5 to 5 at all temperatures over 12°C. For example, at 15°C three fruit from Lot C were fully red coloured at the end of storage life, two were fully green, and all others were intermediate. Similar results were found for Lot D. Several fruit that coloured during the first week at 15°C maintained quality for longer than fruit that remained predominantly green throughout storage. This suggests that colour development of capsicums during storage cannot be regarded as a senescence process.

It had been expected that waxing would decrease the O₂ concentration inside the capsicum cavity, thereby retarding changes in capsicum colour. This effect has been reported for capsicums held in storage atmospheres containing reduced O₂ (Luo and Mikitziel, 1996). Waxing significantly inhibited colour development in fruit from Lot C, but did not affect colour development in fruit from Lot D (α = 0.05). Lerdsathanakul and Krochta (1996) trialled 6 different coatings on capsicum fruit, none of which significantly affected colour development. This study has also found that waxing does not have a consistent effect on fruit colour.
Colour development appears to be relatively independent of other physiological processes. Variability may be due to varietal or cultural factors, or be related to maturity at harvest. More work is needed to clarify the factors that affect red colouration of capsicums.

5.1.3.4. **Effects of Temperature and Waxing on Respiration**

The rate of O$_2$ consumption increased logarithmically with temperature (Figure 5.1.6.). The majority of the data fitted closely to log equations. Given that y is the rate of O$_2$ consumption (mol.kg$^{-1}$.h$^{-1}$), and x is the temperature (°C), the relationships were:

- Lot A: \( y = 0.2119\ln(x) - 0.2091 \quad R^2 = 0.9901 \),
- Lot B: \( y = 0.3924\ln(x) - 0.4661 \quad R^2 = 0.9914 \),
- Lot C: \( y = 0.5529\ln(x) - 0.7993 \quad R^2 = 0.9817 \),
- Lot D: \( y = 0.2019\ln(x) - 0.1983 \quad R^2 = 0.9862 \).

The only values that did not fit these relationships were O$_2$ consumption rates at 20°C by Lot A and B capsicums. These values were higher than expected. Respiration was particularly high in Lot B fruit, for which it was nearly double the anticipated rate. It is likely that this was due to the early onset of rots (Figure 5.1.6.).

Respiration varied significantly between the replicates at temperatures >10°C (\( \alpha = 0.05 \)). At 15°C Lots A and D and Lots B and C were the same, but at 20°C all four replicates were different (\( \alpha = 0.05 \)). Rahman _et al._, (1993) also found considerable variability in the rate of capsicum respiration, mean values at 20°C ranging from 0.6 to 1.2 mol CO$_2$.kg$^{-1}$.h$^{-1}$ for groups of three fruit from a single harvest. However, variations over time were not significant (Rahman _et al._, 1993). Similar results were found in this trial, although a slight decrease during storage was observed in some cases, and respiration rates increased with the onset of rots.
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Figure 5.1.6. - The relationship between temperature and the rate of O$_2$ consumption by capsicums from different harvest dates and locations. Each point represents a mean value calculated over the products storage life; log regression lines shown for each data set (calculations do not include values at 20°C from Lots A and B); Capsicums from Lot A (●), Lot B (●), Lot C (△) and Lot D (■); error bars represent the standard deviation of each mean value (n=4–8).

The rate of O$_2$ consumption by waxed fruit was significantly lower than that of unwaxed fruit for Lots C and D during the first 4 days of measurements ($\alpha = 0.05$). However, after 2-3 days, this difference disappeared for both replicates, and the respiration rates of all fruit were similar (Figure 5.1.7.). This suggests that the wax did not fully adhere to the fruit surface, and became detached during storage. This result may also explain the lack of effect of waxing on either colour development or weight loss.
Chapter 5 - Respiration Life of Capsicums

![Graph showing O₂ consumption over time for waxed and unwaxed capsicums](image)

Figure 5.1.7. - Rates of O₂ consumption by 3 separate samples of waxed (●), or unwaxed (▲) capsicums stored at 20°C; data are presented from Lot D, measurements used 3 groups of 3 capsicums, changed on days 3 and 6, points are mean values calculated at 12 hour intervals, error bars represent the standard deviation of each mean (n=120).

5.1.3.5. Total O₂ Consumption during Storage

Total O₂ consumption varied between the replicates and the different storage temperatures used (Table 5.1.2.). As differences between the replicates were significant (α = 0.05), each was analysed separately. Storage at 5°C reduced total respiration during storage in all cases, while 7°C also reduced total respiration for Lot C fruit (α = 0.05). This effect was likely to be due to chilling injury, which prematurely ended storage life.
## Table 5.1.2. Total $O_2$ consumed (mmol kg$^{-1}$) during acceptable storage life by capsicums from different harvest dates and locations and stored at various temperatures. Letters indicate mean values that are significantly different within each Lot ($\alpha = 0.05$).

<table>
<thead>
<tr>
<th>Storage Temperatures</th>
<th>$5^\circ$C</th>
<th>$7^\circ$C</th>
<th>$10^\circ$C / $12^\circ$C</th>
<th>$15^\circ$C</th>
<th>$20^\circ$C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lot A - Markets</td>
<td>51.7 a</td>
<td>273.8 b</td>
<td>232.4 c</td>
<td>213.7 d</td>
<td></td>
</tr>
<tr>
<td>Lot B - Ebenezer</td>
<td>139.3 a</td>
<td>273.6 b</td>
<td>168.9 a</td>
<td>212.5 c</td>
<td></td>
</tr>
<tr>
<td>Lot C - Sudbery</td>
<td>154.9 a</td>
<td>233.8 b</td>
<td>247.8 b</td>
<td>250.3 b</td>
<td></td>
</tr>
<tr>
<td>Lot D - Camden</td>
<td>73.6 a</td>
<td>144.8 b</td>
<td>184.7 c</td>
<td>130.8 b</td>
<td>152.9 b</td>
</tr>
</tbody>
</table>

Lot C fruit appeared to have a "respiration life". That is, total $O_2$ consumed before the fruit became unacceptably deteriorated was similar at all non-chilling temperatures. Lot D fruit also had some similar results, total respiration being the same at 7, 15 and $20^\circ$C. Total $O_2$ consumption by Lot A fruit at non-chilling temperatures was consistently within a small range. However, in this case there were insufficient replicates to properly analyse the data. In contrast, results from Lot B were extremely variable, possibly due to the onset of blossom end rot in many of the fruit. In this case, there did not appear to be any real pattern in total $O_2$ consumption during storage.

It was expected that total $O_2$ consumed by Lot C capsicums would be less than that of the other fruit due to the greater maturity of this group at harvest. This did not prove to be the case. Overall, total $O_2$ consumption by Lot C was not significantly different to that of Lot B, and was higher than Lots A and D ($\alpha = 0.05$). The results therefore did not appear to be affected by maturity at harvest.

Total $O_2$ consumption by waxed capsicums was less than that of the control fruit, but this difference was not statistically significant ($\alpha = 0.05$). The difference was due to the lower initial respiration of the waxed fruit, as waxing did not significantly affect storage life. It was expected that waxing would both reduce respiration rate throughout storage, and extend storage life due to both modification of the internal atmosphere and restriction of water loss (Lerdthanangkul and Krochta, 1996). However, incomplete adhesion of the wax to the capsicum pedicels made the treatment ineffective.
It could be concluded from the data that capsicums have a respiration life of 200-250 moles O$_2$.kg$^{-1}$. This was estimated by combining all the data from the four replications (Figure 5.1.8.). However, this is a considerable generalisation. Differences between the replicates were significant, making the combined data statistically invalid. Also, total O$_2$ consumption varied greatly between individual fruit. For example, total respiration by Lot C fruit ranged from 0.05 to 0.39 mol.kg$^{-1}$ at 10°C and 0.08 to 0.49 mol.kg$^{-1}$ at 15°C.

![Total O$_2$ consumed (mol.kg$^{-1}$) vs Temperature (°C)](image)

*Figure 5.1.8. - Total respiration during acceptable storage life of capsicum fruit from different harvest dates and locations, stored at various temperatures, points represent mean values from Lot A (●)(n=4), Lot B (○)(n=12), Lot C (▲)(n=18) and Lot D (■)(n=16); error bars indicate the standard error of each mean value; the approximate mean of all replications (——) is also shown.*

Capsicum storage life is usually ended by the appearance of decay, or water loss leading to softening. The rate of deterioration due to these factors is strongly influenced by environmental and cultural factors. Cuts, abrasions and micro-cracking can increase disease incidence and water loss, especially if there is a high initial spore load (Fallik et al., 1999). Keeping quality is also affected by daily temperatures and soil nutrient levels during growth (Janse, 1988). In this study, storage life of some fruit was ended prematurely by blossom end rots, while others appeared to have an increased sensitivity to chilling injury. Deterioration of bell peppers may therefore be determined to some extent by extrinsic, rather than intrinsic factors.

With regard to the determination of a "respiration life" for capsicum fruit, these results are inconclusive. Even though a positive relationship between quality changes and O$_2$ consumption can be demonstrated in some cases, the results varied between replicates as well as between individual fruit. This lack of consistent results makes it unlikely that respiration could be reliably...
used to predict capsicum storage life. Other factors, such as plant nutrition, disease pressure and injury levels, may influence the rate of deterioration more than the rate of respiration. Furthermore, no evidence was found in this study that there was an absolute limit to storage life of this fruit. It is entirely possible that fungicidal treatments or moisture barriers could extend storage life while having little effect on respiration rates.

### 5.1.4. **Key Points**

- Changes in quality, colour, and weight loss were evaluated for capsicums that were either stored at different temperatures or partially coated with wax.

- Despite the variability observed between individual fruit, differences between the replicates were significant for all measured parameters. For example, weight loss varied approximately ten-fold between some samples (Lots A and D).

- Red colour developed on some fruit at the higher storage temperatures, but was not correlated with changes in weight loss or other quality measurements.

- Total respiration at non-chilling temperatures was similar for 3 of the replicate groups. However, the totals varied significantly between the replicates, so cumulative respiration during capsicum storage was not constant.

- These results are inconclusive due to the large amount of variability that was observed. This makes it seem unlikely that total respiration could be used as a reliable indicator of capsicum storage life.
5.2 Relationship between respiration rate and storage life of melons (*Cucumis melo* L., Reticulatus Group)

5.2.1. Introduction and Aims

Similarly to capsicums, melons are both susceptible to rots and chilling sensitive, and are therefore perishable products. Melons are also climacteric, and deterioration can occur rapidly once the fruit are fully ripened. This is particularly the case with "Reticulatus" type melons. These have higher ethylene production and a more pronounced respiratory climacteric than "Inodorus" melons, a characteristic which may be linked to their shortened storage life (Kitamura *et al.*, 1975, Lester, 1988).

The primary cause of loss of melons in Australia is the onset of rots. The causes include various species of *Fusarium*, *Gectrichum candidum*, *Rhizopus oryzae*, and *Cladosporium cladosporioides* (Wade, 1982). Whereas good control of decay organisms on other fruits may be achieved using a hot water scrub alone (Fallik *et al.*, 1999), hot water and / or chlorine have not been found effective at controlling rots on netted melons (Carter, 1981, Joyce *et al.*, 1989, Lester, 1989).

Wade and Morris (1983) recommended the use of a combination of fungicides, particularly prochloraz combined with fenapanil or imazalil, while guazatine plus prochloraz was also found to be effective at high concentrations (Morris and Wade, 1983). Imazalil alone has also been recommended (Carter, 1981, Lester, 1989). However, increasing the delay between infection and treatment substantially reduces the effectiveness of the fungicide, especially if infection occurs postharvest (Wade, 1982).

As well as treating with fungicides, several researchers have examined the use of modified atmospheres (MA) to extend melon storage life. An atmosphere containing 10 kPa O₂ + 10 kPa CO₂ has previously been reported to increase storage life of Galia type melons (Aharoni *et al.*, 1993), while 10 kPa of O₂ combined with hydrocooling after harvest increased the storage life of melons cv. Tendral Negro (Perez-Zuniga *et al.*, 1983). However, this latter variety has also been reported as gaining no benefit from MA, 10 kPa O₂ + 12 kPa CO₂ having no effect on decay or overall storage life (Martinez-Javega *et al.*, 1985). Likewise, reducing the O₂ concentration to 1, 2, 4 or 8 kPa did not reduce subsequent decay in melons cv. PVR no. 45 (Stewart, 1979), although
2.5 kPa O₂ did give a positive benefit when used with melon cv. “Earls Favourite” (Zhao and Murata, 1988).

In this section I have examined whether there is a relationship between respiration rate and storage life of rockmelons, a “Reticulatus” type melon. Commercially obtained fruits were used so as to assess whether total respiration was similar despite the natural variability within the population. Rather than using temperature or waxing, as was trialled with capsicums, I have altered respiration rates and storage life by storing the fruit under various controlled atmospheres. It was also interesting to examine the effects of reducing the O₂ concentration on the respiration rates of melons. These are bulky fruit, and the concentrations of O₂ and CO₂ inside the central cavity are generally different to those in the surrounding air (Lyons et al., 1962). The limited gas permeance of melons would seem likely to affect their response to low O₂ storage atmospheres.

5.2.2. Materials and Methods

5.2.2.1. Plant Material

Rockmelons were obtained from commercial suppliers in Sydney. Melons were selected which were of regular, well rounded shape and had been harvested at full slip. That is, they were predominantly orange – yellow coloured, with typical melon aroma and a natural abscission zone around the pedicel. Melons were purchased on 4 separate occasions, with 12 fruit used from each group. The fruit were dipped in a mixture of 1.3 mL L⁻¹ Panoctine, 1g L⁻¹ Benlate and 0.55 mL L⁻¹ Protak 450 EC, with 1-2 ml detergent added as a surfactant. After air drying, the melons were weighed and their volume was estimated from measurements of height and diameter.

The fruit were examined daily during storage for evidence of rots. No other significant changes were observed, so this was the sole criterion used to evaluate storage life. The fruit were considered unacceptable once rots greater than 20mm diameter became evident anywhere on the melon surface, or when juice leakage indicated internal breakdown.

5.2.2.2. Measurements of O₂ Consumption

O₂ demand was monitored over the duration of each experiment using respirometers, as described in Chapter 3. One melon fruit was placed in each respirometer vessel. A sachet made from Tyvec® and containing 20g NaCl (table salt) was also placed inside each vessel to reduce relative humidity. This was intended to prevent condensation which would encourage rots. The
respirometers themselves were placed in water baths constructed from foam insulating containers. Storage was in a room controlled at 20°C ± 1°C, and data were recorded at 6 minute intervals.

Initially, the O2 concentration inside the bowls was maintained between 18 - 20kPa. After 24 hours, 6 of the 12 respirometers were opened, and a second Tyvek® sachet containing approximately 100g of lime was placed inside each vessel. After resealing, three of the vessels were flushed with N2 until the O2 concentration fell to 2kPa, the others being flushed to an O2 concentration of 10kPa. The setpoints for these containers were re-entered on the computer as 1.5 - 2.5kPa O2 and 9 - 11kPa respectively. Three of the remaining containers were also reset to allow O2 to fluctuate from 9 – 11kPa, while the remainder continued at 18 – 20kPa. This gave atmospheres which approximated 2kPa O2 and 10kPa O2 with 0kPa CO2, 10kPa O2 with 10kPa CO2 and air. Measurements continued until the end of storage life.

The atmospheres inside the respirometers were tested periodically during the experiment. Two ml samples were extracted from each vessel using a hypodermic syringe, and then analysed by gas chromatograph using a thermal conductivity detector (model 580, Gow Mac Instrument Co., USA). If CO2 increased over 12kPa inside any vessel it was opened, the sachet of lime was replaced, and the atmosphere regenerated.

5.2.2.3. Calculations

Respiration rates were calculated from the changes in O2 concentrations inside the vessels as previously. However, control of the low O2 atmospheres was not perfect, as the pumps failed to turn off quickly enough to prevent the O2 concentration rising past the setpoints. Values recorded while O2 was >0.8 kPa higher than the nominal concentration were therefore discarded when determining mean rates of O2 consumption. However, these values were included when calculating the cumulative respiration during storage for each individual melon. The mean rate of O2 consumption was calculated from the first 24 hours of storage, while mean rates in the various atmospheres were calculated using values recorded from 48 - 120h after the experiment began.

Mean rates of O2 consumption, storage life, and total O2 consumption by each replicate were compared by two way ANOVA with randomised complete blocks (CoStat statistical software). Significant differences among the means were determined using Duncan’s Multiple Range Test.
5.2.3. Results and Discussion

5.2.3.1. Storage Life Evaluation

Storage life was ended by the onset of rots. The time taken until this occurred did not vary significantly between the different atmospheres used ($\alpha = 0.05$). These results confirm those reported by Stewart, (1979) and Martinez-Javega et al., (1985), both of whom reported no significant benefit from the use of controlled atmospheres with different melon varieties.

However, there were significant differences among the replicates ($\alpha = 0.05$), with storage life ranging from a mean of 13 days (replicate 3) to 7 days (replicate 2). As the melons were obtained through commercial suppliers, there is no way of knowing the physiological age at harvest, time elapsed since harvest, or what treatments (eg. cooling, fungicides etc) have been used. Better defined experimental material could possibly have overcome this problem, and given more consistent results.

5.2.3.2. Gas Exchange

Placing sachets of lime inside the respirometer vessels effectively scrubbed CO$_2$ from the internal atmosphere. Concentrations of CO$_2$ inside these vessels generally did not rise above 0.5 kPa while the melons remained in acceptable condition. In contrast, the concentration ranged from approximately 9 – 12 kPa inside the vessels in which CO$_2$ was not controlled and O$_2$ was reduced to 10 kPa.

CO$_2$ concentrations inside a number of the 10 kPa O$_2$ / 10 kPa CO$_2$ treatment vessels increased during the trial, some containing nearly 19 kPa CO$_2$ after a week of measurements. This suggests that RQ was >1 for these fruit, indicating that some respiration may have been occurring by the anaerobic pathway. This is possible because of the limited gas permeance of the fruit tissue. Lyons et al., (1962) found that O$_2$ concentrations inside melons ripening in air were 10-13 kPa. Reducing the O$_2$ concentration by up to 12 kPa inside the bowls would have also reduced the internal O$_2$ levels, potentially inducing anaerobic conditions at the fruit centres. It would have been interesting to monitor CO$_2$ production by the melons exposed to 2 kPa O$_2$, as fermentation may have been occurring in these fruit.

Decreasing the O$_2$ concentration reduced the rate of respiration in all cases (Figure 5.2.1.). Reducing the O$_2$ concentration to 2 kPa restricted the rate of O$_2$ consumption to around one seventh of its rate in air. An O$_2$ concentration of 10 kPa also inhibited respiration significantly
compared to rates in air. This O$_2$ level had the same effect on respiration rate irrespective of the presence or absence of CO$_2$ ($\alpha = 0.05$).

![Diagram showing respiration rate over time for different conditions.](image)

**Figure 5.2.1.** - Respiration rates of netted melons initially in air, but transferred after 24 hours to atmospheres containing 2 kPa O$_2$ (♦), 10 kPa O$_2$ (●), 10 kPa O$_2$ + 10 kPa CO$_2$ (▲) and air (■); points represent mean values, error bars indicate the standard error of each mean (n=12).

Initial rates of O$_2$ consumption were the same for the first three replicate groups of melons. However, the fourth group of melons proved significantly different ($\alpha = 0.05$). These melons continued to respire faster than the other replicates even after the fruit were placed under modified atmospheres. In order to compare the effects of the treatments using all the replicates, the respiration data were normalised. That is, the respiration rate in reduced O$_2$ was calculated as a percentage of each melons initial rate in air. The percentage changes in O$_2$ consumption did not vary significantly between the replicates ($\alpha = 0.05$). Mean changes in O$_2$ consumption are shown in Table 5.2.1.
Table 5.2.1. - Rates of O$_2$ consumption under modified atmospheres as a percentage of the initial rates in air, mean values from 12 replications, letters indicate results that are significantly different ($\alpha = 0.05$).

<table>
<thead>
<tr>
<th></th>
<th>Percentage of initial rate in air</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 kPa O$_2$</td>
<td>14.9 a</td>
<td>5.1</td>
</tr>
<tr>
<td>10 kPa O$_2$</td>
<td>53.2 b</td>
<td>8.2</td>
</tr>
<tr>
<td>10 kPa O$_2$ + 10 kPa CO$_2$</td>
<td>54.6 b</td>
<td>5.4</td>
</tr>
<tr>
<td>air</td>
<td>94.9 c</td>
<td>8.8</td>
</tr>
</tbody>
</table>

The virtual halving of respiration rate by reducing the O$_2$ concentration to 10 kPa is a larger effect than usually observed with plant tissues. A polynomial equation describing the relationship between O$_2$ consumption and O$_2$ concentration was fitted to the data (Figure 5.2.2.). As a storage atmosphere containing pure O$_2$ did not significantly increase the respiration rates of netted melon cv. "Goldstar" relative to fruit stored in air (Altman and Corey, 1987), the maximum respiration rate (equivalent to maximum enzyme / substrate affinity - K$_{max}$) is likely to occur at close to 21 kPa O$_2$. Using the polynomial equation, it can therefore be calculated that, for the melons in the current study, half the maximum rate (K$_{0.5}$) occurred at approximately 9.2 kPa O$_2$. The K$_{0.5}$ for other plant products is generally in the range of 0.25 – 5 kPa O$_2$ (Beaudry, 2000). Even at the relatively high storage temperature of 20°C that was used, this value is higher than found for other fruits.

The sensitivity of melon respiration to low O$_2$ and the possible induction of anaerobic respiration in storage atmospheres containing around 10 kPa O$_2$ is likely to be due to their low gas permeance. This means that relatively small alterations in the melons' storage atmosphere may result in internal O$_2$ concentrations that significantly inhibit the activity of cytochrome oxidase and the alternative oxidase. These results are consistent with those of Edwards and Blennerhassett, (1990), who found that coating honeydew melons with wax resulted in anaerobic tissue breakdown in air at 8°C. The subject of melon permeance and the effects on respiration will be addressed in Section 7.6 of this thesis.
Chapter 5 - Respiration Life of Melons

![Graph showing the relationship between O₂ consumption and O₂ concentration](image)

Figure 5.2.2. - Effect of decreasing the O₂ concentration on the respiration rate of ripe rockmelons, respiration calculated as a percentage of initial rate in air, data described by the equation \( y = -0.06x^2 + 6.011x \), where \( x \) is the O₂ concentration (kPa), and \( y \) is the O₂ consumption rate, \( R^2 = 0.997 \); error bars indicate the standard deviation of each mean value (\( n=12 \)).

The lack of effect of high CO₂ on respiration rate was an unexpected result. Ten kPa of CO₂ has been shown to restrict respiration by about the same amount as 2 kPa O₂ in other fruit and vegetables, with the effects of low O₂ and high CO₂ being additive (Kader, 1986a). Kubo et al. (1990) found that exposure to 60 kPa CO₂ had little effect on the respiration rates of non-climacteric products such as carrots, mandarins, grapes and lemons. It was suggested that this was because the reduction of respiration by increased CO₂ is primarily due to the suppression of ethylene. However, rockmelons are climacteric fruit. As such, they produce significant amounts of ethylene and undergo a 2-3 fold increase in respiration rate during ripening (Lyons et al., 1962). McGlasson and Pratt (1963) demonstrated that continuous treatment of mature netted melons with ethylene caused substantial increases in respiration. However, once fully ripened, rates of respiration returned to levels similar to those in untreated fruit. This suggests that ripened melon fruit are relatively unresponsive to ethylene. The fruit used in this study were already postclimacteric when measurements started. This may explain the lack of effect of high CO₂ concentrations on respiration that was observed in this trial.

5.2.3.3. Total O₂ Consumption

Cumulative O₂ consumption before the end of acceptable storage life was not constant. This was due to significant differences in the rates of O₂ consumption (\( \alpha = 0.05 \)), that were not matched by changes in storage life. Also, both storage life and respiration rate varied among the replicates.
For example, melons from the fourth replicate respired faster than those from the other groups, and consumed an average of around 165 mmol O₂ kg⁻¹ by the end of storage life. In contrast, melons from the second replicate consumed approximately 89 mmol O₂ kg⁻¹ before their early deterioration. This was significantly less than the other groups. Only replicates 1 and 3 consumed similar mean amounts of O₂ (α = 0.05), being 118 and 134 mmol O₂ kg⁻¹ respectively.

Because the replications varied significantly, treatment effects were analysed separately for each replicate group of melons (Table 5.2.2.). Total O₂ consumption was significantly lower in 2 kPa O₂ than in air in all cases, the effects of the other treatments being intermediate.

<table>
<thead>
<tr>
<th></th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Replicate 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 kPa O₂</td>
<td>62 a</td>
<td>39 a</td>
<td>36 a</td>
<td>79 a</td>
</tr>
<tr>
<td>10 kPa O₂</td>
<td>125 b</td>
<td>89 b</td>
<td>110 b</td>
<td>162 ab</td>
</tr>
<tr>
<td>10 kPa O₂ + 10 kPa CO₂</td>
<td>130 b</td>
<td>106 bc</td>
<td>145 b</td>
<td>177 ab</td>
</tr>
<tr>
<td>air</td>
<td>155 b</td>
<td>122 c</td>
<td>245 c</td>
<td>242 b</td>
</tr>
</tbody>
</table>

Table 5.2.2. - Mean cumulative O₂ consumption during storage life by rockmelons purchased on four different dates and held in various atmospheres at 20°C; letters indicate results that are significantly different for each replicate group (α = 0.05).

The lack of consistency in these results suggests that respiration rate does not indicate the storage potential of melon fruit. While it had not been expected that the supply of carbohydrates would be limiting, the loss of resistance to pathogens is a senescence process and therefore potentially related to metabolic activity. Clearer results may have been obtained using properly defined fruit. Potentially, a large part of the variability observed in this experiment could have been due to differences in maturity, and variations in the respiratory climacteric of individual fruit. Ideally, the respiration rates and quality of mature green fruit should have been measured from harvest through the respiratory climacteric and ripening, until final senescence.
5.2.4. **Key Points**

- The respiration rates and storage lives were measured for mature rockmelon fruit stored in atmospheres approximating 2kPa O$_2$ + 0kPa CO$_2$, 10kPa O$_2$ + 0kPa CO$_2$, 10kPa O$_2$ + 10kPa CO$_2$ or air.
- Storage life varied significantly between the replicates, but was unaffected by the different treatments.
- The rate of O$_2$ consumption was significantly reduced by lowering the O$_2$ concentration, but was unaffected by a concurrent increase in CO$_2$ concentration.
- It was estimated that reducing the O$_2$ concentration to around 9 kPa would halve the rate of O$_2$ consumption by the melons. The sensitivity of melon respiration to O$_2$ concentration is likely to be due to their low gas permeance.
- Respiration rate was not found to be an indicator of storage life for these melons.
5.3. Relationship between respiration rate and storage life of table grapes (Vitis vinifera cv. ‘Red Globe’)

5.3.1. Introduction and Aims

Grapes are different to both capsicums and melons in that they are both non-climacteric and non-chilling sensitive. They have a low rate of respiration, which may decline gradually during postharvest storage (Filatov, 1973, Kumar and Gupta, 1987). Like melons, they contain substantial carbohydrate reserves in the form of sugars. Although rots are also a major cause of loss in table grapes, fungicidal treatments can result in a long storage life when combined with appropriate storage conditions. Some varieties can remain acceptable for 5-6 months (Hedberg, 1977, Eris and Turkben, 1989). However, although the grapes may appear in good condition after this time, much of their flavour is lost (Eris and Turkben, 1989). A more realistic storage life is therefore around 8 – 10 weeks for thick-skinned and strongly-flavoured varieties (Mustonen, 1992) while those with delicate flavours (such as muscat types) are limited to 2 weeks storage in good condition (Ginsburg et al., 1977).

Rots caused by Botrytis cinerea are the most common cause of wastage in grapes. Infection generally occurs during flowering, the fungus remaining dormant until later in the season or after harvest (Hedberg, 1977, Berry and Aked, 1996). Other rots may be caused by Penicillium sp., Cladosporium herbarum and Alternaria sp. (Harvey, 1977). High harvest temperatures (>30°C) will increase the onset of rots postharvest (Morris et al., 1979). Control of decay is usually achieved by rapid cooling to close to 0°C (Nelson, 1978) combined with treatment with sulfur dioxide. Traditionally, this was applied as a 20 minute initial fumigation with 1% sulfur dioxide (SO₂), followed by weekly applications of 0.25% SO₂ for 20-30 minutes (Harvey, 1977).

However, most Australian table grapes are now treated using pads containing sodium metabisulphite powder. These are placed inside the grape boxes where the moisture within the package reacts with the sulphite, releasing SO₂ by the following reaction;

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Dual release pads give a flush of SO\textsubscript{2} immediately after insertion in the box, followed by slow release over 8-10 weeks at 0°C (Mustonen, 1992). As well as controlling rots, sulfur treatment has been reported to significantly reduce respiration rate. This, combined with reductions in ethylene production and increases in IAA and GA, may further extend storage life (Pentzer et al., 1933, YiQiFang et al., 1997).

Other causes of deterioration in grape quality include berry softening, stalk browning, loss of sugars and acidity and shatter (berry drop) (Ginsburg et al., 1977). While susceptibility to shatter is genetically determined, water loss will increase the incidence of these faults (Nelson, 1979).

Development of grapes on the plant has been successfully predicted for some varieties by calculating accumulated heat units with a base of 7-10°C (Brink, 1974, Anderson et al., 1980). A similar method was proposed by Brockwell et al. (1999) to estimate storage life of table grapes. In this case the optimum storage temperature of -1°C was used as a base level. However, this study found that cumulative respiration was a better predictor of stalk quality than heat exposure (Brockwell et al., 1999). The current study therefore aims to measure the total O\textsubscript{2} consumed during storage of table grapes cv. ‘Red Globe’ at different temperatures and with and without sulfur dioxide treatment.

### 5.3.2. Materials and Methods

#### 5.3.2.1. Plant Materials

Grapes were obtained from 3 different growers in the Mildura region in Victoria. The grapes were packed into bunch bags, then into boxes overlayed with sulfur pads. Two of the growers also supplied boxes of untreated grapes. The fruit were then forced-air cooled to approximately 1°C, transported to Sydney by refrigerated truck and stored at 0°C until commencement of each experiment. Grapes were supplied at weekly intervals to allow initial measurements to be made on each batch of grapes.
5.3.2.2.  **Gas Exchange**

The rate of O₂ consumption was measured using respirometers, as previously described. One respirometer was used at each of the storage temperatures tested. As free water greatly increases the rate of decay (Broome et al., 1995), it was important to avoid condensation inside the bowls. Each respirometer vessel was therefore placed in a larger bowl half filled with water. The equipment was also covered with a piece of thick black cloth so as to reduce temperature fluctuations and, therefore, condensation (Figure 5.3.1.).

Approximately 1.6 kg ± 0.1g of grapes was placed in each respirometer. To maintain sulfur fumigation, sections of fungicidal pad and protective liner were placed on top of the sulfur treated fruit. The size of the pads used was calculated to reflect the standard fruit weight : pad area ratio. Although the SO₂ levels inside sealed respirometers may have initially risen higher than would normally occur inside gas permeable cardboard cartons, this factor was offset by the periodic flushing of each bowl with air. The containers were sealed, the O₂ limits set to 18 – 20 kPa, and readings initiated at 15 minute intervals. Because of the long time taken for oxygen to fall to 18 kPa at the three lowest temperatures, these setpoints were changed to 18.5 - 19.5kPa O₂ after the first week of measurements.

There had been some concern that the SO₂ released during storage would affect the oxygen sensors. To eliminate this possibility activated charcoal filters were placed over the inlets to the O₂ sensors (Figure 5.3.1.). Initial tests showed that the oxygen sensors equipped with filters suffered only a short lag (<1min) in their response time compared to oxygen sensors without filters (data not shown). There was no overall difference in readings between sensors with and without filters.
Figure 5.3.1. - Respirometer vessel containing grapes. The system has been modified from the original (Section 3) by placing the bowl in a water bath, including sulfur pads with the produce, placing a charcoal filter over the O₂ sensor, and covering the device with blackout cloth.

As a separate experiment, two additional respirometers were set up at 20°C. As these were to be used to test the effect of sulfur treatment on both apparent O₂ demand and the respiratory quotient (RQ), CO₂ sensors were added to the equipment already described. Vaisala non-linear infra red sensors model GMM 11C were used for this purpose, as described in Section 3. These are externally mounted, so peristaltic pumps were used to circulate the respirometer atmosphere through the sensors and back into the sealed containers.

