USE OF MODIFIED ATMOSPHERE PACKAGING TO EXTEND THE SHELF-LIFE OF WHOLE AND PRE-CUT LETTUCE

Submitted by

Dino Dioguardi

B.Agr.Sci. (Hons.), The University of Melbourne, Victoria, Australia

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Victoria University of Technology

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Abbreviations

CAS	Controlled atmosphere storage
MAP	Modified atmosphere packaging
CA	Controlled atmosphere
MA	Modified atmosphere
EMA	Equilibrium modified atmosphere
RH	Relative humidity
O_2	Oxygen
CO_2	Carbon dioxide
N_2	Nitrogen
C_2H_4	Ethylene
MP	Minimally processed
RS	Russet spotting
BS	Brown stain
Trt	Treatment
IHD	Institute for Horticultural Development, Knoxfield, Victoria
LSD	Least Significant Difference

Abstract

This thesis investigated the potential of using modified atmosphere packaging (MAP) to maintain the quality of whole and pre-cut lettuce for longer periods than that currently obtained in air. Achieving this goal will enable whole lettuce to be exported to South-East Asia and Japan by ship rather than expensive air freight. Furthermore, additional shelf-life of pre-cut lettuce on domestic markets will provide greater flexibility during distribution and marketing, since storage life of these products decreases rapidly after processing.

To achieve this, it was necessary to determine the optimum gas atmospheres (O_2/CO_2) and respiration rates of both forms of lettuce under such gas regimes and at specific storage temperatures. Controlled atmosphere storage (CAS) trials were used to investigate the physiological and biochemical responses of lettuce to such conditions and found that modification of the gas atmosphere surrounding lettuce can have beneficial effects in maintaining the quality of intact and pre-cut lettuce, provided gas concentrations were within the limits tolerated by the produce. This was achieved by slowing down the respiration rate of tissue and inhibiting other deteriorative processes associated with senescence. The optimum gas atmospheres varied between whole and pre-cut lettuce, presumably because of anatomical differences which affected the gas-diffusion characteristics of the tissue. Physiological responses of whole lettuce to CAS also varied among cultivars.

Information gathered during controlled atmosphere trials was used to design and test modified atmosphere (MA) packages for whole Crisphead lettuce and pre-cut Romaine lettuce. This involved selecting a polymeric film with correct gas transmission rates and deriving a balance between produce weight and surface area of the film. These packaging systems, after initial gas flushing, maintained a desirable equilibrium MA and extended the shelf-life of whole lettuce at 1.5°C and 5°C to approximately 30 and 20 days respectively, significantly longer than that achieved using conventional packaging. Likewise, a MA package was successfully developed for pre-cut Romaine lettuce and this extended the shelf-life to approximately 10-14 days, a significant improvement from the five days achieved when stored in air. Both these package systems are currently being trialed in commercial situations throughout Australia.

Statement of Authorship

Except where reference is made in the text of the thesis, this thesis contains no material published elsewhere or extracted in whole or part from a thesis by which I have been awarded another degree or diploma.

No other person's work has been used without due acknowledgement in the main text of the thesis.

Due to an agreement reached between the East Gippsland Vegetable Industry Board and Victoria University of Technology, this thesis may not be reproduced without permission from the author or the university.

Dino Dioguardi

17th April 1995

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Chapter 1

Introduction

Australia is uniquely positioned by virtue of its geographical proximity to export horticultural produce to Asia. Opportunities exist to supply South East Asia and Japan with Australian lettuce, provided high quality can be assured and costs contained. Fresh lettuce is currently air freighted from Australia, but freight costs are high and cargo space may be limited. Sea freight offers a lower cost alternative and capacity is much greater but it takes about three weeks from harvest in Eastern Australia to destination in South-East Asia and four weeks to Japan (McGlasson, 1992). These times are necessary to allow for assembly of consignments, co-ordination with shipping schedules, delays at dockside and to retain sufficient residual storage life to enable orderly marketing at destination (McGlasson, 1989).

Vegetables such as lettuce are perishable products with an active metabolism which continues during the postharvest period. The quality of vegetables offered to consumers depends primarily upon growing and careful handling good quality produce (Zagory and Kader, 1988). This involves growing appropriate cultivars, utilizing production techniques that maximize yield and reduce pests and diseases, harvesting at optimal maturity, minimizing physical injuries due to handling and using proper sanitation procedures to reduce the number of spoilage microorganisms. Rapid precooling and storage at the optimum temperature and humidity before and during distribution and marketing also contribute significantly to maintaining postharvest quality of vegetables (Kader *et al.* 1989).

Once these primary requirements have been fulfilled, additional postharvest life may be achieved through modification of the gas atmosphere surrounding the commodity (O'Connor *et al.* 1992). Modified atmosphere packaging (MAP) provides such a means (Zagory and Kader, 1988). MAP has the potential to extend the storage life of vegetables and hence make shipping of horticultural produce to distant markets an alternative to expensive air freight.

This thesis was initiated as part of a research and development program established by the East Gippsland Vegetable Industry Board (EGVIB). One of the main objectives of this board was to undertake research that would increase the productivity of vegetable growers in East

Gippsland, Victoria. Directly associated with this board was Vegco Ltd., a fresh vegetable company located in the region. Realizing that "in order for growers to grow and prosper, they need to increase their market share from other competing food products and seek further opportunities in export markets" (Armstrong, 1992), this company has focused on marketing whole and pre-cut produce such as lettuce for domestic and export markets.

The aim of this thesis was to investigate the effectiveness of using MAP to extend the shelf-life of whole and pre-cut lettuce destined for export as well as for domestic markets. To do this it was necessary to determine the optimum gas atmospheres and respiration rate of lettuce held under such gas regimes. It was also important to determine whether extent of preparation or cultivar type affected the response of lettuce to lowered oxygen (O_2) and/or elevated carbon dioxide (CO_2) levels. All of this information was used to design and test modified atmosphere (MA) packages that could be utilized in commercial situations by Vegco Ltd.

Chapter 2

Literature review

2.1 Nature of fruits and vegetables

In contrast to animal products such as meat and cheese, fruits and vegetables are unique in that they actively continue to respire after harvest (Robertson, 1993). Respiration involves the oxidative breakdown of energy-rich organic substrates normally present in cells such as starch, sugars, and organic acids, to simpler molecules (carbon dioxide and water). There is a concurrent production of energy, which can be used by cells for synthetic reactions (Kader, 1987). Such metabolic reactions are essential for maintaining cellular organization and membrane integrity in living cells and hence are vital for retaining product quality (Gorris and Peppelenbos, 1992).

Postharvest respiration in fruits and vegetables is affected by several intrinsic and extrinsic factors such as the kind of commodity, its stage of maturity, cultivar, size of produce, physical condition, concentrations of oxygen (O_2), carbon dioxide (CO_2) and ethylene (C_2H_4) within the package, temperature and possibly light (Zagory and Kader, 1988; Kader *et al.*, 1989). Respiratory activity, as measured by CO_2 production, is indicative of the rapidity with which compositional changes are taking place within plant material and hence is an excellent indicator of the potential storage life of individual fruits or vegetables (Burton, 1974; Phan *et al.*, 1975; Day, 1988). Generally speaking, the achievable shelf-life of horticultural produce is inversely proportional to the respiration rate (Duckworth, 1966; Robertson, 1993).

2.2 Temperature

Proper temperature management is the single most important factor affecting the postharvest life of fresh fruits and vegetables (Kader *et al.*, 1989). Temperature determines the type and velocity of physiological and biochemical modifications that take place (Saracino *et al.*, 1991). Zagory and Kader (1988) stated that within the physiological temperature range, biological reactions increase two-to-three fold for every 10°C rise in temperature. The temperature coefficient for a 10°C interval is referred to as the Q_{10} (Wills *et al.*, 1989; Cameron *et al.*,

1994). The Q_{10} values for fruit and vegetables vary from about one to seven in the temperature range of 0° to 30°C. Many fresh commodities have higher Q_{10} values in the 0° to 10°C storage temperature range than in the 10° to 25°C range (Powrie and Skura, 1991).

The respiration rate of produce is strongly dependent on temperature, increasing as temperature increases (Robinson *et al.*, 1975; Hotchkiss, 1988). Rapid pre-cooling after harvest is therefore important for maintaining quality of produce by slowing the rates of respiration and senescence (Ryall and Lipton, 1979; Brennan and Shewfelt, 1989) and reducing the growth of microorganisms (Varriano-Marston and Wust, 1987). According to Zagory and Kader (1988) the optimum temperature for storing produce is one that delays senescence and maintains quality the longest without causing freezing or chilling injury.

2.3 Modified atmosphere packaging

When used as an adjunct to proper temperature management, controlled atmosphere storage (CAS) and modified atmosphere packaging (MAP) can maintain the organoleptic quality of horticultural commodities for longer durations than normally encountered in air (Labuza and Breene, 1989). Extending the shelf life of produce while maintaining desirable market quality opens the door to new export markets and allows for increased flexibility in meeting market demands (Brecht, 1980).

MAP is defined by Hintlian and Hotchkiss (1986) as "the packaging of a perishable product in an atmosphere which has been modified so that its composition is other than that of air". O'Connor *et al.* (1992) indicated that the gas compositions used in MAP generally contain O_2 concentrations below atmospheric levels (i.e. less than 20.96% v/v) and CO_2 concentrations above atmospheric levels (i.e. greater than 0.03% v/v) with nitrogen (N_2) used as an inert filler gas to make up the remaining gas volume.

The intentional alteration of the natural gaseous environment around foodstuffs during storage is achieved in CAS facilities where the levels of gases are continually monitored and adjusted to maintain the optimal concentrations (Hintlian and Hotchkiss, 1986; Kader *et al.*, 1989; O'Connor *et al.*, 1992). These technically complex facilities are capital intensive and expensive to operate and thus are more appropriate for long-term storage of large quantities of

produce such as apples, pears and kiwifruit (Irving, 1984; Kader *et al.*, 1989; Mannapperuma *et al.*, 1989; Emond and Chau, 1990; Gorris and Peppelenbos, 1992).

MAP and CAS differ only in the degree of control of atmospheric composition; CAS implies a greater degree of precision in maintaining specified levels of O_2 and CO_2 than MAP (Gorris and Peppelenbos, 1992; Kader, 1992). Ooraikul and Stiles (1991) stated that in the MAP system, the cumbersome nature of continuous control of the atmosphere surrounding the commodity is eliminated. This makes it much cheaper for large-scale operations and enables the process to become practical for smaller retail packs of produce.

The primary goal of MAP for fruits and vegetables is to inhibit the deteriorative changes associated with senescence in the case of entire fruits and vegetables and senescence and injury reactions in the case of pre-cut produce (O'Connor *et al.*, 1992).

2.3.1 Responses of fruits and vegetables to modified atmosphere packaging

The responses of fruits and vegetables to CAS and MAP have been extensively reviewed (Isenberg, 1979; Smock, 1979; Brecht, 1980; Kader, 1986; Zagory and Kader, 1988; Kader *et al.*, 1989; Prince, 1989). In general, reduced O_2 and/or elevated CO_2 levels can be beneficial by reducing respiration, delaying ripening, decreasing C_2H_4 production and sensitivity, slowing down compositional changes associated with ripening, reducing chlorophyll degradation and enzymatic browning, reducing the incidence and severity of decay, alleviating physiological disorders and chilling injury, maintaining colour, and preserving vitamins of fresh produce (McLachlan and Stark, 1985; Day, 1988; Zagory and Kader, 1988). Apart from the favourable physiological responses of CAS/MAP, these techniques offer economic and commercial benefits. These are summarised by several other authors (Lioutas, 1988; Tomkins and Blaesing, 1989; Farber, 1991; Kader, 1992).

These physiological effects reflect a reduced metabolic activity which results in a slowing down of deterioration and thus extension of shelf-life (Hewett, 1990). Shelf-life is the time period that a product can be expected to maintain a predetermined level of quality under specified storage conditions (Shewfelt, 1986).

According to O'Connor *et al.* (1992), the extent of benefits derived from atmospheric modification depend upon the commodity and cultivar, its initial quality and physiological age (maturity stage), the amount of minimal processing given to it, and storage conditions such as concentrations of O_2 and CO_2, temperature and the duration of exposure to such conditions.

2.3.2 Relative tolerance to low oxygen and elevated carbon dioxide concentrations

Fresh fruits and vegetables vary widely in their relative tolerance to low O_2 concentrations and/or elevated CO_2 concentrations (Kader and Morris, 1977). The optimum postharvest O_2 and CO_2 conditions have been identified for the majority of fruits and vegetables (Kader, 1989; Saltveit Jr., 1989). The successful application of MAP technology to produce involves the selection of the optimum O_2 and CO_2 concentrations so that the respiration rate is minimized without increasing other metabolic damage to the commodity (Zagory and Kader, 1988; O'Connor *et al.*, 1992). The effects of depleted O_2 and enriched CO_2 levels on respiration are additive and can be synergistic (Kader *et al.*, 1989). Varoquaux and Wiley (1994) stated that because of this, the optimal concentrations of the two gases in combination are difficult to predict without actual measurements in a variety of atmospheres.

Subjecting a cultivar of a given commodity to O_2 levels below its tolerance limits and/or CO_2 levels above its tolerance limits, at a specific temperature-time combination, will result in stress to the living plant tissue (Kader *et al.*, 1989). Exposure of fresh fruits and vegetables to very low O_2 levels may initiate anaerobic respiration (O'Connor *et al.*, 1992). Anaerobiosis, with its accumulation of ethanol, acetaldehyde and organic acids, is usually associated with undesirable off-odours and flavours and a marked deterioration in product quality (Zagory and Kader, 1988; Day, 1990; Gorris and Peppelenbos, 1992). Myers (1989) indicated that the minimum O_2 concentration to avoid injury is about 2% for most produce.

Exposure of fresh produce to levels above their CO_2 tolerance may induce physiological disorders, such as brown stain on lettuce and blackheart in potato. Tolerance limits to elevated CO_2 decrease with a reduction in O_2 level and, similarly, the tolerance limits to low O_2 concentrations increase with an increase in CO_2 level (Kader *et al.*, 1989).

2.3.3 Principles of modified atmosphere packaging

In recent years, rapid advances in polymeric film technology have resulted in the production of packaging films with a wide range of gas permeabilities (Renault *et al.*, 1994). These films, combined with versatile packaging equipment, have led to renewed interest in the use of MAP as a commercial tool to extend the postharvest life of fruits and vegetables (Ooraikul, 1991). Such MAP could be applied to shipping containers, produce cartons, retail packages containing several intact or sliced commodity units, or retail packages for individual units of a commodity (Kader *et al.*, 1989).

When fruit and vegetables are enclosed within an hermetically sealed package, a modified atmosphere (MA) is created naturally as a result of O_2 uptake and CO_2 production in the respiratory process (Schlimme and Rooney, 1994). Geeson (1990) stated that any packaging system must accommodate this respiratory gas exchange. Using a film with suitable gas permeabilities is crucial for the success of MAP (Gong and Corey, 1992). If the film enclosing the product is insufficiently permeable, then internal O_2 levels will fall to very low concentrations and anaerobic respiration will be initiated. Furthermore, high relative humidity (RH) may lead to increased decay (O'Beirne and Ballantyne, 1987). Conversely, when the package is highly permeable to gases, there is little or no atmosphere modification and little enhancement of shelf-life. In addition, moisture loss will cause undesirable wilting and shrivelling (Day, 1993). However, if film of the correct intermediate permeability is chosen, a desirable equilibrium modified atmosphere (EMA) is established when the rates of O_2 and CO_2 transmission through the package equals the intrinsic respiration rate of the product (Tomkins, 1962; O'Beirne and Ballantyne, 1987).

The EMA conditions created and maintained within a sealed package are the net result of the interplay among several factors, both commodity-generated and environmental (Zagory and Kader, 1988). These factors are related to characteristics of the commodity and include the prepared form and type of vegetable, stage of maturity, the products intrinsic respiration rate and fill weight (Hotchkiss, 1988; Geeson, 1990; Gorris and Peppelenbos, 1992). The extent to which the MA deviates from ambient gas levels is also determined by the permeability of the packaging film, the thickness and surface area of the film, the partial pressure gradients of O_2 and CO_2 inside and outside the package system, temperature, RH, package headspace and degree of illumination (Tomkins, 1962; McLachlan and Stark, 1985; Cameron *et al.*, 1989;

Forney *et al.*, 1989; Kader *et al.*, 1989; Labuza and Breene, 1989; Varoquaux, 1991; Beaudry *et al.*, 1992; Robertson, 1993). These parameters need to be optimized for each commodity so that the full benefits of MAP are realized (Day, 1993).

Other important factors to consider when selecting packaging materials with regard to MAP of vegetables include: the resistance to puncture; water vapour transmission rate; sealing reliability; transparency; antifog properties; and cost-effectiveness (Day, 1992). The key to successful MAP for fresh fruits and vegetables is to select a film of correct permeability so that a desired EMA is maintained within the package (Day, 1988).

2.3.4 Methods of creating modified atmosphere conditions

Modified atmospheres within polymeric film packages can be established via passive or active modification or a combination of the two (Kader *et al.*, 1989).

2.3.4.1 Passive modified atmosphere packaging

Commodity-generated or passive MAP involves the matching of commodity respiratory characteristics with the gas permeabilities of the packaging film so that a suitable EMA can passively evolve within a hermetically sealed package through the consumption of O_2 and the evolution of CO_2 in the respiration process (Zagory and Kader, 1988; Kader *et al.*, 1989; Labuza and Breene, 1989; Day, 1993; Schlimme and Rooney, 1994). When atmospheres are modified passively by produce respiration, days or weeks may be needed for in-package gas concentrations to reach a steady state (Geeson *et al.*, 1985; Ballantyne *et al.*, 1988a; Hewett, 1990).

2.3.4.2 Active modified atmosphere packaging

Active modification involves the partial or complete removal of atmospheric gases from the headspace of the package (by pulling a slight vacuum), and the introduction of a gas mixture of a predetermined composition (Zagory and Kader, 1988; Labuza and Breene, 1989; Smith *et al.*, 1990). The atmosphere is maintained by controlling the influx of O_2 and efflux of CO_2 with a properly selected permeable film (Lee *et al.*, 1991). Kader *et al.* (1989) suggested that this mixture can be further adjusted through the use of absorbers or adsorbers inside the package to scavenge O_2, CO_2 or C_2H_4 and by doing so control the concentration of these gases.

Although active modification implies some additional costs, its main advantage is that it ensures the rapid establishment of the desired atmosphere (Ben-Arie, 1990; Powrie and Skura, 1991; Kader, 1992; Day, 1993) and hence a potentially longer storage life (Zagory and Kader, 1988).

2.4 Lettuce

2.4.1 Botany and quality characteristics

Lettuce (*Lactuca sativa* L.), a member of the family *Asteraceae* (Compositae), is a frost-sensitive annual plant with Mediterranean origins (Philips and Rix, 1993). It was grown by the Romans, and is thought to have been cultivated first by the ancient Egyptians in and around 4500 B.C. (Lipton and Ryder, 1989). Nowadays, lettuce is a popular salad ingredient and is grown to some degree on all continents and in most countries of the world (Ryder, 1979). Lettuce is unique amongst the major vegetables in that it is eaten almost exclusively as a fresh, uncooked product (Snowdon, 1991).

According to Lipton and Ryder (1989), modern lettuce cultivars can be grouped into six morphological types: (1) Crisphead, (2) Butterhead, (3) Romaine or Cos, (4) Leaf, (5) Stem or Celtuce and (6) Latin.

Crisphead lettuce, often referred to as Iceberg lettuce, is the most important commercial type in Australia, with literally scores of different varieties grown (Dioguardi, 1995a). It is recognised by its large, firm heads (approximately 1000g at maturity) and crisp texture that are well suited to withstand the rigours of transport and marketing (Peirce, 1987; Lipton and Ryder, 1989). Crisphead lettuce of high quality as sold at retail should have crisp, green outer leaves that are free from any blemishes (Ryall and Lipton, 1979).

Romaine lettuce is easily recognised by its upright stance and long, narrow leaves with prominent midribs. They are generally dark green on the outside and creamy yellow inside the head and have a coarse but crisp texture. Heads usually weigh between 500 and 1000g (Lipton and Ryder, 1989). Because of the popularity of Crisphead and Romaine lettuce in Australia, research into MAP in this thesis will be focused on these two lettuce types.

2.4.2 Nutritional value

Crisphead lettuce contains approximately 96% water and is not especially rich in vitamins and minerals, ranking 26th amongst the major fruits and vegetables (Peirce, 1987). However, because of the quantity eaten, it has become the 4th most important crop in relative contribution to nutrition (Stevans, 1974 as stated by Lipton and Ryder, 1989). The different types of lettuce vary in their nutrient content, with Leaf and Romaine lettuce providing higher levels of vitamins A and C and mineral content than other types (Table 2.1).

Table 2.1 Vitamins A and C and calcium concentration in various lettuce types

Lettuce Type	*Calcium (mg/100g)*	*Vitamin C (mg/100g)*	*Vitamin A (IU)*[a]
Crisphead	20	6	330
Butterhead	35	8	970
Leaf	68	18	1,900
Romaine	68	18	1,900

Source: USDA *National Food Review* (1978)

[a] 1 IU = 0.3 μg vitamin A alcohol

2.4.3 Lettuce in Australia

Lettuce grows best at a relatively cool temperature and does not take kindly to extreme heat or cold (Ware and McCollum, 1980; Yamaguchi, 1983). Despite this, its tolerance range is sufficiently wide to allow production from every state and territory within Australia (Dioguardi, 1995a). The total Australian lettuce yield in 1992/93 was 98,992 tonnes (Table 2.2) with a gross value of production of $58.5 million (ABS, 1994; Table 2.2).

Table 2.2 Lettuce production in Australia (1992/93)

State	*Number of Growers*	*Area (ha)*	*Production (tonnes)*	*Export (tonnes)*
VIC.	166	1,431.7	33,912.2	1,492.4
QLD.	124	1,185.5	30,575.4	235.8
NSW	128	499.2	6,558.2	2,838.4
W.A.	109	376.8	17,450.8	2,058.3
S.A.	54	329.2	9,217	75.7
TAS.	19	73.5	1,145.4	785.9
N.T	2	18.3	133	0.03
Total	604	3,914.2	98,992	7,486.27

Sources: Australian Bureau of Statistics, 1994
Plant Quarantine and Inspection Branch, AQIS, DPIE, 1994

Victoria was the largest producer accounting for 34% of Australia's total lettuce production (Table 2.2).

2.4.3.1 Australian lettuce exports

There has been an increasing trend in the export of fresh lettuce from Australia over the past few years, with 7,486 tonnes exported in 1992/93 (Figure 2.1). This represented 7.5% of the total lettuce production in Australia.

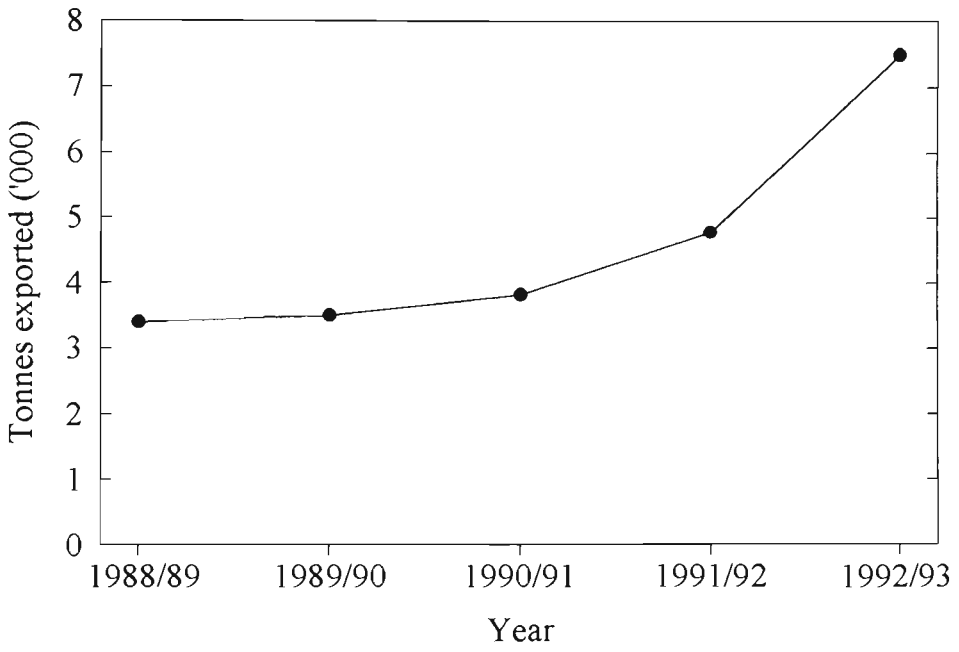


Figure 2.1 Australian lettuce exports

New South Wales and Western Australia are the leading export states accounting for 37.9% and 27.5% of total exports respectively (Table 2.2). Although 38 countries buy Australian lettuce, Singapore, Hong Kong and Japan account for 76.8% of total exports (Table 2.3).

Table 2.3 Major destinations of Australian export lettuce

Destination	***Fresh Exports (tonnes)***	***% Total***
Singapore	2,412	32.2
Hong Kong	1,765	23.6
Japan	1,572	21.0
Other	1,737	23.2
Total	7,486	

Source: Australian Bureau of Statistics, 1994

2.4.4 Postharvest handling and storage of lettuce

In nonclimacteric commodities such as lettuce, quality is optimal at harvest and the task is to minimize quality loss (Yildez, 1994).

2.4.4.1 Temperature effects

According to Lipton and Ryder (1989), prompt and thorough cooling of lettuce soon after harvest (pre-cooling) is one of the essential steps in marketing of lettuce. Lettuce should be pre-cooled to 1° ± 1°C and held at this temperature at high RH (95-97%) for storage and transport (Hinsch *et al.*, 1978; Hardenburg *et al.*, 1986; Snowdon, 1991). Lettuce held under these conditions has a shelf-life of 2-3 weeks (Lipton and Ryder, 1989). Despite this, Gull and Guzman (1988) suggested that under good commercial handling practices, attainment of a product temperature ranging from 2° to 5°C is more economically practical and avoids freezing damage. Extensive research has shown that deterioration is faster at higher postharvest temperatures (Parsons *et al.*, 1960a; Lipton, 1967; Singh *et al.*, 1972). Snowdon (1991) has pointed out that care must be taken to avoid freezing injury, the critical temperature for lettuce being only a fraction of a degree below 0°C (-0.17°C).

2.4.4.2 Controlled/Modified atmosphere storage of lettuce

Crisphead Lettuce

Singh *et al.* (1972) found that whole Crisphead lettuce stored at 1.7°C under a controlled atmosphere (CA) of 2.5% O_2/2.5% CO_2 maintained its quality better than that kept at the same temperature but in normal air for a period of 75 days. This was in contrast to results reported by Watada *et al.* (1964), where no noticeable effects were evident when lettuce heads were stored for 4-8 days at 5°C under a CA of 2.5% O_2/2.5% CO_2. The differences in results could be attributed to the short storage time and the higher storage temperature. Lipton (1971) suggested that if lettuce must be held a month before use, a CA of 3% O_2/2% CO_2 may be superior to normal air, because the combination tends to retard decay development. Haginuma *et al.* (1985) studied the effects of different combinations of O_2 and CO_2 concentrations on the quality of Crisphead lettuce cv. Great Lakes stored at 0-1°C and 95-100% RH for 56-100 days. They found that the optimum conditions for the storage of head lettuce were 10% O_2/4% CO_2 and 5% O_2/2% CO_2. Combinations of 10% O_2/2% CO_2, 3%

O_2/2% CO_2 and 5% O_2/4% CO_2 were also effective. Other research relating to CA storage of Crisphead lettuce is reviewed in section 2.4.4.3 'Postharvest disorders'.

Romaine lettuce

Very limited research has been conducted on CAS/MAP for Romaine lettuce. Aharoni and Ben Yehoshua (1973) tested the effectiveness of modified atmospheres in slowing down deterioration of whole heads of Romaine lettuce cv. Hazera Yellow. Romaine lettuce stored in closed film bags or liners at 1°C, in which CO_2 levels ranged from 2% to 7% and in which O_2 levels ranged from 2% to about 14%, was found to be less yellow and required less trimming than lettuce held in open bags. The same authors also noted that damage in Romaine lettuce only occurred in samples in which the CO_2 concentration rose above 15% and O_2 dropped below 1%. The damage was manifested in two ways: as irregular reddish-brown stains composed of tiny sunken spots in the leaf midrib; and as areas of the leaf base lacking chlorophyll. The young heart leaves also developed a brown colour typical of damage caused by O_2 deficiency; the authors suggest that this damage may be attributed to the high CO_2 concentration.

Lipton (1987) also attempted to characterize the response of whole Romaine lettuce cv. Parris Island to low levels of O_2, high levels of CO_2, and their combined effect at 0°, 2.5° or 5°C. He established that Romaine lettuce can be injured by CO_2 levels greater than 7.5%, but that the degree of injury is not influenced by O_2 levels in the range of 0.5% to 20%. The CO_2-induced injuries were more serious at 0°C than at 2.5°C or 5°C. He concluded that there was potential for using reduced O_2 and/or enriched CO_2 atmospheres at storage temperatures between 2.5° and 5°C.

2.4.4.3 Postharvest disorders

In assigning ratings to various types of postharvest problems, Beraha and Kwolek (1975) considered russet spotting (RS) and brown stain (BS) to be major postharvest disorders along with physical damage and decay. Incidence varied with season, and there appeared to be differences attributable to cultivars and to rates of growth and harvest size.

Russet spotting is a physiological disorder of Crisphead lettuce primarily caused by exposure to low levels (ppm) of ethylene gas at storage temperatures around 5°C (Rood, 1956). The injury usually manifests itself as numerous small, brown, tan or olive, elongated, pit-like spots along both sides of the midrib which may spread over the leaf blade in severe cases (Lipton, 1961; Ryder, 1979; Peirce, 1987). Anatomical studies have indicated cell wall thickening and cell discolouration as two major changes associated with RS of lettuce leaves (Lipton, 1961; Ilker *et al.*, 1977).

Storage temperature has a fundamental role in RS development. Tissue injury can be slowed down or prevented at 0°C, whereas the rate of ethylene-induced damage increases rapidly at 5°C (Rood, 1956; Hyodo *et al.*, 1978; Pratella and Brigati, 1989).

Controlled atmosphere storage is beneficial in reducing RS on lettuce (Stewart *et al.*, 1966; Stewart and Uota, 1976). Lipton (1967) reported that 0.5% to 8% O_2 effectively reduced RS development. A similar result was obtained by Ke and Saltveit (1989a) who found that midrib tissue of Crisphead lettuce exposed to 5°C, 1.5% O_2 and 2 ppm C_2H_4 had significantly lower RS scores than midribs exposed to air containing 2 ppm.

Lettuce cultivars differ in levels of RS tolerance. Ke and Saltveit (1989b) reported significant variations in the extent of RS development among six different cultivars and found that RS could only be induced after the plant tissue had reached a certain developmental stage and developed the ability for the C_2H_4 induction of phenylalanine ammonia-lyase (PAL) and ionically bound peroxidase (POD) and indole-3-acetic acid (IAA) oxidase activities. Differences in cultivar susceptibility were also noted by Pratella and Brigati (1989).

Rood (1956) established that RS susceptibility also depends on lettuce maturity, with firm heads less susceptible to injury than hard lettuce heads. Russet spotting can be controlled by harvesting lettuce at optimal maturity (Patterson *et al.*, 1986), storing and transporting susceptible cultivars at temperatures ranging from 5° to 0°C (Pratella and Brigati, 1989) and segregating lettuce from other commodities with high ethylene production or machinery emitting ethylene (Snowdon, 1991).

Brown stain is a physiological disorder of head lettuce that can be induced by increased CO_2 in the storage atmosphere (Stewart and Ceponis, 1968; Stewart *et al.*, 1970; Brecht *et al.*, 1973 a,b,c). This disorder is usually associated with CO_2 concentrations in the range of 2.5 to 10% (Stewart and Uota, 1971; Kader and Morris, 1977). Symptoms include slightly sunken oval spots on leaves, each possessing a dark brown border (Lipton *et al.*, 1972; Ryall, 1979; Pierce, 1987). Stewart and Uota (1971) state that the affected areas are generally on the outer surface of head leaves, on or near the midrib, and usually extend towards the base of the leaf. The lesions are water soaked when young, but become darker and may coalesce when the injury is severe (Ryder and Whitaker, 1980; Ceponis *et al.*, 1985).

Previous research has shown that the severity of BS increases with increasing concentrations of CO_2 (Stewart and Uota, 1971; Stewart and Uota, 1972; Brecht *et al.*, 1973 a,c; Forney and Austin, 1988) and that low O_2 increases the susceptibility of lettuce to CO_2 injury (Stewart and Uota, 1971; Stewart and Uota, 1972; Brecht *et al.*, 1973c). Carbon monoxide (CO) in the presence of elevated CO_2 increases lettuce susceptibility to BS (Stewart and Uota, 1972). This finding was substantiated by Kader *et al.* (1973a) who found that BS on Crisphead lettuce was increased by exposure to CO combined with elevated CO_2 (2 or 5%) regardless of the O_2 level (2.5, 10 or 21%). Carbon monoxide at 1.3 or 5% added to air without CO_2 did not induce BS.

Brown stain symptoms are not always present under CO_2-enriched atmospheres and become more apparent after lettuce are transferred to air and high temperatures encountered during marketing (Watada *et al.*, 1964; Stewart and Uota, 1971; Siriphanich and Kader, 1985a).

The severity of BS as a symptom of CO_2 injury is highly dependent on the temperature and duration of exposure. Brecht *et al.* (1973b) observed significant levels of BS on lettuce stored in 2% O_2/5% CO_2 at 0°C, whereas little or no BS was found with the same gas mixture at 10°C. Additionally, lettuce held for 5 days in 2% O_2/5% CO_2 at 0°C had substantially less BS than that held under the same treatment for 10 days. Siriphanich and Kader (1985a) reported that CO_2 injury could be induced at increased temperatures (10° and 20°C) with increased CO_2 concentrations (15% CO_2) and concluded that the high CO_2 level

was probably required because of reduced solubility of CO_2 at higher temperatures. With a temperature rise of water from 0° to 20°C the solubility of CO_2 is reduced by approximately 50% (Powrie and Skura, 1991).

Lettuce types and cultivars differ greatly in susceptibility to the BS disorder. Brecht *et al.* (1973a) subjected 11 cultivars of Crisphead lettuce to elevated CO_2 concentrations and noted significant differences in the incidence and severity of BS. They suggested that cultivar differences in BS susceptibility should be given consideration under commercial conditions.

Cultivation conditions such as irrigation, climate, and fertilizer regime can modify plant tissue susceptibility to CO_2 injury (Ryder and Whitaker, 1980). The sensitivity of lettuce to BS also varies with the growing region and time of day at harvest. Lettuce from the California coastal valleys showed more BS under similar storage conditions than those from the desert districts (Ceponis and Kaufman, 1970; Stewart and Matoba, 1972). Forney and Austin (1988) also found that heads picked in the afternoon tolerated high CO_2 levels up to 7.5-10% better than heads picked in the morning.

Butt discolouration: When lettuce are harvested, the cut surface of the stem (the butt) is white. Thereafter, the latex that leaks from the surface is oxidized and turns pink. This is known as butt discolouration. The variables that influence the rate of colour change under constant environmental conditions are unknown (Lipton and Ryder, 1989). Stewart and Uota (1971) found that increased levels of CO_2 (5 and 10%) reduced the severity of butt discolouration in lettuce examined after CAS. However, these differences were not evident after an additional 4 days storage at 10°C in air. Watada *et al.* (1964) also noted that CO_2 inhibited butt discolouration in lettuce. Variable results have been reported on the effect of O_2 concentration on the extent of butt discolouration. Stewart and Uota (1971) found that butt discolouration was not influenced by the O_2 level (3 vs. 21%). On the other hand, Lipton (1967) found that butt discolouration was generally lower in atmospheres of 0.5 or 1% O_2 than in air, but was not affected by 2, 5 or 8% O_2.

Heart leaf injury (HLI), which appears as a reddish brown discolouration of innermost leaves (Kader and Lipton, 1990), is usually the result of increased concentrations of CO_2, though a lack of O_2 may induce similar symptoms (Snowdon, 1991).

2.5 Minimally processed vegetables

During the last decade, consumer's desire for convenience and their ever-increasing concern with eating healthy foods, along with improvements in packaging technology, have given rise to a new category of vegetable products that are called "minimally processed" (MP) (Shewfelt, 1987; King and Bolin, 1989; Labuza and Breene, 1989; Myers, 1989; Dougherty, 1990; Saracino *et al.*, 1991; Klassen, 1994; Yildez, 1994). In general, minimal processing refers to the process of converting harvested fresh vegetables into a convenient peeled, cored, chopped or sliced product that is 100% edible and has retained its fresh quality attributes to a high degree (Bolin and Huxsoll, 1989a; Powrie and Skura, 1991).

Many names are used as synonyms for MP fruits and vegetables; these include: fresh-cut; pre-cut; pre-packed; ready-to-use; lightly processed; semi-processed and value-added (Cantwell, 1992). The range of MP vegetables encompasses both simple products, containing only one variety of vegetable such as packs of broccoli florets and carrot sticks, and more complex products containing several varieties of vegetables such as prepared salad mixes (O'Connor *et al.*, 1992). Huxsoll and Bolin (1989) point out that the forms of these products vary widely, depending on the nature of the unprocessed commodity and how it is normally consumed. The increasing popularity of MP produce has led to a vast array of new commercial products being offered to consumers (Varoquaux, 1991).

Since the tissue integrity of MP commodities is altered during processing, these products are more perishable than their whole counterparts (Bolin *et al.*, 1977; Rolle and Chism, 1987; Shewfelt, 1987; McDonald *et al.*, 1990; Barriga *et al.*, 1991). Even under recommended chill temperatures (0-8°C), enzymic discolouration of cut surfaces and microbial spoilage frequently means that their shelf-life is less than five days at 4°C (O'Connor *et al.*, 1992). According to Bolin *et al.* (1977) these products must have a longer shelf-life than is currently available if they are to realize a wide usage.

2.5.1 Major unit operations of minimally processed vegetables

For MP vegetables, harvesting, processing, storage and distribution need to be accomplished in a fast, highly integrated system to maintain product quality (Yildez, 1994). Many individual processes are required to transform whole vegetables into various MP products. Each sequential step in processing of fresh vegetables may play a negative role in the spoilage mechanisms (Gill, 1990).

The general sequence of operations in the minimal processing of lettuce are summarised in Figure 2.3 and include:

2.5.1.1 Harvesting

Lettuce for processing is usually picked at the lowest possible temperature and transported immediately to the processing plant in bulk bins or crates (King *et al.*, 1991). It is rapidly pre-cooled to remove field heat and the heat of respiration and either processed immediately or held at 1-2°C until required (Yildiz, 1994). Early pre-cooling of raw material dramatically extends the shelf-life of MP produce (Gill, 1990).

2.5.1.2 Trimming/coring

An essential first step in the processing of whole lettuce is removal of the outer leaf layer (2-3 wrapper leaves) and core area to reduce the overall physical and microbial contamination on prepared lettuce (Adams *et al.*, 1989). Outer leaves have a higher bacterial count than inner ones (Maxcy, 1978; Saracino *et al.*, 1991). Bolin *et al.* (1977) found that a high initial microbial load was correlated with a short shelf-life of shredded lettuce.

2.5.1.3 Slicing

In the partial processing of fresh fruits and vegetables, operations such as trimming, peeling, cutting, slicing, and other physical actions cause injury and damage to tissues (Watada *et al.*, 1990). Such wounding results in leakage of enzymes and their substrates from cells which leads to various biochemical deteriorations such as browning, off-flavours and texture breakdown (Rolle and Chism, 1987; Powrie and Skura, 1991; Varoquaux, 1991).

In commercial lettuce processing, piece size reduction is accomplished by sharp rotating knives which slice leaves into the desired pieces (Schwartz per. comm., 1994). To maximize storage

life, cellular damage needs to be minimized. Bolin *et al.* (1977) showed that the stability of shredded lettuce is affected by the way it is cut. For instance, lettuce processed with a sharp blade exercising a slicing action had a storage life about twice that of lettuce cut with a sharp knife using a chopping action. Smaller shred size also reduced storage life. In general, shelf-life for most commodities is enhanced by reducing machine-to-product and product-to-product impacts (Huxsoll and Bolin, 1989; Varoquaux, 1991).

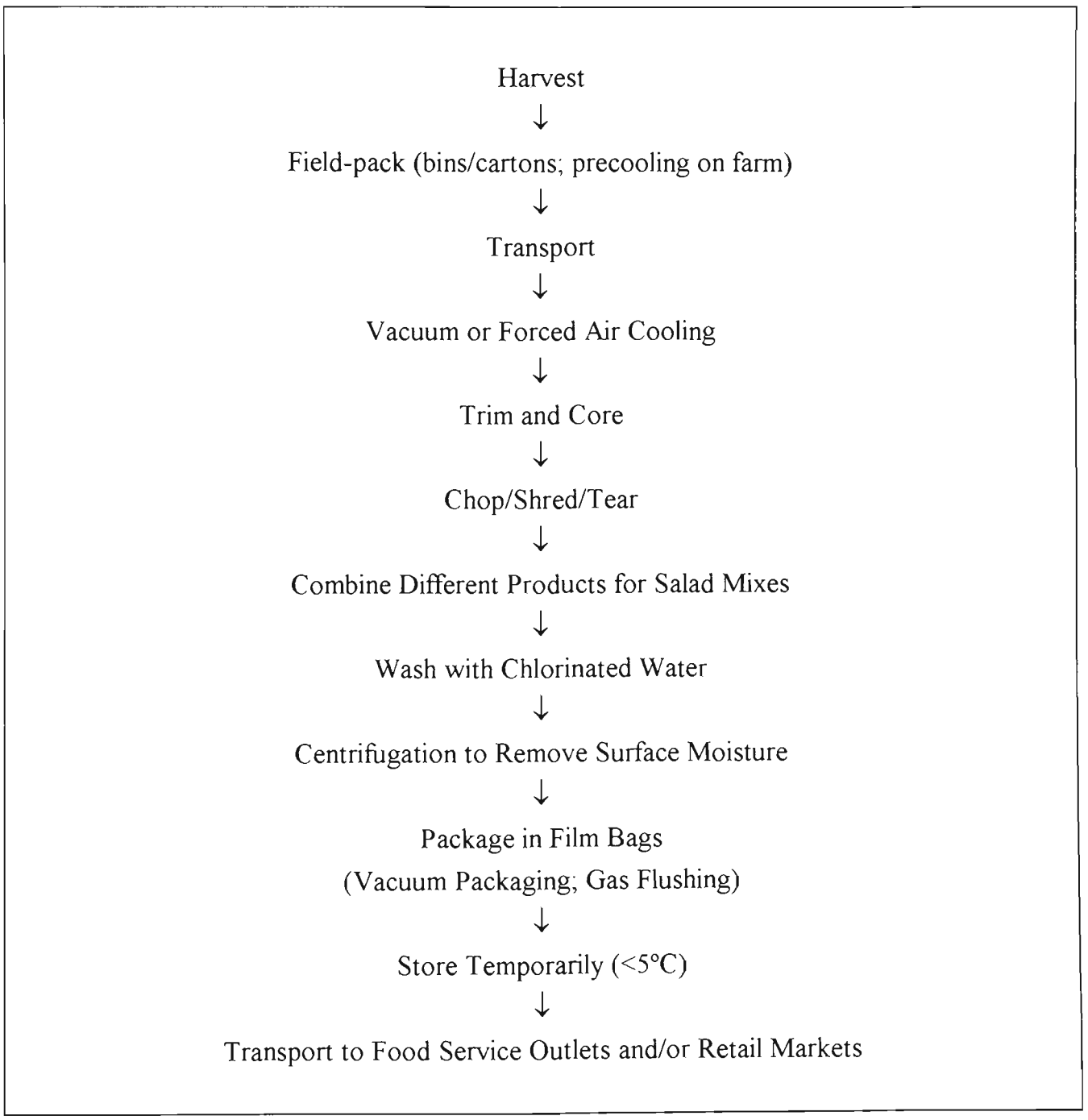


Figure 2.2 Major unit operations of minimally processed lettuce products (Adapted from Cantwell, 1992)

Tissue disruption caused by cutting also results in elevated respiration and transpiration, which leads to rapid deterioration (Watada *et al.*, 1990; Barriga *et al.*, 1991). Cutting of whole heads of broccoli into individual florets resulted in a 40% increase in respiration rate at both 10° and 20°C (McLachlan and Stark, 1985). Further, when carrots were julienne sliced, the respiration rate increased more than seven times. Rosen and Kader (1989) stated that the physiological response to wounding depends on the magnitude of the stress.

Rolle and Chism (1987) indicated that wounding may also induce other biochemical responses in tissues such as the synthesis and accumulation of elevated levels of phenolic compounds. In addition, cuts and punctures allow for microbial contamination of products by providing sites for entry and substrates for growth (Priepke *et al.*, 1976; Yildez, 1994).

2.5.1.4 Washing

The washing of MP fruits and vegetables is common practice and may be carried out by spray techniques (Ewen per. comm., 1994) or by water immersion (Schwartz per comm., 1994), the choice being dependent on the purpose of washing and the delicacy of the tissue (Mitchell, 1985).

Washing cools the cut product and removes sugar and other nutrients at the cut surfaces which favour microbial growth and tissue discolouration (Cantwell, 1992). Thorough washing, to remove contents released by cutting, was found to be important in extending the shelf-life of shredded lettuce (Bolin *et al.*, 1977) and cut carrots (Bolin and Huxsoll, 1991b).

The efficacy of washing is often improved by the inclusion of antimicrobials in the wash water (Shapiro and Holder, 1960; Adams *et al.*, 1989; Saracino *et al.*, 1991). Many anti-microbial wash solutions have been reported in the literature specifically for vegetables, but probably the most widely used is a hypochlorite solution containing 50-100 mg/L available chlorine (Lund, 1983; Brocklehurst and van Bentem, 1990; Yildez, 1994).

2.5.1.5 Drying

After washing, free moisture must be completely removed from the surface of MP produce. Brocklehurst *et al.* (1987) found that free moisture and nutrients on the cut surfaces created

conditions that favoured microbial growth. Similarly, Herner and Krahn (1973) emphasized the importance of keeping cut lettuce dry, even advocating not rinsing at all before storage.

Dewatering is usually accomplished commercially by transporting the lettuce on an extended pierced belt which facilitates the removal of most of the water as well as smaller pieces of product (Saracino *et al.*, 1991). After draining in this way 0.5-1.0% water remains on the lettuce (Bolin *et al.*, 1977). The product is usually further dried by centrifugation, although vibration screens and air blasts can also be used (Bolin and Huxsoll, 1991a; Cantwell, 1992).

2.5.1.6 Modified atmosphere packaging of pre-cut lettuce

The initial preparation treatments for MP produce are usually followed by some kind of modified atmosphere/vacuum packaging prior to distribution (Wiley, 1994). Published information on effective gas levels for MP products is limited compared to information available for whole fruits and vegetables. Cameron *et al.* (1994) suggest that even when data is available, it is difficult to know how the information can be applied during commercial packaging to different cultivars or even the same cultivars grown under different seasons or growing conditions, as these may differ in tolerance to modified atmospheres, particularly to low O_2.

Some information is available on the effects of CAS/MAP on whole heads of lettuce (Refer to Section 2.4.4.2). However, very limited research has been published on the controlled/modified atmosphere storage of MP lettuce. According to Saracino *et al.* (1991), a typical ideal equilibrium for a pre-cut lettuce salad is 2-5% O_2 and 3-8% CO_2.

Barriga *et al.* (1991) found that visual quality and shelf-life of shredded Crisphead lettuce cv. Great Lakes at 4°C was significantly extended by CAS in 3% O_2/10% CO_2. This was probably achieved by limiting plant and microbial enzyme activity. They also suggested that higher CO_2 levels may have additional benefits on shelf-life. Storage in air, 3% O_2 or 3% O_2/5% CO_2 were less effective in retaining visual quality.

Mixed salad vegetables (60% shredded lettuce, 10% each carrot, celery, radish and green onion) packed in polyvinylidene chloride under an initial headspace of 2.25% O_2 and 10.5%

CO_2 had better organoleptic quality after storage at 4.4°C for 10-14 days than vegetables packed under air at the same temperature (Priepke *et al.*, 1976).

Packaging shredded Crisphead lettuce in sealed polyethylene bags at 2.8°C resulted in an atmosphere of approximately 1.5% O_2/11% CO_2 after 14 days which controlled browning, visual quality loss and bacterial buildup during storage (King *et al.*, 1991).

Ballantyne *et al.* (1988a) reported that the least browning and development of off-odours for shredded Crisphead lettuce cv. Saladin resulted from using a 35 µm low density polyethylene (LDPE) film in combination with an initial 5% O_2/5% CO_2 gas flush. An EMA containing 1-3% O_2 and 5-6% CO_2 was established after six days and delayed the onset of browning (a main attribute of end of shelf-life) so that shelf-life was doubled to 14 days at 5°C.

Krahn (1977) found temperature to be the most important variable in the storage of bagged lettuce and reported that the best quality was achieved with a polypropylene film, in which a self-generated MA of 11% O_2/9% CO_2 was attained at 0°C in two weeks.

Of four films tested, McDonald *et al.* (1990) found that chopped Crisphead lettuce cv. Salinas packaged in oriented linear low density polyethylene (LLDPE) developed O_2 levels between 2 and 15% and CO_2 levels less than 20%, and the lettuce was acceptable after 14 days storage at either 1° or 5°C.

The underlying trend in the literature regarding CAS/MAP of MP lettuce is that atmosphere modification needs to be accomplished quickly to retard deteriorative processes. In other words, active MAP is essential for maintaining the quality of pre-cut tissue.

2.5.1.7 Storage temperature

After packaging, MP vegetables should be stored, distributed and marketed at temperatures ranging from 2° to 4°C with an upper maximum limit near 7°C (Lioutas, 1988; Dignan, 1994).

Low temperatures for MP lettuce are essential for prolonged storage (Krahn, 1977; McDonald *et al.*, 1990; Bolin and Huxsoll, 1991a). According to Bolin *et al.* (1977) shredded lettuce held at 2°C remained marketable for about 26 days compared to 10 days for the same product

stored at 10°C, an increase in shelf-life of over 100%. King *et al.* (1991) reported that the ideal storage temperature for shredded lettuce is 1.1°C.

The limited literature on CAS/MAP of whole and pre-cut lettuce suggests that more knowledge is required if these technologies are to be applied to Australian cultivars grown under different climates and cultural and management techniques.

Chapter 3

Materials and Methods

3.1 Controlled atmosphere storage of whole lettuce

For CAS of whole lettuce, each head was trimmed slightly to remove any damaged/diseased leaves, excess stalk or any soil that was present on the lettuce. Each head was then placed in a highly perforated (1.4 mm diameter, 5 mm wide square pitch) 20μm biaxially oriented polypropylene bag (Propafilm MG 20®, ICI Films, Melbourne) for labelling purposes (Plate 3.1b). It was necessary to label each lettuce so that initial lettuce weights could be related to final weights and therefore weight loss over the storage period at 1.5° or 20°C recorded (Refer to Chapter 4).

The gas atmospheres were established in CA chambers (160 L) and maintained by the Oxystat 2 Automated Controlled Atmosphere System (Bishop Instruments Ltd., UK; Plate 3.1 a-d). With this system, periodic analysis (3 hours) of the atmosphere within each chamber was made using infra-red CO_2 and paramagnetic O_2 gas analyzers and feedback was used to adjust the operating conditions of the CA units. Where necessary, 98.5% N_2, from a gas membrane N_2 generator, CO_2 (Food Grade, BOC Gases, Melbourne) or air were added to each chamber to maintain the preset levels of O_2 and CO_2. An interface panel consisting of solid state relays, power supplies and signal conditioning provided electronic switching to sensors and solenoid valves which supported the introduction of complimentary gases into each chamber (Plate 3.1 c,d).

Due to the large number of CA chambers and infrequent sampling times, the desired CA conditions usually took 1-4 days to establish. However, when established the concentration of O_2 and CO_2 within chambers were held within ± 0.5% of the specified concentration.

The respiration rate of lettuce held in air and under CAS was determined by closing off input and output gas lines of each chamber and withdrawing a 2 mL sample through a self-sealing septa located in the lid. The change in CO_2 concentration (usually 0.5-1.0% over 50 hours) was used to determine respiration rate of lettuce as described in Section 3.6 'Gas Analysis'.

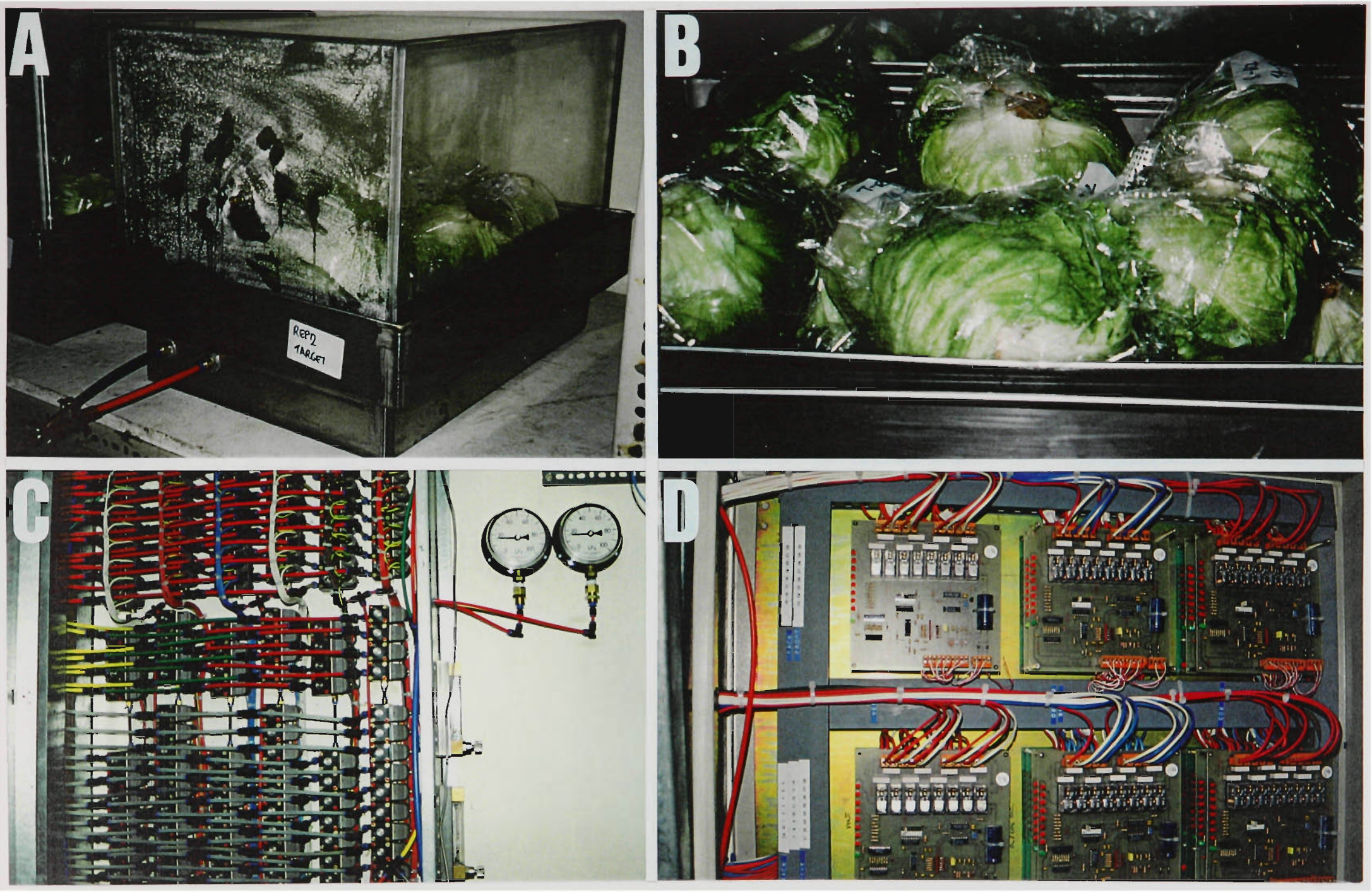
Plate 3.1: System used to establish controlled atmospheres around whole Crisphead lettuce

Plate 3.1A A typical CA chamber used during storage of lettuce. Note input and output gas lines at base of chamber and sampling septa located in the perspex lid

Plate 3.1B Lettuce held inside CA chambers within a highly perforated bag for labelling purposes

Plate 3.1C Gas lines, solenoid valves and flow meters responsible for maintaining the desired gas atmosphere

Plate 3.1D Interface panel responsible for controlling gas levels within CA chambers


A
REPT
TARGET
B
C
D

This length of time between sampling's was necessary because of the low respiration rate of lettuce and the large free volume within containers.

The relative humidity (RH) within CA chambers was determined by inserting a RH probe (Vaisala Humidity Indicator Model HMI 31) into a gas tight socket attached to the input gas line of each tub. The probe had been precalibrated by the manufacturer and had a sensitivity of 0.1% RH and accuracy of ± 2% within the range 15% to 99% RH at 25°C.

3.2 Respiration rate of whole lettuce under 5% O_2/5% CO_2

A 'flow-through' CA system was designed and built by the author to enable a gas atmosphere of 5% O_2/5% CO_2 (± 0.5%) to flow through the CA chambers, each of 129 L capacity (Figure 3.1). Oxygen was delivered into the line through the use of a small aquarium pump (Rena, 301). Carbon dioxide (Food grade) and N_2 were derived from pressurized gas cylinders (BOC Gases, Melbourne). Mini-regulators (SMC Pneumatics Pty Ltd., Melbourne) were used to control the proportion of these gases passing into 8mm plastic tubing. Gases were bubbled through water to facilitate mixing and to humidify the air stream. Before entering the chambers, a portable Servomex gas analyser (Servomex® Series 1400, Servomex Co., Sussex, England) was used to ensure delivery of the correct atmosphere into the chambers. The flow rate of gases into individual chambers was approximately 55 L/h, as determined by a Sho-Rate Purge Meter (Model 1350). The same procedure as described in Section 3.1 was used to determine the respiration rate of lettuce during storage.

3.3 Controlled atmosphere storage of pre-cut lettuce

In order to create varying gaseous environments for pre-cut lettuce, a flow-through CA system was designed and built by the author, so that the system could deliver up to five different gas mixtures (O_2; CO_2; N_2) simultaneously, each to a series of four glass jars (Plate 3.2 a,b,c,d,f). Each jar, considered one replicate, had a void volume of 4.75 L and contained inlet and outlet valves capable of being closed so that respiration measurements could be taken. After lettuce tissue was placed in the jars, they were sealed hermetically by clamping down a glass lid fitted with a rubber O-ring.