5.3.2.3. Quality Assessment

Stem condition and berry quality were considered equally important as consumers often use stem greenness to rate freshness. Both of these factors were assessed weekly for fruits at 0-10°C, with more frequent assessments for fruit at the higher temperatures. Grading scales were as follows;
Chapter 5 - Respiration Life of Grapes

Berries

6 excellent appearance, very turgid, full bloom present, no rots, no sulfur damage (bleached areas around pedicel).
5 very good appearance, turgid, bloom present, no rots, no sulfur damage.
4 fair appearance, fairly turgid, some bloom present, insignificant rots, very slight sulfur damage around fruit pedicels of <10% of berries.
3 mediocre appearance, slightly soft, little or no bloom, rots affecting 10-20% of berries, sulfur damage around pedicels of 10-20% of berries.
2 unacceptable quality, fairly soft, rots affecting 20-50% of berries, significant sulfur damage affecting 10-50% of berries.
1 very poor quality, soft, >50% of berries affected by breakdown, extensive severe sulfur damage.

Stems

6 turgid, green, plump.
5 slightly wilted, green.
4 very wilted, light green, >90% of original thickness remaining.
3 slightly dry, straw coloured, 70-90% of original thickness.
2 mostly dry, fairly brown, 50-70% of original thickness.
1 very dry, very brown, <50% of original thickness, shrivelled.

These grade standards were combined with photographs were taken during storage to ensure consistency of grading. An example, showing severe sulfur damage (grade 1 berries), is shown in Figure 5.3.2. As berry and stem quality deteriorated at a similar rate, the grades were added together giving an overall mark out of 12 at each assessment. Changes over time were analysed by linear regression. These regression equations were used to estimate the number of days until total quality scores fell to 6 units.
5.3.2.4. **Treatments**

Grapes were initially stored at 0, 2, 5, 10, 20 and 30°C. However, the extremely rapid rate of deterioration at 30°C made it necessary to reduce this room to 25°C for grapes from growers 2 and 3. Temperatures were monitored using the thermocouples inside the respirometers as well as independent data loggers ('Tiny Tag', Gemini Dataloggers, UK) placed next to each sample. Changes in quality were assessed using 1.6 kg samples of grapes. Each sample was placed inside a plastic bag along with appropriately sized sulfur pads and protective paper. Each bag was supported inside a small cardboard box, the end being turned over to prevent excessive water loss. Storage conditions were therefore analogous to those inside the respirometers. The grapes inside the respirometers were also examined at each sampling time to ensure that their condition was the same as those inside the bags.

Growers 2 and 3 had supplied non-sulfur treated grapes. Changes in RQ due to sulfur treatment were measured at 20°C using around 1.6 kg of treated and untreated grapes in each respirometer. Fungicidal pads were included with the sulfur treated fruit, as previously described. Respiration was measured for a maximum of 40 hours due to the rapid development of rots and diseases in the untreated fruit. The fruit from each grower was measured three times using new batches of fruit on each occasion, giving a total of six replications.

The effect of sulfur treatment was also tested at the various temperatures used. Non-treated grapes from Grower 3 were placed in respirometers at each of the six temperatures under study. Results were compared with those for sulfur treated grapes from the same grower.
5.3.2.5. **Chemical Analysis**

Ten berries were removed randomly from the storage bags at each assessment time. The fruit were then frozen at -80°C until the end of storage life for all samples. They were then individually defrosted and crushed. Soluble solids concentration (SSC) in the juice was measured using an Atago refractometer. The samples were then centrifuged, and the clear supernatant titrated against 0.1M NaOH to calculate titratable acidity (TA).

5.3.2.6. **Calculations**

Grapes respire slowly, especially at low temperatures. As a result, small fluctuations due to temperature and electrical interference can substantially affect the results when calculating mean changes per time interval. To minimise this effect, the rate of O₂ consumption was calculated as a mean value per day for each replication. Another method also used to reduce variability in the results was to calculate the linear regression line for each block of decreases in O₂. This was expressed as $y = mx + b$. The rate of O₂ consumption can be calculated by multiplying m by the vessel volume, then dividing by (time interval x product weight). As respiration rates of the grapes were generally constant while the fruit remained sound, the results from this method were used to estimate a mean value for respiration during storage for each replication. These data were linearised against temperature by transforming to logarithmic values. The relationship between O₂ consumption rate and temperature was then expressed as a regression equation.

Over the first week of measurements the rate of O₂ consumption was not constant. This was because it took several days for the fruit to thoroughly cool and equilibrate. Total respiration was therefore calculated by adding the sum of O₂ consumption during the first week of storage to the remaining days of storage life x mean respiration rate. Cumulative respiration values could be compared by ANOVA using CoStat statistical software, and differences between treatments determined using Duncans Multiple Range Test.

5.3.3. **Results and Discussion**

5.3.3.1. **Effect of Temperature and Sulfur Dioxide Treatment on Respiration**

The mean respiration rates of the grapes supplied by the different growers were not significantly different ($\alpha = 0.05$), so the replicates were combined. Previous researchers have reported that grape respiration rates decrease during storage (Filatov, 1973, Eris and Turkben, 1989). In this
case respiration rates did not change significantly over time while the fruit remained in sound condition. However, \( \text{O}_2 \) consumption rose markedly with the onset of decay, with rates increasing significantly for grapes stored at 0, 2 and 5\( ^\circ \)C after 6-7 weeks \((\alpha = 0.05)\). Similar increases in respiration rate have been noted after 30 days at 1\( ^\circ \)C (Kumar and Gupta, 1987) and 40 days at 0\( ^\circ \)C (Yahia et al., 1983). It is possible that changes in respiration may be a more sensitive indicator of grape condition than subjective assessments. As shown in the example in Figure 5.3.3., the rate of \( \text{O}_2 \) consumption by grapes at 5\( ^\circ \)C began to rise 2-3 weeks before the end of storage life. This is likely to be due to internal breakdown of the berries, even though deterioration was not visible.

![Graph showing O2 consumption and quality score over time](image)

**Figure 5.3.3.** - Respiration rate (●) and quality grade (■) of grapes stored at 5\( ^\circ \)C from grower 1; each point represents the mean rate of \( \text{O}_2 \) consumption from one day of measurements \((n=240)\), or a subjective assessment based on stem and berry quality; respiration rates began to increase at least 15 days before subjective quality assessments showed the fruit to be unacceptable.

Temperature inside each room was controlled to within ±1\( ^\circ \)C. The exception was the 0\( ^\circ \)C room, which showed more significant, irregular fluctuations, ranging from −1.3 to 2.1\( ^\circ \)C (Figure 5.3.4.). This resulted in greater variability in the results from that room.
Chapter 5 - Respiration Life of Grapes

Figure 5.3.4. - Temperatures in storage rooms held nominally at 6°C (----), 2°C (--), 5°C (---), 10°C (-----), 20°C (-----) and 25°C (-----).

The respiration data were linearised against changes in temperature by calculating the natural log (Figure 5.3.5.). The resulting regression line was described by $y = 0.1153x + 3.5997$, where $y$ is Ln respiration rate (mmol.kg$^{-1}$.h$^{-1}$), and $x$ is the temperature (°C).

Figure 5.3.5. - Respiration rates of grapes stored at different temperatures. Each point represents the mean rate measured during storage for grapes from one of three growers. Linear regression line also shown ($R^2 = 0.98$).

Sulfur dioxide (SO$_2$) treatment has previously been shown to reduce respiration rate (Pentzer et al., 1933, YiQiang et al., 1997). On the other hand, oxidation of sulfur in the pads to SO$_2$ could
have increased apparent oxygen demand. However, neither of these factors had an effect in the
current trial, as the rate of O2 consumption was not significantly affected by SO2 treatment at any
of the storage temperatures used (α = 0.05). CO2 production was also unaffected by SO2
treatment at 20°C (α = 0.05) (data not shown). The only observed difference between the
treatments was that respiration rate increased sooner in the untreated fruit. This was likely to
have been caused by the earlier onset of decay. Observed reductions in respiration rate as a
result of SO2 treatment could therefore be due to suppression of latent infections, rather than
interference with grape metabolism.

The respiratory quotient (RQ) ranged from 1 to 1.3, the mean being 1.2. Although RQ values
were slightly higher in the treated fruit, this difference was also not significant (α = 0.05), and
mean RQ did not change during the measurement period. RQ was greater than 1, suggesting
that some organic acids were being used, in conjunction with sucrose, as respiratory substrates
(Wills et al., 1998).

5.3.3.2. **Effect of Temperature and Sulfur Dioxide Treatment on Storage Life**

Storage life was calculated for grapes in the same way as for capsicums (Section 5.1), using a
regression line to estimate the time at which quality was no longer acceptable. However, changes
in grape quality were not a constant function of time, and showed considerable fluctuations
(Figure 5.3.6.). Although linear regression fitted the data best, R2 values ranged from 0.99 to
0.72. This variability reflects the difficulty in consistently evaluating grape quality. Grapes do not
undergo the distinct changes in colour, firmness or weight that can be used to monitor changes in
products such as bananas, kiwifruits or carrots. Changes in grape quality are generally subtle,
and occur gradually over time.
Figure 5.3.6. - Changes in total quality score during storage of grapes from grower 1 at 0°C (●), 2°C (◆), 5°C (■), 10°C (▲), 20°C (○) and 25°C (◇). Each point represents an assessment of one treatment unit (1.6 kg grapes); linear regression lines are shown for each data set.

Initial calculations of storage life found that the number of days of acceptable quality was less at 0°C than at 2°C. The main cause of the end of storage life in this case was rots. This may have been related to the greater temperature fluctuations, including occasional exposure to temperatures below zero, which occurred in this storage room. It was also observed that the grapes that were examined only twice during storage at 0°C were better quality than those that were assessed weekly. This suggests that handling may have increased damage to these fruit, as has been demonstrated for peaches and nectarines stored at low temperatures (Dahlenburg, 1999). The storage life of these fruit was therefore estimated using both the fruit examined weekly, and that which had been assessed only twice during storage.

Ballinger and Nesbitt (1982) found that the rate of decay of muscadine grapes doubled between 10 - 20°C, while between 0 and 10°C the decay rate was tripled. This is different to the results from the current study, in which storage life was less than doubled at 10°C compared to 0°C, but was virtually quadrupled between 10 - 20°C. The red globe grapes that were used are less susceptible to shatter than muscadine grapes, and so the sample may have contained fewer loose berries with open peduncle scars. Such an effect would have reduced the number of potential infection sites, potentially also reducing infection levels at moderate temperatures. However, such an explanation is purely speculative.

Storage life did not vary significantly among the replicates (α = 0.05), so the data were combined. As with respiration, the data were transformed into a natural logarithm. However, in this case the
data remained non-linear against temperature (Figure 5.3.7.), and was better described by a polynomial regression line \( R^2 = 0.99 \) than a linear regression line \( R^2 = 0.97 \).

![Graph showing the relationship between temperature and the natural logarithm of storage life.](image)

*Figure 5.3.7. - Time of quality retention by fruit stored at different temperatures; each point represents the storage life of grapes from one of three growers; polynomial regression line also shown \( R^2 = 0.99 \).*

Storage life of grapes is affected by a number of factors. Unlike many other fruits, grapes consist of two very different tissue types – the berry and the stem. Each of these components will respire and undergo moisture loss at a different rate. Stems are likely to dehydrate even under high RH due to the high osmotic potential of the attached berries. Furthermore, rates of disease growth are strongly temperature dependant (Nelson, 1978). As grapes commonly carry latent infections (Berry and Aked, 1996), their deterioration will be a function of not only berry robustness and injury levels, but also of the growth rate of the pathogen.

Storage life was significantly shorter in grapes that were not treated with SO\(_2\) \((\alpha = 0.05)\). These fruit remained in acceptable condition for only 2-3 days, whereas SO\(_2\) treated grapes remained in good condition for up to a week. Quality loss was due to rapid fungal growth under the relatively warm, high RH storage conditions.

### 5.3.3.3. Biochemical Changes During Storage

Neither TA or SSC of grapes changed during storage life. TA varied between samples, but changes over time were not significant. Average TA was 4.4 meq, with a standard deviation of 0.32. SSC remained at approximately 14-16%. The low respiration rate of grapes means that approximately 1-2g of carbohydrates per kg are likely to be consumed before the end of storage.
life. This means that <15% of their initial sugar content will be oxidised to fuel respiration. It seems likely that the measurement methods used were not accurate enough to detect such small compositional changes, and that the samples used were not large enough to eliminate variation due to differences among individual berries.

5.3.3.4. **Effect of Temperature and Sulfur Dioxide Treatment on Total O₂ Consumption**

Cumulative O₂ consumption during storage life differed between SO₂ treated and untreated fruit, as well as between the various storage temperatures. Total O₂ consumption was significantly higher at 10°C than at other temperatures, and was also greater at 5°C compared to 0°C and 25°C (α = 0.05). However, differences between 0°C, 2°C, 20°C and 25°C were not significant (α = 0.05). As indicated in Figure 5.3.8., mean values increased as temperature rose to 10°C, then subsequently decreased. If there were a relationship between respiration rate and storage life, this line would be approximately straight or a regular curve. The results therefore indicate that respiration rate is not a good indicator of the potential storage life for grapes. Factors that influence the development of rots are more likely to be important when determining grape storage life.

![Figure 5.3.8.](image)

*Figure 5.3.8. - Total O₂ consumed by grapes before the end of storage life at various temperatures; each point represents the result from a single treatment unit; the line indicates mean values at each temperature.*
5.3.4. Key Points

- Respiration rates and changes in quality of grapes cv. ‘Red Globe’ were measured using fruit from 3 different growers and stored at various temperatures. Both SO₂ treated and untreated grapes were used, and RQ of grapes at 20°C was also measured.

- Respiration rates were transformed by calculating their natural logs, and these data were linear against temperature.

- The natural log of storage life was close to linear against temperature, but was better described by a polynomial regression.

- Leaving grapes untreated with SO₂ had no effect on respiration rate or RQ, but significantly reduced storage life.

- Mean RQ was 1.18, which suggests that organic acids were used in conjunction with glucose as respiratory substrates.

- Total respiration during storage was reduced in fruit that had not been SO₂ treated, and varied significantly between several of the storage temperatures. The results suggest that respiration is not a reliable indicator of potential storage life for grapes.
5.4 Relationship between respiration rate and storage life of broccoli (*Brassica oleracea* L.).

5.4.1. Introduction and Aims

The other plant organs that have been examined so far have been mature fruits. While these may differ considerably in their carbohydrate reserves and mode of ripening, changes are likely to occur relatively slowly (King and Morris, 1994a). In contrast, broccoli is an immature inflorescence, and is actively growing and developing at harvest. It has a high metabolic rate combined with relatively low carbohydrate reserves (Irving and Joyce, 1995). Perhaps as a result of these factors, broccoli deteriorates rapidly after harvest, having a storage life of only 2-3 days at 20°C (Irving and Joyce, 1995).

A number of methods of extending broccoli storage life have been studied, several of which are used commercially. The most important is temperature. At 0°C, broccoli quality can remain acceptable for up to 8 weeks (Klieber and Wills, 1991). Low temperatures are sometimes combined with modified atmospheres. Although reports are mixed regarding the benefits of reducing O₂ (Lipton and Harris, 1974, Klieber and Wills, 1991, Izumi et al., 1996), atmospheres containing >5 kPa CO₂ have been widely reported to delay broccoli senescence (Lebermann et al., 1968, Forney et al., 1989, Bastrash et al., 1993, Izumi et al., 1996). High CO₂ inhibits loss of chlorophyll as well as reducing the rate of respiration (Matthook, 1996). Although increased CO₂ levels are associated with the development of off-odours during extended storage (Lipton and Harris, 1974, Wang, 1979, Makhlof et al., 1989a, Klieber and Wills, 1991), these generally dissipate after a period in air (Kasmire et al., 1974). As a result, this method of slowing the rate of broccoli deterioration is used commercially.

As well as inhibiting loss of chlorophyll, modified atmospheres have the potential to reduce the synthesis and response to ethylene (Burg and Burg, 1967, Kader, 1986a). Ethylene significantly increases the rate of broccoli senescence (Makhlof et al., 1991, Tian et al., 1994). Production of ethylene by broccoli is substantially reduced by low temperatures (Ohta et al., 1993), modified atmospheres (Wang, 1979, Makhlof et al., 1989b) and hot water dips (Forney, 1995, Tian et al., 1997). Cytokinins are thought to interfere with perception of ethylene by broccoli (Tian et al., 1997).
1995). Application of cytokinins has been demonstrated to slow down chlorophyll loss (Shewfelt et al., 1983, Downs et al., 1997), with up to 90% extension of storage life reported in some cases (Rushing, 1990). More recently, the compound 1-methylcyclopropene (1-MCP) has been found to inhibit the binding of ethylene to the ethylene receptors of various fruits and flowers (Abdi et al., 1998, Golding et al., 1998, Jiang et al., 1999, Mullins et al., 2000). By inhibiting the action of ethylene, treatment with 1-MCP more than doubled the storage life of broccoli at 20°C (Ku and Wills, 1999, Fan and Mattheis, 2000).

In general, treatments reported to improve broccoli storage life also reduce respiration rate. Broccoli contains relatively small amounts of soluble sugars and starch compared to most fruit and vegetables, and these levels may be approximately halved after 24 hours at 20°C (King and Morris, 1994b, Downs et al., 1997). Cytokinin treatment extended broccoli storage life without reducing carbohydrate depletion (Downs et al., 1997). However, an exogenous supply of sucrose delayed yellowing of broccoli florets, suggesting that sucrose supply may be one factor controlling broccoli senescence (Irving and Joyce, 1995).

The effects of low temperatures and increased CO₂ concentrations on broccoli storage life could be due to ethylene inhibition, restriction of metabolic activity or both. This section examines the effects of these treatments on cumulative respiration during storage, as well as the mechanisms by which these treatments extend storage life. The first two experiments examine the effects of temperature and modified atmospheres on storage life and total O₂ consumption by broccoli. The final experiment compares the effects of 1-MCP and an atmosphere containing 2 kPa O₂ on broccoli stored at 0°C or 7°C. These treatments were chosen because it was estimated that the respiration rate in 2 kPa O₂ at 7°C would be similar to the rate in air at 0°C. If respiration rate is linked to broccoli storage life, the rate of senescence should be the same in air at 0°C and 2 kPa O₂ at 7°C, but decreased by 2 kPa O₂ at 0°C. However, if ethylene is the chief cause of senescence, then storage life in both air and 2 kPa O₂ at 0°C should be similar to that of 1-MCP treated heads at 7°C. The results are discussed in the context of managing broccoli quality during storage.

5.4.2. Materials and Methods

5.4.2.1. Plant Materials

Broccoli cv. 'Green Belt' was sourced from two growers in the Richmond area near Sydney, and one grower in northern NSW. Broccoli from the first two sources was harvested in the early
morning and transported to the CSIRO North Ryde laboratory within two hours. The third batch was packed in ice, and shipped overnight to Sydney. All the heads were dipped in a 200 ppm calcium hypochlorite solution and patted dry with paper towels. They were then sorted to select heads of even size, colour and shape for each treatment unit. Ten treatment units were prepared, each consisting of six broccoli heads.

For the final experiment using 1-MCP, broccoli cv. 'Green Belt' was obtained from a grower at Castlereagh, near Sydney. The heads were transported immediately after harvest to the laboratory at the University of Western Sydney, Hawkesbury. They were then disinfected and sorted as previously. The heads were sorted into six treatment units consisting of six heads to be used for measurements of respiration and monitoring of quality changes. An additional four heads were added to each unit to be used for destructive analysis at intervals during storage.

5.4.2.2. Treatments

The first six treatment units from each grower were used to examine the effects of temperature. Two heads from each unit were used for respiration measurements at 0, 2, 5, 10, 20, and 25°C, with the remainder stored in perforated plastic bags on cardboard trays. After a week, the heads used for respiration measurements were changed, so that after 3 weeks all heads still in sound condition had been measured.

The remaining four treatment units were used in the modified atmosphere experiment. All the broccoli heads from each unit were placed inside respirometers at 20°C, each vessel containing two heads. The rate of O₂ consumption was measured in air for 24 hours, after which the respirometers were reset to generate modified atmospheres. Respiration measurements were then continued until the end of storage life.

1-MCP was generated for the final experiment using 1g of EthylBloc® powder (Floralife, Burr Ridge, IL) dissolved in 20 ml of potassium hydroxide solution and placed in a sealed drum. According to the product specifications, this quantity was sufficient to generate >1 μl l⁻¹ gaseous 1-MCP, the concentration at which the ethylene receptors are fully saturated (Ku and Wills, 1999). Two of the treatment units were exposed to the gas for 24 hours at 7°C, the remaining four units being stored in air at the same temperature. The 1-MCP treated heads were then returned to air for storage at either 0 or 7°C. Of the untreated heads, two units were placed in an atmosphere containing approximately 2kPa O₂ + 0kPa CO₂ at either 0 or 7°C, the remainder placed in air at the same temperatures. Two heads from each unit were placed in a respirometer,
the remainder being stored in plastic drums ventilated with either air or the special gas mixture. Respiration was measured for a week, then the heads were swapped over as previously.

5.4.2.3. Measurements of O₂ Consumption

Oxygen demand was monitored over the duration of each experiment using respirometers, as previously described. For the experiments examining the effects of temperature and modified atmospheres, the respirometers were placed in water baths constructed from foam insulating containers. This was unnecessary during the final experiment due to the higher rate of air circulation inside the rooms. All the bowls were covered with black cloth to prevent photosynthesis, and data were recorded at 6 minute intervals.

The O₂ concentrations inside the vessels held at different temperatures were maintained between 18 - 20kPa until the broccoli reached the end of storage life. However, for the experiment on modified atmospheres, the atmosphere inside each vessel was altered after 24 hours to either 2kPa O₂ + 0kPa CO₂, 10kPa O₂ + 0kPa CO₂, 10kPa O₂ + 10kPa CO₂, or left as air (control). These atmospheres were generated as previously described for melons in Section 5.2. This included placing Tyvec® sachets containing approximately 100g of lime inside each vessel that had 2-10 kPa O₂ with 0 kPa CO₂. The atmospheres were checked periodically by injecting samples into the gas chromatograph (model 580, Gow Mac Instrument Co., USA). If CO₂ increased over 12kPa inside any vessel the sachet of lime was replaced, and the atmosphere re-established.

The low O₂ atmosphere used in the final experiment was generated using both the respirometers and a flow through system. At each of the two temperatures used, two heads were placed inside a respirometer, the remainder being stored inside large plastic drums. Respirometers were set up as previously, the O₂ fluctuating between 1.5-2.5 kPa, and excess CO₂ removed by reaction with lime. The drums were connected to a humidified gas stream containing 2 kPa O₂ in N₂. The composition of the gas stream was checked automatically every 12 hours by gas chromatography (David Bishop Instruments, UK).

5.4.2.4. Quality Assessment

For the experiment on storage temperatures, broccoli heads stored in air at >10°C were assessed daily, those at 10°C were examined every 3-4 days, while the heads at the lowest storage temperatures were examined weekly. Full daily assessments were not possible for the heads stored in modified atmospheres. Instead, quality was assessed initially, after 24 hours in air, and
at the end of storage life. Colour was measured with the Minolta Colorimeter (CR-300, Osaka, Japan). L, a and b values were recorded, and the Hue angle ($H^o$) calculated as previously. Hue angle is generally the best indicator of broccoli colour, as it indicates the relationship between different tones, rather than yellowness alone (Shewfelt, 1993). In the final experiment, weight was also measured, and quality was assessed subjectively by comparison with a photographic scale (Appendix 7). This scale designated broccoli as grade 1 – freshly harvested to grade 5 – completely yellowed or with severe stem rots. Storage life was considered over once grade increased to 3 – slightly yellowing or with minor rots.

To create the photographic scale, freshly cut broccoli was stored at 20°C and photographed at least once each day with either a SLR film camera (EOS 1000, Canon) or a digital camera (Photogenie, Agfa). This was repeated three times. At the same time as the heads were photographed, their colour was measured using the colourimeter. A grade of 3 was equivalent to an $H^o$ value between 109 and 117 (Figure 5.4.1.). As all broccoli was unacceptable at this grade, the highest $H^o$ reading (117) was designated the end of storage life. The number of days until $H^o = 117$ was estimated to within 0.1 days using polynomial regression lines calculated from the data (Figure 5.4.2.).
Figure 5.4.1. - Changes in hue angle (H°) of broccoli compared to subjective quality grade during senescence at 20°C; error bars indicate the standard deviation of each mean value (n=9).

Figure 5.4.2. - Changes in H° value of replicate 1 broccoli stored at 20°C (●), 10°C (▲), 5°C (■), 2°C (●), and 0°C (○); each point represents a mean value calculated from 6 measurements x 6 broccoli heads; error bars indicate standard error of each mean (n=6); days until H° = 117 estimated from polynomial regression equations; R² values shown for lowest 3 temperatures.

5.4.2.5. **Analysis of Carbohydrates**

Carbohydrates were analysed in the final experiment using the method described by McConchie et al., (1991). At each assessment time, branchlets were removed from the four broccoli heads that had been added to each treatment unit, and immediately frozen at –70°C. After completion of the experiment, individual florets were excised from the branchlets and freeze-dried for 48 hours.
They were then ground into a powder, and the soluble carbohydrates extracted twice in 80% ethanol. The samples were freeze dried a second time, and redissolved in a further 1 ml of 80% ethanol. Sugars were then determined by high performance liquid chromatography (HPLC). Sucrose, glucose and fructose were identified and quantified based on areas under the peak of standard 2.5g.L⁻¹ solutions.

5.4.2.6. Calculations

Respiration rate was calculated as described in Chapter 3. Storage life was calculated on a treatment unit basis for the first experiment on the effects of temperature, and for the broccoli held at 0°C in the final experiment. In the second experiment on modified atmospheres both respiration rate and storage life were calculated for each pair of heads, while storage life was calculated for each individual head stored at 7°C in the final experiment. Data on storage life and respiration rates at different temperatures were linearised by transformation into natural logarithms. The regression lines from this data were used to predict the rates of O₂ consumption and storage life in air for the final experiment, and these estimations compared to the actual data.

Total O₂ consumed during storage was calculated by summing the data for all treatments. Where data had not been continuously recorded (broccoli stored below 5°C for >3 weeks), mean values were substituted for this period. The results were subjected to ANOVA (1 - 3 way, randomised complete blocks) using CoStat statistical software, the results being compared by Duncan’s Multiple Range Test.

5.4.3. Results and Discussion

5.4.3.1. Effect of Temperature

The mean temperature inside each room during the experiment were calculated. In most cases, temperatures remained within ±0.5°C of the original setpoint. The exception to this was the 5°C room, which had shut down for several days due to an electrical fault. This affected data from the first two replicates. On restarting, the temperature fell lower than required, remaining at 3°C during the storage of replicate 3. Calculations involving the broccoli from this room therefore used the mean air temperature from the relevant storage period.
5.4.3.1.1. Respiration Rate

The mean daily O₂ consumption rate over the first 3 weeks of storage was calculated for broccoli from each grower and at each storage temperature. Respiration rates stabilised after <3 days of measurements, and did not change significantly over the experimental period. When all the daily results were compared, there were found to be numerically small but nonetheless significant differences among the replicates (α = 0.05). However, when the overall mean rates during storage were considered these differences were not significant. Therefore, data from the different growers were combined.

Respiration increased significantly as the temperature rose. Q₁₀ values (the degree to which respiration increases for every 10°C rise in temperature) was constant at approximately 3.5 over the temperature range used. This is at variance with previous work suggesting that Q₁₀ values are usually higher between 0-10°C than between 10-20°C (Wills et al., 1998). The relationship between respiration rate and temperature was described by the equation; \( \ln(\text{\(\mu\text{mol O}_2\text{kg}^{-1}\text{.h}^{-1}\)}) = \text{temperature (°C)} \times 0.1248 + 5.7785 \) (Figure 5.4.3.).

![Graph showing the relationship between respiration rate and temperature for broccoli](image)

*Figure 5.4.3. - Relationship between the rate of O₂ consumption and temperature for broccoli cv. "Green Belt". Each point represents the mean rate of measured during storage for broccoli from one treatment unit, data from 3 replications; the linear regression line is also shown (R² = 0.986).*

5.4.3.1.2. Storage Life

Storage life of broccoli was also temperature sensitive. At 0.3°C, broccoli heads remained green for up to 80 days, although rots were observed from around 60 days. However, at 25°C, the
heads yellowed after less than 2 days. Storage life was not linearised against temperature as respiration rate had been. However, the data could be described by two separate linear regression lines (Figure 5.4.4.). The equation for broccoli held at <6°C was:

\[
\ln \text{ (days life)} = \text{ temperature (°C)} \times -0.3054 + 4.6005,
\]

For broccolis stored at >6°C the equation was:

\[
\ln \text{ (days)} = \text{ temperature (°C)} \times -0.1171 + 3.4218.
\]

Simultaneously solving these equations showed that they intercepted at 6.3°C. This suggests that the relationship between temperature and storage life of broccoli is different below around 6°C compared to above this critical temperature.

![Graph showing the relationship between temperature and storage life of broccoli.](image)

**Figure 5.4.4.** Days of acceptable storage life of broccoli held at temperatures above (◆) or below (■) 6°C; each point represents the days of acceptable storage life for broccoli from one treatment unit, data from 3 replications; separate linear regression lines are shown for broccoli at temperatures below \((R^2 = 0.93)\), and above 6°C \((R^2 = 0.97)\).

### 5.4.3.1.3. Cumulative \(O_2\) Consumption

The total \(O_2\) consumed during storage was also different at low temperatures compared to those above 6°C. Cumulative respiration was the same at 25°C, 20°C and 10°C \((\alpha = 0.05)\). It was also the same at 0°C and 2°C \((\alpha = 0.05)\), with the results from 5°C storage being intermediate \((\alpha = 0.05)\). This suggests that total \(O_2\) consumption by broccoli is relatively constant at warmer temperatures, but is more than doubled by storage under 6°C. These results are consistent with those calculated from Klieber and Wills (1991) for broccoli stored continuously at one temperature.
However, even a short exposure to a higher temperature, as experienced by some of the broccoli nominally stored at 5°C, can have a relatively large effect on storage life. This demonstrates the importance of maintaining the cold chain during transport and handling of this perishable product.

![Graph showing O₂ consumed vs. temperature.](image)

*Figure 5.4.5. Total O₂ consumed during acceptable storage life by broccoli held at various temperatures; each point represents the result from one treatment unit; data from 3 replications.*

### 5.4.3.2. Effects of Modifying the Storage Atmosphere

#### 5.4.3.2.1. Respiration Rate

Initial respiration rates in air varied among the broccoli from different growers, replicate 1 being significantly faster and replicate 3 significantly slower than replicate 2 ($\alpha = 0.05$). However, these differences disappeared once the heads were placed under the various low O₂ / high CO₂ atmospheres that were used. All the treatments reduced respiration rates significantly relative to the initial rate in air (Table 5.4.1.).

#### 5.4.3.2.2. Storage Life

High CO₂ (10 kPa) combined with 10 kPa O₂ significantly increased storage life by delaying yellowing of the broccoli florets ($\alpha = 0.05$). CO₂ was the primary cause of this effect, as demonstrated by comparison with the atmosphere containing 10 kPa O₂ alone. This atmosphere did not increase storage life relative to the control ($\alpha = 0.05$). CO₂ concentrations of 5 – 20 kPa have previously been reported to improve chlorophyll retention by broccoli (Lebermann et al., 1968, Lipton and Harris, 1974, Wang, 1979, Makhlof et al., 1989a.). While some researchers
have found that O$_2$ must be reduced below 1 kPa to reduce yellowing (Lipton and Harris, 1974). Lebemann et al. (1968) found an atmosphere containing 2 kPa to be beneficial. In the current study, this treatment significantly extended storage life (Table 5.4.1.) without causing the off odours that can develop at lower O$_2$ concentrations (Ballantyne et al., 1988; Izumi et al., 1996).

5.4.3.2.3. **Cumulative O$_2$ Consumption**

Total O$_2$ consumed during acceptable storage life was calculated for each pair of broccoli heads. The results were slightly lower than those found for broccoli stored at over 6°C (Table 5.4.1.). This can be accounted for by the greater delay between harvest and the start of measurements on each occasion (approximately 0.5 days at 20°C). Total O$_2$ consumption was significantly increased by high CO$_2$ compared to the other treatments ($\alpha = 0.05$). This shows that the increase in storage life under these conditions cannot be explained by the effect on respiration. Rather, the increase in life is likely to be due to inhibition of chlorophyll degradation (Kader, 1986a). In contrast, even though 2 kPa nearly halved the rate of O$_2$ consumption, total respiration was the similar to that in air. In this case, respiration rate would appear to be a good indicator of potential storage life.