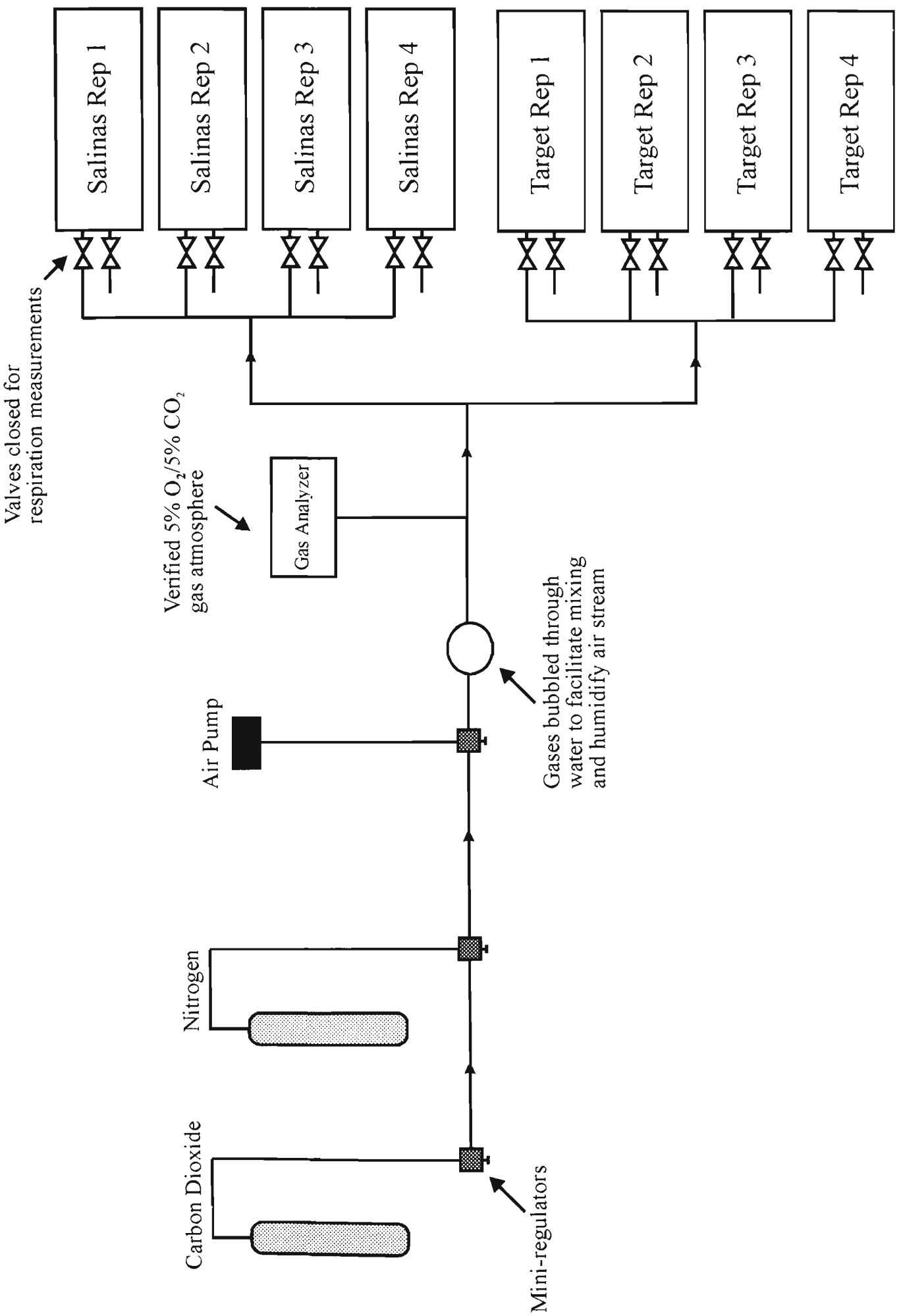


Figure 3.1 Controlled atmosphere system used to measure the respiration rate of lettuce under 5% O_2/5% CO_2

Each gas combination was pre-mixed, calibrated and purchased in pressurized gas cylinders (BOC gases, Melbourne), prior to flushing the jars. Control samples were flushed with air from an aquarium air pump (Rena 301). Flow meters (Fischer and Porter Pty Ltd., Melbourne) were used to control the flow of gases into each jar (Plate 3.2 d). The designated gas mixtures were fed to each jar at a flow rate of 10 L/hr reduced to 5 L/h after 1 hour and so maintained for the rest of the experiment. These were checked daily to ensure that the flow rate was adequate to compensate for the effects of vegetable respiration.

A RH superior to 95% was maintained by bubbling gas mixtures or air through water before entry into the glass jars. For measuring respiration rate of tissue, gas samples (1 mL) were drawn from each jar through a self-sealing silicone septa located in the lid and analysed for CO_2 as described in Section 3.6. Respiration rates were determined by measuring the change in CO_2 concentration (0.5-1.0%) in the sealed jars as a function of time (up to 16 hours). Gas analysis of the atmosphere within each jar was carried out on triplicate samples.

To determine the effect of storage temperature (10° and 20°C) on the respiration rate and quality of shredded lettuce (Chapter 6), two other flow through systems identical to that described above, but consisting of only four glass jars fed with air from an aquarium pump (Rena 301), were constructed by the author. Each jar was considered a replicate. Respiration rates were determined by measuring the change in CO_2 concentration as detailed above.

3.4 Respiration rate of whole Crisphead lettuce and wedges

The respiration rate of whole lettuce heads and wedges (Chapter 6) was determined by measuring the change in CO_2 concentration (0.5-1.0% over 6 hours) within 5.2 L airtight Tupperware® containers. Each container was checked for air tightness by submerging the container under water and checking for air bubbles when supplied with gas under pressure. Gas samples were removed by syringe through self-sealing septa located in the lid of each container and analysed by gas chromatography as described in Section 3.6. Each container was considered a replicate and four replicates were used per treatment. After gas sampling, the lids of containers were opened slightly so that atmospheres could return to levels found in air.

Plate 3.2: Systems used to establish controlled atmospheres and measure respiration rate of pre-cut and whole lettuce

Plate 3.2A CA system used to deliver five different gas atmospheres, each to a series of four glass jars (1 replicate/shelf)

Plate 3.2B CA system used to deliver five different gas atmospheres, each to a series of four glass jars. Note premixed gas bottles in corner

Plate 3.2C Individual jars used to store pre-cut lettuce. Note silicon septa in lid and rubber O-ring used to obtain an air tight seal

Plate 3.2D Flow meters used to control flow rate of gases into jars. Note input gas line into rear of jar at bottom of picture. The premixed gases entered jars at the rear and then flowed over the lettuce before exiting through a fitting located in the side of the jar (depicted in plate 3.2F)

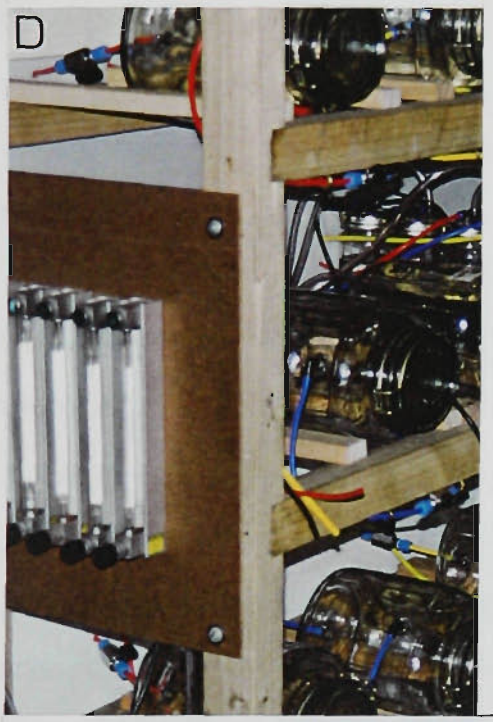
Plate 3.2E System used to measure the respiration rate of whole Romaine lettuce in air. Note input and output connections in lid and ball valves capable of being closed for respiration measurements

Plate 3.2F Output gas lines coming from individual jars. Note ball valves capable of being closed for respiration measurements. The small jars containing water in the centre of picture were used to humidify gas mixtures, before entering jars

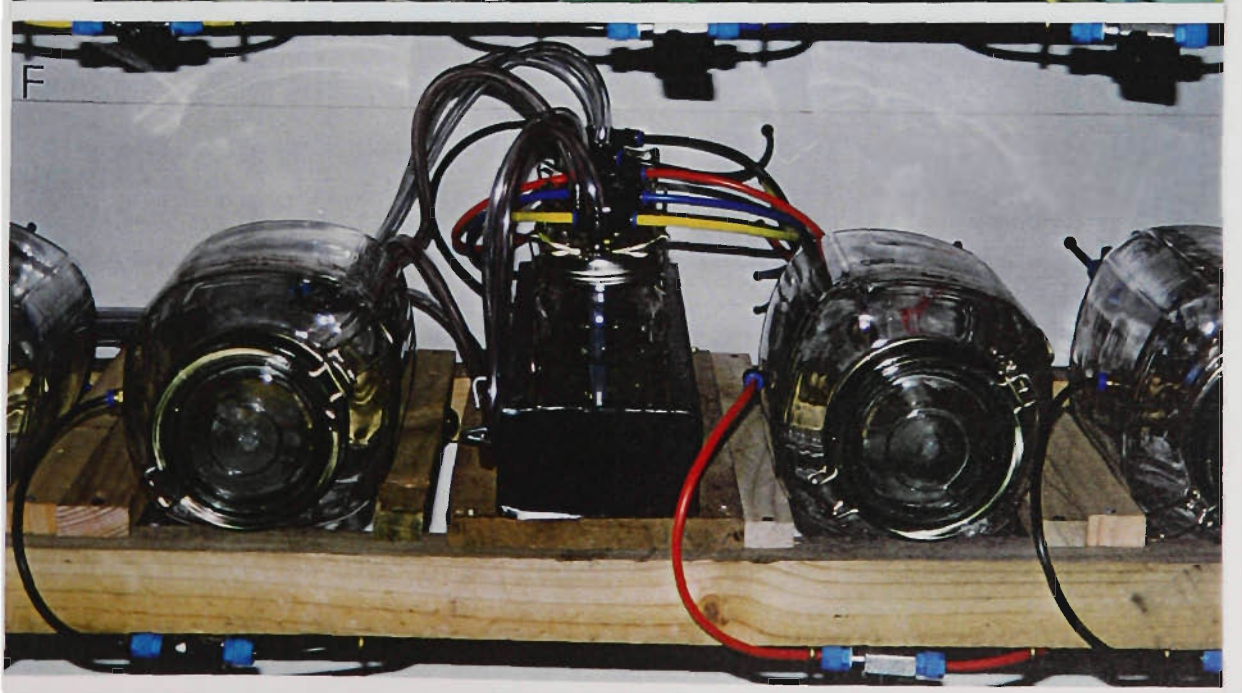

A


B


C


D


E


F

3.5 Respiration rate of whole Romaine lettuce

The respiration rate of whole Romaine heads in air (Chapter 7.2) was determined by measuring the change in CO_2 concentration (0.5-1.0% over 6 hours) within 9.0 L airtight Tupperware® containers as described in Section 3.6 (Plate 3.2e). A RH superior to 95% was maintained by bubbling air through water before entry into the containers. Gas samples were taken by closing inlet and outlet valves and removing a gas sample through a self-sealing septa located in the lid of each container.

3.6 Gas analysis

Gas samples for O_2 (2 mL) and CO_2 (1 mL) determinations within polymeric film packages were removed with gas tight syringes through septa secured to the packages. Self-adhesive septa were prepared by squeezing approximately 1 cm^3 of silicon rubber (Poly Silicone®, Poly Products Padstow NSW) onto 40 x 20mm pieces of plastic adhesive tape and curing at 25°C for at least 3 days. Gas samples were removed from CA units as described earlier.

Gas samples were analyzed for O_2 (Servomex® Paramagnetic O_2 Analyser, Series 1400, Servomex Co., Sussex, England) and CO_2 (ADC Analytical Infra Red CO_2 Analyser, Model 225, Analytical Development Co.) with N_2 as the carrier gas (Flow rate = 100 mL/min). Except where stated otherwise, results of gas analyses are expressed as mean concentrations of duplicate samples. Percentage composition was calculated from peak heights using external standards of reference gas mixtures (BOC Gases, Melbourne). The respiration rate of lettuce tissue was determined as follows;

$$\text{Respiration rate} = \frac{\%\ \text{change in } CO_2 \times 10 \times \text{Free Space Volume in Container (L*)}}{\text{Tissue weight (kg)} \times \text{Time (hr)}}$$

$$= \text{mL } CO_2\ kg^{-1}\ hr^{-1} \text{ (converted to mg } CO_2\ kg^{-1}\ hr^{-1} \text{ to remove the effect of temperature on the volume of gas)}$$

* Lettuce density of 0.89 g cm^{-3} (As quoted by Exama *et al.*, 1993)

3.7 Systems for assessing the quality of lettuce

3.7.1 Visual quality

External visual quality of both whole and minimally processed lettuce was assessed by using a system devised by Kader *et al.* (1973b). The scoring system was slightly modified to permit 1-5 scores based on presumed consumer acceptance and was related to factors such as amount of decay, wilting, mechanical damage and any other disorders/defects (Table 3.1). Lettuce tissue was considered unacceptable above a visual score of 3.

Table 3.1 System used to describe visual quality of lettuce

Score	*Visual quality description*
1	*Excellent*, essentially free from defects
2	*Good*, minor defects; not objectionable
3	*Fair*, slight to moderate objectionable defects, lower limit of sales appeal
4	*Poor*, excessive defects, beyond limit of saleability
5	*Extremely Poor*, not useable

3.7.2 Colour

A Minolta Chroma Meter (CR-200) was used to study the spectral qualities of lettuce during storage. The colorimeter was fitted with an 8mm diameter specimen port and used diffuse illumination and a 0° viewing angle to obtain values for L^* (100=white, 0=black), a^* ($-a^*$=green, $+a^*$=red), and b^* ($-b^*$=blue, $+b^*$= yellow). For measuring butt discolouration of whole lettuce heads, the instrument was calibrated with a standard white tile (CR-210; L^*=97.9, a^*= -0.49, b^*= +1.91) in the CIELAB mode and subsequent readings (Three/lettuce) were achieved by directly applying the colorimeter head to the butt surface. A pale green reference plate (CR-A47; L^*=69.17, a^*= -23.53, b^*= +24.88) was used to calibrate the colorimeter before recording leaf colour of shredded Crisphead and chopped Romaine lettuce.

Butt discolouration is reported in terms of the change in L^* over the storage period as this was found in preliminary experiments to be best correlated with darkening of stem tissue. For minimally processed tissues, leaf colour is reported in terms of hue angle ($\tan^{-1} b^*/a^*$). Hue

angle is related to the greenness/yellowness of the tissue and a decrease in hue angle corresponds to the colour changing from green to yellow (McGuire, 1992).

3.7.3 Amount of Decay

A rating scale, adapted from Kader *et al.* (1973b), was used during postharvest assessments to determine the amount of decay* on each lettuce head (Table 3.2)

Table 3.2 Rating scale for decay

Score	*Decay description*
1	*None*
2	*Slight,* slightly objectionable, may impair saleability
3	*Moderate,* objectionable, definitely impairs saleability
4	*Severe,* salvageable but not normally saleable
5	*Extreme,* not useable

* Due to the large number of lettuce involved, no attempt was made to identify the specific decay organism(s) that developed on lettuce.

3.7.4 Estimation of russet spotting development

A scoring system devised by Kader *et al.* (1973b) was used to determine the extent of russet spotting (RS) development. A RS index was estimated for each lettuce head. This index is a product of scores relating to average distribution per leaf, the number of affected leaves per head and the severity of individual lesions (Table 3.3)

Table 3.3 Basis for calculating a russet spotting index

Score	*Russet spotting index*[a]		
	Average distribution per leaf (P)	Number of affected leaves per head (N)	Severity of individual lesions (S)
1	0-25%	0-2	None
2	25-50%	3-5	Slight
3	51-75%	6-15	Moderate
4	>75%	>15	Severe
5			Extreme

[a] Overall RS Index = Score (P) x Score (N) x Score (S); Range = 0-80
For example, a lettuce head which contained on average 10% moderate RS per leaf on 4 leaves per head would have a RS Index of; 1 (Score P) x 2 (Score N) x 3 (Score S) = 6

3.7.5 Estimation of brown stain development

Table 3.4 shows a scoring system for brown stain (BS). This scoring system was introduced by Kader *et al.* (1973b) and is included here with slight modifications. A BS index was estimated for each lettuce head to describe the severity of BS and was based on the average distribution per leaf, number and degree of discolouration of the lesions.

Table 3.4 Basis for calculating a brown stain index

Score	*Brown stain index*[a]		
	Average distribution per leaf (P)	Number of affected leaves per head (N)	Degree of discolouration (D)
1	0-5%	0-2	None
2	6-15%	3-4	Slight
3	16-20%	5-6	Moderate
4	21-25%		Severe
5	26-35%		Extreme
6	>35%		

[a] Overall BS Index = Score (P) x Score (N) x Score (D); Range = 0-90

3.7.6 Head firmness

Head firmness of the lettuce was recorded before and after storage according to a scale developed by Kader *et al.* (1973b) (Table 3.5; Plate 3.3).

Table 3.5 Rating scale for head firmness

Score	*Firmness description*
1	*Soft*, easily compressed or spongy
2	*Fairly Firm*, neither soft nor firm, good head formation
3	*Firm*, compact but may yield slightly to moderate pressure
4	*Hard*, compact and solid
5	*Extra-hard*, over-mature, may have cracked mid-ribs

Plate 3.3 Rating scale for measuring head firmness*

*Refer to Table 3.5


5
4
3
2
1

3.7.7 Flavour

A rating scale of 1-5, adapted from Kader *et al.* (1973b) was used to describe the flavour of the lettuce after various types of storage (Table 3.6). As flavour is personal, the same person was used for all flavour assessments.

Table 3.6 Rating scale for flavour

Score	*Flavour description*
1	*Excellent,* very fresh flavour
2	*Good,* not objectionable
3	*Fair*, becoming objectionable
4	*Poor,* definitely objectionable
5	*Extremely Poor,* unable to be consumed

3.7.8 Amount of wilting

The amount of wilting on pre-cut tissues was scored on a 1-5 scale as described in Table 3.7.

Table 3.7 Rating scale for wilting

Score	*Wilting description*
1	*None*
2	*Slight,* not objectionable
3	*Moderate*, becoming objectionable
4	*Severe,* definitely objectionable
5	*Extreme,* not acceptable

3.7.9 Determining browning intensity

The amount of browning on shredded lettuce tissue was determined according to the method of Couture *et al.* (1993). Forty grams of lettuce tissue was homogenized with 40 mL water and the homogenate filtered through Whatman No. 1 filter paper and centrifuged at 25,000× g (Sorvall® RC-5B superspeed centrifuge) for 15 min. The supernatant was measured immediately for absorbance at 346nm with a spectrophotometer (GBC 911Δ UV-Visible Spectrophotometer) to estimate the degree of tissue browning. Preliminary experiments

showed that this wavelength gave the greatest difference in absorbance between brown and fresh tissue.

Because browning in Romaine lettuce was mainly confined to the cut midribs, insufficient sample material of this lettuce type meant that a different method for assessing the amount of browning on lettuce samples was required. The method used was a rating index, as detailed in Table 3.8.

Table 3.8 Rating scale for browning

Score	*Browning description*
1	*Absent*
2	*Slight*, not objectionable
3	*Moderate*, becoming objectionable
4	*Excessive*, objectionable
5	*Severe*, highly objectionable

3.7.10 Determination of chlorophyll content

For determining the chlorophyll content of pre-cut Romaine leaves, 2 mL of N,N-dimethylformamide (DMF) was added to aluminium foil-wrapped test tubes each containing one leaf disc (5% w/v). These discs were removed from leaf samples using a 12mm hole punch as described in Chapter 7.2. The test tubes were shaken for 24 h, then absorbance was measured spectrophotometrically (GBC 911Δ UV-Visible Spectrophotometer) at 647 nm and 664.5 nm with DMF as the reference blank (Moran and Porath, 1980). The extinction coefficient reported by Inskeep and Bloom (1985) was used for the quantification of total chlorophyll (Chl a + Chl b):

$$\text{Total Chlorophyll (mg/L)} = 17.90A_{647\ nm} + 8.08A_{664.5\ nm}$$

Where A = absorbance in 1.00 cm quartz cuvettes

3.7.11 Determination of fermentative volatiles

The concentrations of acetaldehyde and ethanol in pre-cut Romaine lettuce tissues were measured according to the method of Mateos *et al.* (1993a). On each sampling day, approximately 40g of fresh midrib tissue from each experimental unit was frozen in liquid N_2 and stored at -70°C until analyzed. Ten grams of midrib tissue was then homogenized at 0°C with a Polytron® tissue homogenizer in 15 mL water. The homogenate passed through filter paper (Whatman No. 1) and a 5 mL sample was placed in a 27 mL jar and hermetically sealed. After 1h incubation at 60°C (Ratek Instruments water bath), 1 mL headspace gas samples from each jar headspace were injected into a Shimadzu gas chromatograph (GC-14B) with a flame ionization detector (at 210°C) and a stainless steel column (3 mm x 1m) containing 100/120 Porapak PS (at 100°C). The injector temperature was 105°C and the carrier gas was N_2 at 100 kPa.

The amount of acetaldehyde and ethanol in the headspace of bags of pre-cut Romaine lettuce was determined by withdrawing a 1 mL headspace sample, through a self-sealing silicon septa adhered to the film, and injecting into a Shimadzu gas chromatograph (GC-14B) under the conditions described above.

3.7.12 Detection of off-odours

The presence or absence of off-odours from pre-cut lettuce was noted immediately after opening jars and recorded on a scale of 1-4 (Table 3.9).

Table 3.9 Rating scale for off-odours

Score	*Off-odour description*
1	*None*
2	*Slight*
3	*Moderate*
4	*Severe*

3.8 Statistics

All data* were analysed by analysis of variance (ANOVA) using the GENSTAT (version 5.0) statistical program (Payne *et al.*, 1989). The least significant difference comparing treatment

means (P=0.05) are presented as bars or numerical values in figures and tables. Differences between treatment means were only reported when they were significant at the P<0.05 level.

*Hue angle for Romaine lettuce (Section 7.3.1.2) was analysed by analysis of covariance using the initial hue angle as the covariate.

Chapter 4

Controlled atmosphere storage of whole lettuce

4.1 Introduction

Although considerable research has been conducted on CAS/MAP of whole Crisphead lettuce, most studies have been confined to cultivars grown overseas under a range of climates, cultural practices and farm management techniques. No research has been reported on CAS/MAP of lettuce cultivars grown under Australian conditions.

The literature suggests that low storage temperatures (0-2°C) combined with gas atmospheres of 2-5% O_2/0-4% CO_2 can extend the shelf-life of intact lettuce over storage in air (Singh *et al.*, 1972; Haginuma *et al.*, 1985) with optimal O_2 and CO_2 concentrations dependent on the individual produce and its storage temperature (Kader *et al.*, 1989).

The objective of the study reported in Chapter 4 was to establish whether or not CAS is useful in maintaining the post-harvest quality of Australian Crisphead lettuce cultivars compared with storage in air at 1.5°C. This temperature represents a realistic transit temperature that can be achieved and maintained in refrigerated (reefer) containers carrying whole lettuce by ship to South-East Asia. If Australian lettuce cultivars respond favourably to CAS, then knowledge of the optimal O_2 and CO_2 concentrations could be used to design MAP for cartons of bulk lettuce. This would enable export of whole lettuce by sea instead of costly airfreight.

The design of MAP requires knowledge of the product (optimum gas concentrations and respiration rates), the package (surface area, film thickness, and film permeability), and the main environmental factors, including storage temperature and RH (Song *et al.*, 1992).

The research into CAS of lettuce reported below involved three types of experiments:

(1) Experiment 1 aimed to identify the optimum O_2 and CO_2 concentrations that prolong the shelf-life of one Australian Crisphead cultivar (Greenway) and determine the effect of a number of different controlled atmospheres on the respiration rate of lettuce. This experiment

identified that a 5% O_2/5% CO_2 atmosphere was effective in maintaining the quality of cv. Greenway (see Section 4.2.1 below). Whether other lettuce cultivars respired differently under this particular gas atmosphere was not determined in this experimental series.

(2) Experiment 2 examined the effect of cultivar type on respiration rate of lettuce held under 5% O_2/5% CO_2. Respiration rates have previously been found to vary between cultivars and are affected by temperature (Robinson *et al.*, 1975). This experiment was necessary to determine if a different MA liner was needed for each cultivar.

(3) Experiment 3 tested the most effective controlled atmospheres identified in experiment 1 plus additional gas atmospheres on the shelf-life of two different cultivars to identify whether cultivars respond differently to CAS.

4.2 Experimental design and procedure

4.2.1 Experiment 1: Controlled atmosphere storage of cultivar Greenway

4.2.1.1 Plant Material

Commercially grown Crisphead lettuce (*Lactuca sativa* L. cv. Greenway) were used as the trial material for the experiment. Cultivar 'Greenway' is classed as a 'Vanguard' type of Crisphead lettuce with a firm, round, well covered head 15 cm (10-18cm) in diameter and weighing on average 886g (750-1150g) at maturity (Yates, 1990).

Lettuce were harvested, packed and transported by truck from Hay, New South Wales, to the Melbourne Wholesale Fruit and Vegetable Market on the 9th August 1993. The journey took approximately five hours and the lettuce were not refrigerated during this period. Twenty-four hours after harvest, the lettuce were transported by non-refrigerated truck to the Institute for Horticultural Development (IHD), Knoxfield, Victoria.

The average core temperature of the lettuce upon arrival was 13.2 ± 0.5°C. Each lettuce was trimmed and placed in a perforated bag as described in Section 3.1. A random subsample of 15 lettuce was used to record the number of leaves trimmed, weight of trimmings, initial visual quality and butt colour of the lettuce (For details refer to Section 3.7). On average, seven leaves weighing approximately 357g were trimmed off each lettuce prior to storage. After

trimming, the average weight, visual quality and butt colour of each lettuce was 877g, 1.4 and 74.64 respectively. All other sensory attributes were assigned a value of 1.0 (Section 3.7).

4.2.1.2 Storage conditions

Lettuce were stored for five weeks at 1.5° ± 0.5°C under five different controlled atmospheres (i.e. treatments 2-6 in Table 4.1) to simulate combined storage and transit conditions i.e. what would happen to the lettuce immediately after harvest. Establishment of CA conditions is described in Section 3.1. The control (treatment 1) consisted of lettuce stored in CA chambers at 1.5°C but continually flushed with air from the coolroom via an aquarium pump (Rena 301) at 100 L/h. Although the ethylene concentration in the coolroom was not measured, the likelihood of ethylene in the air from another source was low as no other type of vegetable was stored in the room. The experiment was randomized, with four replicates per treatment and each replicate consisting of one CA chamber containing 20 individually bagged lettuce. To establish lettuce weight at Day 0, five randomly selected lettuce from each CA chamber were weighed at the start of the trial (Figure 4.1).

The respiration rate of lettuce stored in air and under each CA treatment was determined on days 6, 10, 14, 17, 21 and 27 as detailed in Section 3.1.

Table 4.1 Experimental conditions for controlled atmosphere tests on cv. Greenway

Treatment	***Gas concentration***[a]	
	O_2 (%)	**CO_2 (%)**
1 (Control)	20.96	0.03
2	2.5	2.0
3	5.0	0*
4	5.0	5.0
5	10.0	5.0
6	10.0	10.0

[a] Balance of gas composition was N_2

* For treatment 3, 500g of hydrated lime was enclosed in a paper bag and placed in each chamber to eliminate any CO_2 in the atmosphere. Because the CO_2 was absorbed by the lime, the respiration rate of this treatment was not determined.

The RH levels within CA chambers was measured as described in Section 3.1 and ranged between 77% and 82% throughout the five week storage period. The variation in CA chamber RH between experiments 1, 2 and 3 made it difficult to compare weight loss between the same treatments in different experiments. Despite this, there were distinct trends in individual experiments.

4.2.1.3 Ex-store and ex-market assessments

After the simulated transit period at 1.5°C for five weeks (Section 4.2.1.2), the CA chambers were opened for ex-store assessment. The five heads previously weighed were reweighed to determine weight loss over the storage period (Figure 4.1). Lettuce were also evaluated for amount of butt discolouration, decay, russet spotting, brown stain, overall visual quality and flavour (As described in Section 3.7). These lettuce represented what the wholesaler or agent would see upon arrival after transport from site of production.

At the ex-store assessment, another five heads were randomly selected from each chamber to ascertain the weight of trimmings necessary for the lettuce to reach a marketable retail state. Most unwrapped lettuce is trimmed again at the wholesale or retail level to remove wrapper leaves and damaged or decayed leaves (Lipton and Ryder, 1989). The weight and butt colour were recorded for these lettuce and along with the remaining ten lettuce in each chamber, were transferred to air at 20° ± 1°C and 65 ± 5% RH for one day to simulate the market conditions common for Australia and South East Asia. The remaining procedure for evaluating the quality of lettuce over the ex-market period is summarised in Figure 4.1.