<table>
<thead>
<tr>
<th>O$_2$ consumption (mmol.kg$^{-1}$.h$^{-1}$)</th>
<th>Respiration rate (% of initial rate in air)</th>
<th>(days of acceptability)</th>
<th>Total O$_2$ consumed (mol.kg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 kPa O$_2$, 0 kPa CO$_2$</td>
<td>2.19 c</td>
<td>51.8 c</td>
<td>3.6 c</td>
</tr>
<tr>
<td>10 kPa O$_2$, 0 kPa CO$_2$</td>
<td>3.68 b</td>
<td>87.0 b</td>
<td>2.7 b</td>
</tr>
<tr>
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<td>2.48 c</td>
<td>65.1 c</td>
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</tr>
<tr>
<td>control (air)</td>
<td>4.41 a</td>
<td>102.7 a</td>
<td>2.4 b</td>
</tr>
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</table>

*Table 5.4.1. - Effect of different atmospheres on the rate of O$_2$ consumption both as mmol.kg$^{-1}$.h$^{-1}$ and as a proportion of initial measurements in air, storage life (days) and the total O$_2$ consumed during storage (mol.kg$^{-1}$); values are means calculated from 3 pairs of heads x 3 replications (n=9); letters indicate values that are significantly different ($\alpha = 0.05$).*

5.4.3.3. **Effects of 1-MCP Treatment Compared to a Low O$_2$ Atmosphere**

5.4.3.3.1. **Weight Loss**

Weight loss was significant for all treatments at each measurement time. Both the 1-MCP treated and heads stored in 2 kPa O$_2$ lost significantly less weight than the control ($\alpha = 0.05$). Weight loss
was faster at 7°C than at 0°C, but this difference was not significant over the whole storage period (α = 0.05).

5.4.3.3.2. Respiration Rate

The mean respiration rate was calculated for each week of measurements. Differences between the heads used in successive weeks were not significant. The broccoli respired more rapidly than had been predicted from previous data, being 0.46 and 1.08 mol.kg⁻¹.h⁻¹ compared to 0.32 and 0.77 mol.kg⁻¹.h⁻¹ as expected at 0 and 7°C respectively.

Fan and Mattheis (2000), found that 1-MCP treatment reduced respiration rate at 10°C. In this case, respiration rate was not significantly lower in 1-MCP treated heads at either temperature. It would seem likely that 1-MCP treatment does not reduce respiration. Rather, low levels of ethylene in the storage atmosphere increase the respiration rate in air, and this effect is blocked by 1-MCP treatment.

In contrast, the low O₂ atmosphere significantly reduced the rate of O₂ consumption relative to the control at both storage temperatures. At 7°C, this reduction meant that the respiration rate was similar to that in air at 0°C (α = 0.05), as had been predicted (Table 5.4.2.). In the previous trial at 20°C, it was shown that reducing the O₂ concentration to 2 kPa nearly halved respiration. At 7°C the effect was similar, respiration being reduced by approximately 60%. However, at 0°C, low O₂ decreased respiration by only 30%. It has been proposed that low O₂ reduces respiration due to the limited rate at which O₂ can diffuse into cells, rather than by direct inhibition of the activity of cytochrome oxidase (Cameron and Reid, 1982). At low temperatures metabolic activity is reduced, and the partial pressure gradients between the inside and outside of cells are likely to be less significant. This may account for the reduced effects of modified atmospheres at low temperatures.

Lowering the O₂ concentration can also reduce respiration by inhibiting the effects of ethylene. The data of Burg and Burg (1987) suggested that reducing the O₂ concentration to 2-3 kPa would approximately halve the effects of ethylene on respiration. This effect is likely to be most apparent at low ethylene concentrations (Beaudry, 1999), as are commonly found in many storage environments (Willis et al., 1999). However, if this was the mechanism with broccoli, the 1-MCP treated heads stored at 7°C should have respired at a similar rate to those in 2 kPa O₂. This was not the case, suggesting that respiration was inhibited by factors other than ethylene.
5.4.3.3. Storage Life

Estimation of storage life at 0°C was complicated by the appearance of rots. These resulted in quality becoming unacceptable (grade = 3) after 53 days for the control heads. Both 1-MCP treatment and low O₂ extended storage life to 60 days. Although Ku and Wills (1999) found that 1-MCP treatment inhibited rotting, this effect was small in the current study. After 50-60 days the heads were still green, and the H² value had not decreased (Figure 5.4.6.). Due to the onset of rots, storage life was the same for all heads in each treatment unit. As a result this data could not be analysed statistically.

Whereas broccoli stored at 0°C had a shorter than anticipated storage life, broccoli at 7°C remained acceptable for approximately a week more than the 13 days predicted. Treatment with 1-MCP and low O₂ storage further extended storage life, both treatments significantly delaying yellowing relative to the control (Table 5.4.2.). In this trial, storage life was determined individually for each broccoli head. This was done by calculating a polynomial regression line for each data set, and using this to estimate the number of days until H² = 117. Figure 5.4.6. demonstrates this calculation using the mean values from each treatment unit. The results are compared with those at 0°C, for which the H² values from all three treatments were similar and therefore combined.

![Figure 5.4.6.](image)

*Figure 5.4.6. - Changes in H² of broccoli stored at 7°C; heads treated with 1-MCP (▲), stored in 2kPa O₂ (●), and untreated control (○), or heads stored at 0°C under various conditions (○). Points are mean values for each treatment unit; error bars indicate the standard error of each mean (n=6); polynomial regression lines are also shown for each data set.*
Storage life at 7°C may have been extended due to the high rate of ventilation of the heads in this trial. Whereas broccoli was previously stored either in the respirometers or in perforated plastic bags, on this occasion storage was in drums ventilated with a continuous stream of air or 2 kPa O₂. This may have swept away the ethylene that would otherwise have accumulated around the heads. Reduced exposure to ethylene might also account for the relatively small effect of 1-MCP treatment. Ku and Wills (1999) found that 1-MCP treatment more than doubled broccoli storage life at 5°C. In this case the increase was closer to 40%.

5.4.3.3.4. Cumulative O₂ Consumption

Total respiration during storage life was calculated using mean rates of O₂ consumption. At 0°C, cumulative respiration by the control and the 1-MCP treated heads were similar to those previously calculated at 0-2°C. Total respiration was least for the broccoli stored in 2 kPa O₂ at 0 and 7°C, and greatest for the broccoli treated with 1-MCP and stored at 7°C. Total O₂ consumed by the untreated controls was the same at both of the storage temperatures used. This was an unexpected result, and was due to both a higher than predicted respiration rate and extended storage life in the 7°C stored heads.
Table 5.4.2. - Mean respiration rates, storage life, and cumulative O₂ consumption during storage of broccoli at 0°C and 7°C; respiration rates calculated from weekly measurements (n=4-6); storage life and total O₂ consumption determined for whole treatment units at 0°C, but for individual heads at 7°C (n=6), letters indicate mean values that are significantly different (α = 0.05).

5.4.3.4. Biochemical Analysis

The major soluble sugars were found to be glucose and fructose. Sucrose was present initially as approximately 10% of the total, but had decreased below measurable levels after 2 weeks at either 0°C or 7°C. Pogson and Morris (1997) reported a similar proportion of total sugars as sucrose at harvest, although Tian et al., (1997) found that sucrose initially accounted for 50% of total sugars. Both Pogson and Morris (1997) and Downs et al., (1997) found that sucrose levels fell by >90% after 2 days at 20°C.

Total soluble sugars were initially 33mg.g⁻¹ DM (Figure 5.4.7.). Previously published figures vary by several orders of magnitude in their estimations. On the basis that broccoli is around 10% DM (Barth et al., 1993), initial soluble sugars range from 120 mg.g⁻¹ DM (King and Morris, 1994b) to 60 mg.g⁻¹ DM (Tian et al., 1997) or 0.07 mg.g⁻¹ DM (Pogson and Morris, 1997). Irving and Joyce (1995) and Downs et al., (1997) found initial sucrose concentrations of approximately 20 mg.g⁻¹ DM and 100 mg.g⁻¹ DM respectively. Differences may be due to cultivar, time of sampling after harvest, or the method of measurement.

Glucose and fructose decreased at a similar rate over time. Sugars had fallen significantly after 2 weeks at either 0°C or 7°C (α = 0.05). At the end of storage life, broccoli contained less carbohydrate if stored at 0°C than 7°C (α = 0.05). This is consistent with the results of Pogson...
and Morris (1997), who found that low temperature storage allowed respiration to continue at very low carbohydrate concentrations. Although the atmosphere containing 2 kPa O₂ resulted in lower carbohydrate levels than the control at 0°C, the results were only different at the lowest significance level tested (α = 0.05). Respiration rate was reduced in this treatment relative to the control, so the opposite result had been expected. Also, even though reserves of soluble sugars decreased during storage, respiration remained stable over this time for all treatments. This supports the view that broccoli respiration is tightly regulated, being unaffected by substrate supply (Irving and Joyce, 1995, Pogson and Morris, 1997). The interactions between temperature and treatment, temperature and time and treatment and time were not significant. Effectively, the different treatments had no effect on levels of soluble sugars.

![Graph showing changes in levels of total soluble carbohydrates during storage of broccoli at 0°C (a) or 7°C (b) in air (•), in 2 kPa O₂ (■) or in air following treatment with 1-MCP (▲); error bars indicate standard deviation of each mean value (n=2-3).](image)

**Figure 5.4.7.** Changes in levels of total soluble carbohydrates during storage of broccoli at 0°C (a) or 7°C (b) in air (•), in 2 kPa O₂ (■) or in air following treatment with 1-MCP (▲); error bars indicate standard deviation of each mean value (n=2-3).

### 5.4.4. Conclusions

Pogson and Morris (1997) proposed that respiration was the primary factor limiting broccoli storage life at high temperatures. Under these conditions, carbohydrates are rapidly exhausted in the fast respiring florets. As the supply cannot be replenished sufficiently quickly from reserves in the stem tissues, chlorophyll membranes are broken down and used as a respiratory substrate. When respiration is reduced by low temperatures, transport is no longer a limiting factor, and carbohydrate reserves may be used more efficiently. Irving and Joyce (1995) found that carbohydrate reserves in the stalk remained unchanged at 22°C despite depletion of sucrose and yellowing in the florets. In this study, both yellowing and sucrose loss were reduced when an exogenous supply of sucrose was provided. This result appears to support the suggestion that respiration can limit storage life of broccoli.
Another theory is that ethylene is the critical factor regulating broccoli senescence. The extended storage life of broccoli at low temperatures and in controlled atmospheres could be related to reduced sensitivity to ethylene (Makhlouf et al., 1989b, Makhlouf et al., 1991, Tian et al., 1994). Forney (1995) found that a dip in water at 50°C inhibited broccoli yellowing, a treatment that may have caused denaturation of one or more enzymes involved in ethylene synthesis. Cytokinins treatments have an effect on carbohydrate loss which may be small (Irving and Joyce, 1995) or nil (Downs et al., 1997). However, they are effective inhibitors of the effects of ethylene, and can significantly increase broccoli storage life (Batal et al., 1982, Zacarias and Reid, 1990, Tian et al., 1995, Irving and Baird, 1996).

In this series of experiments, it was found that total O₂ consumed during acceptable storage life was doubled when the temperature was reduced below 6°C. This result demonstrates the importance of keeping broccoli below this critical temperature in order to maintain quality. Cumulative respiration could be increased during storage at higher temperatures by an atmosphere containing high CO₂ or by 1-MCP treatment. Calculations from Irving and Baird (1996) showed that treatment with BAP (a cytokinin) had a similar effect (Table 2.3.1.). Low temperatures, high CO₂, 1-MCP and cytokinins all inhibit ethylene sensitivity. Moreover, treatment with 1-MCP and exposure to 2 kPa O₂ were equally effective at maintaining broccoli quality, even though 1-MCP treatment did not reduce respiration rate. These factors suggest that ethylene is the critical factor controlling broccoli senescence.

Even though respiration is unlikely to be the primary cause of the end of broccoli storage life, there was a correlation between respiration rate and senescence under some conditions. Total O₂ consumption during storage was relatively constant at around 0.3 mmol.kg⁻¹ for broccoli stored at over 6°C in air or in an atmosphere that did not contain high CO₂. At low temperatures total O₂ consumption was around 0.6 mmol.kg⁻¹. However, these results need to be treated with care, as different results were found at 7°C in the final experiment.

Rather than loss of carbohydrates being the cause of senescence, or even an indirect trigger for changes in gene expression (Pogson and Morris, 1997), respiration rates may reflect responsiveness to ethylene. One of the major effects of ethylene is to stimulate respiration, and all metabolic processes are affected by temperature. It may therefore be concluded that measurements of respiration may be a useful guide to storage potential of broccoli. However, it should not be assumed that a reduction in respiration rate automatically produces an equivalent increase in broccoli storage life.
5.4.5. Key Points

- The effects of temperature, storage atmosphere and ethylene on respiration rates and storage life of broccoli florets were determined.

- Respiration rates and storage life data were transformed to linearise them against temperature. Although respiration rate could be predicted from a linear regression line, two regression lines intercepting at approximately 6°C were required to describe storage life data. This suggested that senescence occurred differently above this critical temperature compared to below it.

- Total O₂ consumption was similar at all temperatures above 6°C, but was almost doubled at lower temperatures.

- Modifying the storage atmosphere (10kPa O₂ + 0kPa CO₂, 10kPa O₂ + 10kPa CO₂, or 2 kPa O₂ + 0kPa CO₂) significantly reduced respiration rate. However, storage life was not affected by reducing the O₂ concentration to 10kPa unless it was combined with an increase in CO₂. This second atmosphere also increased storage life more than 2kPa O₂ alone.

- Cumulative O₂ consumption was similar in the low O₂ atmospheres and the control, but was increased by the atmosphere containing high CO₂.

- Respiration rate was reduced relative to the control by 2kPa O₂ at both 0°C and 7°C, but was not changed significantly by 1-MCP treatment at either temperature.

- Storage life of all broccoli at 0°C was ended by rots, so could not be statistically analysed. At 7°C, both 1-MCP treatment and low O₂ significantly increased storage life relative to the control.

- These results suggest that storage life of broccoli is primarily limited by exposure to ethylene rather than by respiration.

- Respiration rate is sensitive to the effects of ethylene, and so may be a useful indicator of storage life of broccoli under certain conditions.
5.5. **Progress Note**

The original hypothesis of this thesis was to determine whether respiration was directly linked to the storage life of fresh produce. If this were true, then total respiration occurring between harvest and the end of storage life should be the same, irrespective of the storage conditions. This would be the "respiration life" of the fruit or vegetable. Initial calculations from published data found some consistencies in the "respiration life" of various products. Total O\textsubscript{2} consumption was also similar under some storage conditions for 3 of the 4 products examined in the previous chapter. However, although the results indicate that respiration rate may sometimes be an indicator of storage life it appears unlikely to be the main factor determining the rate of senescence of all fresh products.

If respiration is not a function of senescence, what are the factors that control produce respiration rate? For example, ethylene is likely to have an important influence on respiration, but the response to ethylene varies between products. Responsiveness is also likely to be affected by product maturity, temperature, the composition of the internal and external atmospheres and even, possibly, attachment to the plant. The remainder of this thesis therefore examines several of the factors that influence respiration rates of fruit. These include the influence of photosynthesis on respiration, the effect of the harvest process itself on respiration during the climacteric, and the role of fruit permeance in limiting respiration rate.
Pre-Harvest Factors Affecting
Respiration Rates of Fruit and
Vegetables

6.1. Refixation of Respiratory CO₂ by
Developing Capsicum Fruit.

The production of fruit and flowers by plants represents a substantial energy cost. However, many fruit remain green during development, and so can directly contribute to their own carbon and energy requirements by photosynthesis (Bazzaz et al., 1979, Cipollini and Levey, 1991). In many cases, photosynthesis refixes the carbon dioxide that has been liberated by respiration. This is possible because of the limited permeability of such bulky plant organs, which results in internal atmospheres that contain elevated CO₂ concentrations (Smillie, 1992). Indeed, Knee (1995) estimated that without an internal source of CO₂, tomato fruit would be unable to photosynthesise at atmospheric CO₂ concentrations less than 3kPa.

Fruit photosynthesis has been shown to occur by the carboxylation of phosphoenolpyruvate (PEP). CO₂ is initially fixed by PEP carboxylase into oxaloacetic acid, which is then converted into malic acid by NADP malate dehydrogenase (Wilmer and Johnstone, 1976). Although photosynthesis by fruit has been compared to that occurring in C₄ and CAM plants (Phan, 1970), a number of differences suggest that it is not the same as either of these processes. However, it is also different to the C₃ metabolism that occurs in the leaves, suggesting that fruit photosynthesis does not fit into existing categories (Willmer and Johnston, 1976, Blanke et al., 1986, Blanke and Lenz, 1989).

The conservation of respired CO₂ by photosynthetic refixation is likely to be a significant source of carbon for many plant organs. This process has been documented for developing fleshy fruit including cherries (Kappes and Flore, 1986), blueberries (Birkhold et al., 1992), apples (Blanke and Lenz, 1988, Lenz, 1989), peaches (Pavel and DeJong, 1993) and tomatoes (Willmer and Johnstone, 1976, Laval-Martin et al., 1977, Hetherington et al., 1998). Refixation of respired CO₂ by
Chapter 6 - Pre-Harvest Respiration and Photosynthesis

Photosynthesis has also been demonstrated in pea pods (Atkins et al., 1977, Flinn et al., 1977, Price and Hedley, 1980), cotton fruit (Wullschleger and Oosterhuis, 1990, Wullschleger et al., 1991), and immature cereal grains (Araus et al., 1993). In some cases, chlorophyll retention in ripening fruit is increased by low light levels, as shown by the development of 'green shoulder' in tomatoes (Smillie et al., 1999).

Many studies have assumed photosynthetic rate to be the difference in CO₂ evolution between the dark and the light, or been based on the uptake of C¹⁴ labelled CO₂ (Willmer and Johnston, 1976, Marcelis and Hofman-Eijer, 1995). However, both of these methods may have underestimated photosynthesis (Blanke and Lenz, 1989). Changes in the internal CO₂ concentration which are not reflected in CO₂ production rates (Hetherington, 1997), and dark fixation of CO₂ (Farineau and Laval-Martin, 1977) could potentially have introduced errors into previous calculations of total photosynthetic activity.

Hetherington (1997) proposed that chlorophyll fluorescence might be a better measurement of photosynthetic capacity in fruit. Using this technique it was shown that the rate of photosynthesis in mango fruit peel was equivalent to or higher than that in the leaves. This is different to previous studies, which found that CO₂ fixation by mangoes was only 1% of that in the leaves, and therefore contributed little to development (Chauhan and Pandey, 1984). Fixation of ¹⁴CO₂ in the pericarp of green cherry tomatoes also occurs at a higher rate than in the leaves when it is expressed on a chlorophyll basis (Laval-Martin et al., 1977). This suggests that calculations by Steer and Pearson (1976) showing that the photosynthesis contributed only 12% towards the total carbon requirement of capsicum fruit may have underestimated the importance of photosynthesis, especially considering the relatively high degree of light penetration into capsicum flesh.

Capsicums differ from most other fruit in that up to 50% of their total volume consists of a large air-filled space within the fruit. In comparison, free air space accounts for 20 to 25% of the volume of apples (Ulrich & Marcellin, 1955), while potato tubers contain as little as 1-2% of their volume as air space (Banks and Kays, 1988). As has been demonstrated for these other products, the composition of the internal atmosphere of a capsicum is likely to be different to that in the outside air. Under conditions of constant temperature and darkness the gas composition inside the cavity is essentially constant (Banks and Nicholson, 2000). However, it seems less likely that the internal atmosphere composition would stabilise under conditions in the field, where there are pronounced variations in light and temperature.

It has been suggested that capsicums are able to photosynthesize under high light conditions (Czarnowski, 1995). However, capsicums lack the lenticels or stomata that enable gas exchange through the cuticles of other fruit (Blanke and Holthe, 1997). It therefore seemed possible that that
the internal cavity acted as a carbon sink for respired CO₂. This carbon could subsequently be re-fixed by photosynthesis.

To investigate this possible relationship between respiration and photosynthesis, I decided to examine the internal atmospheres of mature capsicum fruit under field conditions. The experiments were conducted using a commercial crop located at Ebeneezer, an area north-west of Sydney. Capsicums are grown during the summer months, and so large diurnal fluctuations in temperature and light intensity occurred over the experimental period. The results are discussed in relation to the adaptations of bell pepper fruit that allow the recycling of respiratory CO₂.

6.1.2. Materials and Methods

6.1.2.1. Plant Material

Field experiments were located at the property of Mr Steve Vella at Ebeneezer, NSW during January-February, 1999. The capsicum plants had been grown in a silty loam, mulched with white plastic and watered by overhead irrigation. Equal numbers of mature green fruit were selected from the north and south sides of rows running east-west.

For each experiment eight capsicum fruit were monitored over a three day period. Initially, fruit 1-4 were covered with black cloth to prevent photosynthesis. After two days, the treatment was reversed, with fruit 5-8 being covered. This process was repeated three times in successive weeks, using new fruit each time.

6.1.2.2. Measurement of Internal O₂ and Fruit Temperature

A hole (5mm diameter) was punched in the uppermost surface of each fruit using a septum cutter. Into this was inserted a plastic tube (10 mm long, 6mm diameter) which had been sterilised with 70% ethanol. A KE-25 O₂ sensor (Japan Storage Battery Co. Ltd, Osaka, Japan) was glued onto the protruding end of the tube. The sensors were protected from water and direct sunlight using a plastic liner and aluminium foil (Figure 6.1.1.).

The temperature and CO₂ concentration inside each fruit were also recorded. A thermocouple was attached to individual fruit using plastic insulating tape, and a septum constructed for gas sampling. The septum consisted of three layers of 2cm² fabric tape attached to the capsicum skin. The top layer of tape could be peeled back and a hypodermic syringe inserted, then the tape re-adhered after sampling to prevent leakage.
Measurements of O₂ and temperature were taken every 15 minutes using two data loggers (1200 Series Squirrels, Grant Instruments Ltd, Cambridge, England). CO₂ concentration was sampled twice a day using hypodermic syringes. To prevent leaks during transport to the laboratory, the syringes were pushed firmly into a rubber stopper then immersed in water. Samples were taken at 6:00 a.m. and 4:00 p.m. to correspond with expected minimum and maximum periods of photosynthesis respectively. Analysis was by gas chromatography (model 580, Gow Mac Instrument Co., USA).

![Diagram of measurement setup]

*Figure 6.1.1. - Measurement of the internal O₂ and CO₂ concentrations and skin temperature of a capsicum growing in the field. O₂ measured using a KE-25 O₂ sensor, CO₂ determined by withdrawing 1 ml samples for gas analysis, temperature measured using a T-type thermocouple.*

### 6.1.2.3. Calculations

Cameron and Yang (1982) demonstrated that approximately 97% of gas exchange in tomatoes occurred through the stem scar. A similar effect has been found in capsicums, with 85-90% of gas diffusion occurring through pores in the fruit pedicel (Bower et al., 2000). Whereas pores in the pedicel tissue allow approximately equal exchange of O₂ and CO₂, the capsicum cuticle is differentially permeable to gases. In this case, CO₂ has been found to diffuse approximately 10 x (Banks and Nicholson, 2000) or 2.6 x (Bower and Patterson, 2000) as rapidly as O₂. By comparing increases in internal CO₂ with decreases in internal O₂, it is possible to estimate the proportion of permeation through each diffusive pathway.
If it is assumed that RQ = 1, the relative permeability of any area to CO₂ and O₂ can be calculated using the relationship:

\[ \text{kPa O}_2 + R \times (\text{kPa CO}_2) = 2! \]

where R is the ratio of CO₂ permeation to O₂ permeation. For example, if the internal atmosphere of a fruit contained 15kPa O₂ and 3kPa CO₂, R would equal 2, indicating that the fruit was twice as permeable to CO₂ as to O₂.

For a fruit like a capsicum, in which gas exchange occurs through two different pathways, the R value for the whole fruit will be the sum of the R values for each area of the fruit multiplied by the relative contribution of that area to total gas diffusion;

\[ R_{\text{total}} = (R_{\text{pedicel}} \times \alpha) + (R_{\text{cuticle}} \times \beta) \]

Where \( \alpha \) is the proportion of total gas exchange that occurs through the pedicel, and \( \beta \) is the proportion of total gas exchange occurring through the cuticle. As \( \alpha + \beta = 1 \), the equation can be rearranged to find the value of \( \alpha \):

\[ \frac{R_{\text{total}} - R_{\text{cuticle}}}{R_{\text{pedicel}} - R_{\text{cuticle}}} = \alpha \]

\( R_{\text{pedicel}} \) may be assumed to equal 1, as the diffusivities of O₂ and CO₂ through pores are approximately equal. \( R_{\text{cuticle}} \) was found to equal approximately 2.6 by Bower et al. (2000) and 10.4 by Banks and Nicholson (2000). \( R_{\text{total}} \) was calculated from the internal atmosphere of each fruit.

For example, for a fruit which contained 17kPa O₂ and 3.33kPa CO₂:

\[ 17 + (R_{\text{total}} \times 3.33) = 21 \quad \therefore \quad R_{\text{total}} = 1.2 \]

Using the value of 2.6 for \( R_{\text{pedicel}} \) combined with \( R_{\text{total}} = 1.2 \)

\[ \frac{(1.2 - 2.6)}{(1 - 2.6)} = \alpha = 0.875 \]

Alternatively, using the value of 10.4 for \( R_{\text{pedicel}} \) combined with \( R_{\text{total}} = 1.2 \)

\[ \frac{(1.2 - 10.4)}{(1 - 10.4)} = \alpha = 0.979 \]

In this case, either 87.5% or 97.9% of diffusion into the capsicum is occurring through the pedicel tissue. Calculations using actual results were calculated using \( R_{\text{pedicel}} = 2.6 \) only.

The results were subjected to analysis of variance (ANOVA) using Statistica analytical software (Kernel Release 5.5A, StatSoft Inc.). As O₂ concentration had been recorded every 15 minutes over a 3 day period, it was not practical to examine each datum individually. Instead, mean O₂ concentrations from each 6 hour interval were analysed. All recorded CO₂ concentrations were also subjected to ANOVA, and the standard error of each mean was calculated for both O₂ and CO₂.
6.1.3. Results and Discussion

6.1.3.1. Diurnal Fluctuations in Temperature

The surface temperatures of the capsicum fruit changed by up to 15°C during the experiment (Figure 6.1.2). It was thought that covering capsicums to block out sunlight would reduce diurnal temperature fluctuations. However, this did not occur, and surface temperatures of both light and dark fruit were similar. Weather conditions were similar during the three repetitions of the experiment. The exception was day 3 repetition 2, when conditions were cloudy resulting in relatively low daytime temperatures.

![Graph showing diurnal temperature fluctuations.]

Figure 6.1.2 - Diurnal changes in skin temperature of field grown capsicums initially covered with blackout cloth (-----) or remaining in the light (- - -); after 48 hours the blackout cloths were transferred from the covered capsicums to the uncovered fruit; mean values shown; error bars indicate the standard error of each mean at 12 hour intervals (n=12).

6.1.3.2. Changes in the Composition of the Internal Atmospheres

The internal atmospheres of fruit kept in the dark became progressively depleted in O₂ as the temperature rose. Such a relationship was expected, because temperature affects respiration rates more than gas permeance. However, where photosynthesis was able to continue the internal O₂ concentration remained relatively constant over a wide range of fruit temperatures (Figure 6.1.3.). The tendency towards a constant partial pressure of O₂ was demonstrated repeatedly by separate fruit and under different diurnal temperature changes.
The internal partial pressure of CO$_2$ rose in synchrony with falls in internal O$_2$. Covered capsicums had significantly lower internal O$_2$ concentrations and higher CO$_2$ concentrations than capsicums which remained in the light, both during the day and as mean values over the experimental period ($\alpha = 0.01$). This is consistent with the results of Knee (1995), who found higher internal CO$_2$ concentrations inside tomatoes shaded with aluminium foil. There was a significant interaction between time and treatment for both O$_2$ and CO$_2$ concentrations ($\alpha = 0.01$).

Figure 6.1.3 - Concentrations of O$_2$ (line only) and CO$_2$ (■) inside capsicums initially covered with blackout cloth (— — —) or remaining in the light (— — —). After 48 hours the blackout cloths were transferred from the covered capsicums to the uncovered fruit; data are mean values from 12 capsicum fruit recorded over 3 different time intervals; error bars indicate the standard error of each mean.

6.1.3.3. Routes of Gas Exchange

The proportion of total gas exchange occurring through the pedicel area was estimated by comparing the changes in O$_2$ and CO$_2$ concentrations (Table 6.1.1.). It has previously been estimated that around 85-90% of gas diffusion occurs through the pedicel area in harvested capsicum fruit (Bower et al., 2000). In this experiment it was found that approximately 78-90% of gas diffusion occurred this pathway, suggesting that attachment to the plant may reduce gas exchange by this pathway.

Preventing fruit from photosynthesising during the day appeared to decrease the proportion of gas diffusion occurring through the pedicel to 65-70% of the total. Changes in the partial pressure difference between the inside and outside of the fruit would not be expected to affect the proportion
of diffusion through the pedicel tissue. The results also cannot be explained by temperature changes, as heating was similar in covered and uncovered capsicums. Possibly, the smaller than expected changes in internal CO₂ during the day may actually be due to fixation of CO₂ into malate, rather than a change in the route of gas permeation.

<table>
<thead>
<tr>
<th>Time and day</th>
<th>Capsicums initially covered</th>
<th>Capsicums initially in light</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean O₂ (kPa)</td>
<td>Mean CO₂ (kPa)</td>
</tr>
<tr>
<td>4 pm, day 1</td>
<td>15.1 a</td>
<td>3.8 a</td>
</tr>
<tr>
<td>6 am, day 2</td>
<td>18.1 b</td>
<td>2.2 b</td>
</tr>
<tr>
<td>4 pm, day 2</td>
<td>15.1 a</td>
<td>3.8 a</td>
</tr>
<tr>
<td>6 am, day 3</td>
<td>17.9 b</td>
<td>2.3 b</td>
</tr>
</tbody>
</table>

Changeover – capsicums in light covered up, darkened capsicums uncovered

<table>
<thead>
<tr>
<th>Time and day</th>
<th>Capsicums initially covered</th>
<th>Capsicums initially in light</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean O₂ (kPa)</td>
<td>Mean CO₂ (kPa)</td>
</tr>
<tr>
<td>4 pm, day 3</td>
<td>18.0 b</td>
<td>2.4 b</td>
</tr>
<tr>
<td>6 am, day 4</td>
<td>18.1 b</td>
<td>2.0 b</td>
</tr>
</tbody>
</table>

Table 6.1.1. - Internal O₂ and CO₂ concentrations and approximate percentage of total gas permeation occurring through the pedicel area in unharvested capsicum fruit either remaining exposed to light or covered with blackout cloth; letters indicate means that are significantly different (α = 0.05); permeation (%) calculated from mean values.

6.1.4. Conclusions

Steer and Pearson (1976) found that assimilation of C¹⁴ by capsicum fruit represented a fixation rate of 70ng CO₂h⁻¹ g⁻¹. This was lower than rates of photosynthesis detected in leaves, and it was suggested that translocated carbohydrates supply approximately 7.5 times as much carbon to the fruit as can be directly fixed by the fruit itself. Czarnowski (1995) also stated that photosynthesis by capsicum fruit supplied little carbon for biomass production, although it could reduce the demand for respirable substrate by 60-80%. However, neither of these studies considered the role played by the internal cavity. Recycling of respired CO₂ could have lead to a significant underestimation of total photosynthesis by capsicum fruit. Photosynthesis may also improve fruit quality, as capsicums grown in full sun develop higher levels of capsaicins and sugars than shaded fruit (Jeong et al., 1995).
The bell pepper fruit appears to be well-adapted for refixing CO₂. Its developing seeds, where metabolic activity is greatest, liberate CO₂ into a continuous gas phase (Figure 6.2.4). Gas mixing within this open space is likely to be more efficient than in fruit such as the apple, where the atmosphere is contained between cells. The layer of photosynthetic tissue that surrounds this internal atmosphere is thereby exposed to a uniform concentration of CO₂. This anatomy recalls that described for the developing pea pod, in which photosynthetic layers contained within a cuticle surround an inner gas space and the developing seeds (Atkins et al. 1977). In addition, a cuticle with no stomata and low permeance to gases limits loss of CO₂ from the capsicum cavity.

Figure 6.1.4 - Section diagram of capsicum fruit showing routes of gas diffusion, sources of respired CO₂ and photosynthetic tissues.