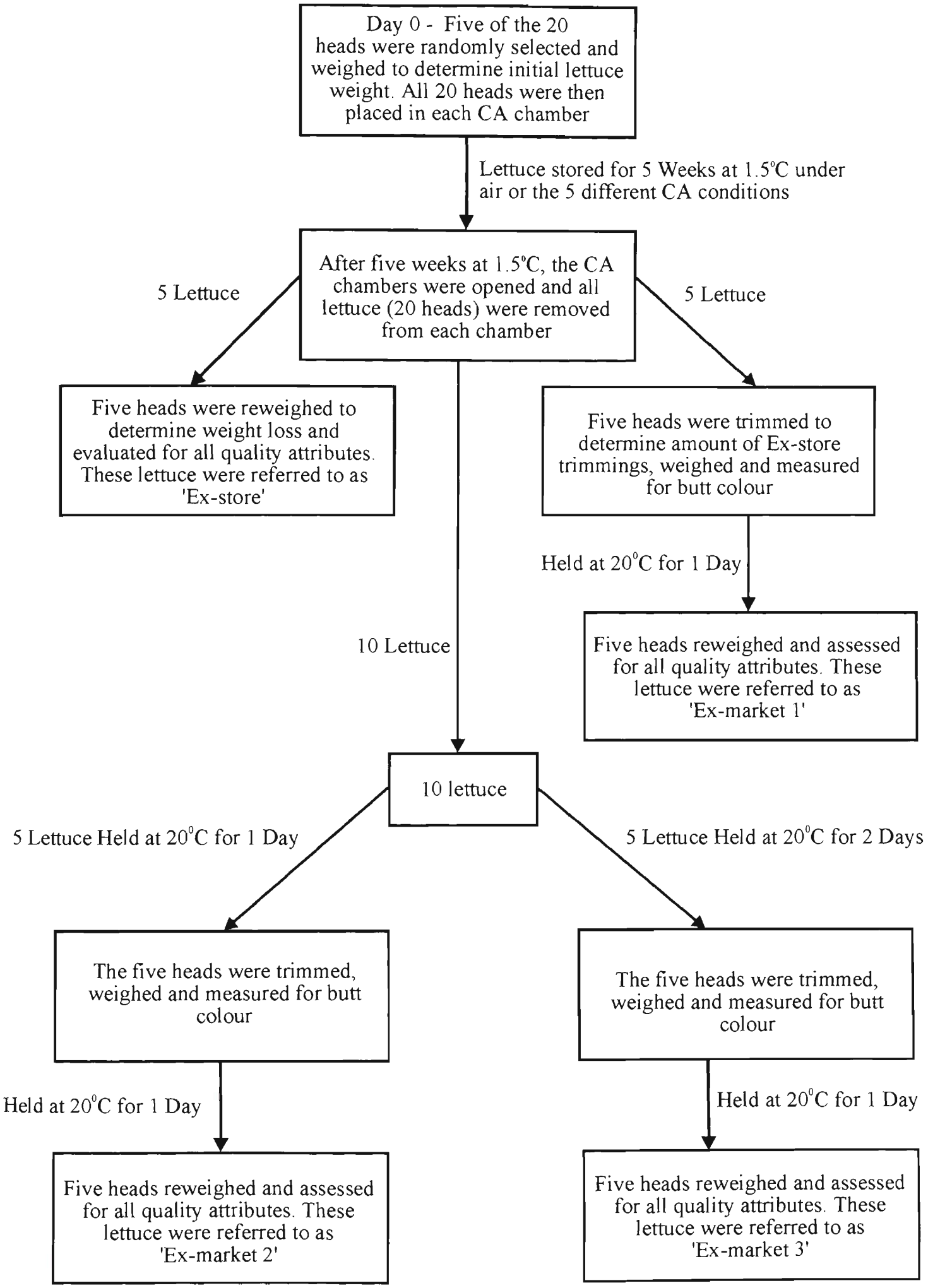


Figure 4.1 Procedure followed for analysing quality of Greenway lettuce in experiment 1

4.2.2 Experiment 2: Effect of cultivar on the respiration rate and quality of lettuce stored under a controlled atmosphere of 5% O_2/5% CO_2

4.2.2.1 Plant Material

Two commercial Crisphead cultivars (*Lactuca sativa* L. cvs. Salinas and Target) were used. Target is classed as a 'Vanguard' type of Crisphead lettuce with a firm, spherical, semi-covered head averaging 17 cm (17-19) in diameter and weighing on average 995g (800-1250) at maturity. It is medium maturing, taking approximately 53 days (52-57) to mature. Similarly, Salinas has a mean head weight of 951g (800-1150) at maturity, takes approximately 52 days (51-56) to mature and has an average head diameter of 16.8 cm (16-18) (Yates, 1988). Both are spring/summer cultivars suitable for the Melbourne region.

Lettuce were harvested, packed and then transported in an air-conditioned van from Cranbourne, Victoria to the IHD, Victoria on the 1st December 1993. The journey took approximately 30 minutes and the lettuce were not refrigerated during this period.

The core temperature of the lettuce upon arrival at IHD was 22° ± 0.5°C. This was because the lettuce were harvested on a particularly hot day (32°C) and not pre-cooled before dispatch. Lettuce were pre-cooled at IHD using conventional room cooling to 10°C before the experiment commenced. Precooling took approximately 2 hours. Each head was trimmed, weighed and placed in a perforated bag as described in Section 3.1. Lettuce were designated an initial visual score of 1.0 (Section 3.7).

The CA flow-through system described in Section 3.2 was used to produce a 5% O_2/5% CO_2 atmosphere to each of eight CA chambers. Four of these chambers, each considered a replicate, contained cv. Salinas, whilst the other four contained cv. Target. Twelve individually bagged lettuce heads were held within each CA chamber.

The lettuce were stored for five weeks at 1.5° ± 0.5°C. During this time, the RH was maintained between 91 and 95% (Refer to Section 3.1 for measurement of RH). The respiration rate of lettuce held within each CA chamber was determined on days 2, 5, 13, 16, 22, 27, 30 and 33 as described in Section 3.1.

After five weeks storage, each head was reweighed to determine any weight loss during storage. A visual score to determine consumer acceptability (Refer to Section 3.7) was also given to each head (not including butt discolouration).

4.2.3 Experiment 3: Controlled atmosphere storage of cultivars Salinas and Target

4.2.3.1 Plant Material

Two commercial Crisphead cultivars (*Lactuca sativa* L. cvs. Salinas and Target) were used. The lettuce were harvested from Cranbourne, Victoria on the morning of the 7th December, 1993 and transported by non-refrigerated truck directly to the IHD, Victoria. The lettuce were not precooled before delivery. The average core temperature of the lettuce upon arrival was 11.7° ± 0.5°C. Each lettuce was trimmed and placed in a perforated bag as described in Section 3.1. A random subsample of 10 lettuce of each variety was used to record the number of leaves trimmed, weight of trimmings, initial visual quality and butt colour of the lettuce (Details of assessment described in Section 3.1).

For cultivar Salinas, on average five leaves weighing approximately 250g were trimmed off each lettuce prior to storage. After trimming, the average weight, visual quality, head firmness and butt colour of each lettuce was 909g, 1.0, 4.0 and 59.02 respectively. For cultivar Target, four leaves weighing approximately 227g were trimmed off each lettuce prior to storage. After trimming, the average weight, visual quality, head firmness and butt colour of each lettuce was 928g, 1.0, 4.0 and 62.24 respectively. All other sensory attributes were assigned a value of 1.0 (Section 3.7).

4.2.3.2 Storage conditions

Lettuce were stored for five weeks at 1.5° ± 0.5°C under four different controlled atmospheres (i.e. treatments 2-5 in Table 4.2) to simulate transit conditions. These gas atmospheres were similar to those trialed with cultivar Greenway in Experiment 1 and were repeated to see if cultivars Salinas and Target responded differently to these gas regimes. Experiment 1 showed that increased CO_2 levels (5%) were effective in inhibiting butt discolouration (Refer to Section 4.3.1.2). For this reason, lettuce were stored in 5% O_2/2% CO_2 to test if this CO_2 level delayed butt discolouration. Furthermore, 7.5% O_2/5% CO_2 was trialed to determine whether or not this atmosphere was beneficial in maintaining lettuce quality. Establishment of

CA conditions is described in Section 3.1. The control (treatment 1) consisted of lettuce stored in CA chambers at 1.5°C but continually flushed with air via an aquarium pump (Rena 301) at 100 L/h. Each CA chamber contained eight heads of each variety which were kept physically separate within the chamber but with a shared atmosphere. Results of previous experiments (Section 4.3.2.1) showed that the respiration rate of these two cultivars was generally not significantly different under CAS. The experiment was completely randomized, with five replications per treatment and each replicate consisting of one CA chamber. Four lettuce of each variety from each chamber were weighed at the start of the trial (Figure 4.2).

The RH levels within CA chambers was measured as described in Section 3.1 and ranged between 80% and 85% throughout the five week storage period.

Table 4.2 Experimental conditions for controlled atmosphere tests on cvs. Salinas and Target

Treatment	***Gas concentration***[a]	
	O_2 (%)	**CO_2 (%)**
1 (Control)	20.96	0.03
2	2.5	2.0
3	5.0	2.0
4	5.0	5.0
5	7.5	5.0

[a] Balance of gas composition was N_2

After the simulated transit period at 1.5°C for five weeks (i.e. at Ex-store assessment), the CA chambers were opened and the four heads of each cultivar previously weighed were reweighed to determine weight loss over the storage period (Figure 4.2). Lettuce were also evaluated for amount of butt discolouration, decay, russet spotting, brown stain, overall visual quality, head firmness and flavour (as described in Section 3.7). These lettuce represented what the wholesaler or agent would see upon arrival.

After the ex-store assessment, the remaining four heads of each cultivar were trimmed to ascertain the weight of trimmings necessary for the lettuce to reach a marketable retail state. These lettuce were then transferred to air at 20° ± 1°C and 65 ± 5% RH for two days to simulate the market conditions in Australia and South East Asia. After two days at 20°C (Ex-market) the lettuce were evaluated as with the ex-store assessment.

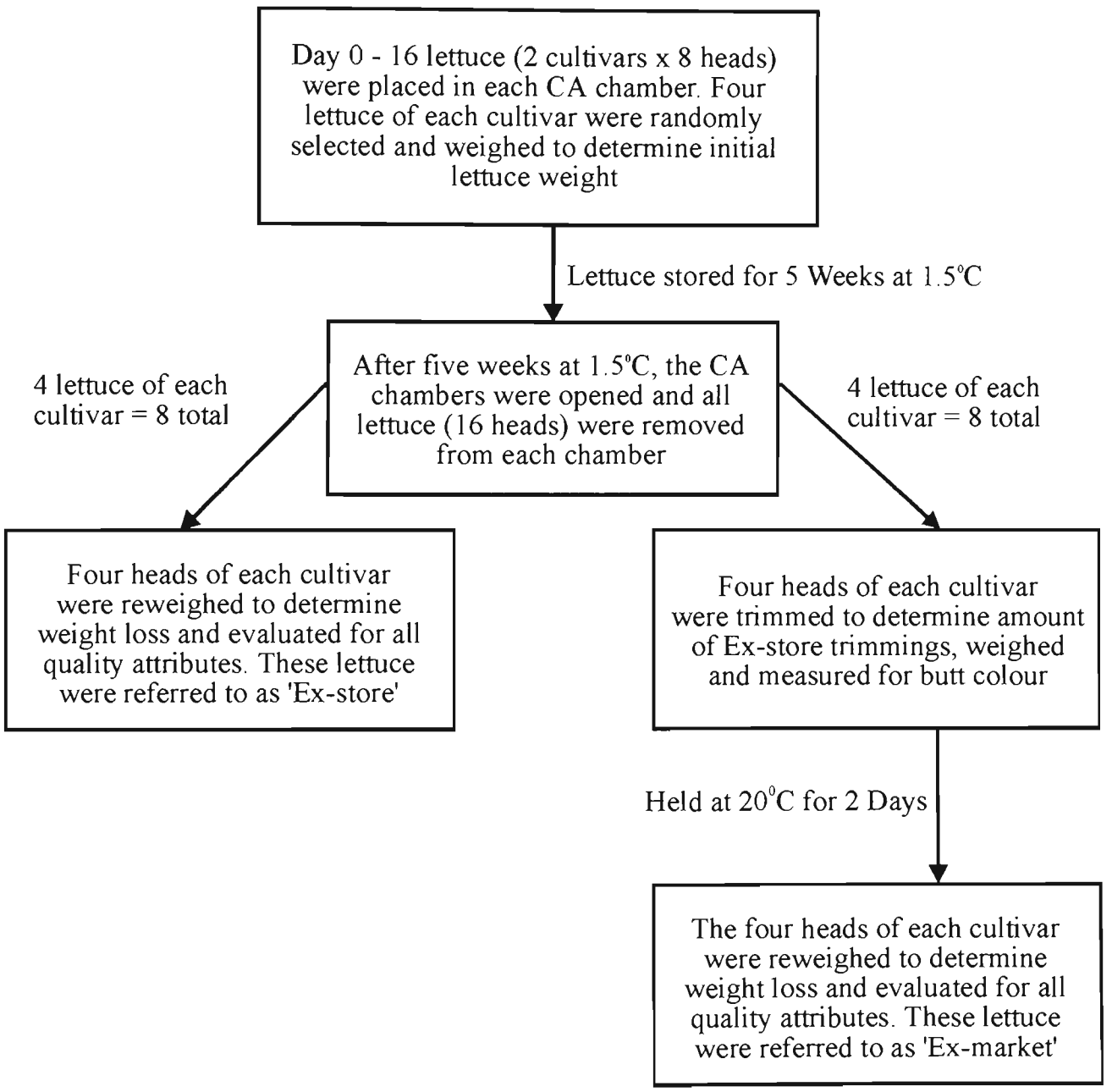


Figure 4.2 Procedure followed for analysing quality of cvs. Salinas and Target lettuce in experiment 3

4.3 Results

4.3.1 Experiment 1: Controlled atmosphere storage of cultivar Greenway

4.3.1.1 Respiration rate of cv. Greenway under air and CAS

The respiration rate of lettuce cv. Greenway stored under four controlled atmospheres and air is presented in Figure 4.3. Respiration measurements commenced on day six, allowing time for a stable CA to establish in each storage chamber. During days six to ten, all CA treatments had a significantly lower respiration rate than the control (treatment 1). Of the CA combinations trialed during this time, treatment 5 appeared to reduce the respiration rate of lettuce the least, being significantly higher than treatments 2 and 4 on day six and treatments 2, 4 and 6 on day ten (Note: 'treatment' refers to lettuce subjected to a particular gas atmosphere). The respiration rate of treatment 5 on day 27 was 3.3 mg CO_2 kg^{-1} hr^{-1}, significantly higher than for treatments 2 and 4 at the same time period (Reminder: respiration was not measured in treatment 3 as this contained CO_2-absorbing lime).

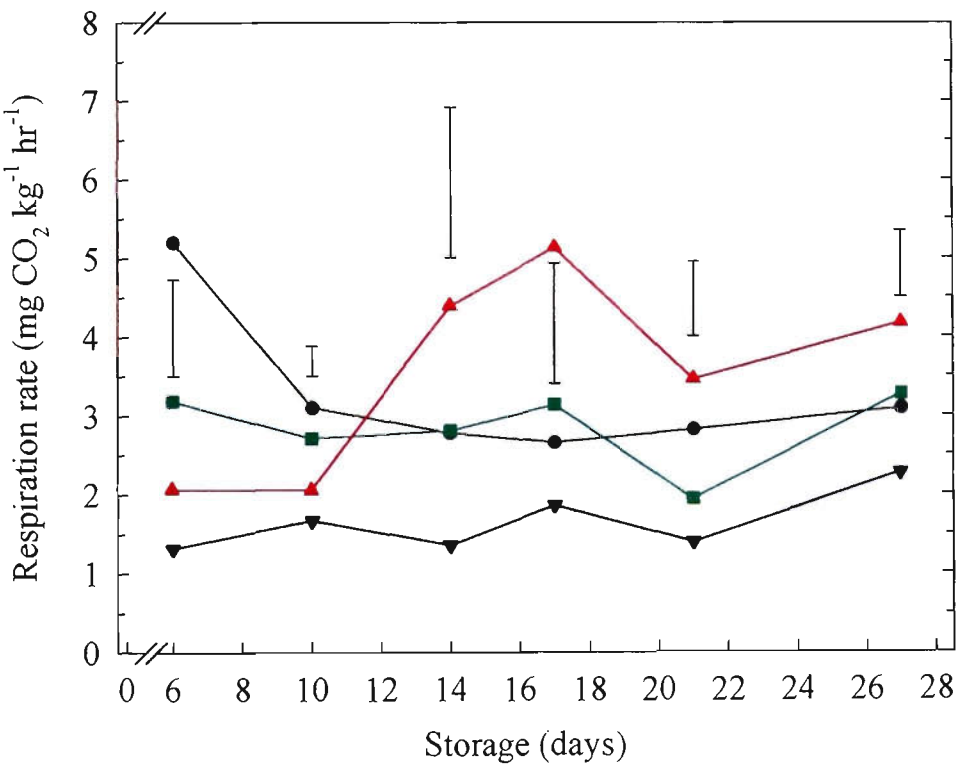


Figure 4.3 Effect of storage atmosphere on the respiration rate of lettuce cv. Greenway. Vertical bars = LSD (P=0.05) (Trt 1; Trt 2; Trt 4; Trt 5; Trt 6)

Trt 1 = Air (Control)	Trt 2 = 2.5% O_2/2% CO_2	Trt 3 = 5% O_2/0% CO_2
Trt 4 = 5% O_2/5% CO_2	Trt 5 = 10% O_2/5% CO_2	Trt 6 =10% O_2/10% CO_2

Although the respiration rate of lettuce in treatment 6 was relatively low initially, it began to rise after 10 days storage and became significantly higher than tissue in treatments 2 and 4 for the duration of the experiment. Lettuce in treatment 6 also had a significantly higher respiration rate than treatment 5 on days 17 and 21 and the control on days 17 and 27 and in general increased the respiration of whole lettuce heads.

Treatments 2 and 4, both containing tissue with respiration rates of approximately 2 mg CO_2 kg^{-1} hr^{-1}, were the most effective of the CA treatments in lowering product metabolism. Both treatments had a significantly slower respiration rate than the control on days 6, 10 and 21, with treatment 2 also lower than the control on day 27.

4.3.1.2 Amount of butt discolouration on cv. Greenway under air and CAS

After five weeks storage at 1.5°C (Ex-store), treatments 4, 5 and 6 had less butt discolouration than the control (treatment 1) or other CA treatments, as evidenced by a smaller change in L^* value (Figure 4.4). Of these, treatment 5 was significantly less discoloured than treatments 4 and 6.

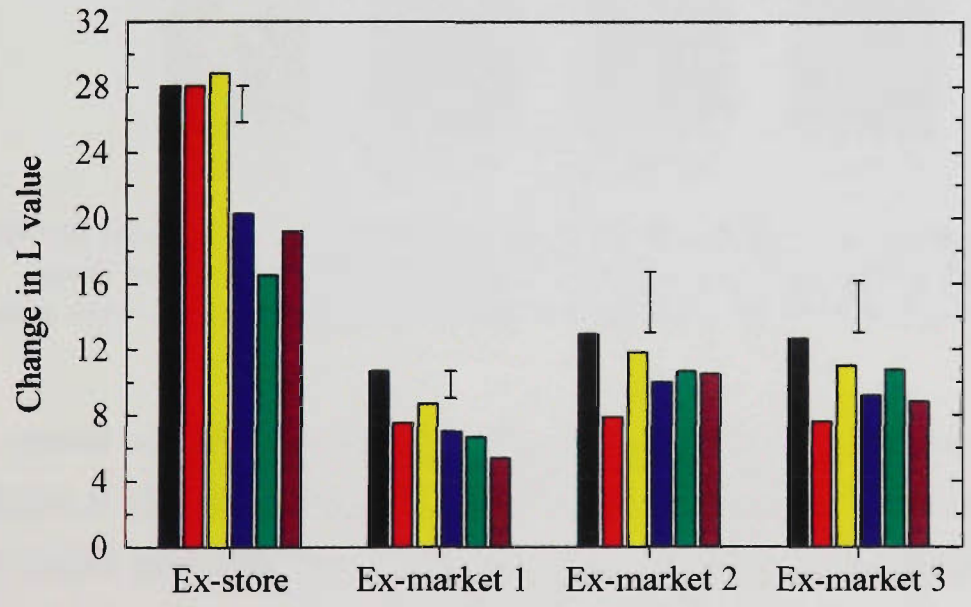


Figure 4.4 The effect of storage atmosphere on the amount of butt discolouration on lettuce cv. Greenway stored for 5 weeks at 1.5°C followed by one, two or three days at 20°C. Vertical bars = LSD (P=0.05) (■ Trt 1;■ Trt 2;□ Trt 3;■ Trt 4;■ Trt 5;■ Trt 6)

After storage in air for one day at 20°C (Ex-market 1), all CA treatments discoloured less than the control. Treatments 4 and 5 discoloured less than treatment 3, whilst treatment 6 stayed

significantly less discoloured than treatments 2 and 3, indicating that a high CO_2 environment may inhibit butt discolouration. This effect was less noticeable as the ex-market period was extended. After two and three days at 20°C (Ex-market 2 and Ex-market 3 respectively), treatment 2 was less discoloured than either the control or treatment 3. The change in L^* value was also significantly smaller in treatments 2, 4 and 6 than in the control after three days at 20°C.

4.3.1.3 Amount of decay on cv. Greenway under air and CAS

When compared to the control, CAS did not significantly alter the amount of decay present on lettuce after five weeks storage (Ex-store; Figure 4.5).

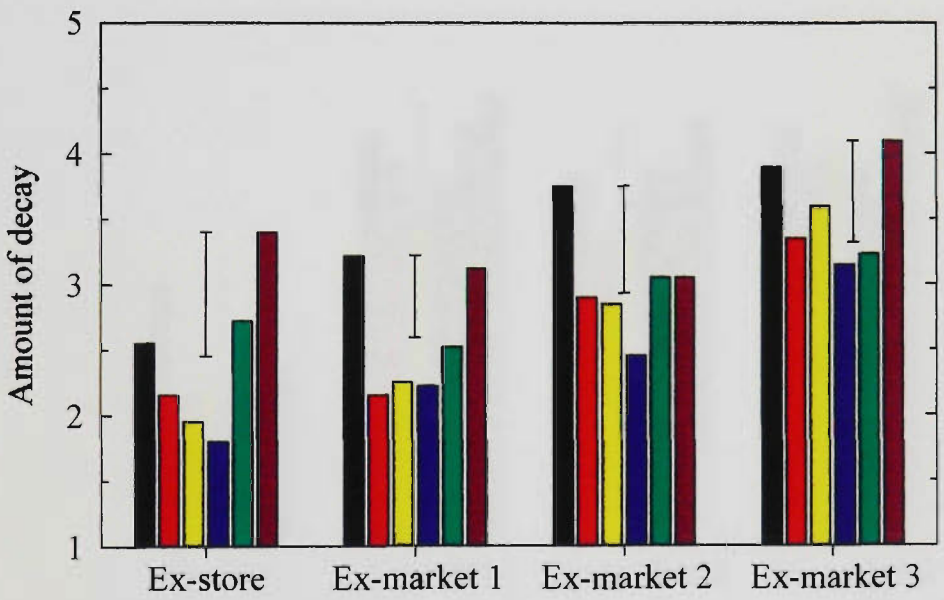


Figure 4.5 The effect of storage atmosphere on the amount of decay on lettuce cv. Greenway stored for 5 weeks at 1.5°C followed by one, two or three days at 20°C.
Vertical bars = LSD (P=0.05) (■ Trt 1; ■ Trt 2; ■ Trt 3; ■ Trt 4; ■ Trt 5; ■ Trt 6)

This is in contrast to results obtained during the ex-market period where treatments 2, 3, 4 and 5 after one day at 20°C and treatments 2, 3 and 4 after two days at 20°C had significantly less decay than lettuce previously stored in air. Despite this, there was no significant difference between CA treatments and the control after three days of the simulated marketing period, when all treatments showed considerable decay. Of the CA treatments trialed, treatment 6 appeared to be least effective at inhibiting decay forming organisms, as evidenced by the presence of significantly more decay than for treatments 2, 3 and 4 at the ex-store and ex-market 1 assessments and significantly more than treatments 4 and 5 after 3 days at 20°C.

4.3.1.4 Weight loss of cv. Greenway under air and CAS

During the five week storage period, lettuce held in air (control) lost approximately 8g/head, approximately double that of lettuce held under any of the controlled atmospheres (Figure 4.6). This figure represented approximately 1% of the initial weight. No significant difference in weight loss was noted between CA treatments at the ex-store assessment.

Weight loss increased dramatically when lettuce were stored in air at 20°C during ex-market trials. Weight loss was high for most treatments and after 1 day at 20°C there was no significant difference in weight loss between CA treatments and the control. Within the CA combinations, treatment 3 lost significantly less weight than treatment 6.

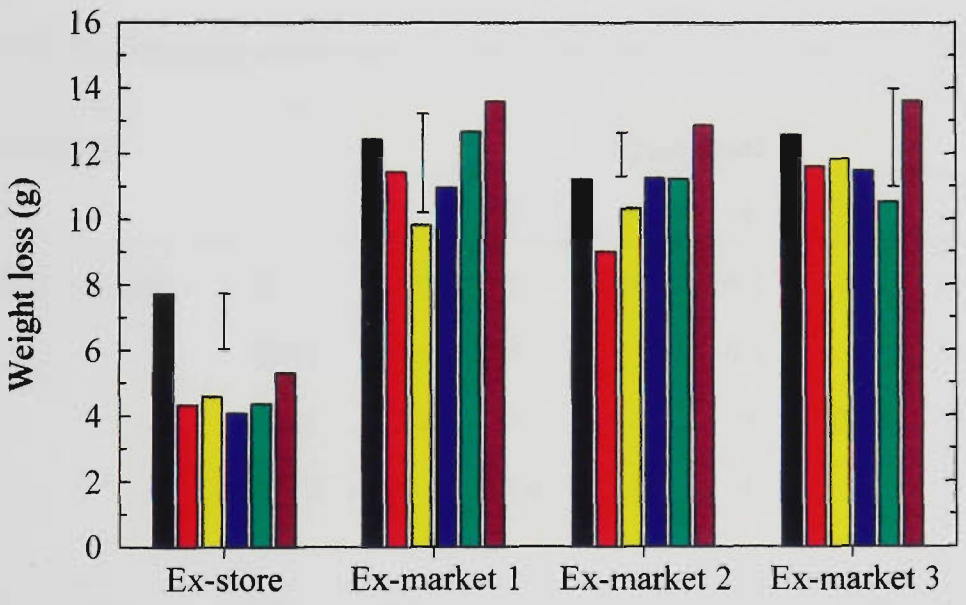


Figure 4.6 The effect of storage atmosphere on the amount of weight loss of lettuce cv. Greenway stored for 5 weeks at 1.5°C followed by one, two or three days at 20°C. Vertical bars = LSD (P=0.05) (■ Trt 1;■ Trt 2;□ Trt 3;■ Trt 4;■ Trt 5;■ Trt 6)

After two days storage at 20°C, treatment 2 had the lowest recorded weight loss, being significantly smaller than the control and treatments 4, 5 and 6. Treatments 4 and 5 lost about the same amount of weight as the control, but less than treatment 6. At the ex-market 3 assessment, CA treatments were not significantly different from the control; however, treatment 5 lost significantly less weight than treatment 6.

4.3.1.5 Russet spotting and brown stain on cv. Greenway under air and CAS

The low russet spotting (RS) scores evident for all treatments in Table 4.3, indicate that RS was not a major postharvest disorder in this experiment. Because scores were all less than

two, the significant differences noted between treatments at the ex-store assessment do not warrant further clarification.

On the other hand, a high CO_2 storage environment, as reflected in treatments 5 and 6, induced objectionable amounts of brown stain (BS) on lettuce tissue (Table 4.3). Although not significant after five weeks storage at 1.5°C (Ex-store), BS became more prevalent in these treatments during the ex-market assessments. This disorder was most noticeable in treatment 6 which had a BS index of 20.67 and 15.62 after storage at 20°C for one and two days respectively, significantly higher than for any other treatment. This index decreased to 5.97 at the end of the storage period, still higher than treatments 1-4.

Table 4.3 Effect of storage atmosphere on the quality attributes of lettuce cv. Greenway.

Quality attribute		***Treatment***						***LSD***
		1	2	3	4	5	6	$P = 0.05$
Russet spotting index	ES	0.25	0	0	0.1	0.6	0.25	0.49
	EM1	1.06	0	0	0.1	0	0	1.43
	EM2	1.50	0	0	0	0.08	0.14	2.01
	EM3	0	0.10	0.15	0	0	0.42	0.49
Brown stain index	ES	0	0	0	0.3	1.28	0.55	1.32
	EM1	0	0	0	0.75	2.07	20.67	3.04
	EM2	0.04	0.75	0	0.6	4.75	15.62	5.95
	EM3	0	0.02	0	0.81	5.03	5.97	3.81
Amount of trimming (g)	ES	107.1	103.5	109.4	116.9	83.1	106.6	42.2
	EM1	148.0	138.9	128.4	134.5	118.0	128.0	47.7
	EM2	147.8	122.4	141.6	150.5	119.6	117.7	37.17
Flavour	ES	3	2.17	2.42	2	2.7	3.92	0.59
	EM1	2.92	2.58	2.42	2.33	2.87	3.76	0.51
	EM2	3.42	2.25	2.75	2.42	2.62	4.51	0.64
	EM3	3.25	2.94	2.92	2.33	2.77	4.16	0.75

ES = Ex-store; EM1 = Ex-market 1; EM2 = Ex-market 2; EM3 = Ex-market 3

Treatment 5, with a BS index of 5.03 after three days storage at 20°C, also had significantly more BS than for treatments 1-4.

4.3.1.6 Amount of trimming required on cv. Greenway

The amount of trimming necessary for heads to reach a marketable state was not significantly different between CA treatments and the control throughout the trial (Table 4.3).

4.3.1.7 Flavour of cv. Greenway under air and CAS

Controlled atmosphere storage had mixed results on the flavour of lettuce tissue (Table 4.3). At the ex-store assessment, treatments 2 and 4 tasted better than the control, with treatment 4 also tasting better than treatment 5. The superior flavour of tissue under treatment 4 also carried over to the ex-market period, tasting better than treatment 5 after one day at 20°C and better than the control on all three sampling days. Except for lettuce held for two days at 20°C, where treatments 2, 3 and 5 tasted significantly better than the control, no difference in flavour was noted between these CA treatments and the control during the marketing period. Of the CA treatments trialed, treatment 6 tasted consistently bitter and had an inferior flavour compared with other treatments throughout the experiment.