The capsicum cuticle represents a cohesive barrier to gas exchange between the seeds and flesh and the external atmosphere. This is analogous to an artificial modified atmosphere package, where respiring tissue is contained within a gas-permeable bag (Kader et al., 1989). However, in artificial systems of this kind, the composition of the internal atmosphere is sensitive to changes in respiration rate caused by temperature changes (Kader et al., 1989, Flodin et al., 1999). Because a rise in temperature results in a greater increase in respiration rates than in package permeability, internal levels of O₂ will decrease. My results show that pepper fruit behave in a similar way when they are prevented from photosynthesising. However, under normal field conditions a balance develops between respiration, photosynthesis and diffusion, resulting in a relatively constant internal atmosphere. In other words, capsicums appear to maintain some degree of homeostasis in their internal atmosphere, despite variations in temperature.

Fruit such as capsicums are similar to CAM plants in several ways. Their structure is bulky and fleshy, with a waxy cuticle of limited permeability to gases. Both green fruit and CAM plants are not
only able to photosynthesize during the day, but can continue to fix CO$_2$ in the dark (Willmer and Johnstone, 1976, Singal et al, 1986). Accumulation of malic acid within the cell vacuoles allows high rates of CO$_2$ recycling in CAM plants during the night, especially where temperatures are relatively high (Luttge and Ball, 1987). Many green fruit have been shown to contain reserves of malate that may be metabolised both during the light and in darkness (Farineau and Laval-Martin, 1977, Smillie, 1992).

If fruit do use malic acid to accumulate respired CO$_2$, it might be expected that this would result in an increase in the titratable acidity of the flesh during dark conditions. However this has not been shown for any fruit. For example, Willmer and Johnston (1976) did not find any diurnal fluctuation in organic acid concentrations in tomatoes. This has been cited as a major difference between CAM and fruit photosynthesis (Blanke and Lenz, 1989). Fruit therefore appear to metabolise CO$_2$ in a manner which resembles that in CAM tissues with regard to a number of the compounds involved, but which differ in several anatomical and physiological characteristics (Willmer and Johnston, 1976).

**Acknowledgement**

I wish to thank Mr S. Vella for allowing the use of his commercial crop of bell peppers at Ebenezer, NSW.

**6.1.5. Key Points**

- The internal atmospheres of developing capsicum fruit were monitored. Half of the fruit were covered in black cloth for two days, then the covers transferred to the remaining fruit for the third day of measurements.
- Internal O$_2$ and CO$_2$ concentrations did not change significantly during measurements of fruit in the light, despite changes in temperature of up to 15°C. However, when the fruit was covered, O$_2$ and CO$_2$ underwent significant diurnal fluctuations.
- This suggests that photosynthesis allows the fruit to maintain a constant internal atmosphere.
- Permeation through the pedicel tissue was reduced in attached fruit compared to published data on detached fruit, suggesting that attachment may reduce gas exchange by this pathway.
6.2 Changes in \( \text{O}_2 \) Consumption by Standard and Genetically Modified Charantais Melons During Ripening On and Off the Vine in France.

6.2.1. Introduction and Aims

There is considerable evidence that ripening is accelerated once fruit are detached from the plant. Detachment stimulates ripening in apples (Smock, 1972, Sfakiotakis and Dilley, 1973, Yang et al., 1986, Pooter et al., 1989, Blanpied, 1993), passionfruit (Shiomi et al., 1996), plums (Abdi et al., 1997) and melons (Pratt et al., 1977, Bliss and Pratt, 1979, Hadfield et al., 1995). Avocados provide an extreme example of the effect of detachment on ripening, as cultivars such as Hass and Fuerte do not ripen on the tree even if treated with exogenous ethylene (Gazit and Blumenfeld, 1970). After detachment from the tree, avocados can remain insensitive to ethylene for several hours, or days if harvested immature (Adato and Gazit, 1974, Eaks, 1980).

It has been suggested that although fruit ripening on the plant undergo a climacteric increase in ethylene production, there is no corresponding increase in respiration rate (Shellie and Saltveit, 1993, Saltveit, 1993, Shiomi et al., 1996, Rogiers and Knowles, 1999). The presence or absence of a climacteric increase in respiration during ripening has been used to classify fruit for more than 70 years (Abeles et al., 1992). At about the time of the peak in respiration, fruit become edible-ripe, with changes occurring in flesh texture, pigment and aroma production (Biale and Young, 1981). It has, therefore, often been assumed that the respiratory climacteric occurs to provide energy for ripening (Abeles et al., 1992). The absence of a respiratory climacteric during ripening on the plant challenges this assumption.

However, all workers in this area are not in agreement on this point. The opposite result has also been found, the ethylene and respiratory climacterics both occurring as fruit ripened on the plant even though this process was delayed relative to harvested fruit of the same physiological age (Andrews, 1995; Hadfield et al., 1995). Knee (1995) suggested that previous measurements that did not find a respiratory climacteric in attached fruit could have been affected by fruit photosynthesis. Given the importance placed on the respiratory climacteric, its presence or absence while fruit are attached to the plant is of considerable interest.
A team at the Ecole Nationale Superieure Agronomique de Toulouse (ENSAT) has generated a transgenic Charantais melon plant (*Cucumis melo* cv. Reticulatus F1 Alpha) (Ayub et al., 1996). These melons express an antisense gene for ACC oxidase. As ACC oxidase catalyzes the last step of ethylene biosynthesis, ethylene production is effectively blocked in these fruit. However, the fruit remain ethylene sensitive, and will ripen when treated with exogenous ethylene (Ayub et al., 1996). As ripening can be controlled by the timing of ethylene application, these fruit are a useful tool for studying the effects of ethylene on respiration and the ripening process. Furthermore, development of the respirometer (as described in Chapter 3) has made it possible to measure respiration rates of developing fruit in the glasshouse without the need for bulky or complex equipment. I therefore decided to study ripening in attached and detached melon fruit. Initial trials were conducted at the ENSAT laboratories in Toulouse, France. These experiments examined the differences in ripening physiology between the transformed and non-transformed fruit, and how ethylene and respiration were affected by detachment from the vine.

6.2.2. Materials and Methods

6.2.2.1. Plant Materials

Both transformed (AS) and the non-transformed, normal ripening (NR) Charantais melon plants (*Cucumis melo* cv. Reticulatus F1 Alpha) were grown during the summer of 1997, with fruit reaching maturity in September. The trial was conducted inside a ventilated, but not temperature-controlled glasshouse. The plants were grown hydroponically, and trained vertically on a trellis to maximize air movement and prevent injury from abrasion. Sulfur dust was applied at least once a week to control powdery mildew. Female flowers were hand-pollinated and date-tagged at anthesis. Where necessary, fruit were thinned to no more than two per plant. At the completion of the experiment, the melons were weighed and their volume measured by immersion in water.

6.2.2. Measurements of Gas Exchange and Temperature

To monitor internal ethylene concentrations, flexible cups were attached to the outsides of the developing melons with elastic strapping. Rubber bottle stoppers were found to be useful for this purpose, being cheap, easily obtained, and with an air space of approximately 10ml. After a time, the atmosphere inside each stopper came to equilibrium with the atmosphere inside the fruit. Ethylene was measured at least daily once the melons reached 32 days after pollination. A 1 ml sample of air was withdrawn from the sampling space inside each rubber cup and injected into a gas chromatograph (Flame ionisation detector, machine developed by INRA Department of...
Electrical Engineering). The onset of the climacteric was signalled by internal ethylene concentrations increasing to >0.5 ppm.

Measurements of respiration commenced once ethylene production started to rise in NR fruit, or 35-40 days after pollination (DAP) in AS fruit. Fruit respiration was measured using respirometers (Chapter 3). A 5mm wide slot was cut in each respirometer lid to allow respiration measurements while melons were attached to the vine. The stems were sealed into these slots using flexible, oil based, modelling clay. The O₂ concentration inside each respirometer was monitored continuously, but recorded at 6 minute intervals. O₂ concentrations were set to cycle between 19–20kPa. Ethylene was measured twice daily by withdrawing 1 mL samples from the respirometer bowls and injecting them into the gas chromatograph as previously. Black cloth was placed over each respirometer to prevent photosynthesis by the developing fruit.

Temperatures were measured using LM 335 temperature sensors with a 2.5 volt offset, as previously described. The length of the cables was adjusted so that the sensors hung in the centre bottom 1/3 of each respirometer. Sensors were calibrated in melting ice before use, and readings at high temperatures compared with measurements made with a pre-calibrated digital thermometer.

6.2.2.3. Treatments

At the beginning of measurements, AS fruit were used that were approximately the same age (DAP) as the NR fruit. The rate of O₂ consumption of all fruit was measured for at least 24 hours after sealing inside the respirometers. Half of the NR and half of the AS melons were then detached from the vines and measurements continued. To avoid systematic errors, fruit that had been detached were left in the same position inside the greenhouse for the duration of the experiment. The effect of attachment and detachment on the respiration rates and ethylene production of AS and NR melons were compared.

A second set of measurements was made using AS melons treated with ethylene. Detached melons were placed in respirometers controlled to 20°F ± 2°F. Half of the pumps used to replenish the vessel atmospheres were placed inside sealed tanks continually flushed with a mixture of air and ethylene. Refreshment of the respirometer atmospheres thereafter was with air containing approximately 10ppm ethylene. The exhaust from these vessels was piped away from the room so as to avoid exposing the control fruit to ethylene.
6.2.2.4. **Calculations**

During the experimental period, the temperature inside the glasshouse changed from 17 to 33°C. These fluctuations in temperature had a considerable effect on respiration, although changes in respiration rate lagged behind temperature changes inside the respirometers because of the thermal mass of the fruit. An example of data from an AS melon are shown in Figure 6.2.1. This delay was generally around 3 hours, but varied between melons. Temperature data were shifted in time for each melon so as to correspond with maximum and minimum respiration values.

Mean respiration rates at temperatures between 18°C and 28°C were calculated at 0.5°C intervals from six replications of NR and AS type melons. Data from the afternoons and evenings were used, as O₂ consumption was more stable when temperature was falling. The relationship between respiration and temperature was approximately linear over this interval (Figure 6.2.2). Regression lines were calculated for the data, the slope of each line representing the increment in respiration per degree rise in temperature. Using this relationship, the raw respiration data were normalised to calculate the approximate rate at 20°C (Figure 6.2.1). Although results still fluctuated considerably during periods of rising temperatures, this method removed the diurnal variations from the data.

![Graph showing changes in temperature and O₂ consumption](image)

**Figure 6.2.1.** - Changes in temperature (---) and O₂ consumption by an AS Charantais melon; O₂ consumption presented as both initial data (---) and data normalised to approximate the rate at 20°C (---); data recorded at 6 minute intervals.
Figure 6.2.2. - Effect of temperature on respiration rates of NR (•) and AS (♦) Charantais melons; points represent mean values calculated during periods of decreasing temperature; error bars indicate the standard deviation of each mean value (n=6).

As pollination dates varied, the NR melons reached their peak levels of ethylene production on different days. Therefore, data were shifted in time so that maximum ethylene levels inside the respirometers occurred on day 4. Differences between attached and detached fruit, NR and AS melons, and changes over time were analysed using CoStat statistical software and means compared by Duncan's Multiple Range Test. Analysis of differences in the rate of O₂ consumption compared mean values from day four of measurements.
6.2.3 Results and Discussion

6.2.3.1 Ethylene Measurements

Ethylene concentrations inside respirometers containing NR fruit reached up to 40 ppm during the ethylene climacteric. Concentrations were significantly higher inside the respirometers containing attached fruit than those containing detached fruit ($\alpha = 0.05$). This was despite considerable variability in the data due to periodic flushing of the vessels. The AS melons produced negligible amounts of ethylene during the experimental period, regardless of whether they were attached or detached from the vine, and the data for these fruit were combined (Figure 6.2.3).

![Ethylene concentrations inside respirometer vessels containing detached NR ( ), attached NR ( ), and attached/detached AS ( ) melons; error bars indicate the standard deviation of each mean value (n=3).](image)

6.2.3.2 Rates of $O_2$ Consumption by Attached and Detached Fruit

It was expected that the preclimacteric rate of $O_2$ consumption would be similar in both AS and NR melons. However, respiration by AS fruit was significantly lower than that of NR fruit, being approximately 0.83 compared to 1.25 mmol $O_2$·kg$^{-1}$·h$^{-1}$ ($\alpha = 0.05$). This difference was similar throughout the temperature range measured.

Detached NR melons showed the expected climacteric increase in respiration rate during ripening. This occurred concurrently with the increase in ethylene concentration inside each respirometer. The AS melons were not exposed to detectable levels of ethylene, and respiration did not change during the experimental period (Figure 6.2.4.).
Figure 6.2.4. - Rates of O₂ consumption by detached NR (●) and AS (◆) melons during the ripening period; each point represents the mean rate of O₂ consumption over a 1 hour period; error bars indicate the standard deviation of each value (n=3).

In contrast to these results, O₂ consumption by attached NR melons did not increase during ripening. Changes in colour and aroma during the experimental period were similar in attached and detached NR melons. However, respiration rates of attached melons were significantly lower during ripening than those of detached fruit (α = 0.05). These melons were therefore similar to AS melons, respiration remaining constant over the experimental period (Figure 6.2.5). Differences in O₂ consumption between detached and attached AS melons were not significant (α = 0.05).

Applying ethylene to detached AS melons induced similar changes in colour and aroma as had been observed for ripening NR fruit. O₂ consumption was significantly increased by exposure to 10ppm ethylene (α = 0.05). This confirmed the sensitivity of AS melons to exogenous ethylene (Figure 6.2.6).
Figure 6.2.5. - Rates of O₂ consumption by attached NR (◼) and AS (♦) melons during the ripening period; each point represents the mean rate of O₂ consumption over a 1 hour period; error bars indicate the standard deviation of each value (n=3).

Figure 6.2.6. - Rates of O₂ consumption by detached AS melons treated with approximately 10ppm ethylene (♦) or remaining in air (◼); each point represents the mean rate of O₂ consumption over a 1 hour period; error bars indicate the standard deviation of each value (n=3-4).

Previous studies that compared the physiology of melons allowed to ripen on the vine with those that have been detached produced conflicting results. Shellelie and Saltveit (1993) showed that although netted muskmelons allowed to ripen on the vine developed a distinct ethylene climacteric there was no concomitant increase in respiration. Harvested melons, however, underwent both ethylene and respiratory climacteries. In contrast, Hadfield et al. (1995) showed that respiration rates of both attached and detached Charentais melons increased during ripening, although this
was delayed in fruit still connected to the vine. The authors of the latter study suggested that differences in ripening behaviour of the two melon types were responsible for discrepancies between the sets of results.

In this trial, the results supported the findings of Shellie and Saltveit (1993). That is, a respiratory climacteric was not detected in Charantais melons that remained attached to the vine during ripening. This was despite the presence of significant levels of ethylene. These results suggest that a substance or substances translocated from the vine counteracted the effects of ethylene on respiration rate.

The results from this trial are far from conclusive, however. The large diurnal fluctuations in temperature that occurred in the greenhouse added considerable variability to the results. Moreover, only 3 replicates were measured for most of the treatments, and rates of ethylene production could not be calculated from the data recorded. Given the controversial nature of the results, there was a clear need to repeat the experiment under more controlled conditions and with greater replication.

### 6.2.4. Key Points

- Charantais melons, antisense for ACC oxidase (AS) and normal ripening (NR), were grown in Toulouse, France.
- Respiration rates and ethylene concentrations inside the respirometer vessels were measured for NR fruit that were either detached or left attached to the plant during ripening.
- Respiration rates of detached NR fruit showed the expected climacteric increase during ripening. However, respiration of attached fruit remained unchanged. This was despite higher concentrations of ethylene inside these vessels than were found for detached fruit.
- Respiration rates of AS fruit were lower than those of NR fruit, and were unaffected by detachment from the vine.
- Detached AS fruit treated with ethylene underwent an increase in respiration rate and other ripening related changes similar to those occurring in NR fruit.
6.3 Changes in $O_2$ Consumption by Charantais Melons During Ripening On and Off the Vine in Australia

6.3.1. Introduction and Aims

Seeds of Charantais melon plants (Cucumis melo cv. Reticulatus F1 Alpha) were imported from France during 1998. The plants were genetically identical to those that had been used for similar experiments in Toulouse, France the previous year. The aim was to examine differences in rates of respiration and ethylene production between fruit ripening on and off the vine.

6.3.2. Materials and Methods

6.3.2.1. Plant Materials

The genetically modified melon seeds were permitted into Australia following examination by AQIS, and on the condition that they were grown only in a locked room in the PC-2 approved glasshouse at the University of Western Sydney, Richmond, and that all organic materials were destroyed at completion of the experiment. Charantais melon seeds were planted during December 1998 (P1), with a further group sown six weeks later (P2). The glasshouse was temperature controlled, providing average temperatures of 25°C during the day and 18°C at night. During maturation of the melons this was changed to a constant temperature range. Both sets of plants were grown in 20 L pots of standard potting mix and watered by drip irrigation. An initial application of slow release fertiliser (Osmocote mini®) was supplemented by weekly applications of a double-strength hydroponic solution (416 ppm N, 124 ppm P, 664 ppm K) during and after flowering.

The humid environment of Richmond makes it difficult to grow cucurbits due to persistent infection by powdery mildew. Humidity was reduced inside the glasshouse used by heating the air slightly, and avoiding having free water present in the room. As in France, the plants were grown on a trellis to maximize air movement around the leaves as well as prevent injury from abrasion. Either Benlate® or Mancozeb® fungicide was applied weekly in an attempt to control disease. Despite this, the incidence of mildew increased over the experimental period.
As previously, female flowers were hand-pollinated and date tagged at anthesis. Where necessary, fruit were thinned to no more than two per plant. At the completion of the experiment, the melons were weighed and their volume measured by immersion in water.

6.3.2.2. Measurements of Gas Exchange and Temperature

The rate of O\textsubscript{2} consumption was measured using respirometers (Chapter 3). Temperature was also measured in the same way as previously. O\textsubscript{2} concentrations were allowed to fluctuate between 19-20%. Measurements of O\textsubscript{2} and temperature were recorded every six minutes for the duration of the experiment.

Ethylene was measured using either a 10S Plus digital gas chromatograph (Photovac Inc., New York, USA) or a chromatograph (Gow Mac Instrument Co., USA) equipped with a flame ionisation detector. To measure the rate of ethylene production, the lids were removed from the respirometers and the vessels ventilated for 5-10 minutes. The containers were then resealed for 1 hour before 1 mL samples were withdrawn for analysis. Ethylene was measured in this way once the melons reached 32 days after pollination (DAP). Half of the melons were detached from the vine once their rates of ethylene production started to increase.

6.3.2.3. Calculations

The rates of O\textsubscript{2} demand by individual melons during ripening were calculated as previously (Chapter 3). As the melons were produced over several weeks, the data from each fruit were shifted in time so that maximum ethylene production occurred on what was nominally day 4.

The data were initially analysed using CoStat statistical software. Three way analyses of variance were used to determine significant differences due to planting date, attachment to the plant, and time of measurement. Regression analyses comparing ethylene production with respiration rates were also performed using Statistica (Kernel Release 5.5A, StatSoft Inc.), with regression lines compared using the method of Mead et al. (1993).

6.3.3. Results and Discussion

Initial analysis of the results found significant differences between results from the two planting dates (\(\alpha = 0.05\)). The results from each planting (P1 and P2) were therefore analysed and presented separately.
6.3.3.1. Ethylene Measurements

A climacteric increase in ethylene production was observed during ripening of both attached and detached fruit from the two planting dates (Figures 6.3.1 and 6.3.2). However, ethylene production during the climacteric was significantly higher for attached fruit ($\alpha = 0.05$). This difference was particularly obvious in melons from P2 (Figure 6.3.2), where mean rates of ethylene production rose to 1.3 \( \mu \text{mol.kg}^{-1}.\text{h}^{-1} \) in attached fruit, compared to 0.4 \( \mu \text{mol.kg}^{-1}.\text{h}^{-1} \) in detached fruit. Rates of ethylene production by detached fruit from P1 and P2 were similar.

These results are consistent with those described in Section 6.2, where higher concentrations of ethylene were detected inside containers with attached fruit. Hadfield et al. (1995) found higher internal ethylene concentrations in Charantais melons ripening while attached to the plant relative to harvested fruit. A similar situation has been described for attached and detached 'Red Delicious' apples (Sfraktotakis and Dilley, 1973), while rates of ethylene production were higher in attached saskatoons compared to harvested fruit (Rogiers and Knowles, 1999).

However, other studies have not recorded increased rates of ethylene production by attached fruit. Detachment from the plant did not affect ethylene production by non-netted muskmelons (Lacan and Bacou, 1996), internal ethylene in netted melons (Shellie and Saltveit, 1993) or internal ethylene in ripening tomatoes (Saltveit, 1993). Harvested passionfruits produced ethylene at a rate up to 20 times higher than fruit which ripened on the vine (Shiom et al., 1996). Charantais melons have also been found to have higher internal ethylene concentrations when detached during ripening compared to attached fruit (Ayub et al., 1996). Such differences among studies are difficult to satisfactorily explain, and indicate the need for more detailed studies with consistent methods of ethylene measurement.

6.3.3.2. Rates of O₂ Consumption by Attached and Detached Fruit

All of the melons underwent a respiratory climacteric during ripening, irrespective of attachment or detachment from the vine. Attached melons from both planting dates had similar peak levels of respiration (1.5-1.8 mmol O₂.kg⁻¹.h⁻¹). However, the rate of O₂ consumption by detached fruit from P1 was significantly higher during the climacteric than that of attached fruit ($\alpha = 0.05$) (Figure 6.3.1). There were no significant differences in respiration rates from P2 (Figure 6.3.2). Although analysis of variance of the data from these two trials showed little overall effect of trial date or attachment on respiration, there was a highly significant interaction between trial, attachment and time ($\alpha = 0.05$).
Figure 6.3.1. - Changes in gas exchange during ripening of Charantais melons, results from planting date 1; O₂ consumption by attached (——) and detached (---) fruit; C₂H₄ production by attached (—_—_—) and detached (—_—_—) fruit; error bars indicate the standard error of the mean (n=3-4).

Figure 6.3.2. - Changes in gas exchange during ripening of Charantais melons, results from planting date 2; O₂ consumption by attached (——) and detached (---) fruit; C₂H₄ production by attached (—_—_—) and detached (—_—_—) fruit; error bars indicate standard error of the mean (n=3-5).

These results suggest that the growing environment may influence the development of the respiratory climacteric. The plants grown in France during the summer were produced under ideal
conditions. These vines produced large, healthy melons with an average weight of 1.2-1.6 kg each. Vines grown in Australia during the autumn were less healthy, being affected by shortened day-lengths, reduced light intensity and disease pressure. The melons matured more slowly than those grown in France, and the ethylene climacteric commenced at approximately 40 DAP rather than 35 DAP as previously. Although the melons still developed normal colour, flavour (>10% SSC) and aroma, they were less than half the weight of the French grown fruit. Under these more marginal conditions, differences in respiratory behaviour between harvested and unharvested melons diminished. The health of the melon plant may therefore have had an effect on the ripening physiology of the fruit.

6.3.3.3. Interactions Between Ethylene Production and O₂ Consumption by Attached and Detached Fruit

Increases in ethylene production have been found to correlate closely with increased concentrations in fruit flesh (Lyons et al., 1962). In this study, the fruit allowed to ripen on the vine may therefore be assumed to have had higher internal ethylene concentrations than harvested fruit. Changes in internal ethylene concentration are strongly associated with increases in respiration during the climacteric (Burg and Burg, 1965). Therefore, up until the point when the ethylene receptors become saturated, increases in internal ethylene concentration should lead to increases in respiration. Consequently, fruit attached to the vine should have had higher respiration rates and a more distinct climacteric than harvested fruit. However, the opposite was found, suggesting that attachment to the plant reduced the effects of ethylene on respiration.

To clarify the relationship between ethylene and respiration rate, increases in O₂ consumption during the climacteric were compared to ethylene production rates (Figure 6.3.3). Comparison of the residual sums of squares from the regression ANOVA showed that the relationship between these factors did not vary significantly between the planting dates for either attached (p = 0.845) or detached (p = 0.429) melons and the data from the two trials were combined. Further regression analysis demonstrated a significant, positive, linear relationship between the rate of ethylene production (x) and the change in respiration relative to pre-climacteric rates (y). For attached melons this could be described by the equation \( y = 0.626 x + 0.032 \) \( (r^2 = 0.82, p < 0.0003) \), while the equation for detached melons was \( y = 1.966 x + 0.05 \) \( (r^2 = 0.71, p < 0.0001) \). Comparison of the regression lines showed that the relationship between ethylene production and respiration rate was distinctly different for attached and detached melons (p < 0.001). Melons that remained attached to the vine during ripening, therefore, had a smaller increase in respiration for each unit increase in ethylene production.
Although the results of this experiment initially appeared to be quite different from those from experiments in France, the conclusions from both sets of results were similar. That is, attachment to the vine reduced the effects of ethylene on respiration. This was despite higher levels of ethylene production in fruit ripening while attached to the plant. However, this effect did not occur to the same extent suggested by Shellie and Saltveit (1993). On the other hand, the thesis of Hadfield et al., (1995) that attachment has no effect on respiration during the climacteric is also not supported by these results. The response found for Charantais melons was intermediate between these published results.

6.3.4. Key Points

- Charantais melons were grown in the glasshouse at UWS Hawkesbury.
- Respiration rates and ethylene production increased during ripening of both attached and detached fruit. However, in attached fruit the respiratory climacteric was smaller and the ethylene production rate was higher.
- Inhibition of the respiratory climacteric was more pronounced in early maturing fruit than in late maturing fruit from plants that were suffering from extensive powdery mildew infection.
- The effect of ethylene on respiration was significantly reduced by attachment to the plant. This inhibition may be due to substances translocated through the plant. The supply of these substances is likely to be affected by plant vigor.
6.4 Effects of Ethylene on O$_2$ Consumption On and Off the vine by Genetically Modified Charantais Melons

6.4.1. Introduction and Aims

Seeds of genetically modified Charantais melon plants (*Cucumis melo* cv. Reticulatus F1 Alpha) were imported from France during 1998. As described in Section 6.1, these melons have been antisensed for ACC oxidase, effectively blocking their production of ethylene. However, the melons remain able to respond to exogenously applied ethylene, as was demonstrated in Section 6.1.3.

The results from previous experiments suggested that attachment to the vine reduced the effects of ethylene on the respiration rates of Charantais melons. The genetically modified plants varied from these only in their capacity to generate ethylene. These melons were therefore an ideal tool for examining the effects of applying ethylene to attached and detached fruit.

6.4.2. Materials and Methods

6.4.2.1. Plant Materials

Both NR (normal ripening) and AS (antisensed) Charantais melons were grown in the PC2 approved glasshouse at the University of Western Sydney, Hawkesbury during the late summer of 2000. The plants were grown in large plastic pots, and trained on a trellis as previously. To reduce humidity in the room, the plants were watered using a wicking system. One end of each wick was buried into the potting mix, the other placed in a covered water reservoir. The central part of each wick was encased in black plastic to prevent dehydration. Water moved along the wick into the drier potting soil by capillary action. At least 2 cotton wicks were used per pot. The water provided by this method was sufficient for seedlings, but needed to be supplemented with weekly watering when the plants were mature.

Rain during the experimental period resulted in humid conditions inside the glasshouse. Benlate® and Mancozeb® fungicides were sprayed weekly to control downy mildew. However, it soon
became evident that the disease had affected many of the vines. To monitor the effects on vine
growth, the length of each main leader was measured several times during development. At the
completion of the experiment the dry weights of the plants aerial parts were also measured.

Flowers of both the NR and AS vines were hand pollinated and date tagged at anthesis. However,
none of the NR melons developed normally, these fruit either aborting completely or failing to
devolve to a reasonable size. This was almost certainly due to disease stress. While the plants
were developing, the room temperature was controlled to 16-20°C at night and 20-26°C during the
day. During measurements of respiration rate this range was changed to 20-24°C for both day and
night.

6.4.2.2. Measurements of Gas Exchange and Temperature

Fully mature AS melons (40-45 days after pollination) were used for measurements of O₂
consumption with respirometers (Chapter 3). Half of the melons were detached before sealing into
the respirometer vessels, while the remainder remained attached to the vine throughout the
experiment. O₂ demand in air was monitored for 3 days. All the fruit were then treated with ethylene
for a further 3 days, while measurements of O₂ consumption continued. Before treating with
ethylene, the containers were thoroughly ventilated with air by removing the lids for several minutes.
1 ml of ethylene was then injected into each vessel. This was sufficient to raise the internal ethylene
concentration to approximately 200 ppm. The pumps were turned off for at least the next 12 hours,
and the computer used to record the rate of O₂ depletion by each melon. The vessels were then re-
opened, and the procedure repeated each day for 3 days.

To ensure that measurements of melons in air and in air + ethylene were consistent, respiration
was only calculated when the O₂ concentration inside the vessel was 18-20 kPa. Despite this, the
data showed wide fluctuations during the experimental period. This effect was probably due to
electrical interference inside the room as peaks corresponded closely with cooling cycles. As a
result, O₂ consumption was calculated as a mean rate per day for each melon.

6.4.3. Results and Discussion

6.4.3.1. Vine Growth

There was a distinct difference in susceptibility to powdery mildew between the NR and AS melon
plants. The main leaders of the AS melon plants were significantly longer than those of the NR
vines throughout their growth (α = 0.05). Also, the 15 AS vines produced an average of 1 fruit per

161
vine over the experimental period, whereas the 10 NR vines produced only two fruit in total, both of which weighed less than 200g. The AS vines had significantly greater dry weight than the NR vines ($\alpha = 0.05$), being more than 50% heavier on average.

The reduced susceptibility of AS melon plants to powdery mildew was an unexpected result. Differences between AS and NR vines were not apparent in France, where disease pressures were less. Plant tissues normally produce ethylene rapidly in response to pathogen attack (McGlasson, 1970). Ethylene itself causes additional stress, and may stimulate growth of some pathogens (Knee et al., 1985). Inactivation of the ethylene receptor sites significantly reduced some disease symptoms on grapefruit infected with *Penicillium digitatum* (Mullins et al., 2000). It would appear from these results that blocking the production of wound ethylene reduced pathogen vigor and/or assisted the melon plant's other defenses. Further experimentation is certainly needed to clarify the effects of ethylene on growth of powdery mildew.

### 6.4.3.2. Changes in $O_2$ Consumption

Detachment from the vine did not significantly affect the rates of $O_2$ consumption by AS melons ($\alpha = 0.05$). However, applying ethylene increased the respiration rate of detached melons, while that of attached melons remained unchanged ($\alpha = 0.05$). This showed that the stimulation of respiration by ethylene was prevented by attachment to the vine (Figure 6.4.1). These results therefore support the hypothesis that attachment to the plant reduces the effects of ethylene on respiration rates.

![Graph showing $O_2$ consumption](image)

*Figure 6.4.1. - Effects of ethylene on detached (■) and attached (□) AS melons; values calculated from 6 melons, each measured on 3 separate days, error bars indicate the standard deviation of each mean value (n=6).*
6.4.4. Key Points

- AS melons were grown in a glasshouse at UWS Hawkesbury. Respiration rates of attached and detached fruit were measured, initially in air, then in an atmosphere containing ethylene.
- Respiration rates of attached fruit were unaffected by ethylene treatment. However, ethylene significantly increased respiration rates of detached fruit.
- These results support the hypothesis that attachment to the plant inhibits the effects of ethylene on respiration rates of Charantais melons.
- NR melon plants were also grown. These plants were more affected by powdery mildew than the AS vines. As a result, these plants were shorter, contained less dry matter, and produced fewer and smaller fruit than the AS melons plants. This suggests that blocking ethylene production by the plant may increase resistance to disease.
Conclusions on the Effects of Detachment on $O_2$ Consumption by Charantais Melon Fruit

Charantais melon fruit had consistently higher rates of ethylene production when they remained attached to the vine during ripening. The comparatively lower levels in detached fruit may be due to limited availability of compounds required for ethylene synthesis. In attached fruit, such compounds would have been translocated to the fruit throughout ripening. An alternative explanation is that increased respiration in response to ethylene inhibits further ethylene production. Such an effect would be expected if increased respiration in response to ethylene is a homeostatic response as proposed by Romani (1987).

Attachment to the plant could be analogous with the effects of the compound 1-MCP, which has been used to reduce the ethylene sensitivity and extend storage life of various commodities (Jiang et al., 1999). One side effect of MCP use has been to significantly increase ethylene production in fruit at some developmental stages (Golding et al., 1998; Mullins et al., 2000). This is thought to occur because MCP blocks the ethylene receptors, thus preventing normal feedback inhibition of continued ethylene synthesis (Mullins et al., 2000). If attachment to the plant also blocks the effects of ethylene, this could likewise affect ethylene synthesis.

Detachment from the plant has been shown to accelerate ripening in apples (Smock, 1972; Sfakiotakis and Dilley, 1973; Yang et al., 1986; Pooter et al., 1989; Blanpied, 1993), passionfruit (Shiomi et al., 1996), plums (Abdi et al., 1997) and some melon varieties (Pratt et al., 1977; Bliss and Pratt, 1979; Hadfield et al., 1995). ‘Hass’ avocados provide an extreme example of this phenomenon, as they are unable to ripen while attached to the tree (Adato and Gazit, 1974). The reasons for this effect are still not well understood, although clearly there are a number of physiological differences between attached and detached fruit. In detached fruit, turgor is likely to fall, gas permeance may increase and the translocation of biochemical factors from the plant is interrupted. Any or all of these factors may affect the ripening process.

Turgor is unlikely to have significantly influenced the development of the climacteric in this case. Melons were kept inside respirometers, in an atmosphere that would have approached 100% RH.
Detachment of a fruit from the plant involves wounding. The creation of this wound may significantly increase gas permeance of some fruit, particularly those with otherwise relatively impermeable cuticles. For example, most gas permeation has been shown to occur through the calyx scar in tomatoes (Cameron and Yang, 1982), capsicums (Burg and Burg, 1965, Bower et al., 2000) and avocados (Ben-Yehoshua et al., 1963). Burg and Burg (1965) found that resealing the calyx scar of harvested cantaloupe melons with wax reduced their respiration rate by 10%. This suggests that the limited gas permeance of fruit tissues may be one factor restricting their respiration rate. Harvesting the fruit, and thereby increasing permeance, may facilitate a larger respiratory climacteric during ripening than would otherwise be possible.

A number of studies have suggested that differences between ripening behavior on and off the plant are due to ‘plant factors’. In their study on the ripening of apples, Sfakiotakis and Dilley (1973) showed that a substance produced by the leaves and translocated to the fruit via the phloem inhibited ripening. The production of different amounts of these factors may account for variations in ripening physiology seen among cultivars of the same species. Different amounts of plant factors could also be responsible for the variations in ripening behavior found in this study.

However, none of the above published works can fully explain the results observed in this series of experiments. In this case, ripening proceeded normally while fruit was attached to the plant. However, the effect of ethylene on O₂ consumption was partially or fully blocked. Romani (1987) expressed the view that the respiratory climacteric is primarily a stress response that occurs due to the presence of ethylene, rather than being due to increased energy demands during ripening. Reduction of ethylene related stress in vines antisensed for ACC synthase could help explain the greater disease tolerance of these plants. Attachment of melon fruit to the plant could also reduce the stress caused by ethylene, whether by ‘plant factors’ translocated from the main vine or other, as yet unknown response mechanisms. However, the ability of the plant to respond to stress in this way may be affected by its own health and vigor, a factor which could explain the variability of results from different planting dates.

Together, the results of these experiments suggest that the respiratory climacteric may not be essential for ripening in climacteric fruit. Increases in respiration rate do not appear to be required to fuel the biochemical changes that occur during ripening. Rather, it is the necessary accompaniment of ethylene action in detached fruit. This confirms the proposal of a number of authors that it is changes in ethylene production that characterise climacteric fruit (McGlasson et al., 1978), the respiratory climacteric itself being primarily an artifact of harvest.
Measuring the Gas Permeance of Capsicums and Melon Fruit

7.1 Introduction

7.1.1. Mechanisms of gas permeation

Fruit and other bulky plant organs offer significant resistance to diffusion of gases. As a result, they develop internal atmospheres that are different from the outside air. Internal atmospheres are created as respiration depletes O₂ from the internal atmosphere while enriching it in CO₂. The principal resistance to the entry of O₂ and exit of CO₂ is due to the cuticular surface of the epidermis (Burton, 1950, Fidler and North, 1971, Burton, 1974, Solomos, 1994a, Perez and Beaudry, 1998). The internal tissues are generally much more permeable to O₂. However, significant gradients may still develop between the skin and the fruit centre. This has been demonstrated for several products including pears and nectarines (Rajapakse et al., 1990), potatoes (Banks and Kays, 1988) and avocados (Ben-Yehoshua et al., 1963).

Gas exchange in bulky plant tissues essentially follows Fick's first law of diffusion. This states that the rate of gas permeation is proportional to the partial pressure difference across a barrier, multiplied by the area of the barrier, and divided by the resistance of that barrier. Previous work has confirmed the validity of this rule by showing that the principle route of gas movement in plants is through the intercellular spaces (Burg and Burg, 1965, Burton, 1974, Solomos, 1987). Calculations with apples have shown that if O₂ diffused purely through an aqueous medium, the largest fruit that would maintain 1kPa O₂ at its centre would be only 7 mm in diameter (Solomos, 1987). Similar figures have been presented for potatoes, demonstrating that cells more than about 5mm from an air space containing 18kPa O₂ would be anaerobic (Burton, 1974). It may therefore be accepted that gas diffusion is through spaces within plant tissue rather than directly through the tissue itself. These spaces are necessarily continuous and interconnected, even though they may represent as little as 1-2% of the total volume in some plant organs (Burton, 1950).

The primary route of diffusion into and out of plant tissue is through pores, lenticels, and other openings in the skin. Wigginton (1974) showed that virtually all gas diffusion into potatoes occurs through the lenticels, the periderm being far more resistant to gas exchange. Blocking the pores with applied surface coatings can reduce the rate of gas exchange by up to 95% in
Some fruit (Banks et al., 1993, Perez and Beaudry, 1998). Alternatively, increases in the number of pores over the fruit surface, or micro-cracking of the fruit cuticle can increase gas exchange with the external atmosphere. Variability in the number of natural openings on the fruit surface may account for many of the differences in internal atmospheres observed between individual fruit (Maguire et al., 1999).

The composition of the internal atmosphere may indicate the degree to which gas exchange occurs directly through the cuticle. Gas exchange through holes such as stomates and lenticels is primarily a function of partial pressure, these spaces being approximately equal in permeance to O₂ and CO₂. In contrast, cuticle tissue is a cohesive barrier, and is differentially permeable to CO₂ and O₂. Banks et al., (1997) estimated that capsicum cuticle tissue was around 10 times less permeable to O₂ than it was to CO₂. Similar results were found for washed Granny Smith apples, which were nearly four times more permeable to CO₂ than to O₂. It was suggested that washing blocked many of the fruit pores, thus increasing the proportion of gas flux occurring through the cuticle (Dadzie et al., 1995).

For this reason fruit may be compared to modified atmosphere packages. Diffusion through pores is analogous to gas exchange through a microporferated film, while diffusion through the cuticle may be similar to that which occurs through permeable barriers such as LDPE (low density polyethylene). In such packages a steady state develops in which the rate of O₂ consumption equals the rate of O₂ permeation through the film (Figure 7.1.1.). Similarly, the O₂ consumption of a fruit may be limited by the rate at which O₂ is able to permeate it. As a result, the respiration rates of fruit are influenced by their permeance to gases (Banks et al., 1993, Dadzie et al., 1995).

![Figure 7.1.1. - Changes in respiratory O₂ demand by respiring fruit inside a permeable barrier and supply of O₂ by permeation through that barrier. As O₂ concentrations fall, the respiration rate of the product decreases, but the rate of permeation increases. Where O₂ consumption equals O₂ permeation a steady state occurs.](image-url)
7.1.2. The relationship between gas permeance and metabolism of plant tissues

7.1.2.1. The Relationship Between Gas Permeance and Fruit Ripening

The rate of fruit ripening is likely to be influenced by fruit permeance. This is because decreased levels of \( O_2 \) and increased levels of \( CO_2 \) generally delay the climacteric and other changes due to senescence. Wardlaw and Leonard (1940) suggested that the progress of the climacteric is limited by the internal concentration of \( O_2 \). Treatments that reduce fruit permeability, thereby changing the internal atmosphere, also affect ripening and respiration rates. For example, surface coatings have been shown to reduce or delay ripening in tomatoes (Brooks, 1937), bananas (Perez and Beaudry, 1998), pears (Amarante et al., 1997) avocados (Ben-Yehoshua et al., 1963, Durand et al., 1984) and apples (Kerbel et al., 1989, Dadzie et al., 1995). Leaving the peduncle attached to harvested tomatoes increased the internal \( CO_2 \) content and slowed ripening of the fruit (Bergevin et al., 1993). Conversely, increasing permeability by removing natural skin wax or peeling hastened the onset of the climacteric in avocados (Ben-Yehoshua et al., 1963).

Gas permeance is likely to change during ripening as fruit soften and intercellular spaces become waterlogged (Cameron and Reid, 1982). Permeability decreased following ripening and softening of bananas (Leonard and Wardlaw, 1941, Vendrell, 1970), avocados (Rodriguez et al., 1989) and cantaloupe melons (Lyons et al., 1962). Respiration rates also decrease following ripening in many fruit.

7.1.2.2. The Relationship between Gas Permeance and Physiological Disorders

Gas permeance may also affect the development of physiological disorders. A decrease in gas permeance during storage of 'White Rosemary' apples was shown to be associated with the onset of superficial scald, whereas the more permeable cultivar 'Reinette de Champagne' was found to be scald resistant (Boitsova, 1972). Decreased internal \( O_2 \) concentrations were also associated with increased levels of scald in 'Granny Smith' apples (Bauchot et al., 1995). This suggests that fruit permeance may be one factor in the development of scald for some apple varieties.

Accumulation of ethylene during storage has been shown to increase not only the incidence of apple scald, but also the sensitivity to chilling injury of some fruit (Lee and Young, 1984, Yuen et al., 1995, Guis et al., 1997). Bergevin et al. (1993) found that tomatoes were more chilling sensitive if the peduncle was left attached. As most gas exchange of tomato fruit occurs through
the stem scar (Brooks, 1937, Burg and Burg, 1965, Cameron and Yang, 1982), it might be expected that the attached peduncle would reduce total fruit permeability. This would be likely to increase the accumulation of internal ethylene, resulting in the effect described. It is possible that differences in permeance could be one reason for variability in chilling sensitivity between different cultivars of fruit.

7.1.2.3. The Effects of Gas Permeance on the Use of Modified Atmospheres

Determining the permeance of fruit to gases is of more than theoretical interest. Resistance to gas diffusion can result in gas concentration gradients in not only between the epidermis and the centre of larger fruit (Solomons, 1987), but also between cells and the surrounding intercellular spaces (Burton, 1978, Cameron and Reid, 1982). Respiration by each cell is likely to be a function of the gas concentrations around it, suggesting that the response to modified atmospheres will be a function of internal rather than external gas composition (Yearsley et al., 1996, Daidze et al., 1996). This is particularly important when determining the lowest O₂ concentration tolerated by a fruit, and the effects that changes in temperature and CO₂ level will have on that tolerance (Beaudry et al., 1992, Yearsley et al., 1997). For example, recommended storage atmospheres for fruit rarely contain less than 2kPa O₂ (Kader, 1980). Aerobic respiration by individual cells can continue at O₂ concentrations much lower than this (Cameron and Reid, 1982). However an external atmosphere containing 2kPa O₂ may result in an internal O₂ concentrations that are close to 0kPa.

Variability in fruit permeance is an important factor restricting the use of modified atmospheres during storage. An atmosphere containing 3kPa O₂ may be beneficial for one fruit but result in internal anaerobiosis in a less permeable fruit. Resistance to gas diffusion in one apple cultivar varied sevenfold among individual fruit, with different cultivars varying by a similar amount (Daidze et al., 1993, Banks et al., 1993). Naturally occurring differences in internal atmospheres may be increased by surface coatings that have been designed to create a modified atmosphere within the fruit (Banks, 1985a). For example, internal O₂ concentrations ranged from 3 - 16kPa in apples treated with CMC (carboxymethylcellulose) coating (Banks et al., 1997).

7.1.3. Calculating fruit permeance

The permeance of a plant organ to a gas can be defined as the rate at which the gas can diffuse in or out of that organ per unit of surface area for a given gradient of partial pressure. Permeability is calculated by multiplying permeance by the thickness of the diffusion barrier (Banks et al., 1995). However, as most diffusion occurs through a limited number of pores in the fruit cuticle, the thickness of the fruit or even the cuticle itself may have little effect on total gas exchange. The permeance of cuticular tissue is also likely to vary as it usually contains breaks
such as the stem scar, lenticels or growth cracks (Maguire et al., 1999). Such areas are likely to be particularly important in contributing to total fruit permeance. As fruit tissues do not present a uniform barrier to gas diffusion, permeance is likely to be a more appropriate measurement than permeability (Banks et al., 1997).

As long as the respiration rate and the internal atmosphere of the fruit can be monitored simultaneously, it is possible to measure permeance to O₂. However, determining the internal atmosphere without altering it by the measurement process can be difficult. Many methods have been developed to overcome this problem. Hulme (1951) described equipment for measuring the internal atmosphere of apples using a hypodermic syringe and manometric techniques. Later researchers have also directly sampled internal atmospheres using hypodermic needles, the contents being analysed by gas chromatography (Lyons et al., 1962, Burg and Burg, 1965, Bergevin et al., 1993, Solomos, 1994a). A potentially less damaging method was proposed by Solomos (1989). A capped hypodermic needle was inserted through a septum glued onto the calyx of apple fruit. This could be left in place, and the syringe periodically attached so as to sample the atmosphere inside the fruit locules. However, these methods are generally only useful for fruit with internal cavities.

To measure the internal atmosphere of fruit that lacked internal cavities, Wardlaw and Leonard (1940) removed small plugs of tissue and replaced them with glass tubes equipped with sampling ports. After allowing time for equilibration of the tube contents with the fruit internal atmosphere, samples could be withdrawn for analysis. This method of 'cavity creation' was taken further by Burg and Burg (1965), who made a 1cm diameter hole through the centre of a fruit. The ends were sealed with rubber stoppers, thus allowing repeated sampling by insertion of a hypodermic syringe.

The above techniques all involve some level of damage of the plant material being examined. One non-destructive method of measuring the composition of the internal atmosphere of plant materials is to use pressure to evacuate the gases from the product. The fruit or vegetable is placed in a saturated brine solution inside an inverted funnel. The pressure is then rapidly reduced and held constant for 2-3 minutes, pulling the internal atmosphere out of the fruit. The evolved gas can then be collected and analysed by gas chromatography (Fidler and North, 1971, Saltveit, 1982, Rodriguez et al., 1989). This method has the advantage that all the gas previously held within the product is available for analysis. However, the internal gas concentrations cannot be measured continuously, and the method does not reflect gradients within the product, or give information about routes of diffusion. Also, gases that are normally dissolved within the sap may be released along with those from the intercellular spaces (Solomos, 1994a).

All of the above methods rely on measuring the internal atmosphere and the respiration rate separately, and then calculating permeance on the assumption that the product is at a steady
state. Measurements may be affected by gradients within the flesh, and the effects of treatments such as waxing may be difficult to assess because the treatment itself reduces respiration rates (Cameron and Yang, 1982, Banks, 1985b). To measure the permeance of fruit to ethylene, Cameron and Yang (1982) held tomato and cucumber fruit in an atmosphere containing up to 1,000 μL·L⁻¹ of ethane, an inert gas. The fruit were then moved into an air atmosphere, and the rate of ethane efflux measured by gas chromatography. The same method was also found effective for measuring permeance of apples and potatoes (Banks, 1985b). This method appears to be an effective way of measuring the permeance of the skin to ethylene and O₂. However, it does not give information about gradients within the flesh, and may not be accurate where diffusion occurs through differentially permeable membranes rather than through pores in the cuticle.

Wigginton (1974) proposed another, non-destructive method. Small vials were attached to the skins of potato tubers. Movement of gases through the lenticels meant that, after a time, the atmosphere inside the containers equilibrated with that inside the tubers. The atmosphere inside the vial could be analysed by gas chromatography. Chambers with a volume of 0.34 ml came to equilibrium within 2 hours of attachment to potato tubers, and could be sampled repeatedly without affecting the results (Banks and Kays, 1988). As this method allows measurement of changes over time, it can be used to accurately determine the anaerobic compensation point of fruit (Yearsley et al., 1996). Combining these measurements with destructive sampling from the fruit cavity using a syringe provides a way to monitor gradients within the fruit itself (Rajapakse et al., 1990). By allowing simultaneous measurements of the internal atmosphere and respiration, this method could also be used to calculate gas permeance.

I have adapted versions the last two of these methods to measure gas permeance of capsicums and melon fruit. In both cases I used KE-25 O₂ sensors to measure O₂ concentrations, as previously described. Unlike sampling carried out using a gas chromatograph, these sensors require only a small volume of gas for analysis and do not create or destroy O₂. By connecting the sensors to a data logger, they can generate a continuous measurement of changes in O₂ concentrations over time. These data can be used directly, or combined with measurements of respiration to calculate O₂ permeance.
7.2. Measuring the internal atmosphere and
gas permeance of the pedicel and
blossom ends of capsicum fruit

7.2.1. Introduction

The diffusion rate of O₂ may be an important factor limiting fruit respiration rates. Cameron and Yang (1982) determined the resistance to diffusion of ethylene inside fruit by measuring the rate of efflux of preloaded ethane gas. I have developed a similar system to that of Cameron and Yang, but in this case the rate of ingress of O₂ into an internal atmosphere flushed with N₂ was measured. Capsicums make an ideal model for studying fruit permeance due to their large internal air space and thin flesh of relatively uniform thickness.

7.2.2. Materials and Methods

7.2.2.1. Plant Material

Mature green capsicums were obtained from local commercial suppliers. Fruit were chosen that had a regular shape and were free from defects. Using a sharp knife, the stem ends were removed from half the fruit, and the blossom ends removed from the remainder. The central pith and seeds were also removed from each fruit, effectively leaving a cup shaped piece of capsicum tissue (Figure 7.2.1.).

The surface areas of the capsicums were measured using two different methods. Both methods were similar to techniques described by Banks (1985c) and Clayton et al. (1995) for measuring the surface areas of potatoes and apples respectively. The first method involved covering the capsicum exterior with non-overlapping sections of tape. Fruit surface area was calculated by multiplying the tape width x length of tape required. The second method involved covering the capsicum with several layers of masking tape. The tape was then carefully cut from the fruit, sectioned, and adhered to sheets of paper. Photocopies of the sheets were made, and the shape of the tape sections cut out. The surface area of each set of cut outs was calculated from its weight.

A third method of measuring surface area was also used after completion of the initial experiments. Seeds and pith were removed from fruit in order to weigh the cuticle + flesh. This
was then dissected into measurable parts of relatively uniform thickness. Total surface area was estimated using the linear regression equation describing the relationship between weight and surface area.

The internal volume of each capsicum "cup" (Figure 7.2.1.) was measured by filling with distilled water, then weighing the water used (1g = 1ml).

![Section A and Section B](image)

*Figure 7.2.1. - Capsicum "cups" created by removing either the pedicel end (Section A) or the blossom end (Section B) of capsicum fruit.*

7.2.2.2. *O₂ Measurements*

Holes were drilled in the centers of two glass sheets. Into these were mounted KE-25 O₂ sensors (as previously described). The O₂ sensors were connected to the computer via ADAM data loggers and an RS-232 conversion module, as used previously with the respirometers. Two tubes (2mm ID) were also fixed through each hole, one protruding approximately 50 mm, the other shortened to approximately 10 mm. The tubes and sensors were fixed in place using flexible modeling clay. Modeling clay was also used to form a 5mm high circular barrier on the glass. This was around 40-50 mm in diameter larger than the capsicum itself. This formed the wall of a "moat" around the capsicum. To ensure an air tight seal, the cut end of each fruit was coated with silicone grease before being placed onto the glass. Water was then added around the outside of the fruit to ensure an air tight seal (Figure 7.2.2.).
Figure 7.2.2. Apparatus for measuring \( \text{O}_2 \) flux through capsicum skin

Once the capsicum “cups” were in place, the sensor readings were recorded at 6 minute intervals for 24-30 hours at 20\(^\circ\)C. The experiment was then repeated using 0 kPa \( \text{O}_2 \), rather than 21 kPa as an initial, internal concentration. To do this, the insides of the capsicums were flushed with high purity \( \text{N}_2 \) for approximately one hour. The gas supply was then shut off, and the rate of \( \text{O}_2 \) ingress into each fruit was recorded. This was repeated twice for each pair of fruit at 20\(^\circ\)C. The fruit were then placed in a cold room at approximately 0.5\(^\circ\)C for about 3 hours. Once the equipment and fruit were thoroughly chilled, the system was recalibrated and the experiment repeated. Low temperatures reduce respiration exponentially, but have a much smaller effect on permeability. For example, the \( Q_{10} \) value for capsicum respiration between 5-10\(^\circ\)C is approximately 2-3 (using data from Chapter 5.1). However, \( Q_{10} \) values for most packaging films (which are, like capsicum cuticle, primarily composed of lipids) are approximately 1-2 over the same temperature interval (Jacxsens et al., 2000). Differences between measurements at 20\(^\circ\)C and measurements at 0.5\(^\circ\)C should therefore be mainly due to the effects of respiration. This entire procedure was repeated three times using new fruit on each occasion.

7.2.2.3. Calculations

Oxygen concentrations recorded by the sensors were converted into kPa using atmospheric data (Macquarie University Automatic Weather Station). The rate at which the \( \text{O}_2 \) concentrations inside the capsicums increased over time was related to the difference in partial pressure between the inside of the capsicum and the external atmosphere. The integrated rate equation results in an exponential. This can be linearised according to the relationship;
- \ln \left(1 - \frac{P_{O_2,\text{internal}}}{P_{O_2,\text{air}}}\right) = -k \times t

where $P_{O_2,\text{internal}}$ is the O$_2$ partial pressure (Pa) inside the capsicum fruit, $P_{O_2,\text{air}}$ represents the O$_2$ partial pressure in the atmosphere, and $t$ represents time (s). The rate constant $k$ denotes the gradient of the line, and thus represents the rate of change in O$_2$ in the internal atmosphere. $k$ could then be used to calculate the permeance of the tissue by the equation:

$$\text{Permeance (umol.Pa}^{-1}.\text{m}^{-2}.\text{s}^{-1}) = \frac{k \times \text{volume (mol)}}{\text{surface area (m}^2\text{)}}.$$  

(Cameron and Yang, 1982).

Measurements of the rate of O$_2$ diffusion are made more difficult by respiratory activity. As well as conducting experiments at 0.5°C, the effects of respiration were minimised by calculating the rate of ingress only when the partial pressure difference was > 5kPa. Under these conditions the rate of flux across the fruit cuticle should be relatively high compared to the respiration rate. Furthermore, the low O$_2$ concentration inside the capsicum may reduce the rate of O$_2$ consumption by the fruit.

### 7.2.3. Results and Discussion

#### 7.2.3.1. Surface Area Determination

The two methods that used adhesive tape to measure the surface area gave very similar results. Both methods were therefore effective ways of measuring capsicum surface area. A good correlation was found between weight and surface area of segments of capsicum skin (Figure 7.2.3.). Regression analysis of the data found that:

$$\text{surface area (cm}^2\text{) = weight (g) x 1.45}$$

However, estimates of total surface area calculated by this method were consistently 10-20% lower than values calculated using measured sections of tape. This was probably due to the acutely curved areas at the tops and bottoms of the fruit, which had higher surface areas than was indicated by their weight. As a result, this method was not used in the calculations.
Figure 7.2.3. - Relationship between weight and surface area of capsicum segments; each point represents data from one segment of skin + flesh, the linear regression line for the data is also shown ($R^2 = 0.98$).

7.2.3.2. **Composition of the Internal Atmosphere**

Initial results showed that the $O_2$ concentration inside the cut fruit depended upon which end of the fruit had been removed. As shown in Figure 7.2.4., removal of the stem end (Section A) resulted in a rapid decrease in the internal concentration of $O_2$. In some cases, internal $O_2$ fell to $<5$kPa after 30 hours (data not shown). However, the internal $O_2$ concentration decreased by an average of only 3 kPa in fruit from which the blossom end had been removed (Section B).

Figure 7.2.4. - $O_2$ concentrations inside capsicum fruit from which the blossom end (■), or the pedicel end (○) had been removed; points represent mean hourly values; error bars indicate the standard deviation of each mean ($n=3$).
Differences in gas permeance between the stem and blossom ends of the capsicums were further demonstrated following flushing with \( N_2 \). Once flushing was stopped, the previous equilibrium \( O_2 \) concentration was rapidly re-established in those fruit from which the blossom ends had been removed (Section Bs). This was different to the fruit with the pedicel ends removed (Section As) of the fruit, in which \( O_2 \) remained low for the remainder of the experiment (Figure 7.2.5).

![Graph showing O2 concentration over time](image)

Figure 7.2.5. \( O_2 \) concentrations inside capsicum fruit from which the pedicel end (●) or the blossom end (■) had been removed; fruit sections were initially flushed with \( N_2 \); points represent mean values, error bars indicate the standard deviation of each mean (n=3)

### 7.2.3.3. Calculation of Gas Permeance

The rate of \( O_2 \) ingress into each fruit section could be linearised against time using the method of Cameron and Yang (1982) (Figure 7.2.6.). This relationship became non-linear once the \( O_2 \) concentration inside the capsicum halves had risen higher than 16kPa, and these values were not included. \( R^2 \) values were >0.99 for both A and B sections. If \( O_2 \) consumption had been significant under the experimental conditions, then reducing the rate of respiration relative to the rate of permeation by repeating the experiment at 0°C would have given a different permeance result. However, the values were not significantly different (\( \alpha = 0.01 \)), suggesting that \( O_2 \) consumption is unlikely to have greatly affected calculations of gas permeance under the conditions used.
Figure 7.2.6. - Linearised O₂ concentrations inside capsicum fruit from which the pedicel end (A) (●) or the blossom end (B) (■), had been removed; Y values for section A on the right axis, values for section B on the left axis; linear regression lines are shown; $R^2(A) = 0.996$, $R^2(B) = 0.993$; points are calculated from mean values ($n=3$).

Permeance was calculated using the gradients of the linear regression lines. The mean permeance of the B section fruit was $6.5 \times 10^{-2} \ \mu \text{mol.Pa}^{-1}.\text{m}^{-2}.\text{s}^{-1}$ (std. dev. = $2.08 \times 10^{-5}$).

However, permeance was more than a thousand times lower for A sections, which had a mean permeance of $3.6 \times 10^{-5} \ \mu \text{mol.Pa}^{-1}.\text{m}^{-2}.\text{s}^{-1}$ (std. dev. = $1.7 \times 10^{-5}$). These results are similar to those reported by Lendzian (1982), who found that isolated capsicum cuticles had a permeance to O₂ of $1.8 \times 10^{-5} \ \mu \text{mol.Pa}^{-1}.\text{m}^{-2}.\text{s}^{-1}$. The published results were slightly lower than those reported here. This is consistent with the use of excised sections of cuticle, as compared to the whole fruit used in the current study.

It was evident from these results that gas permeation into capsicum fruit occurs mainly through the region of attachment to the plant. Burg and Burg (1965) suggested that at least 65% of gas diffusion into capsicums occurred through the fruit pedicel. Most gas diffusion into tomatoes also occurs through the pedicel area (Brooks, 1937, Burg and Burg, 1965, Cameron and Yang, 1982). Both blocking the stem scar with vaseline (Cameron and Yang, 1982) and leaving the peduncle intact (Bergevin et al., 1993) significantly reduced gas permeance of tomato fruit. Solanaceous fruit such as tomatoes and capsicums lack the stomata or lenticels that allow gas exchange through the cuticle in most other fruit (Blanke and Holthe, 1997). The absence of natural openings accounts for the low permeability of the capsicum skin, and the dominance of the pedicel area as a route for gas diffusion.
7.2.4. Key Points

- The stem end or the blossom end was removed from each of eight capsicums. The cut surface of each capscicum section was sealed onto a glass sheet that had an O₂ sensor mounted in the centre.
- The capscicum halves were flushed with N₂, and the rate of O₂ ingress calculated. Measurements were taken while the internal O₂ was <16kPa at 0 and 20°C.
- At 20°C, the rate of O₂ diffusion through the pedicel section was $6.5 \times 10^{-2} \text{ μmol O}_2 \text{Pa}^{-1} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. Permeability of the blossom end was nearly two thousand times lower, being $3.6 \times 10^{-6} \text{ μmol O}_2 \text{Pa}^{-1} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$.
- The low permeance of capscicum cuticle is likely to be due to the lack of natural openings such as pores, lenticels or stomates.
7.3. Permeance of a wax coating

7.3.1. Introduction

The previous experiments showed that most gas diffusion into capsicums is through the pedicel area. By selectively blocking this area, the permeance of the whole fruit may be compared with that of the cuticle and flesh. A convenient way of doing this would be to apply a mixture of wax and Vaseline®. However, wax coatings are themselves permeable to gases. This made it necessary to test not only the permeance of the wax, but the extent to which it blocked permeation through capsicum tissue.

7.3.2. Materials and Methods

7.3.2.1. Gas Permeance of Wax Alone

A 16cm² hole was cut in the lid of a 200 ml sealable container. This was covered with Tyvec®, then coated with a 1mm thick layer of 50/50 mixture of paraffin wax and petroleum jelly (Vaseline®). By warming this mixture slightly it could be applied as a liquid, ensuring a continuous coating over the surface. A KE-25 O₂ sensor was placed inside the container and connected to a data logger (Grant 200 series squirrel data logger). A second O₂ sensor was placed inside an identical, unaltered container to determine the permeance of the jar itself.

Two tubes were inserted into each container, and the containers were flushed with N₂ until the O₂ concentration inside fell to < 0.5 kPa. The jars were then sealed, and the rate of O₂ ingress was recorded. The experiment was repeated 3 times, and the mean permeability calculated.

7.3.2.2. Permeance of Capsicum Pedicels Coated with Wax

As initial results suggested that flux through the wax could be significant, the effectiveness of the wax in blocking the capsicum pedicel was also tested. Mature green capsicum fruit were obtained from commercial suppliers. Using a circular cutter, the pedicel area was excised and mounted securely in the lid of a sealable container (Figure 7.3.1.). The container was flushed with N₂ then resealed. A data logger was used to record the sensor output at 2 minute intervals. This allowed the rate of ingress of O₂ into the container to be calculated.

The pedicel was then covered with a thick layer of the wax mixture, and the process repeated. This procedure was repeated four times at 20°C, using new plant material on each occasion.
Pedicel permeance was also tested at 0.5°C to ascertain whether respiration significantly affected the results.

Figure 7.3.1. - Excised capsicum pedicel mounted in the lid of a sealable container, the container was flushed with $N_2$, then resealed and the rate of influx of $O_2$ through the plant tissue recorded.

7.3.2.3. *Calculations of Gas Permeance*

The data from the $O_2$ sensor were linearised by the method of Cameron and Yang (1982), as described in Section 7.2.2 and the gradient of the linear regression line used to determine the permeability to $O_2$. Surface area was omitted from calculations of the permeance of the pedicel tissue. This was due to the difficulty of accurately measuring the area of the pedicel surface, as well as the observation that results were similar from pedicel sections that varied in their surface area. Permeance was therefore calculated on a whole pedicel basis. As previously, only values recorded while the $O_2$ partial pressure inside the container was less than 15 kPa were used when calculating permeance of the plant tissue.

The total gas permeance of fruit can be calculated in the same way as the resistance of an electrical circuit, where various barriers to diffusion operate in either parallel or series. This approach has the advantage of separating the effects of the various barriers to permeation, thereby allowing the effect of a change in one barrier to be evaluated in terms of its effect on the whole (Cameron and Reid, 1982). The total permeance of waxed capsicum pedicel, may therefore be calculated by:

$$R_{\text{total}} = R_{\text{pedicel}} + R_{\text{wax}}$$
Chapter 7 - Permeance of a Wax Coating

A more complex model proposed by Banks et al. (1993), suggests that a wax coating will have more effect on fruit permeance if it adheres tightly to the fruit skin. In this case, the resistance of the wax should be added in series to each of the components of total resistance. The components of total resistance of the pedicel tissue would comprise the skin and pores in the skin. These individual resistances can be added in the same way as resistors in parallel. Correction must also be made for the area covered by each component; giving the overall formula;

\[
P_{\text{total}} = \left( \frac{1}{R_{\text{skin}} + R_{\text{wax}}^{\text{total}}} \times \frac{A_{\text{skin}}}{A_{\text{total}}} \right) + \left( \frac{1}{R_{\text{pores}} + R_{\text{wax}}^{\text{total}}} \times \frac{A_{\text{pores}}}{A_{\text{total}}} \right)
\]

To obtain an approximate measurement of the effect of waxing, it was assumed that virtually all diffusion under normal conditions occurred through the pores in the pedicel tissue. Banks et al., (1993) estimated that the pores of a model fruit would cover approximately 0.01% of the surface area (median value). Although low, this figure may still overestimate the area covered by pores. For example, pores accounted for only 0.0006% of the surface area of Russet Burbank potato tubers (Abdul-Baki and Solomos, 1993). Applying wax should block these pores. Calculations of permeance therefore used \(A_{\text{pores}} = 0.0001\).