4.3.1.8 Overall visual quality of cv. Greenway under air and CAS

The control produced an average overall visual quality of 3 after five weeks storage at 1.5°C, whereas treatments 2, 3 and 4 produced lower average scores. However, the large variability in numeric scores within treatments meant that there was no significant difference between CA treatments and the control at this time period (Figure 4.7). Despite this, treatments 2, 3 and 4 were more visually appealing than treatment 6 at the ex-store assessment.

After one day of the simulated marketing period, treatments 2, 3, 4 and 5 were considered to have a significantly better appearance than the control. The control, with a score of 3.79 was considered to be unmarketable from this point on. Treatment 6 was also considered unmarketable at the ex-market 1 assessment, being significantly worse than all other treatments.

Despite treatments 2, 3 and 4 appearing better than the control at the ex-market 2 assessment, the visual quality of these CA combinations was becoming objectionable. Treatment 4 was

significantly better than treatments 5 and 6 which were not acceptable. All treatments were considered unusable at the end of the simulated marketing period.

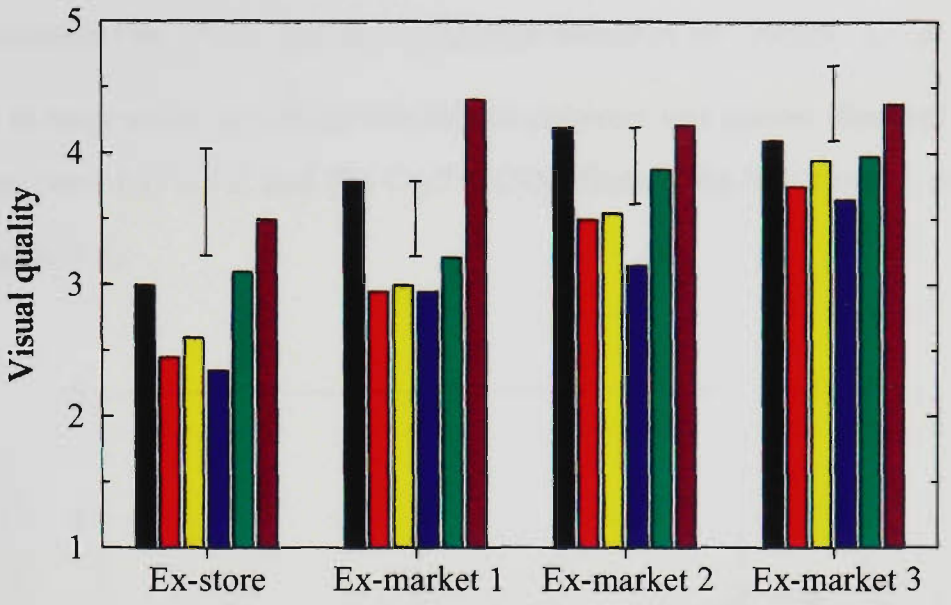


Figure 4.7 Effect of storage atmosphere on the visual quality of lettuce cv. Greenway stored for 5 weeks at 1.5°C followed by one, two or three days at 20°C.
Vertical bars = LSD (P=0.05) (■ Trt 1;■ Trt 2;□ Trt 3;■ Trt 4;■ Trt 5;■ Trt 6)

4.3.2 Experiment 2: Effect of cultivar on the respiration rate and quality of lettuce stored under a controlled atmosphere of 5% O_2/5% CO_2

4.3.2.1 Respiration rate of cvs. Salinas and Target stored in 5% O_2/5% CO_2 at 1.5°C

The pattern in respiration rate of the two lettuce cultivars was almost identical during the five week storage period at 1.5°C and 5% O_2/5% CO_2, fluctuating between 1.5 and 3.5 mg CO_2 kg^{-1} hr^{-1} (Figure 4.8).

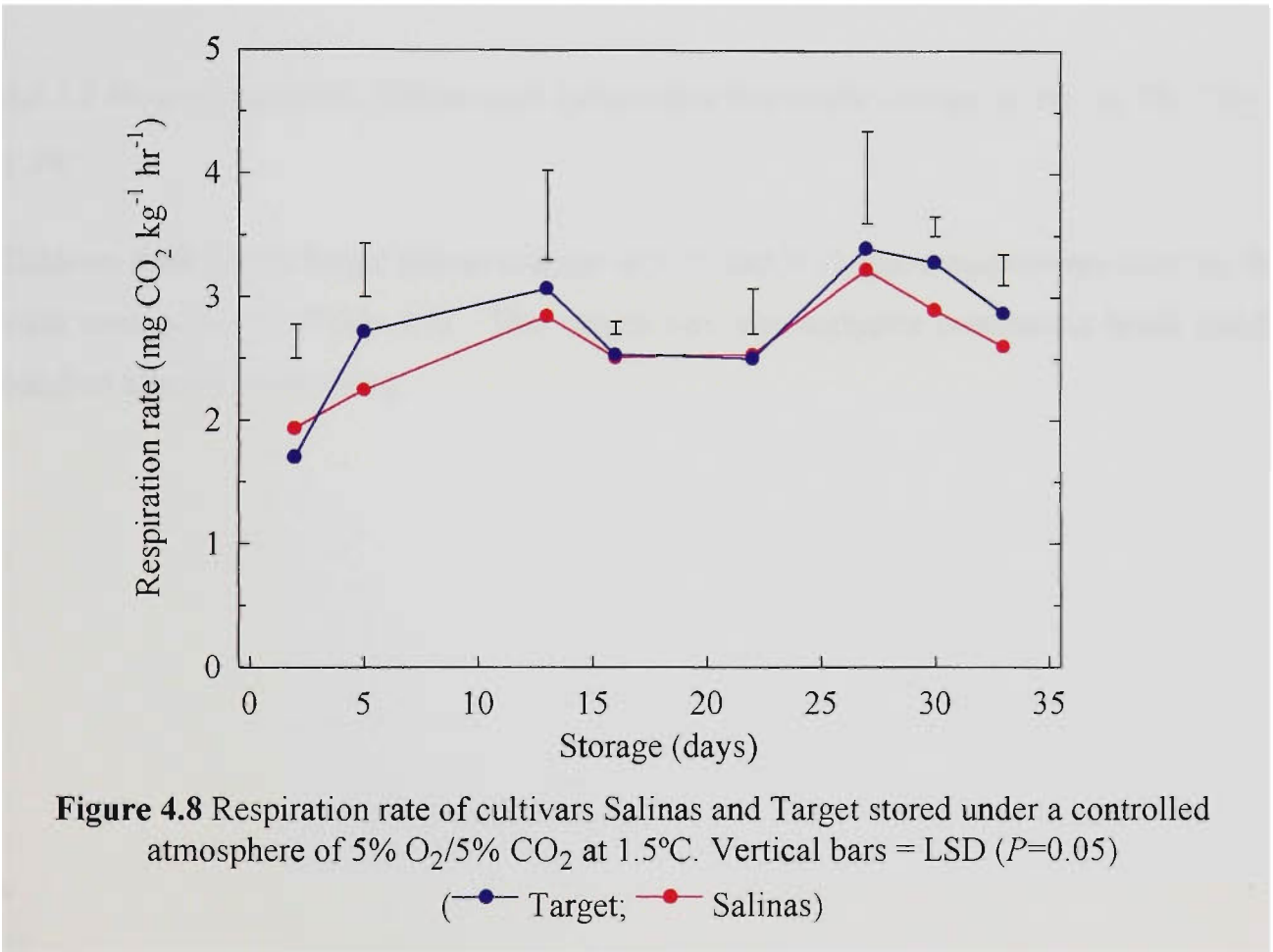


Figure 4.8 Respiration rate of cultivars Salinas and Target stored under a controlled atmosphere of 5% O_2/5% CO_2 at 1.5°C. Vertical bars = LSD (P=0.05) (Target; Salinas)

Generally speaking, there was an upward trend in the respiration rate of both cultivars with time. Cultivar Salinas, the smaller of the two cultivars, had a significantly lower respiration rate than cultivar Target on days 5, 30 and 33.

4.3.2.2 Visual quality of cvs. Salinas and Target after five weeks storage in 5% O_2/5% CO_2 at 1.5°C

After five weeks storage at 1.5°C, Salinas and Target had an external visual quality of 1.83 and 1.75 respectively (Table 4.4; Plate 4.1 a,b,c). In other words, they were rated between excellent and good. There was no significant difference in quality between the two cultivars.

Table 4.4 Effect of cultivar on the visual quality and weight loss of two lettuce cultivars stored for five weeks under 5% O_2/5% CO_2 at 1.5°C.

Cultivar	*Quality attributes*	
	Visual quality	**Weight loss (g)**
Salinas	1.83	5.76
Target	1.75	6.32
LSD at $P = 0.05$	0.42	2.71

4.3.2.3 Weight loss of cvs. Salinas and Target after five weeks storage in 5% O_2/5% CO_2 at 1.5°C

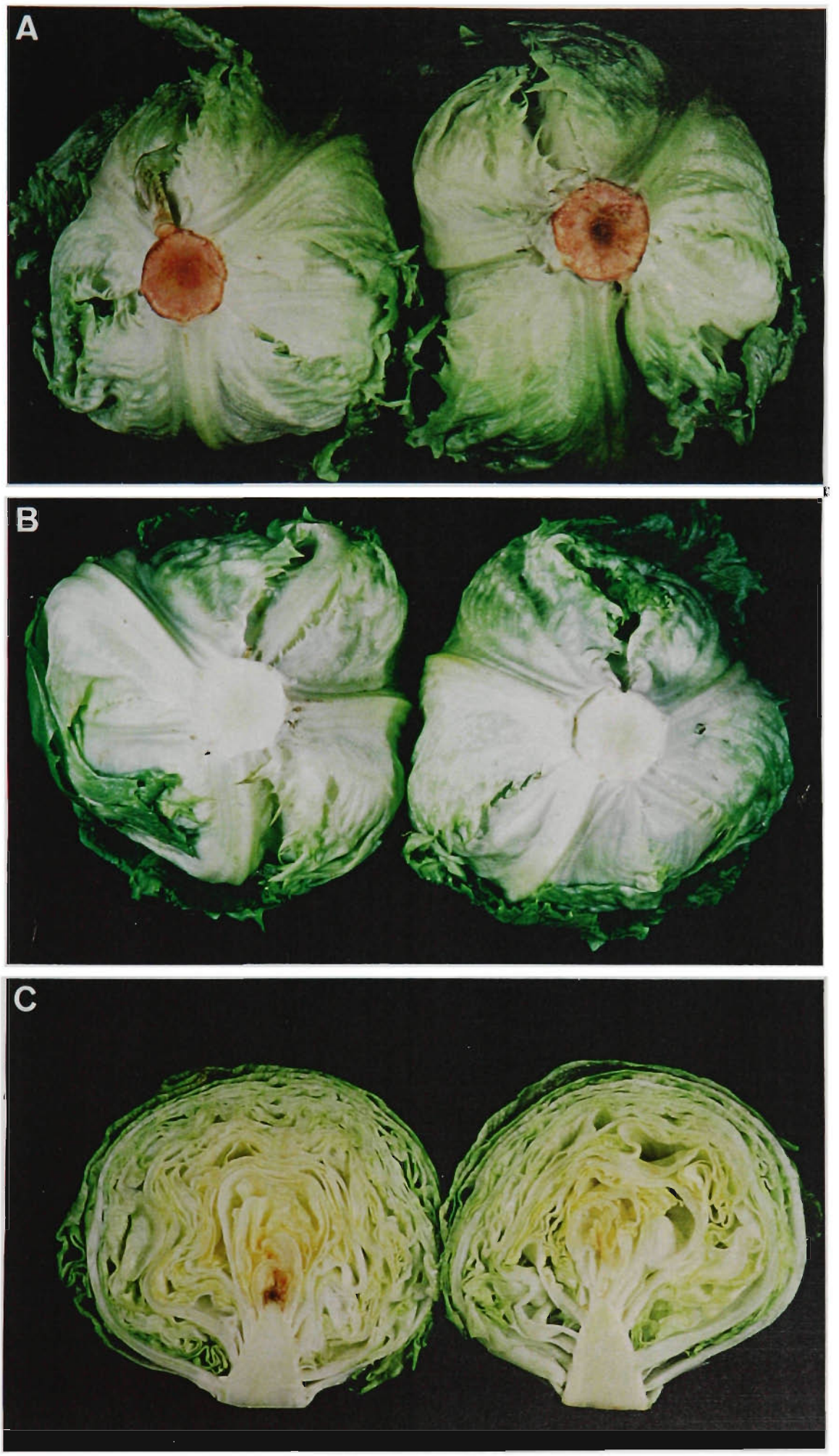
Cultivars Salinas and Target lost an average of 5.76 and 6.32 grams respectively over the five week storage period (Table 4.4). This weight loss was negligible considering heads initially weighed approximately 900 g.

Plate 4.1: Quality of cultivars Salinas and Target after five weeks storage at 1.5°C under 5% O_2/5% CO_2

Plate 4.1A Quality of Salinas (left) and Target (right) after five weeks storage at 1.5°C under 5% O_2/5% CO_2

Plate 4.1B Quality of Salinas (left) and Target (right) following trimming after five weeks storage at 1.5°C under 5% O_2/5% CO_2

Plate 4.1C Internal quality of Salinas (left) and Target (right) after five weeks storage at 1.5°C under 5% O_2/5% CO_2. The brown area at the stem apex of the Salinas lettuce was not a recognisable disorder.


A
B
C

4.3.3 Experiment 3: Controlled atmosphere storage of cultivars Salinas and Target

4.3.3.1 Amount of butt discolouration on lettuce held under air or CAS

After five weeks simulated transport (Ex-store), CAS did not significantly affect the amount of butt discolouration recorded on cultivars Salinas and Target when compared to the control (Treatment 1; Figure 4.9). Furthermore, no difference was noted in the change in L^* values between CA treatments at this time period for both cultivars.

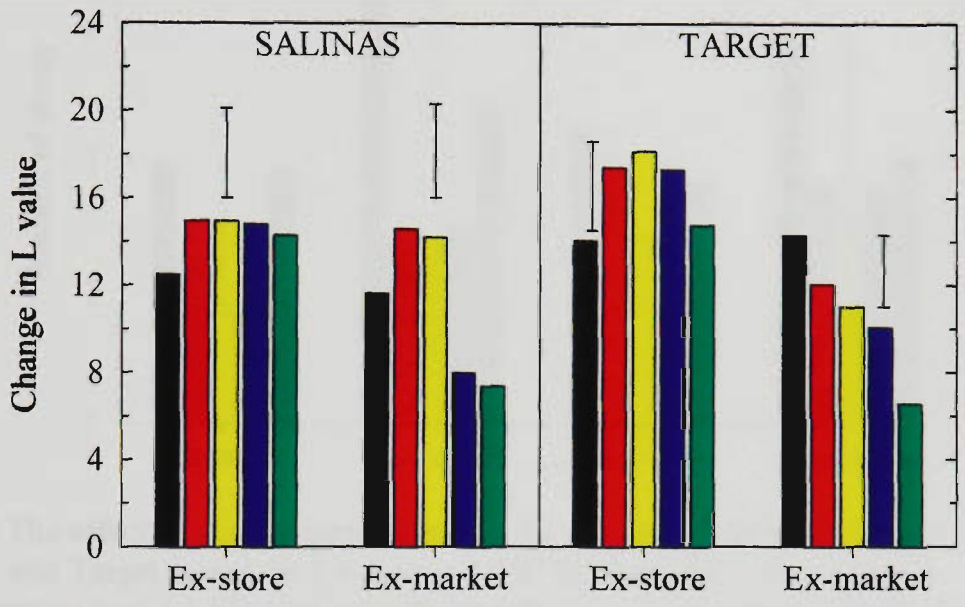


Figure 4.9 The effect of storage atmosphere on the amount of butt discolouration on lettuce cvs. Salinas and Target stored for 5 weeks at 1.5°C followed by 2 days at 20°C. Vertical bars = LSD (P=0.05) (■ Trt 1;■ Trt 2;□ Trt 3;■ Trt 4;■ Trt 5)

Trt 1 = Air (Control)	Trt 2 = 2.5% O_2/2.0% CO_2	Trt 3 = 5% O_2/2% CO_2
Trt 4 = 5% O_2/5% CO_2	Trt 5 = 7.5% O_2/5% CO_2	

However, after a two day marketing period (Ex-market), Salinas lettuce previously held in treatments 4 or 5 had significantly less butt discolouration than treatments 2 and 3. Likewise, Target lettuce held in treatments 4 and 5 were less discoloured than the control, with treatment 5 also significantly less discoloured than treatments 2, 3 and 4. These results suggest that a high CO_2 environment may be responsible for inhibiting butt discolouration, and are consistent with results for cultivar Greenway outlined in Section 4.3.1. There appeared to be a cultivar effect during the marketing period, with treatment 3 of Salinas significantly more discoloured than treatment 3 of Target (lsd=3.04).

4.3.3.2 Amount of decay on lettuce held under air or CAS

For cultivar Salinas, treatments 3 and 4 had significantly less decay than either the control or treatment 5 after five weeks storage at 1.5°C (Figure 4.10). At the ex-market assessment, all CA treatments had less decay than the control. Treatments 2, 3 and 4 were also significantly less decayed than treatment 5.

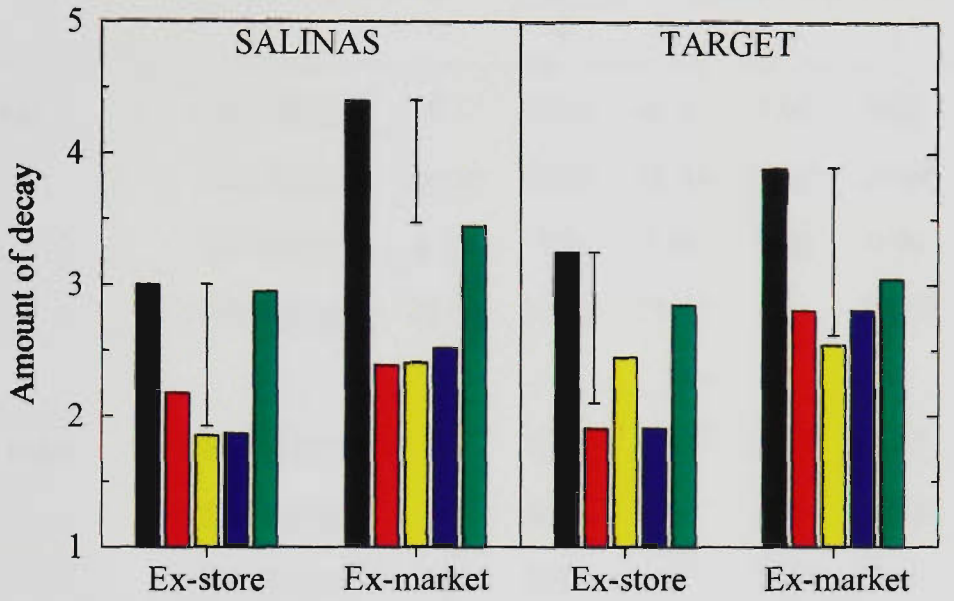


Figure 4.10 The effect of storage atmosphere on the amount of decay on lettuce cvs. Salinas and Target stored for 5 weeks at 1.5°C followed by 2 days at 20°C.
Vertical bars = LSD (P=0.05) (■ Trt 1;■ Trt 2;□ Trt 3;■ Trt 4;■ Trt 5)

Similar results were obtained with cultivar Target at the ex-store assessment, with treatments 2 and 4 having significantly less decay than the control. After the simulated marketing period, only treatment 3 had less decay than the control. No significant differences were noted between CA treatments for cultivar Target at the ex-store and ex-market assessment.

4.3.3.3 Weight loss from lettuce held under air or CAS

Controlled atmosphere storage was effective in reducing weight loss from lettuce. For cultivar Salinas, treatments 2 and 3 lost approximately 6g/head after five weeks storage, significantly less than the 14g/head lost from the control (Table 4.5). Similarly, treatments 3, 4 and 5 of cultivar Target lost approximately 5g/head, significantly lower than the 8.5g and 7.2g/head lost from the control and treatment 2 respectively during the simulated storage period.

Holding lettuce at 20°C for two days increased weight loss from both cultivars dramatically. At the ex-market assessment of Target lettuce, there was no difference in the amount of weight

lost between the control and CA treatments. On the other hand, treatments 3 and 4 of cultivar Salinas lost significantly less weight than the control during the marketing period.

Table 4.5 Effect of storage atmosphere on quality attributes of lettuce cvs. Salinas and Target.

Quality attribute	*Assessment*	*Treatment*					*LSD*
		1	2	3	4	5	$P = 0.05$
Weight loss (g)	ES Salinas	14.01	6.34	6.14	7.02	9.62	7.11
	EM Salinas	30.08	24.3	23.58	23.32	24.44	6.30
	ES Target	8.58	7.2	5.41	5.38	4.96	1.68
	EM Target	25.53	23.61	23.47	24	26.55	5.97
Brown stain index	ES Salinas	0.0	0.02	0.5	0.65	0.20	1.09
	EM Salinas	0.01	0.11	0.3	2.99	0.84	1.9
	ES Target	0	0.14	0	3.08	0.6	3.39
	EM Target	0	0	2.67	1.3	4.43	3.83
Amount of trimming (g)	ES Salinas	57.4	54.1	42.9	33.6	64.4	17.52
	ES Target	70.5	68	55.2	42.6	62.9	24.04
Head Firmness	ES Salinas	3.4	3.37	3.4	3.62	3.47	0.36
	EM Salinas	3.5	3.15	3.3	3.48	3.4	0.59
	ES Target	3.27	3.12	3.2	3.45	3.07	0.5
	EM Target	3.6	3.55	3.27	3.18	3.17	0.59
Flavour	ES Salinas	3.53	2.03	2.07	2.36	3.37	0.74
	EM Salinas	4	2.19	2	2.77	3.4	0.59
	ES Target	3.67	2.11	2.2	2.19	2.87	0.44
	EM Target	4.2	2.31	2.2	2.61	3.67	0.67

ES = Ex-store; EM = Ex-market

There appeared to be a cultivar effect with treatments 1 and 5 of Salinas losing significantly more weight than the same treatments for cultivar Target at the ex-store assessment (lsd=4.07). This effect carried over to the ex-market period with the control of Salinas again losing significantly more weight than the control of Target lettuce (lsd=3.49).

4.3.3.4 Amount of brown stain and russet spotting on lettuce held under air or CAS

The amount of BS present on lettuce of both cultivars after five weeks storage at 1.5°C (Ex-store) was low, with no significant difference noted between CA treatments and the control (Table 4.5). However, the ex-market period appeared to accentuate the development of BS. Treatment 4 of Salinas had more BS than the control or other CA treatments, whilst treatment 5 of Target developed more BS than the control and treatment 2. Despite this, the BS ratings were relatively low for both cultivars throughout the experiment. A comparison of the BS index between cultivars showed that treatment 4 of Target at the ex-store assessment had significantly more BS than treatment 4 of Salinas at the same time period (lsd=1.92). Furthermore, at the ex-market evaluation, treatment 5 of Target had significantly more BS than treatment 5 of Salinas (lsd=2.84). No russet spotting was observed on either cultivar throughout the experiment.

4.3.3.5 Amount of trimming from lettuce held under air or CAS

After five weeks storage (Ex-store), cultivars Salinas and Target held in treatment 4 required less trimming than the control to reach a marketable state (Table 4.5). Treatment 4 of Salinas also required significantly less trimming than treatments 2 and 5, whilst treatment 3 had a smaller trimming loss than treatment 5. Similarly, the trimming requirement was smaller in treatment 4 of cultivar Target when compared to treatment 2.

4.3.3.6 Visual quality of lettuce held under air or CAS

For cultivar Salinas, only treatment 4 was considered significantly more appealing than the control after five weeks storage (Figure 4.11; Plate 4.2 a-f). Lettuce held in treatment 4 also looked better than treatment 5 at the ex-store assessment. The simulated marketing period decreased the quality of the control to a point where it was totally unmarketable. All CA treatments were considered to be more visually appealing than the control at this point, with treatments 2, 3 and 4 also having a better visual quality than treatment 5.

Plate 4.2: Quality of cultivars Target and Salinas after storage in air or controlled atmospheres for five weeks at 1.5°C

Plate 4.2A Initial quality of Target (left) and Salinas (right) on day 0 (untrimmed)

Plate 4.2B Initial quality of Target (left) and Salinas (right) on day 0 (trimmed)

Plate 4.2C Quality of cultivar Target after 5 weeks storage at 1.5°C (Viewed from top). Clockwise from top: Control (Air); 2.5% O_2/2% CO_2; 5% O_2/2% CO_2; 7.5% O_2/5% CO_2; 5% O_2/5% CO_2. Note severe butt discolouration on the control.

Plate 4.2D Quality of cultivar Salinas after 5 weeks storage at 1.5°C (Viewed from top). Clockwise from top: Control (Air); 2.5% O_2/2% CO_2; 5% O_2/2% CO_2; 7.5% O_2/5% CO_2; 5%O_2/5% .

Plate 4.2E Quality of cultivar Target after 5 weeks storage at 1.5°C (Viewed from underneath). Clockwise from top: Control (Air); 2.5% O_2/2% CO_2; 5% O_2/2% CO_2; 7.5% O_2/5% CO_2; 5%O_2/5% CO_2

Plate 4.2F Quality of cultivar Salinas after 5 weeks storage at 1.5°C C (Viewed from underneath). Clockwise from top: Control (Air); 2.5% O_2/2% CO_2; 5% O_2/2% CO_2; 7.5% O_2/5% CO_2; 5%O_2/5% CO_2


A
B
C
D
E
F

Unlike cultivar Salinas, for cultivar Target there was no significant difference between visual quality of CA treatments and the control at the ex-store assessment. However, after two days storage at 20°C, treatments 3 and 4 were significantly more appealing than the control. Despite this, the simulated marketing period generally left all treatments from both cultivars in an unmarketable state.

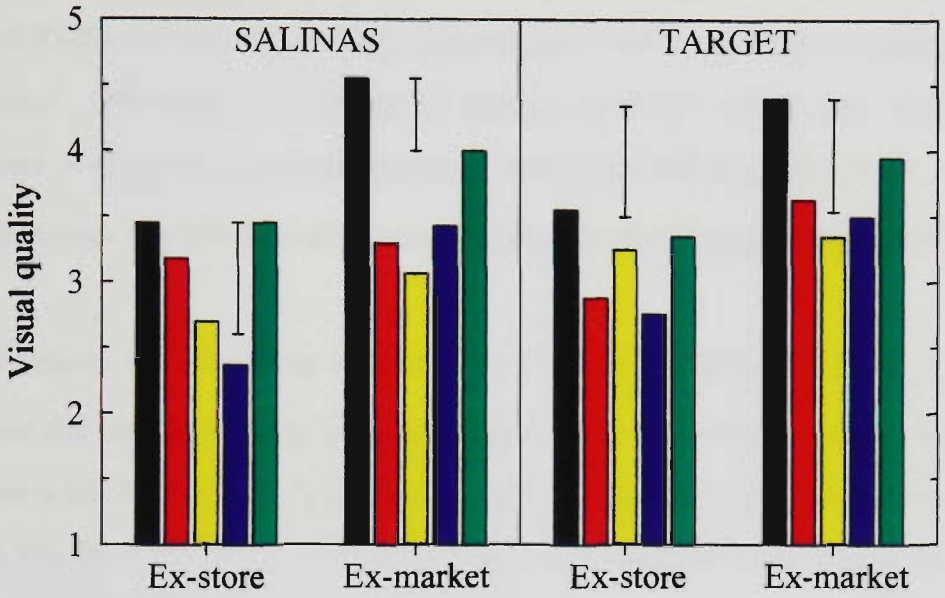


Figure 4.11 The effect of controlled atmosphere storage on the visual quality of lettuce stored for 5 weeks at 1.5°C followed by 2 days at 20°C. Vertical bars = LSD (P=0.05)
(■ Trt 1;■ Trt 2;□ Trt 3;■ Trt 4;■ Trt 5)

4.3.3.7 Head firmness of lettuce held under air or CAS

When compared to the control, CAS did not significantly affect the head firmness of Salinas and Target lettuce throughout the experiment (Table 4.5).

4.3.3.8 Flavour of lettuce held under air or CAS

Lettuce stored in air (control) at 1.5°C was generally unable to be consumed after the five week storage period, as evidenced by the poor flavour ratings (Table 4.5). When sampled, the control tasted dull and had lost its fresh-taste qualities. On the other hand, lettuce stored under CA were generally crisp and tasted sweet. Treatments 2, 3 and 4 of cultivar Salinas and all CA treatments of cultivar Target tasted significantly better than the control after five weeks storage at 1.5°C.

After the simulated marketing period, all CA treatments of cultivar Salinas and treatments 2, 3 and 4 of cultivar Target had a better flavour than the control. Of the CA combinations trialed, treatment 5 tasted somewhat bitter when compared to other CA treatments. Its flavour was

considered worse than other CA treatments for both cultivars at both sampling dates (Table 4.5).

4.4 Discussion

Lettuce is a highly perishable crop with a maximum useful storage life in air of 2-3 weeks (Lipton and Ryder, 1989). This study showed that CAS is useful in extending the shelf-life of whole lettuce. However, responses of lettuce to CAS varied and depended upon the concentration of O_2 and CO_2 in the storage atmosphere and type of cultivar used. Lipton and Ryder (1989) state that CAS entails potential benefits and dangers for intact lettuce.