7.3.3. Results and Discussion

7.3.3.1. Wax Permeance

The sealed container proved to have very low gas permeance. The atmosphere inside the container remained below 1 kPa O₂ for several days following flushing with N₂. In contrast, O₂ concentrations increased rapidly inside the container with the waxed section (Figure 7.3.2.). This data were linearised and the gradient (k coefficient) of the resulting linear regression line was used to calculate the permeance of the wax. This was found to be \(1.9 \times 10^{-3} \mu\text{mol O}_2\text{Pa}^ {-1}\text{m}^2\text{s}^{-1}\).

Figure 7.3.2. Changes in O₂ concentration over time inside a sealed container with a standard lid (---) or a lid with a 16 cm² waxed section (--), following flushing with N₂. Data are
mean values (n=3), (a) shows raw data, (b) shows the same data transformed, with linear regression line ($R^2 = 0.9998$).

As the wax was applied to the fruit pedicel in a layer 2-3 times thicker than was tested, total permeation through the wax when applied to the pedicel should be approximately $3.8 \times 10^{-3} \text{ mmol} \text{ O}_2 \text{ Pa}^{-1} \text{ m}^{-2} \text{ s}^{-1}$. This rate of gas transfer was combined with the permeance of the pedicel tissue to estimate the effects of waxing, and this result compared with actual data.

### 7.3.3.2. Permeance of Waxed Pedicel Tissue

The permeance of the pedicel tissue was calculated in the same way as the permeance of the wax section. That is, the data were linearised to calculate the $k$ coefficient (Figure 7.3.3.). The mean rate of flux of $O_2$ through the capsicum pedicels was $1.83 \times 10^{-5} \text{ mmol} \text{ O}_2 \text{ Pa}^{-1} \text{ s}^{-1}$.

The first model tested was based on the wax coating adhering only loosely to the capsicum surface. It predicted that total permeance after waxing would be approximately $7.3 \times 10^{-7} \text{ mmol} \text{ O}_2 \text{ Pa}^{-1} \text{ s}^{-1}$, a reduction in permeance of around 96%. The second model assumed that the coating adhered tightly to the fruit skin, fully blocking the pores. This estimated that waxing would restrict gas diffusion to approximately $1.47 \times 10^{-7} \text{ mmol} \text{ O}_2 \text{ Pa}^{-1} \text{ s}^{-1}$, a reduction in permeance of nearly 98.5%.

The experimental results showed that waxing did substantially reduce gas exchange through the capsicum pedicel tissues. $O_2$ permeation through pedicels coated with wax occurred at a mean rate of $3.4 \times 10^{-7} \text{ mmol} \text{ O}_2 \text{ Pa}^{-1} \text{ s}^{-1}$ (Figure 7.3.3.). This result was intermediate between those predicted by the two models. This is in agreement with the suggestion by Banks et al. (1993) that most surface coatings neither fully adhere to or are fully separated from the fruit surface. The results are consistent with those predicted by Banks et al. (1993) for a model fruit. Waxing therefore appeared to be a valid method for blocking $O_2$ permeation through capsicum pedicels.
Figure 7.3.3. - Linearised functions of changes in O₂ concentration inside a sealed container with a capsicum pedicel inserted in the lid, before (♦) and following (□) application of wax to the fruit tissue; points are mean values, error bars indicate the standard deviation of each value (n=4), linear regression lines are also shown, (R² > 0.99).

7.3.4. Key Points

- The rate of O₂ diffusion through wax, capsicum pedicel tissue, and waxed capsicum pedicel tissue were measured.
- The data were linearised by the function; \(-\ln(1-(O₂/21)) = k \times \text{time}\). The value of \(k\) was determined by linear regression and used to calculate permeance.
- The permeance of a 1 mm thick layer of wax was \(1.9 \times 10^{-3} \mu\text{mol } \text{O}_2 \cdot \text{Pa}^{-1} \cdot \text{m}^{-2} \cdot \text{s}^{-1}\), while the permeance of the complete pedicel was \(1.84 \times 10^{-5} \mu\text{mol } \text{O}_2 \cdot \text{Pa}^{-1} \cdot \text{s}^{-1}\). Waxing reduced the pedicel permeance to \(4.8 \times 10^{-7} \mu\text{mol } \text{O}_2 \cdot \text{Pa}^{-1} \cdot \text{s}^{-1}\).
- Comparison of the actual result with those from theoretical models showed that the data were intermediate between the result predicted for a loose coating (\(7.3 \times 10^{-7} \mu\text{mol } \text{O}_2 \cdot \text{Pa}^{-1} \cdot \text{s}^{-1}\)) and that for a tightly adhering coating (\(1.47 \times 10^{-7} \mu\text{mol } \text{O}_2 \cdot \text{Pa}^{-1} \cdot \text{s}^{-1}\)).
- Applying wax to capsicum pedicels was an effective method of blocking gas permeation through this area.
7.4. Finding the path of maximum gas diffusion into detached capsicum fruit

7.4.1. Introduction

The results from Section 7.2 showed that the major fraction of gas exchange in capsicum fruit occurs via the stem end. However, the exact path of gas diffusion was still unclear. To investigate this phenomenon further, parts of the capsicum pedicel were selectively blocked with wax, and the resulting changes in the internal O₂ concentration were measured.

7.4.2. Materials and Methods

7.4.2.1. Plant Materials

Fully green capsicums were obtained from commercial suppliers. The stems were trimmed to approx. 20mm in length. Part of each fruit was coated with a mixture of melted paraffin wax and Vaseline®. Care was taken to avoid cracking, and to seal the edges onto the fruit skin. The areas waxed are shown in Figure 7.4.1., being as follows;

a) junction between pedicel and fruit cuticle only
b) pedicel / calyx tissue + (a)
c) stem + (b)
d) no wax – control

Figure 7.4.1. - Selective application of wax to different areas of the capsicum pedicel.
7.4.2.2. \textit{O}_2 \text{ Measurements}

Tubes approximately 15mm long with an internal diameter of 5mm were glued onto the gas exchange ports of four \textit{O}_2 sensors. The ends of the tubes were cut diagonally to maximise gas exchange, and were sterilised with 70\% ethanol. Holes were made in the capsicums using a sharpened, sterilised hole punch of appropriate diameter, and the tubes inserted. The \textit{O}_2 sensors were secured in place and leaks prevented with flexible plastic putty.

The \textit{O}_2 sensors were connected to an ADAM 4018 with output to a computer, as used in section 7.3. Readings from the \textit{O}_2 sensors were recorded every 6 minutes over a 12 hour period. This procedure was repeated four times using different fruit on each occasion.

7.4.2.3. \textit{Calculations}

Changes over time in the mean internal \textit{O}_2 concentrations were subjected to analysis of variance (ANOVA) (two factors, completely randomised) using CoStat statistical software. As the large amount of data collected made it impractical to analyse every value, mean values calculated at hourly intervals were analysed. Differences among the means were evaluated using Duncans Multiple Range Test.

7.4.3. \textit{Results and Discussion}

Internal \textit{O}_2 concentrations decreased rapidly in the fruit which had been fully waxed around the stem and calyx (treatment c) (Figure 7.4.2.). After 5 hours the \textit{O}_2 concentration in treatment c was significantly different from all other treatments (\( \alpha = 0.05 \)). Treatments a and b resulted in internal atmospheres that were not significantly different to the control (\( \alpha = 0.05 \)).

From these results it would appear that partial waxing of the pedicel area has minimal effects on capsicum permeance. There are two possible explanations for this result. The first is that most gas exchange in capsicum occurs through the stem tissue. In this case harvesting of fruit may have a significant effect on capsicum permeability. The length of stem remaining on the fruit may also influence overall fruit permeability, thereby potentially affecting respiration rate.

Alternatively, the large number of pores in the pedicel and open structure of the underlying tissue may mean that this region offers minimal resistance to gas exchange. A high rate of permeation through the pores suggests that the entire area must be blocked to significantly affect gas exchange. This hypothesis was tested by Perez and Beaudry (1998) by partially coating bananas with paraffin. In this case the flesh proved to be a significant barrier to gas diffusion, and large gradients in gas concentrations occurred within the fruit. However, the large
air space inside capsicum fruit ensures thorough mixing of internal gases. As a result, partial coatings have little effect on total gas exchange.

![Graph showing O₂ concentration over time](image)

**Figure 7.4.2.** Mean O₂ concentrations inside capsicums which had wax applied to different parts of the pedicel region; treatment a (---), treatment b (- - - ) and treatment c (-----), and the untreated control (-----); error bars indicate the standard deviation of each mean at hourly intervals (n=4).

### 7.4.4. Key Points

- Changes in O₂ concentration were monitored inside capsicums which had different regions of the pedicel regions coated with wax.
- Partially waxing the pedicel area did not significantly affect the internal O₂ concentration; internal O₂ fell significantly only when the entire pedicel area was waxed.
- These results suggest that either most permeation occurs through the capsicum stem, or that all pores in the pedicel region must be blocked for there to be any effect on the internal atmosphere.
7.5 The permeance to O₂ of detached

*Capsicum annuum* fruit

7.5.1. Introduction

A simple and non-destructive method of measuring the internal atmospheres of plant products is to seal a chamber onto the fruit or vegetable surface (Wigginton, 1974). After a time, the atmosphere inside the chamber will equilibrate with that in the underlying flesh. The length of time taken to reach equilibrium will be a function of the permeance of the fruit cuticle as well as the size of the air space inside the chamber. The gas composition inside each chamber can then be analysed by gas chromatography. This method has been used successfully to measure the internal atmospheres of potatoes (Wigginton, 1974, Banks and Kays, 1988), apples (Yearsley et al., 1996, Dadzie et al., 1996), asian pears and nectarines (Rajapakse et al., 1990).

I have adapted this technique using the KE-25 O₂ sensors previously described. The sensors were attached directly onto the fruit, their sampling space forming the external chamber. The fruit and attached sensors could be placed inside a respirometer, allowing simultaneous measurements of the rate of O₂ consumption and the internal atmosphere. By combining both sets of information, it is possible to calculate the fruit's permeance to O₂. Capsicums were used to test this method because of the ease with which their internal atmosphere could be measured, allowing comparison with other methods. Also, by selectively waxing the pedicel area, the permeance of this area could be compared with that of the fruit body.

7.5.2. Materials and Methods

7.5.2.1. Fruit Characteristics

Green capsicum fruit (*Capsicum annuum*) were obtained from commercial suppliers at the Sydney wholesale markets. Fruit of even size and maturity were selected and weighed. Volume was measured by water displacement. Surface area of the fruit was estimated by covering the exterior with non-overlapping, pre-measured sections of adhesive tape, as described in Section 7.2. After completion of the experiment, the internal volume was measured by filling the fruit with water, as well as by subtracting weight \( \times \) density from the total volume. These methods gave similar results, so the average of the two measurements was used for each fruit.

24 hours after the experiment began, the pedicels and surrounding areas were blocked using the same 50/50 mixture of paraffin wax and petroleum jelly (Vaseline®) as in sections 7.3 and
7.4. After 48 h the mixture was removed by gently flexing the fruit to break up the coating. Measurements then continued for a further 24 hours.

7.5.2.2. **Gas Measurements**

Three KE25 O₂ sensors were used to monitor each capsicum fruit. The first O₂ sensor was attached to the end of a 10 mm long x 6 mm diameter tube. This was inserted into the fruit cavity to monitor the internal O₂ concentration (Figure 7.5.1.). A second sensor was sealed directly onto the capsicum cuticle. At equilibrium, this sensor was expected to indicate the O₂ concentration in the capsicum skin. These two sensors were secured in place using oil-based modelling clay. A third sensor was attached to the respirometer lid, and was used to monitor O₂ concentrations inside the vessel.

Both O₂ and CO₂ were also measured by periodic injection of gas samples into a Gas Chromatograph (Gow Mac series 580). To do this, three layers of 2 cm² electrical insulating tape were adhered to the fruit. The top layer of tape was peeled back to allow insertion of a syringe needle, and then replaced to prevent leakage after sample collection.

The capsicums and O₂ sensors were placed inside respirometer bowls, as previously described, to measure fruit respiration rates (Figure 7.5.1.). Readings from the O₂ and temperature sensors were monitored continuously by computer, and recorded at six minute intervals. Oxygen inside the respirometers was controlled between 19.6 and 20 kPa. To prevent photosynthesis, the entire apparatus was covered with blackout cloth.

![Diagram](image)

Figure 7.5.1. - Respirometer vessel and O₂ sensors used to monitor the respiration rate and internal atmosphere of a detached capsicum fruit.
7.5.2.3. **Calculations**

In a steady state, the rate of O$_2$ permeation into a fruit equals the rate of removal of O$_2$ by respiration. The rate at which O$_2$ permeates the fruit is affected by the partial pressure difference between internal and external atmospheres, and overall fruit permeance. Permeance of the capsicums could therefore be calculated by:

\[
\text{Permeance} = \frac{\text{rate of O}_2\text{ consumption (}\mu\text{mol O}_2\text{.s}^{-1}\text{)}}{\text{partial pressure difference (Pa)} \times \text{surface area (m}^2\text{)}}
\]

O$_2$ consumption was calculated from the decreases in O$_2$ concentration inside the respirometer, combined with reductions in O$_2$ inside the fruit itself. A linear regression line was calculated for each data set between flushing cycles (20-19.6 kPa). The gradient of the regression line was then used to calculate O$_2$ consumption over that time interval. Permeance was calculated both as a value per unit of surface area, and on a per fruit basis.

For the purpose of analysis, mean values of permeance and respiration rate were calculated for 6h blocks of measurements for each fruit and compared by analysis of variance (ANOVA, single factor, completely randomised) using CoStat® statistical software. Differences among means were evaluated using Duncans Multiple Range test.

By combining the data from this experiment with figures for the rate of oxygen flux through waxed and unwaxed pedicel tissue, it was possible to calculate the permeance of the capsicum body alone, rather than in combination with the pedicel area. The total flux of O$_2$ into each fruit was calculated by multiplying the previous permeance value by the surface area. From this was subtracted the mean total O$_2$ flux through the pedicel tissue (1.84 $\times$ 10$^{-5}$ $\mu$mol O$_2$ kPa$^{-1}$ s$^{-1}$), as determined in Section 7.3. The result indicates the approximate amount of O$_2$ entering the fruit through the cuticle. The total rate of O$_2$ flux into each waxed fruit was also determined. From this was subtracted the mean flux of O$_2$ through a waxed pedicel (4.8 $\times$ 10$^{-7}$ $\mu$mol O$_2$ kPa$^{-1}$ s$^{-1}$), also determined in Section 7.3. Total O$_2$ flux of each fruit before, during and after wax application were reconverted to permeance values by dividing by the relevant surface area. These results were analysed by ANOVA (one way, completely randomised).

7.5.3. **Results and Discussion**

7.5.3.1. **Comparison of Internal and External O$_2$ Sensors**

Measurements of O$_2$ by the sensors connected to tubes piercing the capsicum skin were compared to those from O$_2$ sensors attached to fruit cuticles. Although the results from the internal O$_2$ sensors varied by up to 3 kPa among fruit, decreases and increases in internal O$_2$
were of similar magnitude in all cases. Measurements of internal O\textsubscript{2} levels stabilised after approximately 12 hours inside the respirometers. In contrast, the sensors attached to the capsicum skins did not stabilise but continued to register a falling O\textsubscript{2} concentration after 12 hours (Figure 7.5.2.). The mean rate of decrease was 0.1 kPa.hr\textsuperscript{-1} (std. dev. = 0.08).

Internal O\textsubscript{2} fell rapidly after wax was applied to the capsicum pedicels, approaching as low as 3 kPa in some cases. Again, apparent O\textsubscript{2} concentration at the skin surface declined more slowly than measurements inside the cavity. Even after 30 hours, O\textsubscript{2} measurements through the skin continued to decline at a rate of approximately 0.02 kPa.hr\textsuperscript{-1} (std dev = 0.02). Likewise, when the wax was removed, a rapid rise in the measured internal O\textsubscript{2} concentration was reflected in a smaller and slower rise in measured concentrations on the skin (Figure 7.5.2.).

![Graph showing internal O\textsubscript{2} concentration over time](image)

**Figure 7.5.2.** - Recorded readings of O\textsubscript{2} concentration from KE-25 O\textsubscript{2} sensors attached to the capsicum skin (---), and connected to a short tube which had been inserted into the capsicum cavity (—); wax was applied to the pedicel area after 24 hours, then removed after a further 42 hours; data are mean values; error bars represent the standard error of the mean at 22, 62 and 82 h (n=12).

It is evident that an O\textsubscript{2} sensor attached to the skin of a capsicum does not measure the internal atmosphere effectively. This is presumably due to the low permeability of the capsicum cuticle, which delays equilibration between the sampling space of the O\textsubscript{2} sensor and the capsicum flesh. However, some equilibration with the underlying tissues does occur, as O\textsubscript{2} concentrations recorded from the skins of unwaxed fruit were lower than concentrations in the internal cavities. This suggests that a significant gradient in O\textsubscript{2} concentration exists within the flesh and / or the cuticle, as has been previously observed for capsicums (Banks and Nicholson, 2000) as well as other fresh produce (Solomos, 1987, Banks and Kays, 1988, Rajapakse et al., 1990).
7.5.3.2.  **Internal Gas Concentrations**

Internal gas concentrations measured using the $O_2$ sensors were confirmed using gas chromatography (GC) to analyse both $O_2$ and $CO_2$. The mean value of $[CO_2] + [O_2]$ was close to 21 kPa both initially and after the wax was removed (Table 7.5.1.). When wax was applied to the pedicle area, $O_2$ concentration in the internal atmosphere decreased. However, this fall in $O_2$ was not fully balanced by a rise in $CO_2$, resulting in a total of $[CO_2] + [O_2]$ which was less than 21 kPa. This suggests that waxing not only reduced permeance, but changed the way that gas permeation occurred.

<table>
<thead>
<tr>
<th></th>
<th>kPa $O_2$</th>
<th>std. dev.</th>
<th>kPa $CO_2$</th>
<th>std. dev.</th>
<th>Permeance ratio $^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial readings</td>
<td>17.8</td>
<td>1.2</td>
<td>3.2</td>
<td>1.1</td>
<td>1.0 a</td>
</tr>
<tr>
<td>After wax applied</td>
<td>4.3</td>
<td>2.8</td>
<td>6.3</td>
<td>2.2</td>
<td>2.6 b</td>
</tr>
<tr>
<td>After wax removed</td>
<td>17.0</td>
<td>1.0</td>
<td>4.2</td>
<td>1.5</td>
<td>0.9 a</td>
</tr>
</tbody>
</table>

$^2$ Means followed by the same letter are not significantly different ($\alpha = 0.01$)

Table 7.5.1. - Mean composition of the internal atmospheres of capsicum fruit; mean values from 12 replications, permeance ratio calculated by; (change in internal $O_2$ compared to air) / (change in internal $CO_2$ compared to air). Letters indicate values which are significantly different ($\alpha = 0.05$).

Under normal conditions, the permeance ratio of a capsicum was approximately 1, the value for a microperforated bag. As $O_2$ and $CO_2$ diffuse through holes in a barrier at similar rates, any accumulation of $CO_2$ inside the fruit will be balanced by depletion of $O_2$ as long as respiration remains aerobic (Burg and Burg, 1965). However, once wax was applied, the permeance ratio increased to 2.6, a value characteristic of a package made of coherent film. In this type of package gases dissolve into and out of the barrier itself. Similarly, gases dissolve into the cuticular membrane of fruit, entering a liquid phase. The solubilities of $O_2$ and $CO_2$ are different ($O_2 = 0.004339g.100gH_2O$, $CO_2 = 0.1688g.100gH_2O$, Handbook of Chemistry and Physics, 35th edition, Chemical Rubber Publishing, 1953). Therefore, the rate at which they move through the membrane will also vary.

The cuticle of fruit is primarily composed of lipids. $CO_2$ is highly soluble in lipids and dissolves relatively easily into this phase (Solomos, 1987). Polyethylene, which is also a lipid barrier, is approximately four times more permeable to $CO_2$ than to $O_2$. Under aerobic conditions, respiration occurring inside a polyethylene bag will result in an internal depletion of $O_2$ that is four times greater than the accumulation of $CO_2$. Apples washed with Tween 20 closely
approached these values, as their permeance ratio can be calculated as 3.8 (Dadzie et al., 1995). As the permeance ratio in this case was calculated to be 2.6, this suggests that diffusion was by both diffusion and permeation. Banks and Nicholson (2000) found that the capsicum cuticle was around 10 x more permeable to CO₂ than to O₂. Such a high value appears surprising. However, this report measured the rate of influx into sampling vials attached to the capsicum skins and flushed with N₂, as opposed to the steady state method with whole fruit used in the current study. The published results could possibly have been influenced by significant gradients within the capsicum flesh, while those in the current study may have been affected by imperfections in the capsicum surface.

7.5.3.3. Permeance and Respiration Rates of Whole Fruit

The permeance of whole tomato fruit has been reported to be 3.8 x 10⁻⁴ μmol O₂.Pa⁻¹.m⁻².s⁻¹ (Cameron and Reid, 1982). Using the data on respiration rate and internal atmosphere presented by Lyons et al. (1962), cantaloupe melons were estimated to have a mean permeance of 11.7 x 10⁻⁴ μmol O₂.Pa⁻¹.m⁻².s⁻¹. The permeance of capsicums was intermediate between these values, being 6.5 x 10⁻⁴ μmol O₂.Pa⁻¹.m⁻².s⁻¹ (s.d. = 1.8 x 10⁻⁴).

Waxing the capsicum pedicel reduced the fruit’s permeance nearly 8-fold to approximately 0.64 x 10⁻⁴ μmol O₂.Pa⁻¹.m⁻².s⁻¹ (s.d. = 0.2 x 10⁻⁴) (Figure 7.5.3a.). This reduction in permeance due to waxing was highly significant (α = 0.05). The stem scar has previously been shown to be the site of at least 60% of gas exchange for capsicums (Burg and Burg, 1965) and 97% of gas exchange for tomatoes (Cameron and Yang, 1982) respectively. These results show that 85-90% of gas movement occurs through the capsicum pedicel. The total surface area of fruit such as capsicums and tomatoes therefore has less influence on the composition of their internal atmosphere and permeance than the small area of reduced resistance at the point of attachment to the plant.

Application of wax to the pedicel reduced internal O₂ considerably, thus also decreasing respiration rate (Figure 7.5.3b.). Although this reduction in respiration rate was significant (α = 0.05), so was the increase in respiration rate following wax removal when compared to the initial respiration rate (α = 0.05). This effect is similar to the stimulation of respiration reported for cucumbers removed from low O₂ storage (Kanelli et al., 1988). The use of waxing as a postharvest treatment to reduce respiration could therefore have mixed effects on capsicum quality.
Figure 7.5.3. - The mean permeance (a) and respiration rate (b) of 12 capsicum fruit during storage at 20°C, values calculated at 6 hour intervals from mean half hourly measurements of each fruit; wax was applied to the pedicel area after 24 hours, then removed after a further 42 hours; error bars represent the standard error of each mean (n=12).

7.5.3.4. Permeance of the Capsicum Flesh and Cuticle

The permeance of the capsicum body alone was calculated using the data on total flux through pedicel tissue that were obtained in Section 7.3. Permeance did not vary significantly over the duration of the experiment (α=0.05) (Table 7.5.2.). This confirmed the validity of the methods used, as waxing the pedicel was not expected to have affected the permeance of the fruit body. The mean permeance of the capsicum body was 0.97 × 10⁻⁴ μmol O₂.kPa⁻¹.m⁻².s⁻¹. In Section 7.2 measurements of the rate of influx of O₂ into a capsicum half indicated a mean permeance of 0.36 × 10⁻⁴ μmol O₂.kPa⁻¹.m⁻².s⁻¹. The figure calculated using the steady state method is around 3 times higher, possibly due to leaks or inconsistencies in the wax coating. However, these differences are comparatively small given the overall variability of the data.

<table>
<thead>
<tr>
<th>Permeance (μmol O₂.kPa.m⁻².s⁻¹)</th>
<th>Before waxing</th>
<th>Fruit with waxed pedicel</th>
<th>After wax removed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.24 × 10⁻⁴</td>
<td>0.64 × 10⁻⁴</td>
<td>0.99 × 10⁻⁴</td>
</tr>
<tr>
<td></td>
<td>0.59 × 10⁻⁴</td>
<td>0.07 × 10⁻⁴</td>
<td>0.61 × 10⁻⁴</td>
</tr>
</tbody>
</table>

Table 7.5.2. - Permeance to O₂ of capsicum body (pedicel excluded) before waxing, with wax coating, and following wax removal. The three means were not significantly different (α=0.05).

Previous work found the O₂ permeance of capsicum cuticles to be 0.18 × 10⁻⁴ μmol O₂.kPa⁻¹.m⁻².s⁻¹ (Lendzian, 1982), and 0.23 × 10⁻⁴ μmol O₂.kPa⁻¹.m⁻².s⁻¹ (Banks and Nicholson, 2000). Both of these reports used different methods to that in the current study. The method used by Banks...
and Nicholson (2000) has already been discussed. Lendzian (1982) used cuticle tissue that had been killed then placed in solution. As a result, the intercellular spaces through which gas exchange normally occurs may have collapsed or filled with fluid. The fruit used in this experiment, however, were alive and turgid, and the intercellular air spaces would be expected to have remained intact. Also, the whole fruit used in this experiment were more likely to have had slight irregularities or damage than the sections of cuticles used by Lendzian (1982), potentially increasing their gas permeance.

One source of error in the methodology used in this trial is that the differential permeance of waxed capsicum fruit could have lead to a change in internal pressure. While the flexibility in the capsicum itself would seem likely to equalize such an effect, it cannot be assumed that this occurs. Decreases in the partial pressure of $O_2$ may have reduced measurements of $O_2$ recorded by the KE-25 $O_2$ sensors, in turn reducing the calculated permeance values. Future work should therefore include measurements of pressure for the internal and external atmospheres.

Permeability of the cuticle to gases has previously been shown to be reduced by coating with wax (Brooks, 1937, Banks, 1985a, Banks et al., 1993). For capsicums, wax would only need to be applied to a localised area around the pedicel to substantially reduce permeance. However, considerable variations in internal atmospheres and permeance were observed during this experiment, with $O_2$ inside waxed capsicums ranging from 0.5 to 11 kPa. Such variability also occurs for other fruit, and is considered to be one of the principal problems limiting the use of fruit coatings to improve storability (Banks et al., 1993, Banks et al., 1997).

The limited gas permeance of capsicum fruit may in part be an adaptation to allow recycling of respiratory $CO_2$. The results in section 5.2, showed that photosynthesis in capsicum fruit refixes respiratory $CO_2$ accumulated in the internal cavity. This would not be possible were it not for the low rate of gas diffusion through the capsicum cuticle.

In conclusion, two methods for measuring the permeance of capsicums to $O_2$ were tested. Section 7.2 measured the rate of gas flux into capsicum sections that were initially flushed with $N_2$. Data were linearised, and the slope of the regression line used to calculate gas permeance. The second method combined simultaneous measurements of internal $O_2$ concentrations and steady state $O_2$ consumption to calculate permeance according to Ficks First Law of Diffusion. Both methods were effective, and the results were similar. However, the first method is likely to be more appropriate when information on the total permeance is required, and the plant organ contains a central cavity. The second method may be used with a wider range of plant organs, and may be useful when examining changes over time, or gradients within a fruit.
7.5.4. **Key Points**

- Measurements of the internal O₂ concentration of capsicums were made using sensors attached to the fruit skin and inserted into the fruit cavity. Internal CO₂ and the rate of O₂ consumption by each fruit were also measured.

- After 24 hours the pedicel areas were blocked with wax. The wax was removed after a further 42 hours of measurements.

- Fruit permeability was calculated using the combined data on O₂ demand, internal O₂ concentration and surface area.

- Sensors attached to the capsicum failed to equilibrate with internal O₂ concentrations within a reasonable time due to the low permeance of the capsicum cuticle.

- Changes in the O₂:CO₂ ratio in the internal atmosphere indicated that diffusion into unwaxed fruit was primarily through holes, but that waxing increased the proportion of total gas flux that was through the differentially permeable fruit cuticle.

- Permeance to O₂ was decreased nearly 8-fold by waxing, and O₂ consumption was approximately halved.

- By subtracting the total flux of O₂ through the unwaxed and waxed capsicum pedicels (Section 7.3) from the total O₂ flux into unwaxed and waxed whole fruit, it was possible to estimate the total gas diffusion through the remainder of the fruit. The mean permeance of the capsicum body was $0.97 \times 10^{-4} \mu\text{mol O}_2 \cdot \text{Pa}^{-1} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. 

7.6. Differences in internal $O_2$ concentrations of different melon varieties, and changes during ripening

7.6.1. Introduction

In the previous section, it was shown that attaching an $O_2$ sensor to the skin of a capsicum was not an effective method for measuring its internal atmosphere. This was due to the low permeance to $O_2$ of the capsicum cuticle, which greatly delayed equilibration between the sensor sampling space and the underlying flesh. However, a similar method has been shown to be an effective way of measuring the internal atmosphere of other products (Rajapakse et al., 1990, Yearsley et al., 1996, Dadzie et al., 1996). This is because, unlike capsicums, the cuticles of most fruit and vegetables contain pores such as stomata or lenticels, which facilitate gas exchange between the internal atmosphere and the air.

Melons, like apples, are fleshy fruit that have natural openings in their cuticle. However, the characteristics of the melon cuticle vary considerably among varieties. *Inodorus* melons such as honeydews have a thick, waxy cuticle that might be expected to have low permeance to gases. In contrast, the open, corky structure of the surface netting which is typical of *Reticulatus* melons such as rockmelons develops little protective wax and might be expected to have a much higher gas permeance. An $O_2$ sensor attached to the surface of such a melon should therefore reach equilibrium with the internal atmosphere within a reasonable time.

In this study I have tested the efficacy of using externally attached $O_2$ sensors to measure the internal atmospheres of different melon varieties. By comparing measurements from the melon centre with those on the skin, it should be possible to calculate the permeance of the melon flesh. These methods were then used to examine changes during ripening.
7.6.2. Materials and Methods

7.6.2.1. Plant Materials

The initial part of the experiment used both Inodorus and Reticulatus type melons. These comprised 12 ‘Yelo melon’ honeydew melons, 30 ‘Sweet Obsession’ honeydew melons, 20 ‘Planters jumbo’ rockmelons and 12 ‘Tropicale’ rockmelons. All melons were obtained from commercial suppliers in Sydney. Netted melons were selected which had been harvested at half to full slip, indicating that they had fully matured on the vine (Webster, 1975). Inodorus melon fruit are generally non-slipping (Lester and Shellie, 1992), so these were selected on the basis of apparent ripeness (appearance and odour), regularity of shape and freedom from defects.

Rockmelons ‘Planters Jumbo’ were supplied by Sarda Farms in North Queensland for the second part of this experiment. Melons were harvested which were mature green, and had not started to slip from the vine. On arrival at the Sydney laboratories the fruit were placed in a large drum and treated with >100 ppm ethylene in air for approximately 16 hours.

The melons were weighed, and their volume was measured by displacement in water. The volume was then used to calculate the dimensions of the equivalent sphere and, thereby, the approximate surface area of each melon. A second method involved measuring the external dimensions and using this information to calculate the surface area of the equivalent sphere. To test the validity of these methods, several melons were covered with 2-3 layers of adhesive tape. The tape was then removed, sectioned, photocopied and the surface area calculated as previously described for capsicums. This method gave very similar results to calculations of surface area that used the melon volume. Subsequent estimates of surface area used the volume method alone.

7.6.2.2. Gas Measurements

In the first part of this experiment, the O₂ concentration was measured at both the skin and at the centre of each fruit. To measure the O₂ concentration at the fruit centre, a 7 mm diameter PVC tube was glued onto the gas exchange port of an O₂ sensor. The end of the 100 mm long tube was cut diagonally so as to maximise gas exchange, and the entire tube sterilised in 80% ethanol. A hole was made in each melon using a sharpened, sterilised cork borer of appropriate diameter, and the tube inserted. The tube was fixed in place using flexible plastic modelling clay. The same material was used to attach a second O₂ sensor directly to the surface of each melon.
This sensor was additionally secured using an elastic strap placed around the fruit. It was expected that, after a time, the atmosphere in the sampling space of each sensor would reach equilibrium with the underlying tissue.

In the second part of the experiment, internal \( \text{O}_2 \) concentrations were measured using sensors on the fruit skins alone. This avoided damaging the fruit, and was found to be a reliable indicator of changes in the internal atmosphere.