Storage of lettuce cv. Greenway in a low O_2 (2.5-5%)/relatively high CO_2 atmosphere (2-5%) reduced the respiration rate of tissue when compared to that of lettuce held in air. These results agree with the findings of Singh *et al.* (1972) who reported that respiration was reduced by CAS (2.5% O_2/2.5% CO_2). Similar results were obtained by Lipton (1967) and Brecht *et al.* (1973b), in which low O_2 (2%) reduced CO_2 production rate of lettuce heads held at various temperatures. Zagory and Kader (1988) pointed out that the first measurable effect of reduced O_2 concentrations around produce is on CO_2 evolution. This statement is consistent with the results obtained with cultivar Greenway (Section 4.3.1.1). A reduction in respiration rate of produce in response to CAS has been reported by other authors (Lebermann *et al.*, 1968; Makhlouf *et al.*, 1989a).

An O_2 concentration of 10% was less effective in reducing the respiration rate of cv. Greenway than O_2 levels of 5% or lower. The rate of respiration is sensitive to changes in O_2 concentration below about 8% and CO_2 above about 1% (Zagory and Kader, 1988). An atmosphere consisting of 10% O_2/10% CO_2 initially appeared to be more beneficial in reducing respiration than 10%O_2/5% CO_2. Kader *et al.* (1989) stated that the effects of reduced O_2 and elevated CO_2 on respiration are additive and can be greater than the effects of either alone. However, after an initial low respiration period of 10 days, high CO_2 concentrations (10%) increased respiration in lettuce (Figure 4.3). These results are consistent with the findings of other researchers (Siriphanich and Kader, 1985a; Kubo *et al.*, 1990). The increase in respiration by exposure to excess CO_2 may be related to physiological injury (Kader, 1986) or anaerobic respiration (Makhlouf *et al.*, 1989a; See discussion page 141).

Herner (1987) stated that the manner in which horticultural commodities respond to CO_2 depends on the nature of the commodity and on the concentration and length of exposure to the gas. From these studies it appears that gas atmospheres consisting of 2-5% O_2 combined with 2-5% CO_2 were the most effective CA combinations in reducing respiration rate in cultivar Greenway.

The respiration rate of cultivars Salinas and Target under a 5% O_2/5% CO_2 was almost identical, at approximately 3 mg CO_2 kg^{-1} hr^{-1} after 33 days. Although not compared statistically, these two spring/summer cultivars appeared to respire slightly faster than the winter cultivar Greenway, which stayed at approximately 2 mg CO_2 kg^{-1} hr^{-1} throughout the trial when stored under the same atmosphere. This result suggests that respiration rate could vary with cultivars adapted for different growing seasons. Preharvest factors, such as climatic conditions and cultural practices, can affect the morphological and compositional characteristics of a given genotype, which, in turn, influences its respiration rate (Kader, 1987).

The results reported in Section 4.3.1.2 show that CAS can reduce the rate and degree of butt discolouration compared to that of lettuce stored in air. During simulated transport of cultivar Greenway at 1.5°C, an increased CO_2 concentration in the storage atmosphere (5-10%) was effective in inhibiting butt discolouration, supporting the results of other researchers (Watada *et al.*, 1964; Stewart and Uota, 1971; Stewart and Uota, 1976; Lipton, 1987). Reducing O_2 concentration below 10% did not affect the amount of butt discolouration. These results contradict the findings of other researchers who found that low O_2 atmospheres retard butt discolouration (Parsons *et al.*, 1964; Lipton, 1967; Singh *et al.*, 1972).

The beneficial effects of CAS in reducing butt discolouration on cultivar Greenway carried over to the ex-market period, but the difference in severity of discolouration among various treatments became less evident after three days holding in air at 20°C. This observation was similar to that found by Lipton (1967) and Wang *et al.* (1984) who reported that the beneficial effect of CAS/MAP in reducing butt discolouration disappeared after lettuce heads were transferred back to air.

CAS did not reduce the amount of butt discolouration on cultivars Salinas and Target during simulated transit. However a high CO_2 environment during this period appeared to reduce discolouration during the marketing period. These results suggest that cultivars may differ in their response to elevated CO_2, especially when subsequently exposed to very high temperatures.

Russet spotting (RS) is a postharvest disorder that can develop during the transport and storage of Crisphead lettuce (Ke and Saltveit, 1989b). Results with cultivars Greenway, Salinas and Target suggest that these varieties are relatively resistant to RS. Pratella and Brigati (1989) reported differences in cultivar susceptibility to RS at 0°-5°C.

According to Ke and Saltveit (1989b), RS susceptibility depends on the extent of expression of the enzymes phenylalanine ammonia-lyase, ionically bound peroxidase and indole-3-acetic acid oxidase. These researchers concluded that the variation in RS susceptibility between resistant and sensitive cultivars appears to be related to the degree of induction of these enzyme activities.

The low storage temperature (1.5°C) is a possible explanation for the low RS development observed on these cultivars. Other researchers have reported an association between storage temperature and amount of RS (Rood, 1956; Hyodo *et al.*, 1978; Pratella and Brigati, 1989). However, because RS development was not enhanced when lettuce were held at 20°C for three days, this suggests that the types of cultivar used in this study were the most likely reason for low RS scores.

The amount of brown stain (BS) on lettuce cv. Greenway was low after the simulated transit period. The occurrence of BS in different controlled atmospheres varied so much among individual replications that average differences among treatments were not always statistically different. Thus, lettuce appears to vary considerably in its response to elevated CO_2 (Stewart and Uota, 1971).

High CO_2 concentrations (5-10%) combined with high O_2 concentrations (10%) induced significant amounts of BS on cultivar Greenway during the marketing period. Carbon dioxide levels exceeding 1% can induce severe BS (Stewart and Ceponis, 1968; Stewart *et al.*,

1970; Stewart and Uota, 1971; Lipton *et al.*, 1972; Brecht *et al.*, 1973 a,b,c; Kader and Morris, 1977) and injury is almost certain when the CO_2 level exceeds 4% (Stewart and Uota, 1972). Interestingly, in this study, lettuce stored in 5% O_2/5% CO_2 did not develop objectionable amounts of BS. This result differs from other researchers who reported that BS is aggravated when low O_2 and high CO_2 levels coexist (Stewart and Uota, 1971; Stewart and Uota, 1972; Brecht *et al.*, 1973c).

The severity of BS increased as the CO_2 concentration was raised from 5 to 10%. This finding confirms previous studies which showed that BS is caused by increased levels of CO_2, and that in general, the higher the concentration, the greater the incidence of injury (Stewart and Uota, 1971).

Although high CO_2 (5-7.5%) induced significant amounts of BS on cultivars Salinas and Target during the marketing period, these cultivars appear to be less susceptible to BS than cultivar Greenway. The maximum CO_2 concentration tolerated by fruits and vegetables is highly dependent on the species (Renault *et al.*, 1994). Differences in susceptibility to BS between lettuce cultivars has been reported by Brecht *et al.* (1973a). It has been postulated that CO_2 dissolution, which enhances acidity in the cell medium, may participate in this physiological disorder (Varoquaux and Wiley, 1994). Siriphanich and Kader (1985 a,b) report that high CO_2 concentrations affect the metabolism of phenolic compounds in lettuce. Kader *et al.* (1989) attributed differences in cultivar susceptibility to CA/MA-induced physiological disorders to anatomical differences influencing their gas-diffusion characteristics.

Brown stain became more apparent on cultivar Greenway during subsequent holding of lettuce in air at 20°C for 3 days. Marketing conditions can greatly influence the expression of BS (Watada *et al.*, 1964; Stewart and Uota, 1971; Siriphanich and Kader, 1985a; Forney and Austin, 1988). Nevertheless, this study does not seem to support the notion that BS of lettuce may be a form of chilling injury that is aggravated by high CO_2 levels (Brecht *et al.*, 1973b).

The amount of lettuce decay is determined by factors such as weather at harvest, lettuce vigour and condition, microbial contamination, mechanical injuries, and temperatures and humidities encountered during marketing (Ceponis and Kaufman, 1968). Preliminary experiments (data

not shown) have shown that an important step in reducing decay on lettuce was removal of the outer wrapper leaves before storage. This procedure reduces the number of potential pathogens by eliminating the older, less vigorous leaves that may be decayed and carrying pathogens (Harvey *et al.*, 1961; Ceponis *et al.*, 1985) and is particularly advantageous during long transit storage; for example, in shipments overseas (Lipton, 1971).

In general, CAS was effective in retarding the development of decay in lettuce. Oxygen concentrations ≤5% and CO_2 concentrations between 2-5% inhibited decay formation on cvs. Salinas and Target throughout the trial. On the other hand, 2.5-10% O_2/0-5% CO_2 reduced decay on cv. Greenway during the marketing period. It appears that atmospheres low in O_2 (≤10%) are necessary to inhibit decay development on lettuce. This result confirms the findings of Stewart and Uota (1976) who found that low O_2 (3%) inhibited decay more than air. Stewart (1978) found that 5% O_2/3% CO_2, was the most effective gas combination in inhibiting decay, consistent with results reported here. In the present study, the presence of CO_2 in the storage atmosphere also inhibited decay, although its effect was not as important as reduced O_2 concentrations. Other research has found that CAS reduces decay particularly when low O_2 and high CO_2 levels co-exist (Singh *et al.*, 1972). However, these results contradict those of O'Connor *et al.* (1992) who stated that levels of 1-10% O_2 and 1-15% CO_2 have minimal inhibitory effects on spoilage microorganisms.

Controlled atmosphere storage was effective in reducing weight loss from all three cultivars during the simulated transit period. Differences between CA treatments were slight and may be attributed to differences in respiration rate. Despite this, the weight loss within samples of all cultivars stored in air was relatively low and not objectionable. Increasing the storage temperature from 1.5° to 20°C increased weight loss dramatically, more so in cvs. Salinas and Target than in Greenway. This result may have been due to the smaller head size of cultivar Greenway and the smaller surface area:volume ratio. Differences in weight loss between CA treatments and the control decreased during the marketing period.

Firmness is the primary textural attribute measured in fruits and vegetables (Shewfelt, 1993). According to Ryder and Whitaker (1980), the optimum stage for firmness in lettuce is a fully matured head well filled with leaves and yielding slightly to pressure. Postharvest treatments

such as CAS did not significantly alter the firmness of cvs. Salinas and Target when compared to lettuce held in air, agreeing with the finding of Risse (1981).

Flavour is the human perception which includes taste and odour sensations (Powrie and Skura, 1991). Atmospheres low in O_2 (≤5%) and high in CO_2 (2-5%) tended to retain the slightly sweet flavour of lettuce more than air. Increasing the O_2 concentration to 7.5% or 10% and the CO_2 concentration to 10% rendered lettuce slightly bitter and unpalatable. Lettuce stored in air tasted dull, its flavour deteriorating as time progressed. Similarly, Powrie and Skura (1991) stated that the taste profile of a fruit or vegetable changes with storage time, the rate being dependent on temperature and the MA.

In summary, CAS proved to be a useful tool to extend the shelf-life of whole Crisphead lettuce grown under Australian conditions. Of the CA combinations trialed, atmospheres with reduced O_2 (2-5%) and elevated CO_2 levels (2-5%) were the most effective gas combinations for maintaining quality in cultivars Greenway, Salinas and Target. Controlled atmosphere storage became detrimental to lettuce quality when CO_2 concentrations rose above 5%, promoting brown stain and affecting flavour. For this reason, the design of an MA package for whole lettuce destined for export and domestic markets was based upon these recommended atmospheres and the respiration rate of lettuce under such conditions.

Chapter 5

Modified atmosphere packaging of whole lettuce

5.1 Introduction

Modified atmosphere package selection and design aims to achieve a balance between the enclosed produce respiration rate and film permeability to attain and maintain an acceptable equilibrium modified atmosphere (EMA) within the package (Song *et al.*, 1992). Achievement of this goal is dependent on a knowledge of several product and package parameters. As much information as possible must be accumulated about the desired finished product, including specific cultivar characteristics, CO_2 production rate at the target storage temperature and under CAS, low O_2 and elevated CO_2 concentration sensitivity thresholds, product mass and total surface area of the sealed package (Kader, 1986; Schlimme and Rooney, 1994).

Earlier studies (Chapter 4) identified 2-5% O_2 combined with 2-5% CO_2 as being the optimum gas atmospheres for maintaining the quality of Crisphead lettuce at 1.5°C. The respiration rate of tissue under such gas profiles and temperature was approximately 2-3 mg CO_2 kg^{-1} hr^{-1}. O'Connor *et al.* (1992) suggested that once the optimum atmosphere and the respiration rate of produce under this regime and required temperature has been determined, the packaging material should then be selected on the basis of it having a permeability very similar to the respiration rate of the fruit or vegetable.

The objective of the study reported in Chapter 5 was to utilize results from Chapter 4 to design and test a MA package for whole lettuce destined for export and domestic markets. This involved selecting a packaging film with the correct O_2 and CO_2 permeabilities and specifying the area of the film, weight of the produce, and dimensions of the package that result in a desirable EMA.

A range of microperforated films, which had adequate resistance to tearing and puncturing during filling operations, were evaluated in order to determine which was the most suitable for a lettuce carton-lining system, to create modified pack atmospheres at 1.5° and 5°C.

The 1.5°C represents carriage temperatures within reefer containers destined for export markets. Earlier studies (Chapter 4) found that brown stain (BS) may be a problem in Crisphead lettuce held in high CO_2 concentrations at low temperatures (1.5°C). Lipton and Ryder (1989) state that low temperatures (below 5°C) during transit promote BS development on lettuce held under elevated CO_2 and that the susceptibility of Crisphead lettuce to CO_2 injury at low temperatures can be greatly reduced by using temperatures closer to 5°C during short transit periods. For this reason, it was also decided to design and test a MAP carton liner for whole lettuce stored, distributed and marketed within Australia at temperatures around 5°C.

5.2 Experimental design and procedure

5.2.1 Plant material

Commercially grown Crisphead lettuce (*Lactuca sativa* L. cv. Greenway) were harvested, packed and transported by truck from Hay, New South Wales, to the Melbourne Wholesale Fruit and Vegetable Market on the 19th May, 1994. The journey took approximately five hours and the lettuce were not refrigerated during this period. Twenty four hours after harvest, the lettuce were transported by non-refrigerated truck to the IHD, Knoxfield, Victoria.

The average core temperature of the lettuce upon arrival was 12° ± 0.5°C. Each lettuce was trimmed as described in Section 3.1. A random subsample of 10 lettuce was used to record the number of leaves trimmed, weight of trimmings, initial head firmness and butt colour of the lettuce (see Section 3.7 for details). On average, four leaves weighing approximately 345g (25% of initial lettuce weight) were trimmed off each lettuce prior to storage. After trimming, the average weight, head firmness and butt colour of each lettuce was 1016 g, 3.6 and 57.36 respectively. All other sensory attributes were assigned a value of 1.0 as detailed in Section 3.7.

5.2.2 Storage conditions

Bags of a commercially available low density/linear low density polyethylene film (ICI Films, Melbourne) were used as polymeric carton liners for whole lettuce (Table 5.1). The same type of film was used for all treatments, with gas transmission rates varied through number of microperforations per liner. The microperforations allowed the gas transmission rate of the film to be increased without significantly affecting the structural properties of the film.

According to Myers (1989), perforated package systems have the advantage of using one film type for all fresh commodities, and the manufacturing process and gas permeability can be more easily controlled.

Table 5.1 Composition, thickness and gas permeability of unperforated polyethylene film

Film			Gas permeability (ml/m^2/day/atm)	
Temperature	Type	Thickness (μm)	O_2	CO_2
1.5°C	LDPE/LLDPE *	30	6600[a]	12,200[a]
5°C	LDPE/LLDPE	30	6800[b]	12,500[b]

Source: ICI Films Australia

* LDPE = Low density polyethylene; LLDPE = Linear low density polyethylene

[a] Value represents permeability of unperforated film to gases at 1.5°C

[b] Value represents permeability of unperforated film to gases at 5°C

The number of perforations per liner (effective surface area 1.24 m^2) varied from 0, 2 or 60 in liners held at 1.5°C to 4, 8 or 60 for lettuce stored at 5°C (Table 5.2). The diameter of individual perforations was approximately 400 μm, a size reported to be effective by Geeson (1990).

Extensive preliminary experiments at 1.5°C (data not shown) determined the number of perforations necessary to establish predetermined beneficial gas atmospheres mentioned in the introduction. Liners with 60 perforations (60P) were designed to maintain a high RH without establishing any significant MA (Table 5.2). At 1.5°C, liners with zero perforations were designed to maintain a MA low in O_2 (3-5%) and CO_2 (3%).

Table 5.2 Nature of polymeric films used for lettuce stored at 1.5°C and 5°C

Treatment	*Temperature*			
	1.5°C	**Expected MA (1.5°C)**	**5°C**	**Expected MA (5°C)**
1 (Control)	Air	-	Air	-
2	60P *	20% O_2/1% CO_2	60P	20% O_2/1% CO_2
3	0P	5% O_2/3% CO_2	4P	5% O_2/5% CO_2
4	0P/Lime[a]	5% O_2/0% CO_2	8P	9% O_2/5% CO_2
5	2P	8% O_2/5% CO_2	-	-

*P = Perforations/liner (1.24 m^2)

[a] 20 g of hydrated lime was placed within each liner to absorb respiratory CO_2

Bags containing two perforations/1.24 m^2 were expected to establish an atmosphere of approximately 8-10% O_2 and 5% CO_2. This higher O_2 level would provide a 'safety margin' in the case of increased O_2 consumption through temperature abuse.

The liners at 5°C were designed to be more permeable than those at 1.5°C because of the expected increase in respiration rate at the higher temperature. Again, several preliminary experiments were undertaken to determine the number of perforations necessary to establish an atmosphere of approximately 5% O_2/5% CO_2, an atmosphere believed to be beneficial in maintaining lettuce quality at 5°C.

After trimming, twelve lettuce (average total weight of 12 kg) were placed in each liner housed within a standard size lettuce carton. The cartons containing the lettuce were transferred in an non-refrigerated van from IHD to Jorgenson Waring Foods, Scoresby, Victoria, a journey of approximately 5 minutes. Under conditions of < 10°C, a Corr-Vac gas flushing machine equipped with a gas mixer pulled a slight vacuum in order to evacuate air from liners* and then backflushed the bags (4.75 sec) with a premixed gas mixture of O_2 and CO_2 (with balance of gas as N_2). These gas mixtures varied according to stable levels of each gas achieved in preliminary trials. Initial gas flushing was necessary because lettuce respired too slowly to quickly establish an effective MA. Liners were then heat sealed for three seconds at 120°C using a 1 m wide impulse seal (* Note: Treatment 1 (control) was trimmed and placed back in its original box without a carton liner, whilst liners of treatment 2 were not gas flushed, but rather, only heat sealed. Sealing the bags of treatment 2 helped establish a high RH).

The lettuce were then transported back to IHD, weighed and stored at their experimental temperatures (1.5°C or 5°C). The lettuce cartons were completely randomized with eight replications (reps) per treatment and each replicate consisting of a carton containing twelve heads (Plate 5.1). At 1.5°C, lettuce were held for 40 days (Ex-store 2), with half of the lettuce (4 reps) evaluated after 26 days storage (Ex-store 1; Figure 5.1a). At 5°C, lettuce were held for 32 days (Ex-store 2), with half of the lettuce (4 reps) evaluated after 18 days storage (Ex-store 1; Figure 5.1b).

Gas samples were taken from each package every two-to-three days throughout the storage period at both temperatures from all treatments (except treatment 1) as detailed in Section 3.6.

At ex-store evaluations, liners were removed from cartons, reweighed and then opened. Six randomly selected lettuce (three from the top layer and three from the bottom) were removed and assessed for butt colour, amount of decay, russet spotting, brown stain, visual quality, head firmness and flavour as detailed in Section 3.7. These lettuce represented what the wholesaler or agent would see upon arrival. These lettuce were discarded after evaluation. The other six remaining lettuce in each carton were trimmed to determine the amount of trimmings necessary to reach a marketable state, weighed and the butt colour of each was recorded. These lettuce were then held at 20°C for two days (Ex-market 1 or 2), after which they were assessed for all the quality attributes as at the ex-store assessment.

Plate 5.1	Experimental design showing lettuce stored at 1.5°C. Each stack of lettuce was considered a replicate (Eight in total). The five treatments were randomly placed within each stack. Gaps were left between and behind stacks to allow for adequate venting. Good air-flow was also assisted by handle holes in either end of each box.


CRISP
CHOICE

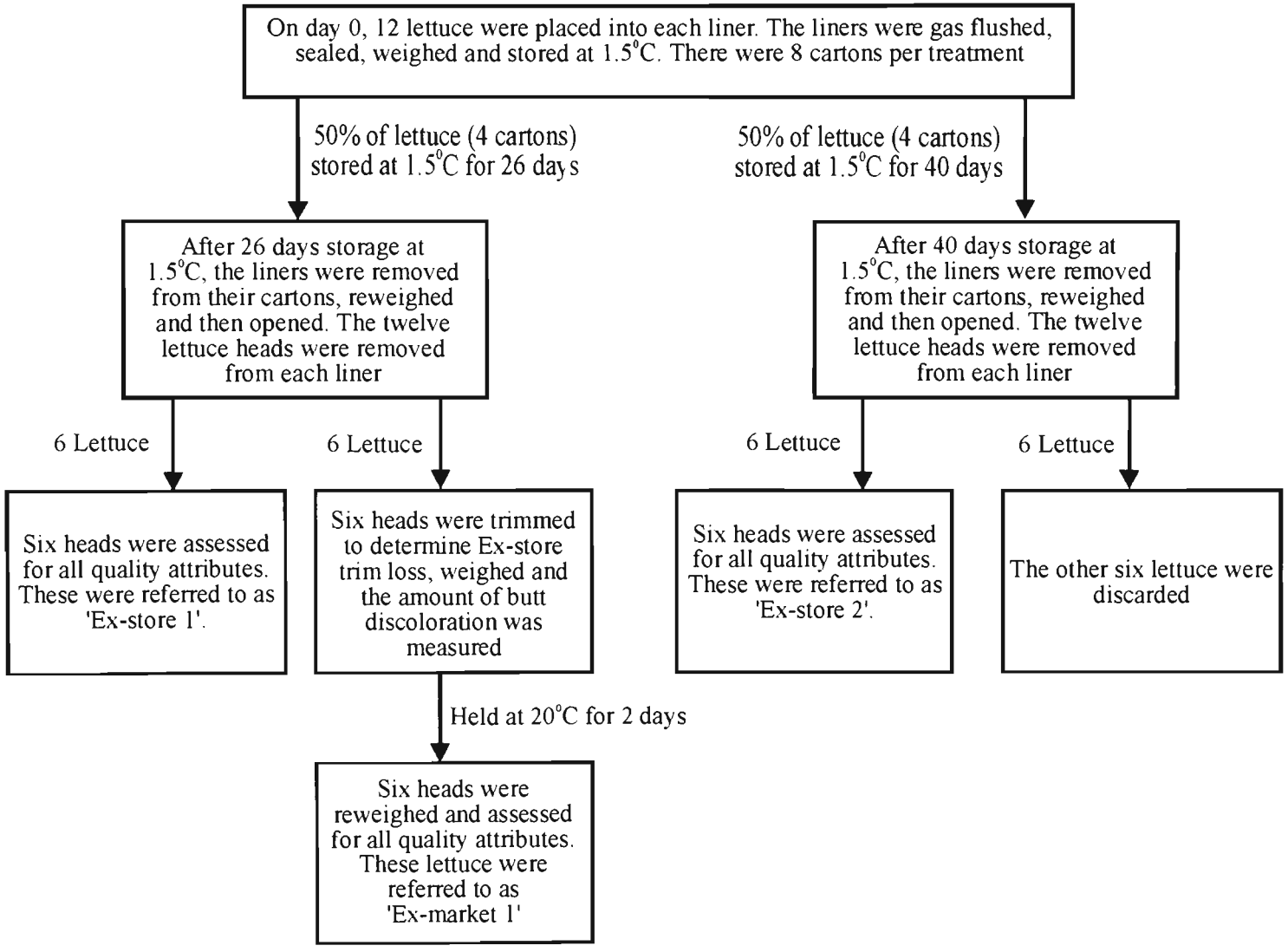


Figure 5.1a. Procedure followed for analysing quality of lettuce at 1.5°C

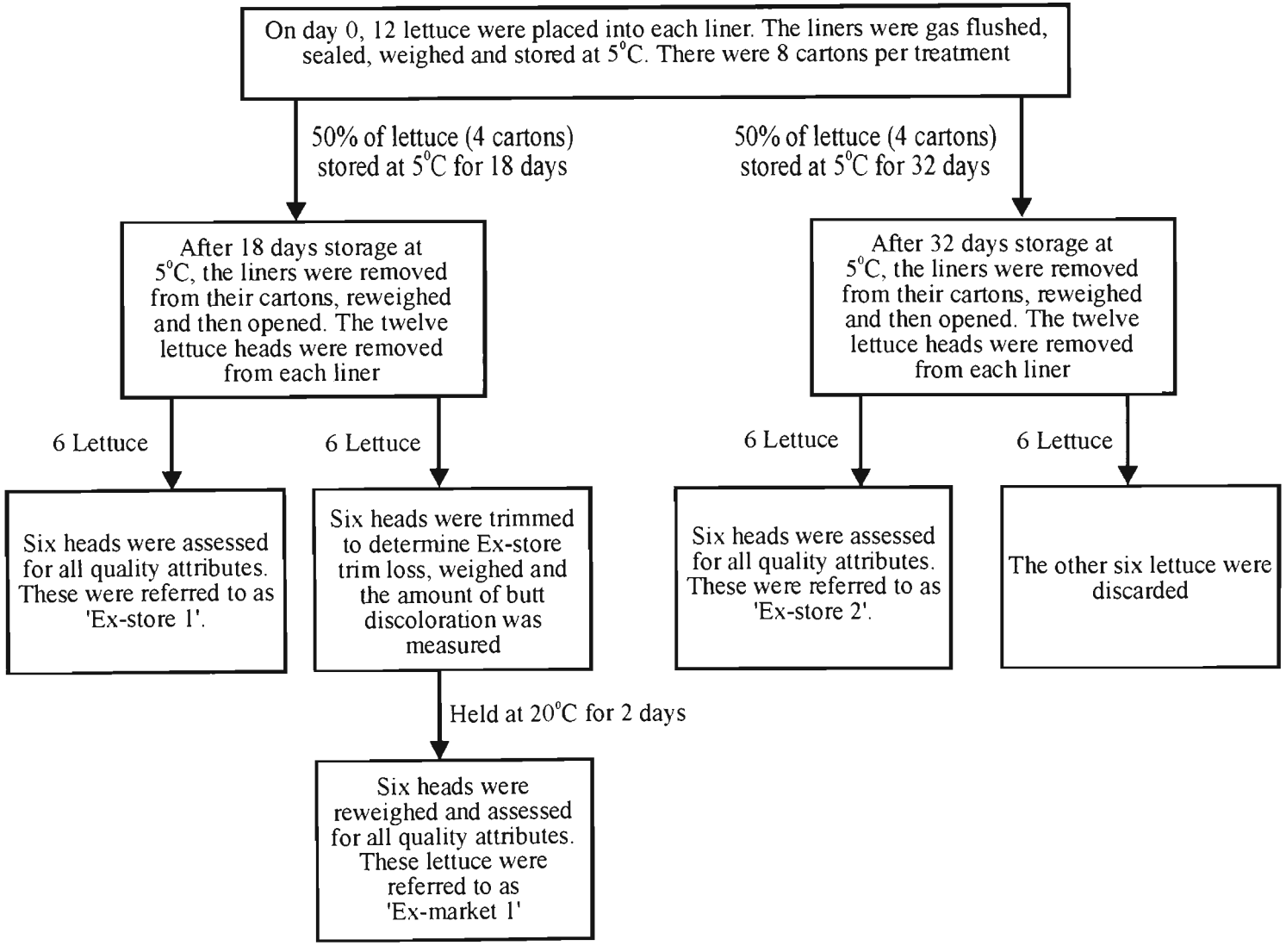


Figure 5.1b. Procedure followed for analysing quality of lettuce at 5°C

5.3 Results

5.3.1 Modified atmosphere packaging of lettuce stored at 1.5°C

5.3.1.1 Gas concentrations within liners

The gas profiles established within the various treatments stored at 1.5°C are presented in Figure 5.2. As expected, treatment 2 did not set up any appreciable MA, with O_2 and CO_2 concentrations equilibrating at approximately 19% and 2% respectively.

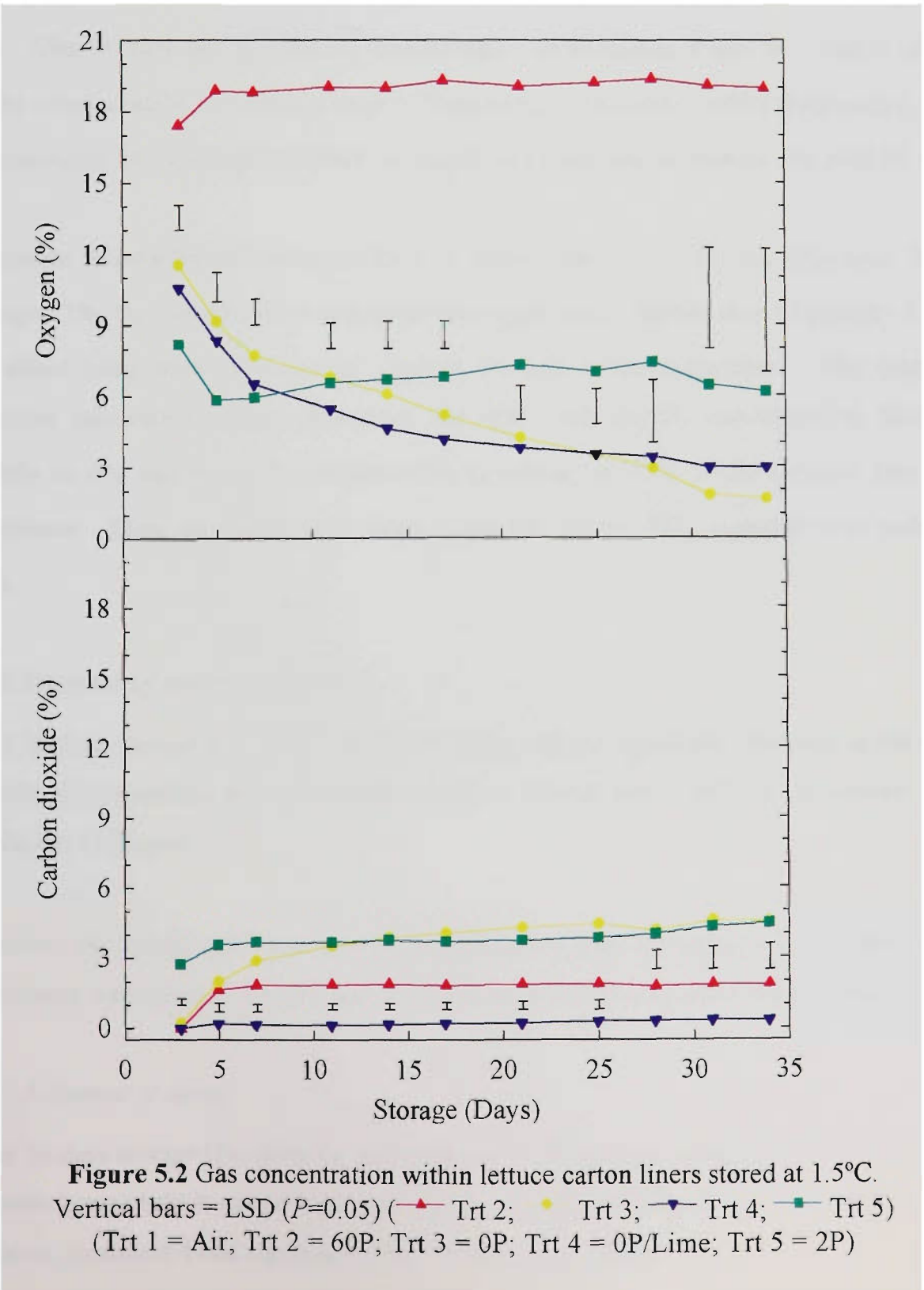


Figure 5.2 Gas concentration within lettuce carton liners stored at 1.5°C. Vertical bars = LSD (P=0.05) (Trt 2; Trt 3; Trt 4; Trt 5) (Trt 1 = Air; Trt 2 = 60P; Trt 3 = 0P; Trt 4 = 0P/Lime; Trt 5 = 2P)

On the other hand, at the first quality evaluation (Ex-store 1; 26 days) treatment 3 had established a MA of approximately 3.5% O_2/4.5% CO_2. After 40 days, the O_2 concentration of treatment 3 had decreased to 1.5%, whilst the CO_2 concentration remained relatively constant at approximately 4.5%. Although CO_2 concentrations within this treatment were close to that predicted (3%), O_2 levels were considerably lower than desired.

Placing hydrated lime in treatment 4 kept the CO_2 concentration below 0.5% throughout the trial. After 26 days storage, the O_2 concentration of treatment 4 was 4%, almost identical to the concentration recorded in bags of treatment 3. However, unlike treatment 3, the O_2 concentration in treatment 4 tended to stabilize and was approximately 3% after 35 days.

Treatment 5 established an atmosphere of approximately 7% O_2/4% CO_2 after 26 days storage. The O_2 concentration was generally significantly higher than treatments 3 and 4, the effect being more pronounced towards the end of the experiment. The atmosphere remained relatively constant throughout the trial, with the O_2 concentration decreasing slightly to 6% and the CO_2 concentration increasing to 4.5% in the last five days of the experiment. These gas levels were close to the 8% O_2/5% CO_2 expected from preliminary trials.

5.3.1.2 Amount of butt discolouration

After 26 days storage at 1.5°C (Ex-store 1), there was no significant difference in the amount of butt discolouration between lettuce held in plastic liners and the air-treated control (treatment 1) (Figure 5.3).

However, the change in L^* was greater in treatment 4 than in treatment 3. At the ex-store 2 assessment, treatments 3, 4 and 5 had all discoloured significantly less than the control.

5.3.1.3 Amount of decay

After 26 days storage (Ex-store 1), no significant difference was noted in the amount of decay between lettuce held in carton liners and lettuce stored in air (Figure 5.4). For this assessment however, treatment 4 had significantly less decay than treatment 2.

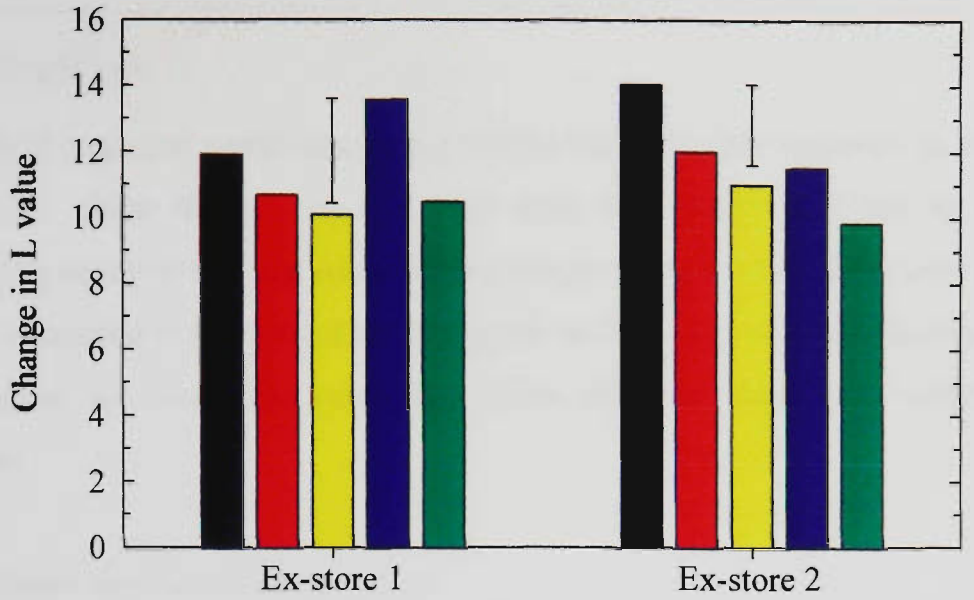


Figure 5.3 Effect of MAP on the amount of butt discolouration on lettuce stored at 1.5°C Vertical bars = LSD (P=0.05) (■ Trt 1;■ Trt 2;□ Trt 3;■ Trt 4;■ Trt 5)

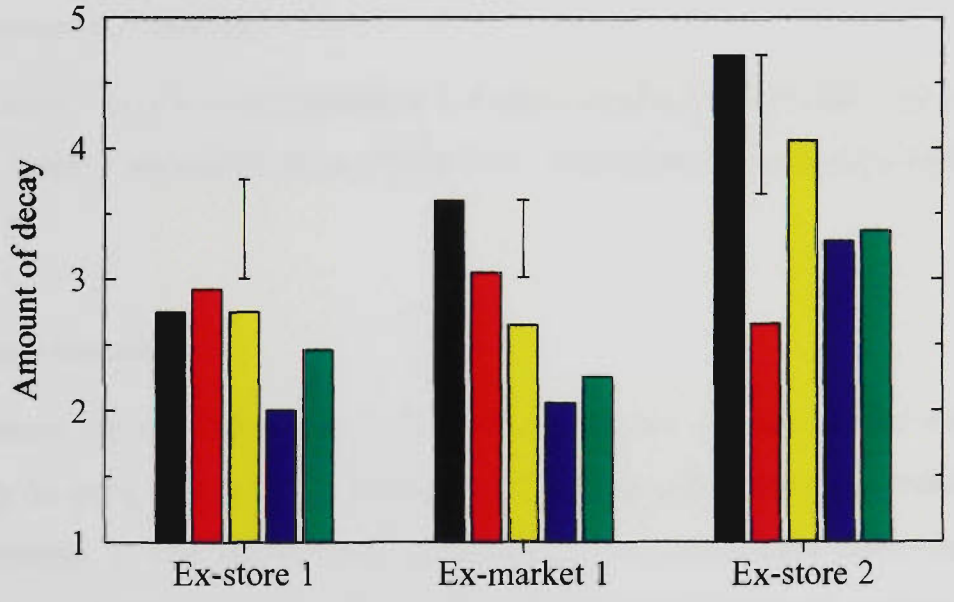


Figure 5.4 Effect of MAP on the amount of decay on lettuce stored at 1.5°C Vertical bars = LSD (P=0.05) (■ Trt 1;■ Trt 2;□ Trt 3;■ Trt 4;■ Trt 5)

The ex-market period at 20°C appeared to accentuate decay development in lettuce stored in air, with treatments 3, 4 and 5 rating lower than treatment 1 (Ex-market 1). Treatment 4 also had less decay than treatments 2 and 3, whilst treatment 5 scored better than treatment 2.

Decay was severe in the control after 40 days at 1.5°C (Ex-store 2). At this time period, lettuce held in treatments 2, 4 and 5 had significantly less decay than the control (air), however the amount of decay was becoming objectionable. Treatment 2 had significantly less decay than treatment 3.

5.3.1.4 Weight loss

Lettuce held in air lost significantly more weight than any other treatment at every assessment (Table 5.3). After 40 days storage, each head from treatment 1 lost on average 79.5g, representing nearly 8% of the initial lettuce weight. Weight loss from other treatments was very low compared to the control and no significant difference was noted between treatment 2-5 throughout the trial. The marketing period increased the rate of weight loss from all treatments.

5.3.1.5 Russet spotting and brown stain

No russet spotting or brown stain was observed on any treatment throughout the storage period.

5.3.1.6 Amount of trimming

At the ex-store 1 assessment, treatments 3, 4 and 5 required significantly less trimming than the control to reach a marketable state (Table 5.3). Treatment 4 also required less trimming than treatment 2.

5.3.1.7 Head firmness

Head firmness did not differ greatly between treatments throughout the experiment (Table 5.3). After 26 days, treatment 3 was slightly softer than other treatments trialed. On the other hand, treatment 2 was significantly softer than other treatments following the simulated marketing period (Ex-market 1). No difference was noted between treatments following 40 days storage.

5.3.1.8 Flavour

Lettuce stored in air generally tasted bitter or was unable to be consumed. It was therefore judged to have a flavour inferior to all liner treatments after 26 days storage, and treatments 3, 4 and 5 at the ex-market 1 assessment and treatments 2, 4 and 5 at the end of the experiment (Table 5.3). These latter treatments also tasted significantly better than treatment 3 after 40 days storage.

Table 5.3 Effect of MAP on quality attributes of lettuce stored at 1.5°C

Quality attribute		*Treatment*					*LSD*
		1	2	3	4	5	$P = 0.05$
Weight loss (g)	ES1	28.04	1.83	0.98	1.6	1.37	5.82
	EM1	28.33	4.67	9.38	9.0	9.0	7.41
	ES2	79.5	1.4	1.5	2.5	2.5	18.28
Amount of trimming (g)	ES1	214	191	125	115	130	72
Head firmness	ES1	3.05	3.1	2.7	3.3	3.15	0.32
	EM1	3.0	2.56	3.02	2.99	3.06	0.39
	ES2	3.0	3.2	3.2	3.05	3.1	0.29
Flavour	ES1	2.25	2.0	2.0	2.0	2.0	0.11
	EM1	2.67	2.41	1.92	2.08	2.16	0.4
	ES2	3.58	2.0	3.17	2.0	2.25	0.67

ES1=Ex-store 1; EM1=Ex-market 1; ES2=Ex-store 2

5.3.1.9 Overall visual quality

After 26 days storage, treatments 4 and 5 were rated as good/fair and were significantly more appealing than the air control which was considered unmarketable (Figure 5.5; Plate 5.2). No significant difference in overall visual quality was noted between the control and treatments 2 and 3 at the ex-store 1 assessment. When plastic liner treatments were compared, treatment 4 appeared significantly better than treatment 3.

The visual quality of the control declined dramatically following two days storage at 20°C (Ex-market 1) and was significantly worse than all other treatments. Only treatments 4 and 5 were considered marketable at this time period. Treatment 4 was significantly better than treatments 2 and 3 as evidenced by a lower visual quality rating.

After 40 days at 1.5°C (Ex-store 2), treatments 2, 4 and 5 were visually more appealing than treatment 1 (Plate 5.3). Despite this, these treatments were considered unmarketable because

of their high relative visual quality scores. Treatment 2 was significantly better than treatments 3 and 5, however it too was a borderline treatment in terms of consumer acceptability.

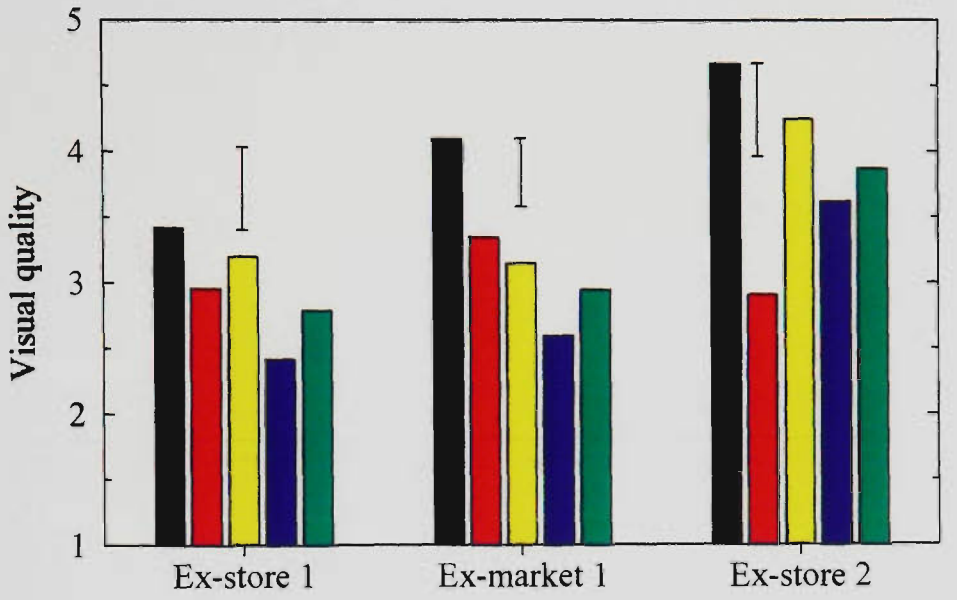


Figure 5.5 Effect of MAP on the visual quality of lettuce stored at 1.5°C Vertical bars = LSD (P=0.05) (■ Trt 1;■ Trt 2;□ Trt 3;■ Trt 4;■ Trt 5)

Plate 5.2: Lettuce stored in air or MAP for 26 days at 1.5°C

Plate 5.2A Initial lettuce quality on day 0

Plate 5.2B Lettuce stored in air for 26 days at 1.5°C. Note moderate decay

Plate 5.2C Lettuce stored for 26 days at 1.5°C in a liner containing 60 perforations

Plate 5.2D Lettuce stored for 26 days at 1.5°C in a liner containing 0 perforations

Plate 5.2E Lettuce stored for 26 days at 1.5°C in a liner containing 0 perforations and a lime sachet to absorb respiratory CO_2

Plate 5.2F Lettuce stored for 26 days at 1.5°C in a liner containing 2 perforations

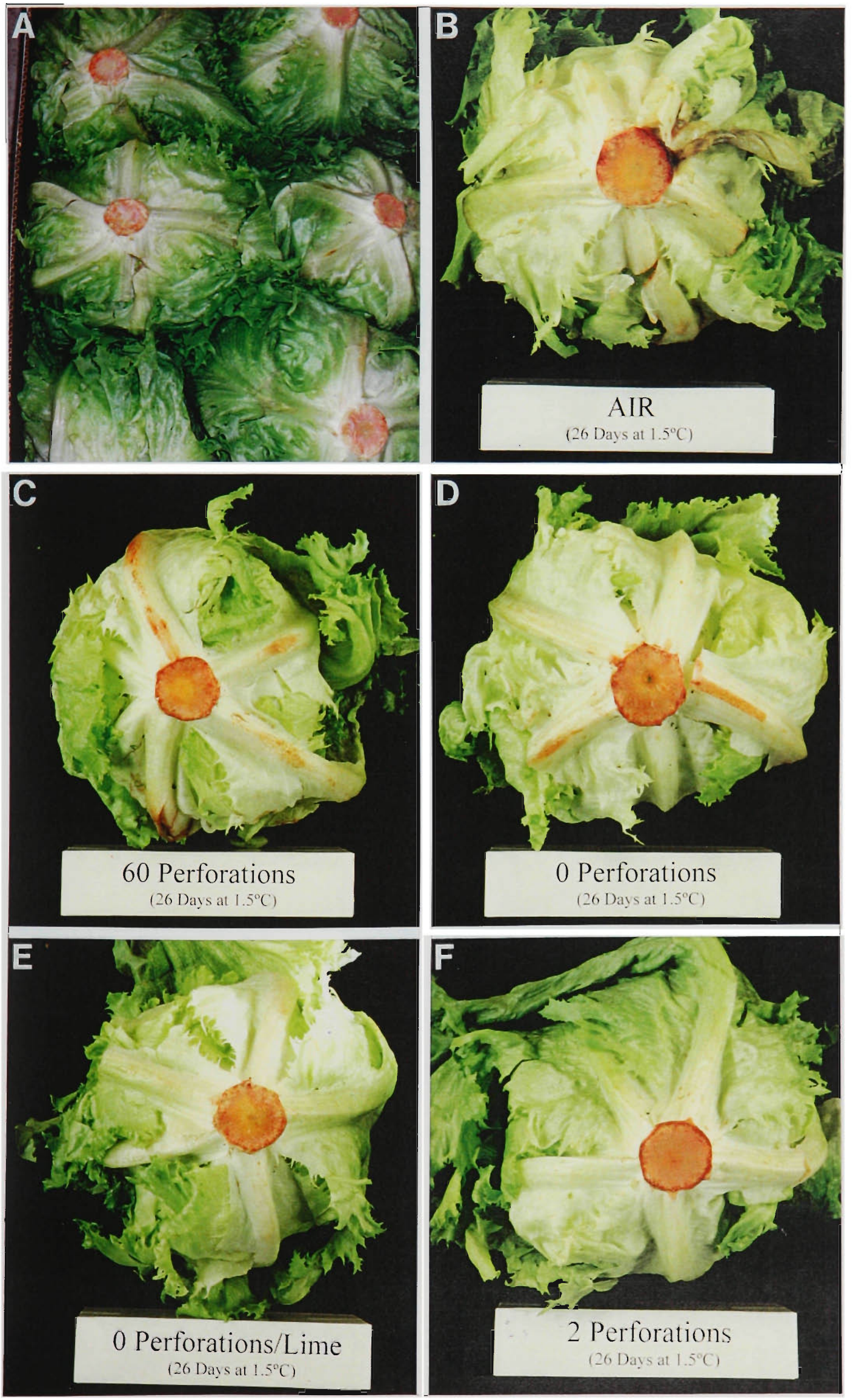

A
B
AIR
(26 Days at 1.5°C)
C
60 Perforations
(26 Days at 1.5°C)
D
0 Perforations
(26 Days at 1.5°C)
E
0 Perforations/Lime
(26 Days at 1.5°C)
F
2 Perforations
(26 Days at 1.5°C)

Plate 5.3: Lettuce stored in air or MAP for 40 days at 1.5°C

Plate 5.3A Initial lettuce quality on day 0

Plate 5.3B Lettuce stored in air for 40 days at 1.5°C. Note severe decay

Plate 5.3C Lettuce stored for 40 days at 1.5°C in a liner containing 60 perforations

Plate 5.3D Lettuce stored for 40 days at 1.5°C in a liner containing 0 perforations

Plate 5.3E Lettuce stored for 40 days at 1.5°C in a liner containing 0 perforations and a lime sachet to absorb respiratory CO_2

Plate 5.3F Lettuce stored for 40 days at 1.5°C in a liner containing 2 perforations


A
B
AIR
(40 Days at 1.5°C)
C
60 Perforations
(40 Days at 1.5°C)
D
0 Perforations
(40 Days at 1.5°C)
E
0 Perforations/Lime
(40 Days at 1.5°C)
F
2 Perforations
(40 Days at 1.5°C)

5.3.2 Modified atmosphere packaging of lettuce stored at 5°C

5.3.2.1 Gas concentrations within liners

The O_2 and CO_2 levels within the various treatments stored at 5°C are presented in Figure 5.6. Very little atmosphere modification occurred in treatment 2, equilibrating at approximately 18% O_2/2% CO_2.

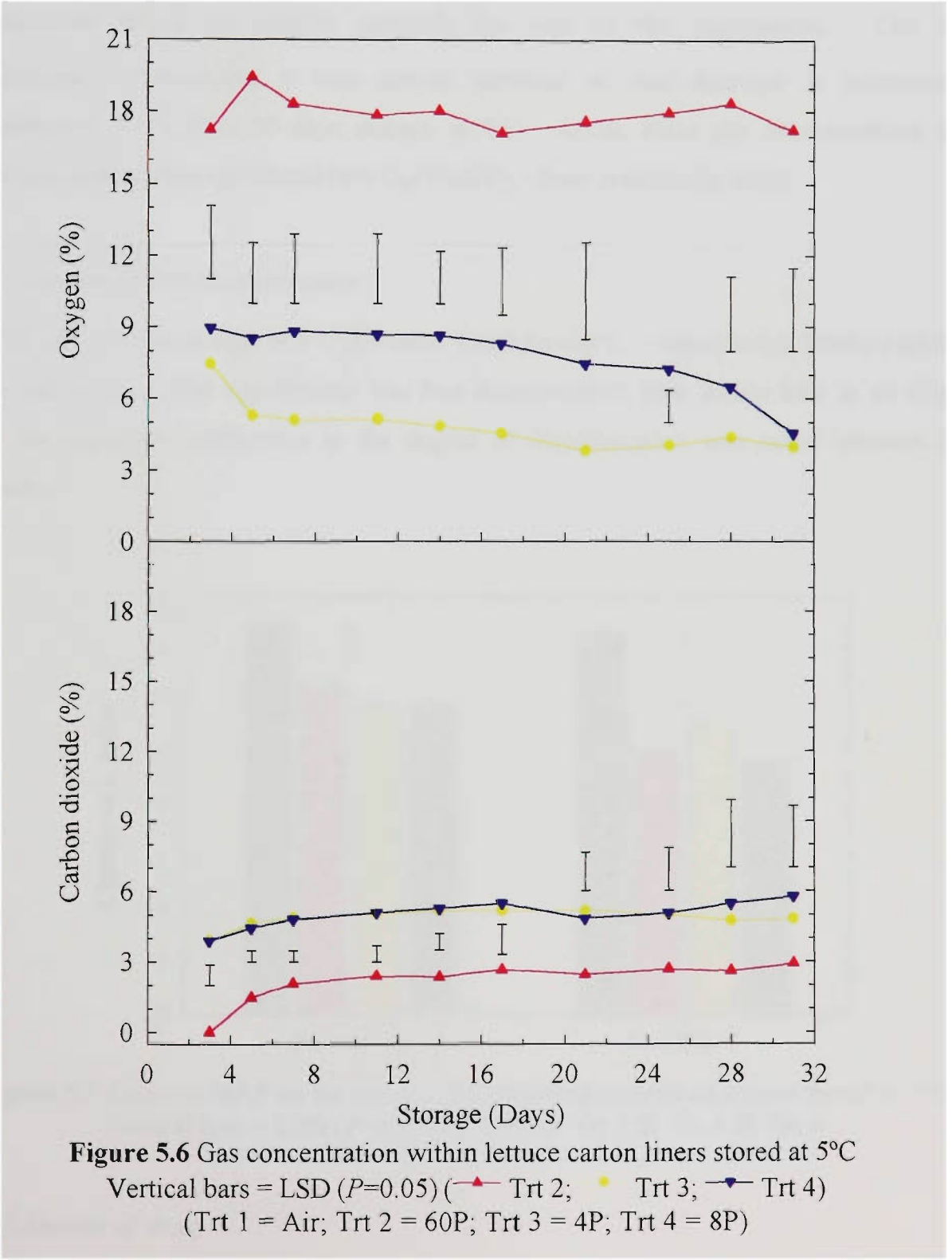


Figure 5.6 Gas concentration within lettuce carton liners stored at 5°C
Vertical bars = LSD (P=0.05) (Trt 2; Trt 3; Trt 4)
(Trt 1 = Air; Trt 2 = 60P; Trt 3 = 4P; Trt 4 = 8P)

On the other hand, treatment 3 did set up an effective MA, equilibrating at approximately 4% O_2/5% CO_2. Except for CO_2 levels during the last five days of the experiment, these gas

levels were significantly different to those established in treatment 2 and very close to those expected (5% O_2/5% CO_2) from preliminary trials.

Increasing the number of perforations per liner to eight (Treatment 4) increased O_2 concentration within liners to between 8 and 9% for the majority of the trial. These O_2 levels were generally higher than those detected in treatment 3, however O_2 concentration began to decline towards the end of the experiment. The CO_2 concentration of treatment 4 was almost identical to that detected in treatment 3, approximately 5-6% after 30 days storage at 5°C. Again, these gas concentrations were almost identical to those predicted (9% O_2/5% CO_2) from preliminary trials.

5.3.2.2 Amount of butt discolouration

After 18 and 32 days storage at 5°C (Ex-store 1 and Ex-store 2 respectively), lettuce packed in plastic carton liners had significantly less butt discolouration than lettuce held in air (Figure 5.7). No significant difference in the degree of discolouration was noted between liner treatments.

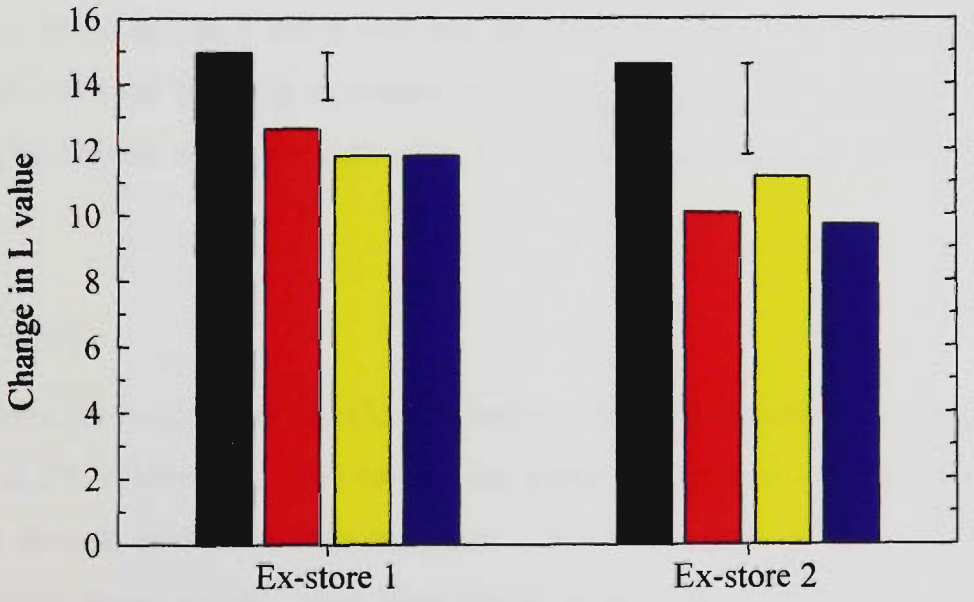


Figure 5.7 Effect of MAP on the amount of butt discolouration on lettuce stored at 5°C Vertical bars = LSD (P=0.05) (■ Trt 1;■ Trt 2;□ Trt 3;■ Trt 4)

5.3.2.3 Amount of decay

The control (treatment 1) decayed rapidly, with lettuce considered unmarketable at the first ex-store assessment (Figure 5.8). The large amount of decay found in the control contributed

greatly to the poor visual quality of the produce. The simulated marketing period after 18 days storage hastened decay development somewhat, however decay levels in the control were already approaching extreme before lettuce were stored at 20°C. At the ex-store 2 assessment, the control was given the maximum decay rating of 5.