Each melon with its attached \( \text{O}_2 \) sensor(s) was placed in a respirometer, as described in section 3 (Figure 7.6.1.). All of the \( \text{O}_2 \) and temperature sensors used were connected to ADAM data loggers, and thence to a computer. The atmosphere inside each bowl was allowed to fluctuate between 19-20 kPa \( \text{O}_2 \), this level being maintained by flushing with air. The computer recorded temperature and \( \text{O}_2 \) readings every 6 minutes for up to 5 days.

![Diagram](image)

**Figure 7.6.1. - Equipment for measuring the internal atmosphere and rate of \( \text{O}_2 \) consumption of a detached melon fruit.**

Data from the attached \( \text{O}_2 \) sensors were discarded if the readings fluctuated by >0.1% during the flushing cycles. Fluctuations of this magnitude indicated that the sampling space between the sensor and the melon was not fully sealed, and that the sensor was therefore not giving a true indication of the internal \( \text{O}_2 \) concentration.
7.6.2.3. Calculations

The permeance of the fruit to gases was calculated as previously, on the basis of Fick's First Law of Diffusion. Permeance was calculated both from O\textsubscript{2} measurements made on the skin, and those made at the centre of each fruit. As the flesh and the skin act as resistors placed in series (Nobel, 1983), it was possible to calculate the resistance to gas diffusion of the flesh;

\[ R^{\text{fruit}} = R^{\text{flesh}} + R^{\text{skin}} \]

Where \( R \) = resistance.

As resistance is the inverse of permeance;

\[ \frac{1}{P^{\text{flesh}}} = \frac{1}{P^{\text{fruit}}} - \frac{1}{P^{\text{skin}}} \]

Where \( P \) = permeance.

Variations in permeance among the different melon varieties were analysed by ANOVA (one way, completely randomised) with CoStat\textsuperscript{®} statistical software, and differences between mean values were determined using Duncans Multiple Range test. For the initial experiment, permeance was compared to the mean rate of O\textsubscript{2} consumption by each melon, the results being analysed by linear regression. The regression lines were then compared by the method described by Mead et al. (1983). Changes in respiration rate and permeance for melons in the second part of the experiment were also subjected to ANOVA (one way, completely randomised) using mean data recorded at 10 hour intervals.

7.6.3. Results and Discussion

A number of the results obtained in the initial part of this experiment were discarded due to leaks between the melons and the attached O\textsubscript{2} sensors. Particular difficulty was experienced with the honeydew melons 'Sweet Obsession'. These melons had a heavily waxed cuticle, making adhesion of the sensor unreliable. Additional repetitions were performed using O\textsubscript{2} sensors attached to the fruit skins only. Leaks also occurred when measuring the 'Tropicale' rockmelons. As there were not enough stable results from the 'Tropicale' melons for reasonable analysis, data from this variety were not used. The remainder of this section deals only with results from 20 'Planters Jumbo' rockmelons, 18 'Sweet Obsession' honeydew melons, and 12 'Yelo melon' honeydews.
7.6.3.1. **Comparison of Internal and External \(O_2\) Sensors**

Both the \(O_2\) sensors attached to the melon skins, and those inserted into the melon cavities, came to equilibrium within 24 hours (Figure 7.6.2.). This was different to the results for capsicums, where attached sensors had still not equilibrated with the internal atmosphere after several days. This suggests that attaching an \(O_2\) sensor to the outside of a melon was an effective way of measuring the \(O_2\) concentration in the underlying flesh. As the sensor with the attached tube stabilised within a similar time to the externally attached \(O_2\) sensor, it seemed that diffusion along the tube was sufficient to enable a reasonably rapid response time.

![Graph showing \(O_2\) concentration over time](image)

*Figure 7.6.2. - Mean \(O_2\) concentrations measured by \(O_2\) sensors attached to the skin (---) or connected to tubes inserted into the cavity (---) of 'Planters Jumbo' rockmelons; error bars represent the standard error of each mean at two hour intervals (n=16).*

7.6.3.2. **Differences in \(O_2\) Consumption and Permeance Between Melon Varieties**

Differences between the melon varieties are summarised in Table 7.6.1. The rates of \(O_2\) consumption of the two *Inodorus* type melons were similar. However, the rockmelons respired significantly more rapidly (\(\alpha = 0.05\)), there being nearly a three fold difference between the *Reticulatus* and *Inodorus* melon types. These results are consistent with those previously reported for postclimacteric cantaloupe fruit (Lyons et al., 1962, McGlasson and Pratt, 1963) and Inodorus group melons (Miccolis and Saltveit, 1995).
O₂ concentrations inside the melons were considerably modified compared to those in the external atmosphere. The honeydew melons generally had higher internal O₂ levels than the other varieties. Differences in internal O₂ concentrations between the ‘Yelo melons’ and the rockmelons were not significant ($\alpha = 0.05$).

Gradients in O₂ concentration existed between the skin and the central cavity for all varieties. Generally these were of the order of 2-4 kPa. However, there was considerable variation between individual melons. Several melons recorded gradients of around 10kPa between the skin and the cavity, while others had similar O₂ concentrations at both locations, or, in several cases, slightly higher O₂ concentrations inside the cavity. These inconsistencies in the data may be the result of incomplete sealing to the fruit surface, blocked lenticels at the point of attachment, or free juice blocking the tube in the case of measurements in the fruit centres. Also, wounding may have increased respiration in the region immediately surrounding the inserted tube, increasing the apparent gradient through the flesh.

Total O₂ permeance was calculated from the measurements made in the fruit cavity, while permeance of the skin was estimated using data from sensors attached to the fruit surface. The permeance of the ‘Yelo’ melons was significantly lower than that of the rockmelons and the honeydew melons. Differences among the varieties were primarily due to the skin, which was more than 3 times more permeable to O₂ in the rockmelons compared to the ‘Yelo’ melons.

The surface of a netted melon is essentially a fractal. This means that the area available for gas exchange is really far higher than the surface area values determined using simple methods. The net on the surface of such melons consists of phellem derm tissue. This initially develops below the surface but expands into a bulging mass of tissue, rupturing the fruit epidermis. In contrast, the *Inodorus* melons epidermis and mesophyll cells are tightly organised and change little during fruit development (Lester, 1988).

Comparison of the permeance of the skin with that of the total fruit allowed estimation of the permeance of the melon flesh. Variability in these results was very high, the standard deviation being larger than the mean value for both the ‘Yelo’ melons and the rockmelons. However, the honeydew flesh was found to be significantly more permeable than that of the ‘Yelo’ melons. This result was surprising considering that the two varieties were morphologically very similar. Even though only results that appeared to be unaffected by leaks had been used when calculating honeydew permeance, these inconsistencies suggest that some of the data may still have been affected by incomplete sealing of the O₂ sensors to the melon skin.
### Chapter 7 - Permeance References

<table>
<thead>
<tr>
<th>Rate of O₂ consumption (mmol kg⁻¹ h⁻¹)</th>
<th>O₂ concentration (kPa)</th>
<th>Permeance to O₂ (µmol. Pa⁻¹ s⁻¹ m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>centre</td>
<td>skin</td>
<td>total</td>
</tr>
<tr>
<td>Honeydew</td>
<td>0.53 a</td>
<td>10.3 a</td>
</tr>
<tr>
<td>'Yelo' melon</td>
<td>0.50 a</td>
<td>7.8 ab</td>
</tr>
<tr>
<td>Rockmelon</td>
<td>1.45 b</td>
<td>6.4 b</td>
</tr>
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</table>

Table 7.6.1. - Respiration rates, internal O₂ concentrations and calculated permeance values for *Inodorus* type melons Honeydew 'Sweet Obsession' and 'Yelo melon', and *Reticulatus* type rockmelon 'Planters Jumbo'; mean values calculated from 12-20 replications, letters indicate results that are significantly different (α = 0.01).

#### 7.6.3.3. Relationship Between O₂ Consumption Rates and Permeance.

It was evident from the results that those melons that had higher respiration rates generally tended to be more permeable to O₂. The permeance of the skin was therefore compared to the respiration rate for each melon (Figure 7.6.3.). Permeance was positively correlated with respiration rate for eachmelon variety, and a linear regression line was calculated for each data set. Comparison of the regression lines showed that similar rates of O₂ consumption were associated with significantly higher permeance values in honeydew melons compared to the other varieties (α = 0.01). However, the relationship between respiration rate and permeance was the same for 'Yelo' melons and rockmelons.
Figure 7.6.3. - Relationship between skin permeance and respiration rate of honeydew melons (●), ‘Yelo’ melons (△) and rockmelons (▲). These relationships are described by the equations $y = 0.7836 x + 0.16$ ($R^2 = 0.53$); $y = 0.5745 x + 0.02$ ($R^2 = 0.84$) and $y = 0.5444 x + 0.1005$ ($R^2 = 0.60$) respectively.

These results suggest that increasing or decreasing the permeance of any of these melon varieties should have a corresponding and similar effect on respiration. Potentially, reducing the permeance of rockmelons could reduce respiration to a rate approaching that of the ‘Yelo’ melons, with corresponding effects on storability. The low permeance of ‘Yelo’ melons may be one factor limiting their respiration and suppressing the climacteric. For example, Altman and Corey (1987) found that the netted melon ‘Goldstar’ had a greater respiratory response to ethylene than the winter melon ‘Tamdew’, a result they attributed to the lower gas permeance of the latter variety.

7.6.3.4. **Changes in $O_2$ Permeance During Melon Ripening**

Unripe rockmelons underwent a climacteric increase in respiration over the 50 hours following ethylene treatment (Figure 7.6.4.). The mean rate of $O_2$ demand was rising at the start of measurements, indicating that the respiratory climacteric had already commenced. Mean respiration rates increased by around 150% during the climacteric, peaking at 2.1 mmol $O_2 \cdot kg^{-1} \cdot h^{-1}$ two days after ethylene treatment.
These rates of $O_2$ demand were consistently higher than the $CO_2$ production rates recorded by Lyons et al., (1962) during ripening of cantaloupe melons cv. Powdery Mildew Resistant no. 45. $CO_2$ production of these melons increased from 0.9 to 1.7 mmol $O_2$.kg$^{-1}$.h$^{-1}$. However, the respiration rates in this trial were lower than those cited by Suslow et al., (2000) who found that cantaloupe melons resired at 1.9 to 2.8 mmol $O_2$.kg$^{-1}$.h$^{-1}$. This considered, it seems probable that ripening proceeded normally, and was not affected by the high dose of ethylene used to initiate the climacteric.

Although the permeance of individual melons decreased during ripening, the mean decrease was not significant ($\alpha = 0.05$). This was due to the high level of variation between individual fruit, which resulted in a large standard error for each mean value. The permeance values were therefore normalised by dividing each measurement by the initial value for the same melon. This meant that the initial permeance of each melon was 1, and that permeance decreased to some fraction of this value during ripening (Figure 7.6.4.). Analysis of the normalised data for melon permeance showed that permeance had fallen significantly 95 hours after measurements began ($\alpha = 0.05$). Final permeance was significantly lower than initial permeance ($\alpha = 0.05$).

Many changes occur during ripening of fruit. One of these changes is softening, brought about by the breakdown of pectins and other structural components of the cell walls. Leakage of the cell solutes is a characteristic of ripening and senescence, and reduces both the size and continuity of the intercellular air spaces (Nooden, 1988). In this study I found significant reductions in fruit permeance, but not until nearly four days after initiation of the climacteric. Electrolyte leakage from netted melons var. ‘Perita’ reaches a maximum at or just before the climacteric (Lester, 1988), while cantaloupe melon fruit undergo loss of pectins and polysaccharides from the cell walls as they reach full maturity (Webster, 1975). This suggests that there may be a gap of several days before the breakdown of individual cells significantly affects gas transfer through the intercellular air spaces.
Figure 7.6.4. - Changes in the rate of \( \text{O}_2 \) demand (——) and \( \text{O}_2 \) permeance (—) during ripening of rockmelon fruit. Data from each melon were normalised to equal a fraction of the initial permeance of the same melon; mean values calculated from eight replications, error bars indicate the standard error of each mean permeance value at 10 hour intervals.

7.6.3.5. Comparison with Published Data

Lyons et al. (1962) published data on the internal atmospheres and rates of \( \text{CO}_2 \) production by four individual cantaloupe melons during ripening. By combining this information with surface areas estimated from the melon weights (McGlasson, pers. comm.), it was possible to estimate the permeance of these melons. The data were moved in time so that maximum respiration occurred on day 5 of measurements for all melons used. Mean values were derived from the current results so as to coincide with the time intervals used in the published work. This allowed comparison between the previous published results and those from the current study (Figure 7.6.5.). The results from the current study were consistent with those derived from Lyons et al. (1962). This similarity in values from studies using different melon varieties and nearly 40 years apart further validates the methods used in the current study.
Figure 7.6.5. - Changes in gas permeance of netted melons during ripening; points are mean values for gas permeance (■) and respiration rate ( — ) estimated from Lyons et al., (1962), or are permeance values derived from the current study at the appropriate time intervals (▲); error bars indicate the standard error of each mean (n=4-8).

### 7.6.4. Conclusions

This work has aimed to clarify the relationship between gas permeance and the respiratory behavior of different melon cultivars. During ripening *Reticulatus* type melons undergo a 2-3-fold increase in respiration rate (Lyons et al., 1962). Subsequently, respiration rate remains high, and storage life is generally less than 14 days (Lester, 1988). In contrast, although a significant respiratory climacteric has been demonstrated in some honeydew melons (Pratt, 1972, Passam and Bird, 1978), many other *Inodorus* melon cultivars show little increase in respiration during ripening (Miccolis and Saltveit, 1995). These melons also respire at half to one third the rate of rockmelons, and have a storage life of up to 4 weeks (Lester, 1988).

Lester (1988) suggested that the intact epidermis of *Inodorus* melons, when compared to the open, fissured epidermis of *Reticulatus* melons, could be responsible for differences in storage life. Changes in membrane permeability were found to be related to the initiation of melon ripening (Lester, 1988). The results of this study show that permeance is positively correlated with fruit respiration rate for different varieties of melons. This suggests that permeance may be one factor limiting respiration rate. However, it was also found that respiration rate increased during ripening despite decreasing fruit permeance. Furthermore, the internal O₂ concentrations
measured during this experiment were mostly >5 kPa, a level generally considered unlikely to significantly limit respiration rate.

Despite these inconsistencies, it seems likely that melons with reduced gas permeance will also have lower respiration rates and somewhat suppressed respiratory climacterics. Fruit with these characteristics are also likely to have improved storage life. However, reduction of the size of the climacteric may also reduce the production of the associated volatiles responsible for the distinctive taste and aroma of different melons. Melon breeding programs could therefore aim to reduce fruit permeance in order to achieve fruit with better storability, but must still consider consumer acceptability.

7.6.5. **Key Points**

- $O_2$ permeance was measured using external and internal sensors on different melon varieties including mature green rockmelons treated with ethylene.
- Readings from $O_2$ sensors attached to the outside cuticles of melon fruit were consistent with measurements of $O_2$ concentrations made at the melon centers, although a significant $O_2$ gradient existed through the fruit flesh.
- *Reticulatus* type ‘Planters Jumbo’ rockmelons were more than 3 times as permeable to $O_2$ as the *Inodorus* type ‘Yelo melons’. Honeydew melons were intermediate between these types.
- Respiration rate was correlated with fruit permeance, with rockmelons and ‘Yelo’ melons conforming to the same relationship between these factors.
- Fruit permeance decreased following the respiratory climacteric. This was likely to be due to breakdown of the cell walls, which reduced the size and continuity of the air spaces available for gas exchange.
- Permeance values were very similar to those estimated from published data.
8

Summary and General Discussion

The initial aim of this thesis was to examine whether there was a direct link between respiration and produce storage life. To answer this question, I first had to address a number of issues. These included how to measure respiration rates and quality, as well as what products would be best suited to this sort of study. As it developed, the project diversified into other areas, including determination of respiration and internal atmospheres of different fruit in the field as well as the glasshouse, and adapting previous methods so as to record permeance to gases.

Measurements of respiration rate generally use either a flow through or a static system. Each system has its advantages and disadvantages. Flow through systems can monitor large numbers of different samples ventilated with any gas mixture, and the system can be automated relatively simply. However, the rate of gas flow is critical, and may require adjusting if respiration rate changes during storage. Carbon dioxide production is usually measured due to its larger signal to noise ratio. However, if RQ ≠ 1 this may not be an accurate indicator of metabolic activity. Static systems often measure O₂ depletion as well as CO₂ production, and can give a quick, simple determination of respiration rate. On the negative side, they are not easy to adapt to special gas mixtures, are susceptible to leaks, and are difficult to automate.

The respirometer combines a number of the features of both systems. As with a flow through system, it is reasonably easy to construct using low cost materials. It is also fully automated, and can generate a modified atmosphere system or use a special gas mixture with minor modifications. However, by using some elements of the static system, the difficulties of measuring and controlling flow rate have been avoided, and both O₂ and CO₂ exchange can be measured. In addition, the system is portable and flexible, and so can be used in the field or glasshouse or adapted to measure O₂ permeance.

The respirometer is now being used in several facilities within Australia, as well as in research laboratories in South Africa, France and Vietnam. The system has proven particularly well suited to use in a developing country such as Vietnam, where regular gas supplies and large, complex
equipment such as gas chromatographs are not available. The respirometer can be constructed and maintained by the researchers themselves and be used for teaching purposes as well as basic research on crop physiology (Figures 8.1 and 8.2).

Figure 8.1. - The group of researchers and lecturers who attended a workshop on constructing and using respirometers at Can Tho University, Vietnam, November 1999, workshop run by Dr Robyn McConchie, Dr Jenny Jobling and myself, funding provided by the Sydney University Good Neighbours Fund and the Crawford Foundation.

Figure 8.2. - Vo-Tong Thanh-Phuong from the National University of Ho Chi Minh City assembles a respirometer during the Can Tho University workshop.

To calculate “respiration life”, I needed to reliably determine the end of storage life. However, changes in quality are often difficult to assess subjectively. It is stated by Shewfelt (1999), that while the term quality has often been defined, there is little agreement as to what it is, how it can
be measured, and how these attributes can be related to consumer acceptability. Quality may refer to a degree of excellence, or simply freedom from major defects (Abbott, 1999).

In this case, the end of storage life did not necessarily need to relate to consumer acceptability. This was important, as while the extent of decay may be difficult to measure the loss of sensory attributes such as flavour are even harder. Similarly, analysis of biochemical attributes was generally less useful than physical methods such as development of rots, changes in colour, softening, and subjective assessments of appearance. One possible exception may prove to be the detection of programmed cell death. The preliminary trials using the ApoAlert kit appeared promising, although considerably more work would be needed to determine whether the results really indicated increased CPP32 activity. Of all the methods used, changes in H^3 of broccoli were the most easily repeatable and objective. However, even this method was ineffective at low temperatures. Both objective and subjective quality measurements were therefore needed to identify the end of storage life.

Cumulative respiration was not constant under all storage conditions for any of the products examined. However, this may be due in part to the methods used to evaluate the end of storage life. Where senescence occurred in definable stages, total respiration was relatively constant. For example, cumulative respiration by broccoli was similar at all storage temperatures above 6°C, although it was doubled at cooler temperatures. However, the end of storage life of grapes was more difficult to define, and cumulative respiration varied over the temperature range used. This suggests that products that change subtly or develop rots during senescence may be less likely to have a "respiration life" than those undergoing more distinct changes.

This observation is supported by the results calculated from published data. Cumulative respiration by climacteric fruit was generally similar under different storage conditions, whereas this was not the case for non-climacteric fruit. Products such as bananas, kiwifruit and tomatoes undergo substantial changes in colour and firmness during ripening, enabling accurate determination of a particular stage of senescence. In contrast, the storage life of non-climacteric fruit such as cherries, loquats and strawberries is usually limited by the onset of disease. In this case storage life is not only a function of product health and metabolism, but is also affected by infection levels, fungicide treatments and pathogen growth rates. When rots were used to determine the end of storage life of a climacteric fruit – melons – there was also no relationship found between respiration rate and storage life. It may be concluded that cumulative respiration may be constant when definable changes in colour or texture determine storage life, but not when it is limited by disease.
The idea that senescence is linked to metabolic activity is not new. A similar theory relating to animals was proposed by Max Rubner, who showed that different species consumed a similar number of calories per gram bodyweight during their life (Austad, 1997). Subsequent experiments demonstrated that the total number of heartbeats of water fleas remained constant at different water temperatures despite significant changes in life span (McArthur and Baillie, 1929). The discovery of free radicals appeared to provide a plausible explanation of why this relationship occurs. Created as a side effect of respiration, free radicals are highly reactive and cause additive damage over time to the surrounding tissue (Austad, 1997).

However, the "Rate of Living" theory is now no longer accepted. Many animals, including birds, bats, and humans, live several times longer than indicated by their metabolic rate. Life spans of animals are now thought to be largely genetically predetermined, and are regulated by the likelihood of predation (Austad, 1997). Similarly, no evidence has been found during this study that respiration determines storage life of fresh produce. Most fruit still contain large reserves of carbohydrates long after they have reached an advanced stage of senescence, and treatments such as 1-MCP can slow senescence while having little effect on respiration rate. Romani (1984) suggested that the rate of respiration is affected by the maintenance of homeostasis. This suggests that respiration rates indicate plant stress, and that this in turn relates to the rate of senescence.

The ability of fruit to maintain homeostasis declines with age and detachment from the plant, resulting in senescence. For example, the internal atmospheres inside capsicums growing in the field remained relatively constant despite large diurnal fluctuations in temperature. However, when the fruit were shaded, the internal atmospheres changed significantly with temperature. In this case, photosynthesis offset the effects of high daytime temperatures on respiration rates, a process aided by the retention of CO₂ inside the capsicums large internal air space.

Homeostasis was also maintained to a greater extent in Charantais melons developing on the vine relative to harvested fruit. The respiratory climacteric was prevented or reduced in fruit that remained attached to the plant, even though ripening proceeded similarly to detached fruit. It was concluded that attachment to the plant inhibited the effects of ethylene on respiration. However, this effect was diminished when the plants were old or diseased, suggesting that these plants had lost the ability to maintain homeostasis.

Another factor likely to significantly affect respiration rates is the permeance of the product to gases (Banks et al., 1993, Dadzie et al., 1995). The cuticle of fruit and vegetables represents a significant barrier to gas exchange between the atmosphere and the underlying cells. In the case
of capsicums, the cuticle contains no natural openings and most gas diffusion is via the pedicel tissue. Melons have many natural openings in their cuticle, but as these are bulky fruits the flesh is also a significant barrier to gas exchange. Differences in permeance may account for some of the variability in respiration rates, storage life, and response to modified atmospheres that have been observed between individual fruit. This could be one reason for the lack of consistent results for melons stored in low O₂ atmospheres.

In conclusion, evidence of a "respiration life" for different fruit and vegetables is tenuous. However, respiration may be a useful guide to storage life for some products under certain conditions. Other positive outcomes from this project include a new device for measuring respiration, and two methods for measuring fruit permeance to O₂. The results have demonstrated that photosynthesis affects the internal atmosphere in developing capsicum fruit, and the inhibition of the effects of ethylene on respiration by Charantais melons attached to the vine. Future work could examine respiration life of other climacteric fruit and leafy vegetables, the effects of ethylene on non-climacteric fruit attached to the plant, and the interaction between ethylene synthesis and disease resistance.
Chapter 9 - Appendices

Appendices
Appendix 1 - Biochemistry of Respiration

Respiration of carbohydrates via glycolysis (□) with either anaerobic (□) or aerobic metabolism in the mitochondria (□). CO₂ production (→), energy producing steps (→) and those that require energy (→) are indicated. Adapted from Willis et al. (1998).
Appendix 2 - Respiration rates and storage lives of various fruits

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<td>6</td>
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<td>14</td>
<td>8</td>
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<tr>
<td>mushrooms</td>
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<td>14</td>
<td>38</td>
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<td>7</td>
<td>71</td>
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<tr>
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<td>28</td>
<td>10</td>
</tr>
<tr>
<td>green onions</td>
<td>10</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>peas</td>
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<td>14</td>
<td>20</td>
</tr>
<tr>
<td>capsicums</td>
<td>8</td>
<td>28</td>
<td>5</td>
</tr>
<tr>
<td>radish topped</td>
<td>0</td>
<td>24</td>
<td>3</td>
</tr>
<tr>
<td>radish bunched</td>
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<td>10</td>
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</tr>
<tr>
<td>radicchio</td>
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<td>18</td>
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<tr>
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<td>0</td>
<td>7</td>
<td>10</td>
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<td>5</td>
<td>20</td>
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<td>7</td>
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<tr>
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<tr>
<td>tomatillo</td>
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<td>8</td>
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<tr>
<td>tomatillo</td>
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<td>7</td>
<td>17</td>
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</tbody>
</table>
### Appendix 4  Advantages and Disadvantages of Methods of Measuring Respiration Rate

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical and Chemical methods</td>
<td></td>
</tr>
<tr>
<td>- May be inexpensive to set up</td>
<td>- Labor intensive</td>
</tr>
<tr>
<td>- Can be accurate over time</td>
<td>- Limited number of measurements are possible</td>
</tr>
<tr>
<td>- Cannot readily show changes in respiration over short time intervals</td>
<td></td>
</tr>
<tr>
<td>Flow-through systems</td>
<td></td>
</tr>
<tr>
<td>- Allows use of many different atmospheres</td>
<td>- CO₂ generally easier to measure than changes in O₂, but if RQ ≠ 1, CO₂ production may not accurately indicate the metabolic activity of the product</td>
</tr>
<tr>
<td>- Can be used to measure a large number of separate samples simultaneously</td>
<td>- Flow rate must be controlled and measured very precisely</td>
</tr>
<tr>
<td>- Can be automated to measure respiration over extended time intervals</td>
<td>- Flow rate must be altered according to the respiration rate</td>
</tr>
<tr>
<td>- Atmospheres can be changed during the experiment e.g. O₂ gradually decreased to measure the point at which anaerobic respiration becomes significant</td>
<td>- Changes in respiration rate e.g. during the climacteric, may change conditions inside the container</td>
</tr>
<tr>
<td>- Nitrogen generators allow systems to be set up relatively cheaply</td>
<td>- Relatively high flow rate is different to usual storage conditions</td>
</tr>
<tr>
<td>Static Systems</td>
<td></td>
</tr>
<tr>
<td>- Sealable containers can give quick, easy measurements of respiration rate</td>
<td>- Non-automated systems are labor intensive, and cannot easily measure changes occurring over time</td>
</tr>
<tr>
<td>- Systems can be automated to give measurements at regular intervals</td>
<td>- Automated systems are complex, difficult to use and susceptible to a number of systematic errors</td>
</tr>
<tr>
<td>- Both O₂ and CO₂ can be measured</td>
<td>- Respiration may be affected by changes in the storage atmosphere</td>
</tr>
<tr>
<td>- Measurement conditions may be similar to those encountered during produce storage</td>
<td>- Cannot easily measure respiration in different atmospheres</td>
</tr>
<tr>
<td>- Permeable containers may be used to give measurements of respiration rate in different atmospheres</td>
<td>- Systems can be affected by leaks.</td>
</tr>
</tbody>
</table>
### Respirometer

- System is inexpensive, and can be constructed without special skills
- Measurements are fully automated
- Respiration rates can be measured in a large range of atmospheres
- Multiple samples may be measured simultaneously and results viewed in real time
- Both O₂ and CO₂ sensors may be used
- System is portable, so can be used in the field as well as the laboratory, and at different temperatures

- If the setpoints are too far apart, respiration may be affected by changing conditions inside the containers
- O₂ sensors are sensitive to atmospheric pressure, so need regular recalibration
- Data set requires processing to remove negative values
Appendix 5  Example of a Quick Basic programme used to run the respirometer

REM  **** Programme to operate 8 respirometers ****
REM This programme is the property of CSIRO Plant Industry - Horticulture, AUSTRALIA

REM However, it can also run a second set of eight respirometers - the ADAMs to control these are currently REM'd out
REM ADAMs are 3-temp, 4-oxy, 5-control
REM extra ADAMs are 6-temp, 7-oxy, 8-control
REM revised October 1999 for malons

DECLARE SUB AppenResultNot (ResultFileS, VesselNo, gap, TotalReadings, startdateS, Weight(), MaxOx(), MinOx(), MaxVol(),
                                  JarVol(), Ostast(), ReadingNo)
DECLARE SUB ResFile (ResultFileS, VesselNo)
DECLARE SUB AppenOxVolFile (ResultFileS, VesselNo, gap, TotalReadings, startdateS, Weight(), MaxOx(), MinOx(), MaxVol(),
                                   JarVol(), Ostast())
DECLARE SUB AppenResultFile (ResultFileS, ReadingNo!, Time!), MVolt(), OxyRate!, OxVol!, FlashS!, VesselNo!, MinOx!,
                               MaxOx!, OxyRate!, Volts!, T5!)
DECLARE SUB CreateFile (ResultFileS, VesselNo, gap, TotalReadings, startdateS, Weight!, MaxOx!, MinOx!, MaxVol!, JarVol!,
                        Ostast!)
DECLARE SUB SetFlashCyc! (MVolt!, OxVol!, MinOx!, MaxOx!, FlashS!, VesselNo!, cmdS!, PSI!, MaxVol!, JarVol!,
                        OxVolOld!, OxyRate!, Weight!, gap)
DECLARE SUB GetTempReading (VesselNo!, temp!, cmdS!, Volt!)
DECLARE SUB AtmosMillVolts (MaxAvt!, and!, MVolt!)}
DECLARE SUB PrintOut (VesselNo!, MinOx!, MaxOx!, MVolt!, OxVol!, OxyRate!, temp!, Volt!, PSI!)
DECLARE SUB AdamCheck (resultS, replyS, cmdS)
DECLARE SUB AllPumpsOff (resultS)
DECLARE SUB DelayTime (delay!)
DECLARE SUB GetOxyReading (VesselNo!, MVolt!, cmdS!, OxVol!, MaxAvV!, JarVol!, OxVolOld!, OxyRate!, Weight!, gap)
DECLARE SUB ReadOxySensor (VesselNo!, cmdS)
DECLARE SUB ReadTempSensor (VesselNo!, cmdS)
DECLARE SUB InitOxyAdam ()
DECLARE SUB InitPumpAdam ()
DECLARE SUB InitTempmad ()
DECLARE SUB CalsOxygenDemand (VesselNo!, MaxAvV!, MVolt!, JarVol!, OxyChange!, Ostast!, OxVol!, OxyRate!,
                               Weight!, OND%! , gap!)
DECLARE SUB GetConstants (ConstFileS, VesselNo, gap, TotalReadings, Weight!, MaxOx(), MinOx(), MaxVol(), JarVol!)
DECLARE SUB FileAwayConstants (ConstFileS, VesselNo, gap, TotalReadings, Weight!, MaxOx!, MinOx!, MaxVol!, JarVol!,
                               Ostast!)
DECLARE SUB WeightSet (Weight!, an!)
DECLARE SUB HighOxSet (an!, MaxOx!)
DECLARE SUB LowOxSet (an!, MinOx!)
DECLARE SUB OXlimits (MinOx!, MaxOx!, MaxAvV!, Weight!, an, MVolt!, JarVol!, vol, anz)
DECLARE SUB Menu1 (MinOx!, MaxOx!, MaxAvV!, vol, Weight!), VesselNo, gap, TotalReadings, MVolt!, JarVol!)
DECLARE SUB ReadingNumber (TotalReadings!)
DECLARE SUB SetGap (gap!)
DECLARE SUB VesselNumber (VesselNo!)
DECLARE SUB VesselConstants (MinOx!, MaxOx!, MaxAvV!, JarVol!, VesselNo!, Weight!, an!, MVolt!, vol, anz)
DECLARE SUB GetYearTime (StartYear, leap, YearTime$, startdate$)
DECLARE SUB GetDate (y, m, d, StartYear, leap, YearTime$, y, m, d, DayOfTheWeek$)

DIM MinOx(30): DIM MaxOx(30): DIM MaxMv(30)
DIM Weight(30): DIM JarVol(30): DIM vol(30)
DIM OxVal(30): DIM OxRate(30): DIM MVolts(30)
DIM OxDexp(30): DIM Oxchange(30): DIM Oxlast(30): DIM OxValOld(30)
DIM Volts(30): DIM temp(30): DIM PS(30): DIM Flash$30
DIM Timer(5000): DIM Ox(30)
DIM OxRate(2000): DIM OxVal(12, 1000): DIM OxRate(30)

ConstFile$ = "c:\melon\Constant.txt"
StartYear = VAL(RIGHT$(DATES, 4)) 'returns 1997 etc

CLS
CALL GetConstants(ConstFile$, VesselNo, gap, TotalReadings, Weight, MaxOx, MinOx, MaxMv, JarVol(),
CALL Menu1(MinOx, MaxOx, VesselNo, gap, TotalReadings, MVolts, JarVol())
CALL FileAwayConstants(ConstFile$, VesselNo, gap, TotalReadings, Weight, MaxOx, MinOx, MaxMv, JarVol(),
Oxlast$)

CLS
CALL GetYearTime(StartYear, leap, YearTime$, startdate$) 'In minutes since the beginning of the year
CALL AppendResultsVessel(VesselNo, gap, TotalReadings, startdate$, Weight, MaxOx, MinOx, MaxMv, JarVol(),
Oxlast$, ReadingNo)

CALL InitOxyAdam
CALL InitTempAdam
CALL InitPumpAdam
CALL AllPumpsOff(result$)
PRINT : PRINT "Setting the flush cycles..."
CALL SetFlushCycle(MVolts(), OxVal(), MinOx(), MaxOx(), Flash$, VesselNo, cmd$, PS(), MaxMv(), JarVol(), OxValOld(),
OxRate(), Weight(), gap)

PRINT
PRINT "Waiting to take the first reading."
REM Next line flags a leap year (1 = TRUE)
IF INT(Year$ / 4) = Year$ / 4 AND Month$ = 2 THEN Yearday$ = Year$ + 1 AND leapy = 0 ELSE leapy = 0

start$ = YearTime$ 'This establishes start of experiment (min since beginning of year)
Finish$ = YearTime$ + (TotalReadings * gap) 'And the end of the experiment in year minutes
NextReading:
CALL SetFlushCycle(MVolts(), OxVal(), MinOx(), MaxOx(), Flash$, VesselNo, cmd$, PS(), MaxMv(), JarVol(), OxValOld(),
OxRate(), Weight(), gap)
CALL GetYearTime(StartYear, leap, YearTime$, startdate$)
IF YearTime$ > Finish$ THEN END 'Start at the beginning & go on to the end
IF INKEY$ = "z" THEN END

ReadingNo$ = CINT((YearTime$ + start$) / gap) + 1 'Next reading number to be taken
ThisReading = start$ = (ReadingNo$ * gap) 'This establishes when reading is to be taken
CALL SetFlushCycle(MVolts(), OxVal(), MinOx(), MaxOx(), Flash$, VesselNo, cmd$, PS(), MaxMv(), JarVol(), OxValOld(),
OxRate(), Weight(), gap)

DO
CALL GetYearTime(StartYear, leap, YearTime$, startdate$)
IF YearTime$ > ThisReading OR YearTime$ = ThisReading THEN EXIT
END
"Exit when time for reading"
References

IF INKEY$ = "z" THEN EXIT DO
CALL SetFlashCycl(MVolts, OxVal(), MinOx(), MaxOx(), Flash$(), VesselNo, cmdS, PS(), MaxMv(), JarVol(), OxValOld(), OxRate(), Weight(), gap)
LOOP
Time(ReadingNo) = (YearTime() - start) / 60  'Time in hours from start
CALL SetFlashCycl(MVolts, OxVal(), MinOx(), MaxOx(), Flash$(), VesselNo, cmdS, PS(), MaxMv(), JarVol(), OxValOld(), OxRate(), Weight(), gap)
REM Take reading now !!!!!!!!!!!!!!!!!!!!!
FOR i = 1 TO VesselNo
  VesselNo = i
  CALL GetOxyReading(VesselNo, MVols, cmdS, OxVal(), MaxMv(), JarVol(), OxValOld(), OxRate(), Weight(), gap)
NEXT i
TS = TIMES
CALL CalcOxygenDemand(VesselNo, MaxMv(), MVols, JarVol(), OxVal(), Oxchange(), Oxlast(), OxVal(), OxRate(), Weight(), OxDent(), gap)
CALL GetTempReading(VesselNo, temp1, cmdS, Volts())
CALL SetFlashCycl(MVolts, OxVal(), MinOx(), MaxOx(), Flash$(), VesselNo, cmdS, PS(), MaxMv(), JarVol(), OxValOld(), OxRate(), Weight(), gap)
CLS
PRINT
PRINT "Reading No "; (ReadingNo()); TAB(20); "Time elapsed = "; Time(ReadingNo()); "(Hrs) "; TIMES
PRINT
CALL PrintOut(VesselNo, MinOx(), MaxOx(), MVols, OxVal(), OxRate(), temp(), Volts(), PS())
CALL SetFlashCycl(MVolts, OxVal(), MinOx(), MaxOx(), Flash$(), VesselNo, cmdS, PS(), MaxMv(), JarVol(), OxValOld(), OxRate(), Weight(), gap)
CALL AppendResultFile(ResultFile$, ReadingNo(), Time(), MVols, OxRate(), OxVal(), Flash$, VesselNo, MinOx(), MaxOx(), OxRate(), Volts(), TS)
CALL SetFlashCycl(MVolts, OxVal(), MinOx(), MaxOx(), Flash$(), VesselNo, cmdS, PS(), MaxMv(), JarVol(), OxValOld(), OxRate(), Weight(), gap)
GOTO NextReading
END

SUB AdamCheck (result$, reply$, cmd$)
REM initialisation commands and checks completed
Initcount = 0  'This limits number of attempts
WHILE result$ <> reply$ AND Initcount < 6  'Repeats if not successful
  Initcount = Initcount + 1
  PRINT #1, cmd$  'sends init command
  'Delay = 2: CALL DelayTime 'delay routine
  result$ = INPUT$(4, #1)  'returns reply from comport
  result$ = MID$(result$, 1, 3)  'strips leading characters from reply
WEND
IF Initcount = 6 THEN PRINT "Unable to initialise modules": END
END SUB

SUB AllPumpsOff (result$)
WHILE result$ <> "=" 'checks command successfully executed
CLOSE #1: OPEN "com1:9600,N,8,S,1,R,SH,CS,CD,DS" FOR RANDOM AS #1
PRINT #1, "#050000" 'sends command to set ADAM 4050 channels to 'off'
CALL DelayTime(delay)
result$ = INPUT$(1, #1) 'returns reply from com port
CLOSE #1: OPEN "com1:9600,N,8,S,1,R,SH,CS,CD,DS" FOR RANDOM AS #1
PRINT #1, "#080000" 'sends command to set ADAM 4050-b channels to 'off'
END
CLOSE #1

END SUB

SUB AppendOxvalFile (ResultFlil$, VesselNo, gap, TotalReadings, startdate$, Weight(), MaxOx(), MinOx(), MaxMv(),
                      JarVol(), Oxlast$)
FOR f = 1 TO VesselNo
  VesselNo = f
  CALL ResFlilVesselNo, VesselNo)
CLOSE #1: OPEN ResultFlil$ FOR APPEND AS #1
PRINT #1, "Result file for Oxygen Demand from "; startdate$
PRINT #1, "Number of Vessel = "; f
PRINT #1, "Sampling interval = "; gap
PRINT #1, "Total Number of readings = "; TotalReadings
PRINT #1, "Weight (Kg): "; ", ", ", "Set MaxOx": "; ", ", ", "Set MinOx": "; ", ", ", "MaxMv at 21% Oxygen": "; ", ", "Jar Volume (mL)
PRINT #1, "PRINT #1, "ReadingNo": "; ", ", "Time (Hrs)": "; ", ", "Mv Oxygen": "; ", ", "%a Oxygen": "; ", ", "Oxygen Demand": "; ", "Temp (°V)
NEXT f
CLOSE

END SUB

SUB AppendResultFile (ResultFlil$, ReadingNo$, Time(), MVolts(), OxRate(), OxVal(), Flus$, VesselNo, MinOx(),
                      MaxOx(), OxRate(), Volts(), TS)
FOR i = 1 TO VesselNo
  VesselNo = i
  CALL ResFlilVesselNo, VesselNo)
CLOSE #1
OPEN ResultFlil$ FOR APPEND AS #1
IF OxRate(i) = 0 THEN OxRate(i) = 0
NEXT i
CLOSE #1

END SUB

SUB AppendResultorNot (ResultFlil$, VesselNo, gap, TotalReadings, startdate$, Weight(), MaxOx(), MinOx(), MaxMv(),
                        JarVol(), Oxlast$, ReadingNo$)
REM CreateFile has a Kill statement. AppendFile does not
PRINT : INPUT "Do you want to overwrite the existing files ? (y/n) ". n$ if n$ = "n" THEN CALL AppendOxvalFile(ResultFlil$, VesselNo, gap, TotalReadings, startdate$, Weight(), MaxOx(), MinOx(),
                                   MaxMv(), JarVol(), Oxlast$)

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References

IF nS = "N" THEN CALL AppendOxValFile(ResultFileS, VesselNo, gap, TotalReadings, startdate$, Weight(), MaxOx(),
        MinOx(), MaxMv(1), JarVol(), Oxlast))
IF nS = "y" THEN INPUT "are you sure about that? (y/n) ", nS
IF nS = "y" THEN INPUT "are you sure about that? (y/n) ", nS
IF nS = "y" THEN CALL Createfile(ResultFileS, VesselNo, gap, TotalReadings, startdate$, Weight(), MaxOx(), MinOx(),
        MaxMv(), JarVol(), Oxlast))
IF nS = "y" THEN CALL Createfile(ResultFileS, VesselNo, gap, TotalReadings, startdate$, Weight(), MaxOx(), MinOx(),
        MaxMv(1), JarVol(), Oxlast))
IF nS = "n" THEN CALL AppendOxValFile(ResultFileS, VesselNo, gap, TotalReadings, startdate$, Weight(), MaxOx(),
        MaxMv(1), JarVol(), Oxlast))
IF nS = "n" THEN CALL AppendOxValFile(ResultFileS, VesselNo, gap, TotalReadings, startdate$, Weight(), MaxOx(),
        MinOx(), MaxMv(), JarVol(), Oxlast))
END SUB

SUB AtmosMillivolts (MaxMv(), an, MVolts())

    VesselNo = an
    CLS
    PRINT : PRINT
    PRINT "If the vessel is open, the computer can measure the sensor mV,"
    PRINT "otherwise you can enter the mV manually."
    PRINT : PRINT "computer measures ""c"""
    PRINT : PRINT "manual input - 'm' : PRINT"
    INPUT "Please answer c or m " ; nS
    IF nS = "m" GOTO 60
    IF nS = "c" GOTO 70
    GOTO 43

60 PRINT : PRINT "What is the MV reading of sensor " ; an ; " in air?"
    INPUT ; MaxMv(an)
    EXIT SUB

70 CLOSE #1: OPEN "c:\1\000\N.B.I.R.S.C.S.C.D.D.S" FOR RANDOM AS #1
    CALL ReadOxySensor(VesselNo, cmdS)
    5270 PRINT #1, cmdS 'sends command to adam 4018
    CALL DelayTime(delay) 'delays comp port read
    result$ = INPUT$(LOC(1), #1) 'reads adam reply
    FOR T = 1 TO 5
    IF LEN(result$) <> 9 THEN GOTO 5270'Check buffer correct length
    NEXT T
    result$ = MIDS(result$, 2, 7)'strips leading characters
    MVolts(an) = VAL(result$)
    MaxMv(an) = MVolts(an)
    CLOSE #1
END SUB

SUB CalcOxygenDemand (VesselNo, MaxMv(), MVolts(), JarVol(), OxChange(), Oxlast(), OxVal(), OxRate(), Weight(),
        OxDem(), gap)

    FOR i = 1 TO VesselNo
    'OxVal(i) = (MVolts(i) / MaxMv(i)) * 21'converts mV TO %O2
    IF (OxVal(i) - Oxlast(i)) < .1 THEN OxRate(i) = 0: GOTO 670
    OxChange(i) = Oxlast(i) - OxVal(i) 'calculates %change in O
    IF Oxchange(i) < 0 THEN OxRate(i) = 0: GOTO 670
    GOTO 670
END SUB
References

IF Oxychange(i) = 0 THEN OxyRate(i) = 0: GOTO 670

'O2 Dem(i) = (Oxychange(i) / 100) * JarVol(i) 'O2 demand = % change x jar volume in mls
OxyRate(i) = (Oxychange(i) * 60 / JarVol(i)) / (Weight(i) - gap * 100) calculates ml/kg/hour

670 Oxlast(i) = OxyVal(i) 'stores current %O2

NEXT i

END SUB

SUB Createfile (ResultFile$, VesselNo, gap, TotalReadings, startdate$, Weight(i), MaxOx(i), MinOx(i), MaxMv(i), JarVol(i), Oxlast(i))

FOR f = 1 TO VesselNo
VesselNo = f
CALL ResFile(ResultFile$, VesselNo)
CLOSE #1: KILL ResultFile$
OPEN ResultFile$ FOR APPEND AS #1
PRINT #1, "Result file for Oxygen Demand from "; startdate$
PRINT #1, "Number of Vessel = "; f
PRINT #1, "Sampling interval = "; gap
PRINT #1, "Total number of readings = "; TotalReadings
PRINT #1, "Weight (Kg)"; ""," Set MaxOx"; ""," Set MinOx"; ""," MaxMv at 21% Oxygen"; ""," Jar Volume (ml)
PRINT #1, "Weight(f)"; "", "MaxOx(f)"; "", "MinOx(f)"; "", "MaxMv(f)"; "", "JarVol(f)
PRINT : PRINT #1, "ReadingNo"; ""," Time (Hrs)"; ""," Mv Oxygen"; ""," % Oxygen"; ""," Oxygen Demand"; ""," Temp (V)

NEXT f
CLOSE

END SUB

SUB DelayTime (delay)

delay = .2
start = TIMER
repeat = 1
73 IF TIMER > (start + delay) THEN EXIT SUB
repeat = repeat + 1
IF repeat > 10 THEN EXIT SUB
GOTO 73

END SUB

SUB FileAwayConstants (ConstFile$, VesselNo, gap, TotalReadings, Weight(i), MaxOx(i), MinOx(i), MaxMv(i), JarVol(i), Oxlast(i))

'KILL ConstFile$
OPEN ConstFile$ FOR OUTPUT AS #1
PRINT #1, VesselNo; "; gap; "; TotalReadings
FOR i = 1 TO VesselNo
PRINT #1, Weight(i); "; MaxOx(i); "; MinOx(i); "; MaxMv(i); "; JarVol(i)
NEXT i
CLOSE

END SUB

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References

SUB GetConstants (ConstFilS, VesselNo, gap, TotalReadings, Weight(), MaxOx(), MinOx(), MaxMv(), JarVol())

OPEN ConstFilS FOR INPUT AS #1
INPUT #1, VesselNo, gap, TotalReadings
FOR i = 1 TO VesselNo
INPUT #1, Weight(i), MaxOx(i), MinOx(i), MaxMv(i), JarVol(i)
NEXT i
CLOSE

END SUB

SUB GetsOxyReading (VesselNo, MVolts(), cmdS, OxVal(), MaxMv(), JarVol(), OxValOld(), OxtrRate(), Weight(), gap)

FOR i = 1 TO VesselNo
'VesselNo = i
CLOSE #1: OPEN "com1:9600.N.8.1.RS:CS:CD:DS" FOR RANDOM AS #1
CALL ReadOxySensor(VesselNo, cmdS)
FOR T = 1 TO 5
78 PRINT #1, cmdS 'sends command to adam 4018
CALL DelayTime(delay) 'delays comp port read
result$ = INPUT$(#1, 1)
PRINT result$
IF LEN(result$) = 8 THEN T = 5
NEXT T
result$ = MID$(result$, 2, 6)strips leading characters
MVolts(VesselNo) = VAL(result$)
OxVal(VesselNo) = (MVolts(VesselNo) / MaxMv(VesselNo)) * 21' converts mV TO %O2
CLOSE #1
NEXT i

END SUB

SUB GetsTempReading (VesselNo, temp(), cmdS, Volts())

FOR i = 1 TO VesselNo
VesselNo = i
CLOSE #1: OPEN "com1:9600.N.8.1.RS:CS:CD:DS" FOR RANDOM AS #1
CALL ReadTempSensor(VesselNo, cmdS)
3270 PRINT #1, cmdS 'sends command to Temp adam 4018
CALL DelayTime(delay) 'delays comp port read
result$ = INPUT$(LOC(1), #1)reads adam reply
FOR T = 1 TO 5
IF LEN(result$) <= 9 THEN GOTO 327W/Check buffer correct length
NEXT T
result$ = MID$(result$, 2, 7)strips leading characters
Volts(i) = VAL(result$)
CLOSE #1
NEXT i

END SUB

SUB GetYearTime (StartYear, leap, YearTime#, startdate$)
PRINT "Getting year time"
Year = VAL(RIGHT$(DATES(4))) 'returns 1997 etc
References

Month = VAL(LEFT$(DATES, 2)) 'numerical month
Day = VAL(MIDS(DATES, 4, 2)) 'numerical day
startdate$ = DATES

REM The following computes the day of the year it is for non-leap years

IF Month = 1 THEN Yearday = 0 + Day
IF Month = 2 THEN Yearday = 31 + Day
IF Month = 3 THEN Yearday = 59 + Day
IF Month = 4 THEN Yearday = 90 + Day
IF Month = 5 THEN Yearday = 120 + Day
IF Month = 6 THEN Yearday = 151 + Day
IF Month = 7 THEN Yearday = 181 + Day
IF Month = 8 THEN Yearday = 212 + Day
IF Month = 9 THEN Yearday = 243 + Day
IF Month = 10 THEN Yearday = 273 + Day
IF Month = 11 THEN Yearday = 304 + Day
IF Month = 12 THEN Yearday = 334 + Day

REM The next line allows for leap years
IF INT(Year / 4) = Year / 4 AND Month = 2 THEN Yearday = Yearday + 1

REM Next line computes the time elapsed since the beginning of the year (minutes)
YearTime# = (Yearday - 1) * 24 * 60 + TIMER / 60

REM Next line corrects for a New Year
IF Year > StartYear THEN YearTime# = YearTime# = 365 * 24 * 60
IF leap = 1 AND Year > StartYear THEN YearTime# = YearTime# = 24 * 60 'Corrects for starting year being leap
PRINT "Got year time"

END SUB

SUB HighOxSet (an, MaxOx$)
CLS
PRINT "Current upper limit for oxygen is "; MaxOx$(an)
PRINT : PRINT
INPUT "What is new limit?"; an$
IF an$ = "" THEN GOTO Again
MaxOx$(an) = VAL(an$)
END SUB

SUB InitOxyAdam
CLOSE : OPEN "com1:9600,N,8,1,RS,CS,CD,DS" FOR RANDOM AS #1
cmd$ = "%w0404006000" 'stores command to initialise control O2 adam 4018
reply$ = "04" 'defines correct reply
CALL AdamChec(res$), reply$, cmd$ 'executes and checks reply
PRINT ""
PRINT "oxygen adam 4 0K"
CLOSE #1
CLOSE : OPEN "com1:9600,N,8,1,RS,CS,CD,DS" FOR RANDOM AS #1
cmd$ = "%w0707006000" 'stores command to initialise control O2 adam 4018
reply$ = "%07" 'defines correct reply
References

CALL AdamCheck (ResultS, replyS, cmdS)$executes and checks reply
PRINT ""
PRINT "oxygen adam 7 OK."
CLOSE #1

END SUB

SUB InitPumpAdam

CLOSE : OPEN "com1:9600,N,8,1,RS,CS,CD,DS" FOR RANDOM AS #1
cmdS = "$a0505400600" 'This initialises pump adam 4050
replyS = "$103" 'defines correct reply
CALL AdamCheck (ResultS, replyS, cmdS)$executes and checks reply
PRINT ""
PRINT "controller adam 5 OK."
CLOSE #1

CLOSE : OPEN "com1:9600,N,8,1,RS,CS,CD,DS" FOR RANDOM AS #1
'cmdS = "$a0808400600" 'This initialises pump adam 4050
'replyS = "$108" 'defines correct reply
'CALL AdamCheck (ResultS, replyS, cmdS)$executes and checks reply
PRINT ""
PRINT "controller adam 8 OK."
'CLOSE #1

END SUB

SUB InitTempAdam

CLOSE : OPEN "com1:9600,N,8,1,RS,CS,CD,DS" FOR RANDOM AS #1
cmdS = "$a03030400600" 'This initialises temp adam 4018
replyS = "$103" 'defines correct reply
CALL AdamCheck (ResultS, replyS, cmdS)$executes and checks reply
PRINT ""
PRINT "temperature adam 3 OK."
CLOSE #1

CLOSE : OPEN "com1:9600,N,8,1,RS,CS,CD,DS" FOR RANDOM AS #1
'cmdS = "$a06060400600" 'This initialises temp adam 4018
'replyS = "$106" 'defines correct reply
'CALL AdamCheck (ResultS, replyS, cmdS)$executes and checks reply
PRINT ""
PRINT "temperature adam 6 OK."
'CLOSE #1

END SUB

SUB LowOxSet (an, MinOx(an))
Again:
CLS
PRINT "Current lower limit for oxygen is ", MinOx(an)
PRINT : PRINT
INPUT "What is new limit": anS
IF anS = "" THEN GOTO Again
MinOx(an) = VAL(anS)
END SUB
References

SUB Menu1 (MinOx(), MaxOx(), MaxMv(), vol, Weight(), VesselNo, gap, TotalReadings, MVols(), JarVol())

    constants for the run

ShowMenu1:
    CLS

PRINT TAB(20): "To alter, enter appropriate number"
LOCATE 4, 1
PRINT "Number of readings............. "; TotalReadings; TAB(50); " ........ 1"
PRINT "Reading interval.............. "; gap; TAB(50); " ........ 2"
PRINT "Number of vessels.............. "; VesselNo; TAB(50); " ........ 3"
PRINT "Vessel constants............... "; TAB(50); " ........ 4"
PRINT: PRINT
PRINT "** Remember that there is only enough memory for a total of 2000 readings."
PRINT "** So be careful to set the time interval accordingly."
PRINT
PRINT "To run program, type RUN & return": inS
IF inS = "run" THEN GOTO FinishMenu1
IF inS = "RUN" THEN GOTO FinishMenu1
IF inS = "1" THEN CALL ReadingNumber(TotalReadings)
IF inS = "2" THEN CALL SetGap(gap)
IF inS = "3" THEN CALL VesselNumber(VesselNo)
IF inS = "4" THEN CALL VesselConstants(MinOx(), MaxOx(), MaxMv(), JarVol(), VesselNo, Weight(), an, MVols(), vol, ansS)

GOTO ShowMenu1
FinishMenu1:

END SUB

SUB OxLimits (MinOx(), MaxOx(), MaxMv(), Weight(), an, MVols(), JarVol(), vol, ansS)

Begin: CLS
    PRINT "Vessel Number ": an
    PRINT: TAB(20); "To alter, enter appropriate number"
    LOCATE 4, 1
    PRINT "Lower oxygen limit (\"o\")........... "; MinOx(an); TAB(50); " ........ 1"
    PRINT "Upper oxygen limit (\"o\")........... "; MaxOx(an); TAB(50); " ........ 2"
    PRINT "Weight in kg........................... "; Weight(an); TAB(50); " ........ 3"
    PRINT "Oxygen sensor at 21\% (mV)........... "; MaxMv(an); TAB(50); " ........ 4"
    PRINT "Void volume (mL).................... "; JarVol(an); TAB(50); " ........ 5"
    PRINT: PRINT
    PRINT "To return to MENU, press M"
INPUT "": ansS
IF ansS = "" THEN GOTO Begin
IF ansS = "1" THEN CALL LowOxSet(an, MinOx())
IF ansS = "2" THEN CALL HighOxSet(an, MaxOx())
IF ansS = "3" THEN INPUT "Weight in kg "; Weight(an)
IF ansS = "4" THEN CALL AtmoSMillivolts(MaxMv(), an, MVols())
IF ansS = "5" THEN INPUT "Respirometer Volume in (L)"; vol
    IF vol = 0 THEN vol = 4.7
    JarVol(an) = (vol - Weight(an)) * 1000
IF ansS = "M" THEN GOTO 750
IF ansS = "m" THEN GOTO 730
GOTO Begin
730 PRINT "Vessel ", an; " finished"

END SUB

SUB PrintOut (VesselNo, MinOx(), MaxOx(), MVolts(), OxVal(), OxRate(), temp(), Volts(), PS())

PRINT "Number"; TAB(10); " Limits"; TAB(25); " MaxM"; TAB(37); " [O2]"; TAB(45); " O2 mL/Kg/h"; TAB(60); " Temp"; TAB(70); " Pump"

FOR i = 1 TO VesselNo
PRINT i; TAB(10); MinOx(i); " "; MaxOx(i); TAB(25); MVolts(i); TAB(37); OxVal(i); TAB(50); OxRate(i); TAB(60); Volts(i); TAB(70); PS(i)
NEXT i

END SUB

SUB ReadingNumber (TotalReadings)
INPUT "How many readings do you want"; TotalReadings$
TotalReadings = VAL(TotalReadings$)
END SUB

SUB ReadOxySensor (VesselNo, cmd$)

h = (VesselNo - 1)
cmda = .48 + h
cmdb = .42 + h
IF VesselNo < 9 THEN cmd$ = CHR$(35) + CHR$(48) + CHR$(52) + CHR$(cmda)
IF VesselNo > 8 THEN cmd$ = CHR$(35) + CHR$(48) + CHR$(55) + CHR$(cmdb)
'PRINT cmd$

END SUB

SUB ReadTempSensor (VesselNo, cmd$)

i = (VesselNo - 1)
cmda = .48 + i
cmdb = .42 + i
IF VesselNo < 9 THEN cmd$ = CHR$(35) + CHR$(48) + CHR$(51) + CHR$(cmda)
IF VesselNo > 8 THEN cmd$ = CHR$(35) + CHR$(48) + CHR$(54) + CHR$(cmdb)
'PRINT cmd$

END SUB

SUB ResFlt (ResultFlt$, VesselNo)

IF VesselNo = 1 THEN ResultFlt$ = "C:\melon\melon1.res"
IF VesselNo = 2 THEN ResultFlt$ = "C:\melon\melon2.res"
IF VesselNo = 3 THEN ResultFlt$ = "C:\melon\melon3.res"
IF VesselNo = 4 THEN ResultFlt$ = "C:\melon\melon4.res"
IF VesselNo = 5 THEN ResultFlt$ = "C:\melon\melon5.res"
IF VesselNo = 6 THEN ResultFlt$ = "C:\melon\melon6.res"
IF VesselNo = 7 THEN ResultFlt$ = "C:\melon\melon7.res"

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IF VesselNo = 8 THEN ResultFile$ = "C:\melon\melon8.res"
IF VesselNo = 9 THEN ResultFile$ = "C:\melon\melon9.res"
IF VesselNo = 10 THEN ResultFile$ = "C:\melon\melon10.res"
IF VesselNo = 11 THEN ResultFile$ = "C:\melon\melon11.res"
IF VesselNo = 12 THEN ResultFile$ = "C:\melon\melon12.res"
IF VesselNo = 13 THEN ResultFile$ = "C:\melon\melon13.res"
IF VesselNo = 14 THEN ResultFile$ = "C:\melon\melon14.res"
IF VesselNo = 15 THEN ResultFile$ = "C:\melon\melon15.res"
IF VesselNo = 16 THEN ResultFile$ = "C:\melon\melon16.res"
END SUB

SUB SetFlushCyl (MVolts(), OxVal(), MinOxI(), MaxOxI(), FlushS(), VesselNo, cmdS, PS(), MaxMv(), JarVol(), OxValOld(), OxrRate(), Weight(), gsp)
FOR i = 1 TO VesselNo
VesselNo = i
CALL GetOxyReading(VesselNo, MVolts(), cmdS, OxVal(), MinMv(), MaxMv(), JarVol(), OxValOld(), OxrRate(), Weight(), gsp)
OxVal(i) = (MVolts(VesselNo) / MaxMv(VesselNo)) * 21' converts mV TO %O2
h = (i - 1)
a = 48 + h
b = 42 + h
IF i < 9 THEN OS = CHR$(35) + CHR$(48) + CHR$(53) + CHR$(49) + CHR$(na) + CHR$(48) + CHR$(49)
IF i < 8 THEN OS = CHR$(35) + CHR$(48) + CHR$(53) + CHR$(49) + CHR$(nb) + CHR$(48) + CHR$(49)
IF i < 9 THEN ce = CHR$(35) + CHR$(48) + CHR$(53) + CHR$(49) + CHR$(na) + CHR$(48) + CHR$(49)
IF i < 8 THEN ce = CHR$(35) + CHR$(48) + CHR$(53) + CHR$(49) + CHR$(nb) + CHR$(48) + CHR$(49)
IF OxVal(i) < MinOx(VesselNo) THEN FlushS(i) = "ON"
IF OxVal(i) > MaxOx(VesselNo) THEN FlushS(i) = "OFF" 'if %O2 > flush limit
IF FlushS(i) = "ON" THEN cmdS = OS ELSE cmdS = cS
CLOSE #1: OPEN "com1:9600,N,8,1,RS,CS,CD,DS" FOR RANDOM AS #1
PRINT #1, cmdS
IF cmdS = OS THEN PS(i) = "ON"
IF cmdS = cS THEN PS(i) = "OFF"
CLOSE #1
NEXT i
END SUB

SUB SetGap (gsp)
INPUT "What is the gap between readings (min)"; gapS
gap = VAL(gapS)
END SUB

SUB VesselConstants (MinOxI(), MaxOxI(), MaxMvI(), JarVol(), VesselNo, Weight(), an!, MVolts(), vol, ass)
Menu2:
CLS
PRINT
FOR i = 1 TO VesselNo
PRINT " Vessel "; i; TAB(50); " ...... "; i
PRINT " "; MinOxI(); "to "; MaxOxI(); " % oxygen "; "Weight is "; Weight(i); " kg"
PRINT " "; MaxMvI(); " MVol at 21% O2 "; JarVol(i); " is the void volume (mL)"
NEXT i
PRINT: PRINT "To alter, enter appropriate number"
PRINT "To return to Main Menu, press 0"
INPUT " "; an

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If n = 0 THEN EXIT SUB
CALL OverLimits(MinOn(), MaxOn(), MaxNv(), Weight(), an, MVolSt(), JarVol(), vol, ans5)
PRINT ; PRINT "To return to Main Menu, Press Return"
GOTO Menu2
END SUB
SUB VesselNumber (VesselNo)
INPUT "How many vessels are there"; VesselNo$ 
VesselNo = VAL(VesselNo$)
END SUB
SUB WeightSet (Weight(), an)
CLS
PRINT "Current weight is "; Weight(an)
PRINT ; PRINT
INPUT "What is new weight (kg)": an$ 
IF an$ = "" THEN GOTO Again
Weight(an) = VAL(an$)
END SUB


Appendix 6. Changes in physical and biochemical attributes of broccoli

<table>
<thead>
<tr>
<th>Day</th>
<th>Weight retained (% of original)</th>
<th>Colour (H^4)</th>
<th>Fluorescence (Fv / Fm)</th>
<th>Protein (mg.g^-1)</th>
<th>pNA activity (umol.mg protein^-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100 a</td>
<td>129.0 a</td>
<td>0.85 a</td>
<td>201 a</td>
<td>0.091 a</td>
</tr>
<tr>
<td>1</td>
<td>99.75 a</td>
<td>128.7 ab</td>
<td>0.79 b</td>
<td>153 a</td>
<td>0.214 a</td>
</tr>
<tr>
<td>2</td>
<td>98.27 b</td>
<td>126.4 b</td>
<td>0.79 b</td>
<td>148 ab</td>
<td>0.185 a</td>
</tr>
<tr>
<td>3</td>
<td>96.32 c</td>
<td>111.9 c</td>
<td>0.76 b</td>
<td>145 ab</td>
<td>0.190 a</td>
</tr>
<tr>
<td>4</td>
<td>94.50 d</td>
<td>92.9 d</td>
<td>0.66 c</td>
<td>89 bc</td>
<td>0.491 b</td>
</tr>
<tr>
<td>5</td>
<td>92.59 e</td>
<td>81.8 e</td>
<td>0.59 d</td>
<td>81 c</td>
<td>0.584 b</td>
</tr>
</tbody>
</table>

Changes in physical and biochemical attributes of broccoli during 5 days storage at 20°C. Mean values calculated from 3 separate batches of broccoli, total number of heads used in determination = n. Significantly different values are indicated by the succeeding letter (α = 0.05).
Appendix 7   Changes in broccoli colour

Photographs of broccoli taken at 24 hour intervals at 20°C.

Day 1

Day 2
References

Day 3

Day 4

Day 5
Appendix 8  Changes in capsicum colour and quality

Colour grades of capsicums

Colour Grade 1  Colour Grade 2

Colour Grade 3  Colour Grade 4

Colour Grade 5
Quality grades of capsicum fruit, photographs taken daily.

Day 1

Day 2

Day 3

Day 4

Day 5

Day 6

Day 7

Day 8

grade 2.5

grade 3
PLEASE NOTE

The greatest amount of care has been taken while scanning the following pages. The best possible results have been obtained.


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