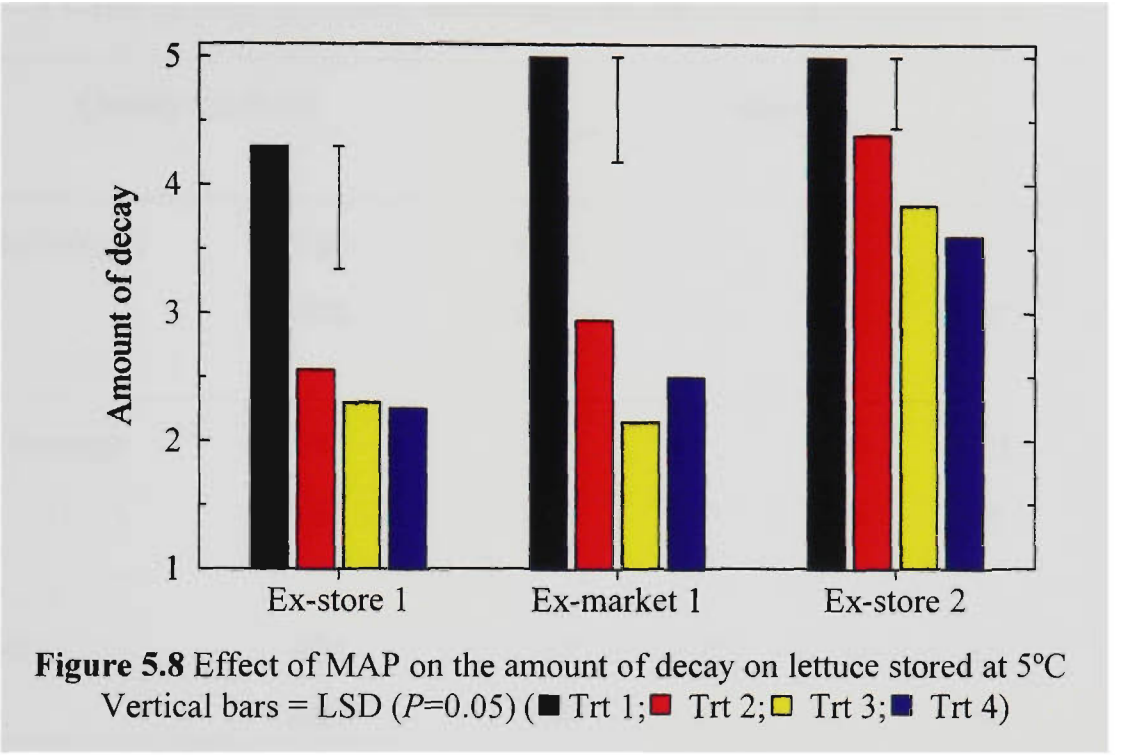


Figure 5.8 Effect of MAP on the amount of decay on lettuce stored at 5°C Vertical bars = LSD (P=0.05) (■ Trt 1;■ Trt 2;□ Trt 3;■ Trt 4)

At every assessment, lettuce stored in plastic liners had significantly less decay than the control (air), with treatments 3 and 4 also less decayed than treatment 2 at the final ex-store evaluation. The amount of decay in treatments 2-4 was considered acceptable after 18 days (Ex-store 1), but levels were too high after 32 days storage (Ex-store 2) to make lettuce marketable.

5.3.2.4 Weight loss

Weight loss from the control was significantly higher than from treatments 2-4 after 18 and 32 days storage at 5°C (Table 5.4). The control lost more weight as storage time increased and after 32 days storage, each head from treatment 1 lost on average 251g, representing nearly 25% of the initial lettuce weight. This compares to the average 0.2% lost from lettuce stored in liners.

5.3.2.5 Russet spotting and brown stain

No russet spotting or brown stain was observed on any treatment throughout the storage period.

5.3.2.6 Head firmness

No significant difference in head firmness was noted between treatments at both ex-store assessments (Table 5.4).

Table 5.4 Effect of MAP on quality attributes of lettuce stored at 5°C

Quality attribute		*Treatment*				*LSD*
		1	2	3	4	$P = 0.05$
Weight loss (g)	ES1	95.41	2.12	1.47	1.87	69.89
	ES2	251.0	3.20	2.10	1.40	56.40
Head firmness	ES1	2.95	3.05	2.90	3.10	0.44
	ES2	3.00	3.05	3.20	2.95	0.48
Flavour	ES1	2.17	2.08	2.00	2.00	0.21
	ES2	5.00	3.00	2.17	2.58	0.27

ES1=Ex-store 1; ES2=Ex-store 2

5.3.2.7 Flavour

After 18 days storage at 5°C, there was no significant difference in flavour between treatments (Table 5.4). However, because treatment 1 was unable to be consumed at the ex-store 2 assessment, treatments 2-4 were considered more palatable than the control after 32 days. Of the liner treatments trialed, treatments 3 and 4 tasted better than treatment 2 at this time, with treatment 3 also more palatable than treatment 4.

5.3.2.8 Overall visual quality

Lettuce stored in air (treatment 1) was unmarketable after 18 days storage at 5°C (Figure 5.9). The simulated marketing period (Ex-market 1) decreased visual quality further and left lettuce in extremely poor condition. A rating score of 5 was also applied to the control after 40 days at 5°C. Unlike the control, the liner treatments were generally still acceptable after 18 days, however treatment 2 was at the lower limit of sales appeal. At the ex-store 2 assessment, treatment 4 appeared significantly better than treatment 2, however all treatments were beyond the level of consumer acceptability (Plate 5.4).

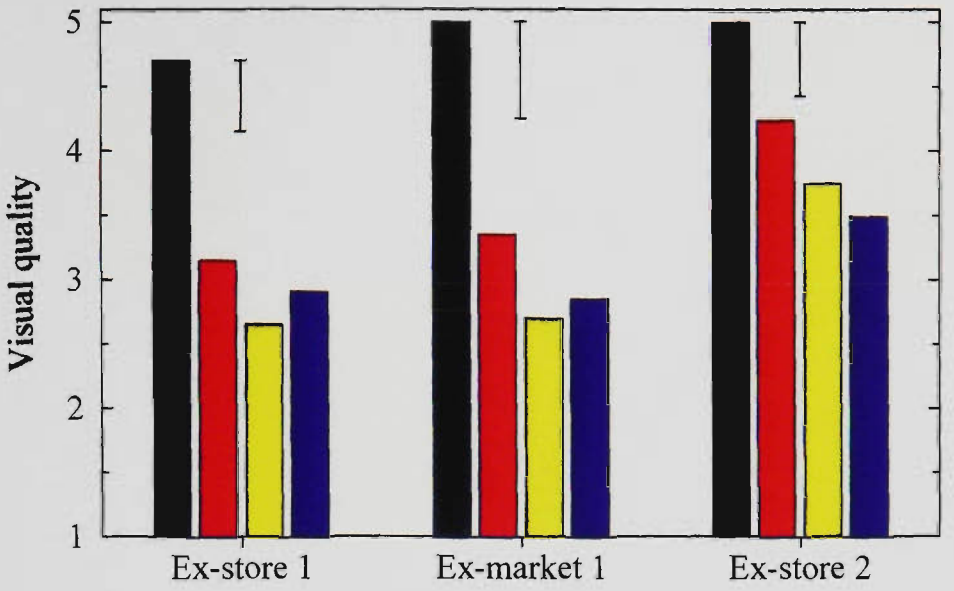


Figure 5.9 Effect of MAP on the visual quality of lettuce stored at 5°C Vertical bars = LSD (P=0.05) (■ Trt 1;■ Trt 2;□ Trt 3;■ Trt 4)

Plate 5.4: Lettuce stored in air or MAP for 32 days at 5°C

Plate 5.4A Initial lettuce quality on day 0

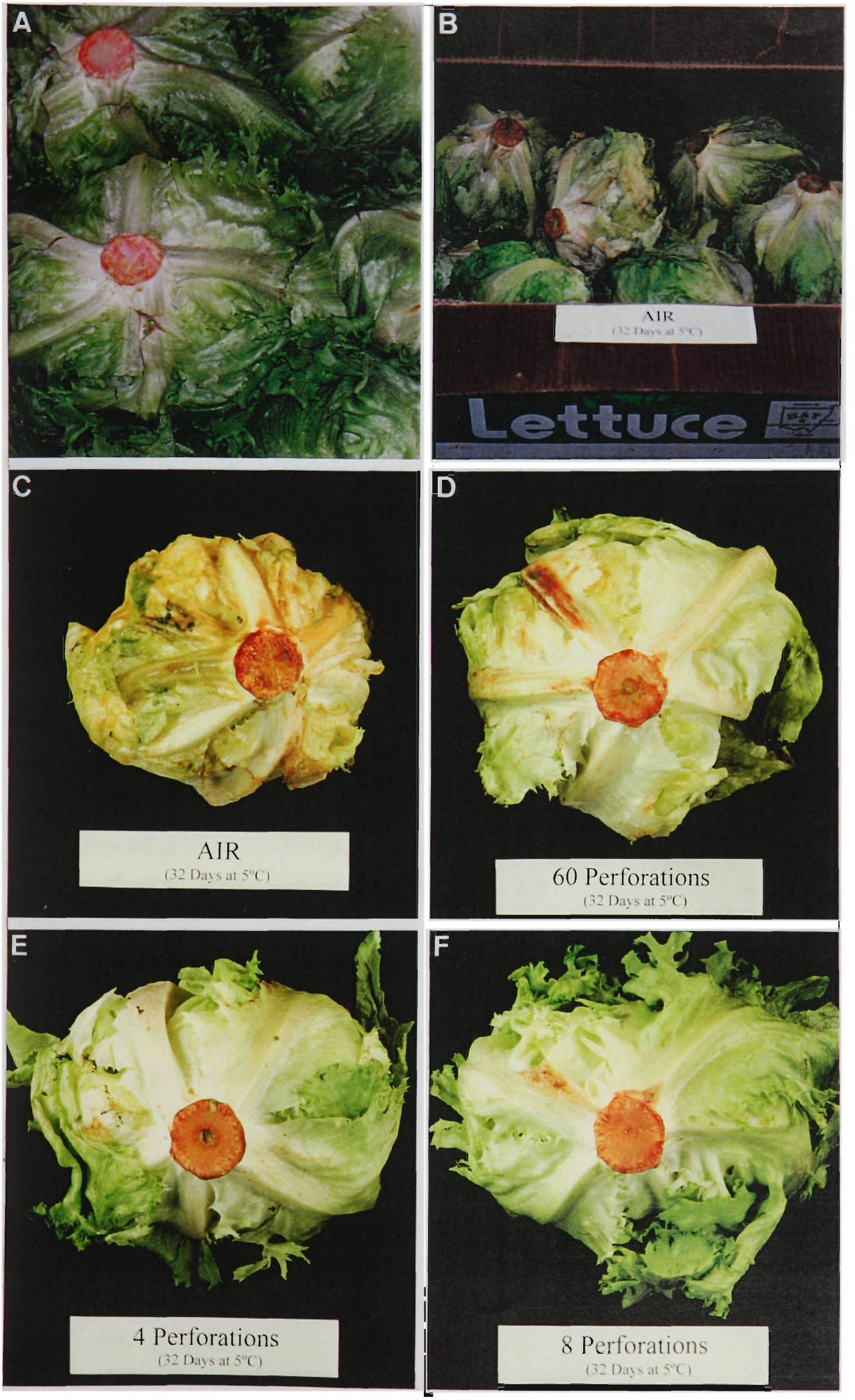
Plate 5.4B Lettuce stored in air for 32 days at 5°C. Note severe decay and wilting

Plate 5.4C Lettuce stored in air for 32 days at 5°C. Note severe decay and butt discolouration

Plate 5.4D Lettuce stored for 32 days at 5°C in a liner containing 60 perforations

Plate 5.4E Lettuce stored for 32 days at 5°C in a liner containing 4 perforations.

Plate 5.4F Lettuce stored for 32 days at 5°C in a liner containing 8 perforations


A
B
AIR
(32 Days at 5°C)
Lettuce
C
AIR
(32 Days at 5°C)
D
60 Perforations
(32 Days at 5°C)
E
4 Perforations
(32 Days at 5°C)
F
8 Perforations
(32 Days at 5°C)

5.4 Discussion

This study showed that MAP can significantly extend the shelf-life of lettuce held at 1.5° and 5°C over lettuce held in air.

Lettuce stored in air wilted rapidly and lost its visual appeal within two to three weeks at 1.5°C. Lettuce is a highly perishable product and is particularly prone to water loss after harvest. Unless care is taken, lettuce develops a wilted condition very rapidly (Story, 1989). Brody (1989) stated that water loss from produce can lead to shrivelling, loss of cell turgor, and other structural defects. Severe wilting was observed in air-stored lettuce when weight loss per head exceeded 5%, confirming results of Ceponis and Kaufman (1968). All vegetables and fruits continue to lose water through transpiration after they are harvested, and this loss of water is one of the main processes that affects their commercial and physiological deterioration (Robertson, 1993).

Lettuce stored within MA carton liners at 1.5°C had a shelf-life of approximately 30-35 days, much greater than that obtained using conventional packaging. Similarly, use of CAS, or MAP, extended the storage life of lettuce by several weeks (Wang *et al.*, 1984; Brecht *et al.*, 1986).

Lettuce responded differently when packaged within different liners. Although polymeric films with 60 perforations per bag were too permeable to establish any appreciable MA, the liners were effective in reducing wilting and weight loss and therefore extended the shelf-life of lettuce to approximately 30 days at 1.5°C. Use of perforated polyethylene wraps for lettuce in storage or transit reduces weight loss and storage defects, thereby increasing saleability (Parsons *et al.*, 1960; Stewart *et al.*, 1967; Wang *et al.*, 1984; Jeong *et al.*, 1990). A reduction in water loss of produce following film wrapping has been reported by other authors (Ceponis and Kaufman, 1968; Forney *et al.*, 1989; Risse and McDonald, 1990).

The beneficial response of storing lettuce within 60 perforation liners was most probably due to a high RH within the bags. Robertson (1993) proposes that to minimize transpiration, produce should be held at low temperature, high RH and as small a water vapour pressure deficit as possible. The primary factor controlling the rate of moisture loss is the water vapour pressure deficit, which reflects the difference between the humidity in the tissue and the humidity of the air in the storage room or container (Kays, 1991a). Lougheed (1977) stated

that some of the benefits attributed to CAS/MAP for leafy vegetables are due to higher humidities within enclosed chambers than around the control samples in room air.

The use of microperforation was effective in altering the gas transmission rates of the polymeric film and proved to be a useful tool in establishing different modified atmospheres within carton liners at both temperatures. Robertson (1993) stated that gas and water vapour transmission through the perforations is proportional to the effective hole diameter, the number of holes per unit film area, and the uniformity of hole distribution over the package surface. The use of micro-perforated packaging techniques have also proved effective in retarding discolouration in parsnips and improving sugar retention in sweetcorn (Geeson, 1990).

At 1.5°C, the most beneficial packaging system was that containing two perforations per bag. This liner established an equilibrium atmosphere of approximately 6% O_2/5% CO_2, very similar to that recommended for lettuce in earlier studies (Chapter 4). This relatively high O_2 concentration remained fairly constant throughout storage. Because the composition of the gas atmosphere is the result of the initial gas composition, the gas exchange through the package, and the respiratory activity of the product, it will change during the course of the storage period until an equilibrium is reached between gas exchange and gas consumption or production (Gorris and Peppelenbos, 1992). An O_2 level of approximately 6% provided a 'safety barrier' in the case of increased O_2 consumption through temperature abuse. To extend the shelf-life of respiring products through MAP, a suitable packaging system must be composed of a gas atmosphere that allows for a basic level of metabolism, which means that a certain level of O_2 should be available (Gorris and Peppelenbos, 1992).

The effect on lettuce quality of 6% O_2/5% CO_2 was similar to that observed under a 5% O_2/5% CO_2 atmosphere in CA trials, namely reducing the amount of butt discolouration, decay and weight loss and maintaining overall visual quality. According to Prince (1989), MAP can elicit similar responses to those observed with CAS, with the major difference being that for MAP the atmospheres are created or at least maintained by the interaction of commodity respiration with the permeation of respiratory gases through packaging films.

Despite this, MA packed lettuce had a slightly shorter shelf-life than CA stored lettuce mainly because of increased levels of decay. Differences between CAS and MAP of horticultural produce are often observed despite the gas composition around the produce being almost identical in both cases, with MAP stored produce being of poorer quality than CAS. According to Robertson (1993) the limiting factor is the appearance of moulds and bacteria in MAP produce as a result of the high (near saturation) in-package RH, a consequence of the low water vapour transmission rate of the films typically used for MAP. Such a problem is not observed in CAS where the flow system presumably results in a lower RH.

Another effective treatment in prolonging the shelf-life of lettuce held at 1.5°C were carton liners containing zero perforations with an enclosed sachet of hydrated lime. This material absorbed respiratory CO_2 within the package. Use of hydrated lime appears to be a practical method of absorbing CO_2 or even controlling the level of CO_2 in film-lined boxes of lettuce during storage (Hardenburg, 1962). This treatment was especially useful in inhibiting decay. Such a result may have been due to the absorption of free water by the hydrated lime.

Although not compared statistically, lettuce stored at 5°C appeared to have a shelf life of approximately 20-25 days, less than lettuce stored at 1.5°C. Undesirable high temperatures accelerate respiration, speed up senescence, cause leaf yellowing and increase moisture loss (Ceponis *et al.*, 1985). Several authors have emphasized the need to cool lettuce thoroughly and to keep the temperature between 0° and 2.5°C during storage, because quality decreases rapidly at higher temperatures (Pratt *et al.*, 1954 as stated by Brecht *et al.*, 1973a; Morris *et al.*, 1955; Parsons and Wright, 1956). According to Lipton and Ryder (1989) the effort and expense of careful harvesting, packing and proper precooling can be negated substantially if lettuce is mishandled or, in particular, if it is inadequately refrigerated during transit.

Nevertheless, MAP of lettuce at 5°C was useful in maintaining the quality for longer periods than that achievable under conventional storage. Carton liners containing four or eight perforations per bag were equally effective in retaining lettuce quality. The beneficial effects of MAP such as reduced butt discolouration, decay and weight loss and retained flavour became more apparent as storage time increased. Despite this, MAP appears to be more effective in inhibiting deterioration when combined with low temperatures (1.5°C). The inhibitory effects of the elevated CO_2/reduced O_2 are improved at low temperatures and are overshadowed by normal respiratory deteriorative processes at ambient temperature and above i.e. the

beneficial effects of CA/MA complement refrigeration and cannot replace reduced temperature (Brody, 1989).

In summary, MAP of lettuce at both 1.5° and 5°C extended the shelf-life of whole Crisphead lettuce over that stored in air. When combined with low temperatures, this preservation technique has the potential of being applied to lettuce shipments destined for export markets and extended storage of lettuce at higher temperatures on domestic markets.

Chapter 6

Controlled atmosphere storage of shredded lettuce

6.1 Introduction

There is a growing demand for prepackaged chopped and shredded lettuce for use in restaurants, institutions, supermarket salad bars, and consumer packs for home use (Brocklehurst *et al.*, 1987; McDonald *et al.*, 1990; Couture *et al.*, 1993). The popularity of these products stems from the decreased need for washing, trimming or cutting and also the reduced transport costs derived by removing waste material, as high as 40-50%, before transit (Bolin *et al.*, 1977; Krahn, 1977). Improved portion control and stable pricing have also contributed to the increasing share of shelf space that these products occupy in retail supermarkets (Unrein, 1993).

The commercial harvesting, handling, processing and distribution of pre-cut lettuce requires a number of steps that are primarily physical in nature, although their effects may contribute to biological, chemical, and physical changes in the product. (Yildez, 1994). For this reason, minimal processing often increases product perishability rather than making it more stable (Rolle and Chism, 1987; Shewfelt, 1987).

Many factors influence quality of pre-cut vegetables including growing conditions, cultural practices, cultivar and maturity at harvest, harvesting and handling methods, inspection standards, and the duration and conditions of storage (Shewfelt, 1987). It has been shown that keeping shredded lettuce cold, using sharp slicing knives, minimizing cellular damage, removing liquid containing active enzymes from the surface by centrifugation, and reducing the initial microbial population all contribute to longer shelf-life (Bolin *et al.*, 1977; Krahn, 1977; Maxcy, 1978; Brocklehurst *et al.*, 1987; Adams *et al.*, 1989; McDonald *et al.*, 1990; Saracino *et al.*, 1991).

Despite this, even under recommended storage temperatures (0-8°C), enzymic discolouration of cut surfaces and microbial spoilage means that the shelf-life of pre-cut vegetables is frequently less than five days at 4°C (O'Connor *et al.*, 1992).

Although a relatively large amount of research with promising results has been conducted on CAS/MAP of whole Crisphead lettuce, the area of MAP treatment of pre-cut vegetables such as lettuce is virtually untouched in the public domain (Wiley, 1994).

The optimal MA conditions for pre-cut vegetables may be different to intact produce. They have a larger cut surface area and are subject to greater wounding stress (Bastrash *et al.*, 1993). According to Yildez (1994), the optimum levels of O_2, CO_2 and temperature must be established for each pre-cut commodity and its individual cultivars. This concept is supported by Labuza and Breene (1989), who feel that it is important to define the end point of high quality life for each type of fruit or vegetable. Both cultivar and maturity play a very important role in determining these endpoint values (Wiley, 1994).

The objectives of the study reported in this chapter were:

- To identify experimentally the optimum atmosphere for preservation of pre-cut lettuce at 4°C. Although the optimum storage temperature for pre-cut lettuce is approximately 1°C (Bolin *et al.*, 1977; Krahn, 1977), 4°C represents a realistic temperature that can be achieved throughout the processing, distribution and marketing of pre-cut vegetables.

- To determine the respiration rates of shredded lettuce in air and under CAS. Several researchers have stated that if MAP is to be designed for the product, it is vital to know the respiration rate of the vegetable in the form it is to be packaged, rather than simply in the unprepared form (Lipton, 1967; Robinson *et al.*, 1975). Empirical studies with MA packages often take the form of "pack-and-pray" if the film permeability and/or rates of respiration are not well characterized (Cameron *et al.*, 1994).

- To determine the effect of storage temperature and degree of processing on the respiration rate of shredded lettuce at 4°C.

Knowledge of the optimum gas atmosphere and respiration rate of shredded lettuce at 4°C would optimize the selection of a polymeric film with the correct gas permeability which, after active MAP, would establish and maintain the desired atmosphere and hence potentially extend the shelf-life of pre-cut lettuce.

6.2 Materials and Methods

6.2.1 Sample preparation

Commercially grown Crisphead lettuce (*Lactuca sativa* L. cv. Greenway) were harvested, packed and then transported by truck from Hay, New South Wales, to the Melbourne Wholesale Produce Market on the 10th July, 1994. The journey took approximately five hours and the lettuce were not refrigerated during this period. Twenty four hours after harvest, the lettuce were transported by non-refrigerated truck to the IHD, Victoria and pre-cooled in a forced-air cooler to 1°C.

The following day, the lettuce (average core temperature 2.7°C) was prepared by removing five-to-six wrapper leaves and the core area, and slicing the solid head with a sharp stainless steel knife into four wedges. Removal of the outer leaf layer has been shown to reduce the microbial load on lettuce (Maxcy, 1978; Adams *et al.*, 1989). Each wedge was further sliced perpendicularly to the midrib into 10 mm × 50 mm segments. The minimally processed tissues were dipped for 1 min in untreated tap water and then disinfected for 2 min in a 50 mg/L available chlorine solution as NaOCl at 4°C. Lettuce was again dipped in tap water for 2 min and 100g portions were centrifuged for 1 min in a 15 cm diameter basket rotating at 1000 rpm to remove surface water. These preparation procedures were carried out in ≤ 5°C conditions.

6.2.2 Controlled atmosphere storage of shredded lettuce

Three different controlled atmospheres plus an air control (treatment 1) were trialed in the experiment (Table 6.1) using the system described in Section 3.3. An O_2 level of approximately 5% was chosen as this has been shown to inhibit browning and retard other deteriorative processes in pre-cut lettuce (Refer to Section 2.5.1.6). Levels below 5% O_2 were not trialed because of the potential of anaerobic metabolism during temperature abuse conditions in commercial MA packages. The CO_2 concentration was varied between 5 and 15% to determine the response of shredded lettuce to high CO_2 levels. To match the weight:volume ratio of existing retail packs of pre-cut lettuce, approximately 350g of shredded lettuce was placed in each glass jar as one replicate and four replicates per treatment were used. To ascertain the effects of relative humidity on storage life, samples of approximately 350g of shredded lettuce were also placed in four open trays in the coolroom (Treatment 2). The relative humidity within the coolroom was 65 ± 5% compared with >95% in the jars.

On day 0, five subsamples of shredded lettuce were analysed for initial leaf colour and browning intensity as described in Section 3.7. The initial hue angle and browning intensity was 116.17 and 0.854 respectively. All other sensory attributes were assigned a value of 1.0 as detailed in Section 3.7.

The lettuce were stored under the designated conditions for 14 days at 4° ± 0.5°C and except for brief periods when samples were taken for gas analysis and sensory evaluations, the lettuce were kept in the dark.

Table 6.1 Atmosphere treatments during the storage period

Treatment	*Gas concentration* [a]	
	O_2 (%)	CO_2 (%)
1(Control)	20.96[b]	0.03
2	20.96[c]	0.03
3	4.60[b]	4.81
4	5.34[b]	9.91
5	4.97[b]	15.01

[a] Balance N_2; [b] High humidity (>95% RH); [c] Low humidity (65% RH)

6.2.3 Respiration rate of shredded lettuce at 4°C

Respiration rates were determined on days 0, 1, 2, 6 and 9 by measuring the change in CO_2 concentration in the jars as a function of time as described in Section 3.3.

6.2.4 Sensory and biochemical evaluations

On days 3, 7, 10 and 14, 60g of lettuce was removed from each sample and used for sensory and biochemical analysis. Jars were reweighed before being connected back to the CA system to determine weight of tissue for respiration measurements. Special care was taken to restore atmospheric conditions immediately after product removal. The control and treatment 2 were terminated after 10 and 7 days respectively because of their poor quality.

On each sample day, samples were evaluated for any off-odours, leaf colour, visual quality, amount of wilting and decay and flavour. Hue angle values were computed as the average of 40 measurements taken from each sample. Lettuce tissue was then frozen in liquid N_2, stored at -70°C and analyzed for browning intensity (Refer to Section 3.7 for details).

6.2.5 Effect of temperature on the respiration rate and quality of shredded lettuce

To determine the effect of storage temperature on the respiration rate and sensory attributes of minimally processed tissue, samples of approximately 350g of shredded lettuce was also stored in glass jars continually flushed with air at 10° and 20°C, using the system described in Section 3.3.

6.2.6 Effect of degree of processing on the respiration rate of lettuce at 4°C

To determine the effect of the degree of processing on the respiration rate of lettuce, whole heads and lettuce quarters, weighing approximately 600g and 160g respectively, were stored in separate 5.2 litre plastic tubs as described in Section 3.4.

6.3 Results

6.3.1 Controlled atmosphere storage of shredded lettuce held at 4°C

6.3.1.1 Respiration rate

Controlled atmosphere storage significantly reduced the respiration rate of shredded lettuce compared to lettuce stored in air at 95% RH (Figure 6.1). Of the three CA treatments trialed, treatment 5 had the lowest respiration rate, equilibrating at approximately 4 mg CO_2 kg^{-1} hr^{-1}. This was approximately 60% lower than other CA treatments and about one-third of the respiration rate of tissue stored in air (control).

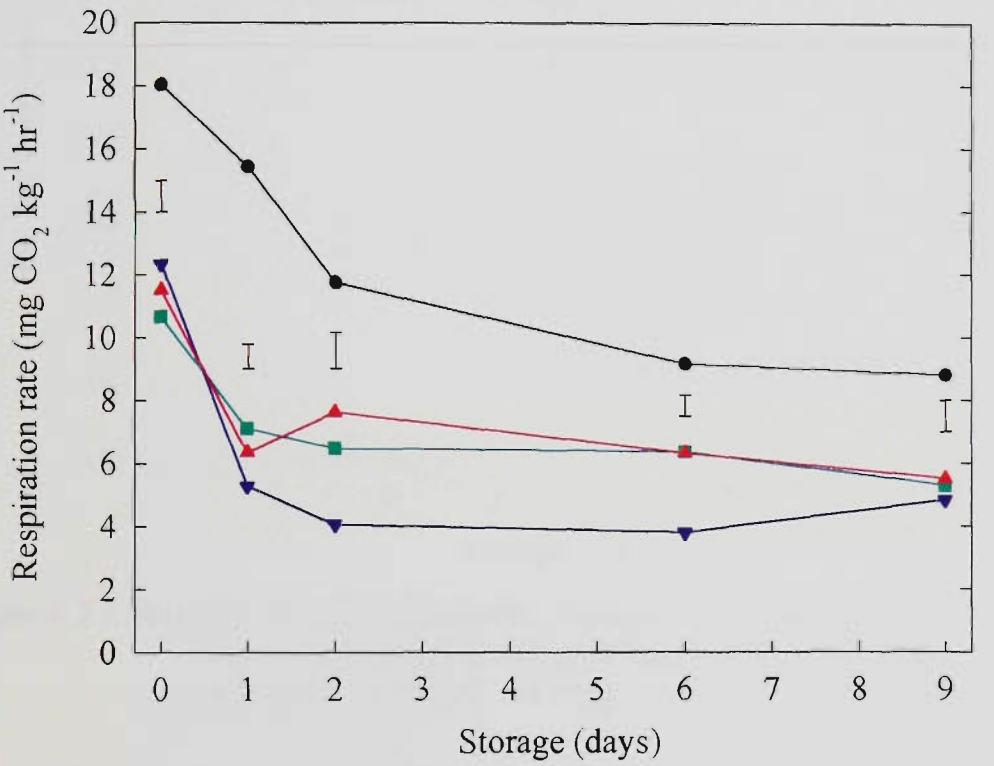


Figure 6.1 Effect of controlled atmosphere storage on the respiration rate of shredded lettuce stored at 4°C. Vertical bars = LSD (P=0.05)
(Trt 1; Trt 3; Trt 4; Trt 5).

Trt 1 = Air (Control; >95% RH)	Trt 2 = Air (65% RH)
Trt 3 = 5% O_2/5% CO_2 (>95% RH)	Trt 4 = 5% O_2/10% CO_2 (>95% RH)
Trt 5 = 5% O_2/15% CO_2 (>95% RH)	

No significant difference in respiration rate was noted between treatments 3 and 4, both respiring at approximately 6.5 mg CO_2 kg^{-1} hr^{-1} throughout the majority of the trial.

6.3.1.2 Change in leaf colour

The hue angle of the control was generally lower than tissue held in CAS (Figure 6.2). The effect was especially pronounced after 10 days storage at 4°C. This smaller hue angle reflected greater colour loss. A significant difference was noted in the rate of colour loss from leaves between the two air treatment samples. Treatment 2, with lower RH, changed from green to yellow at a faster rate than the control.

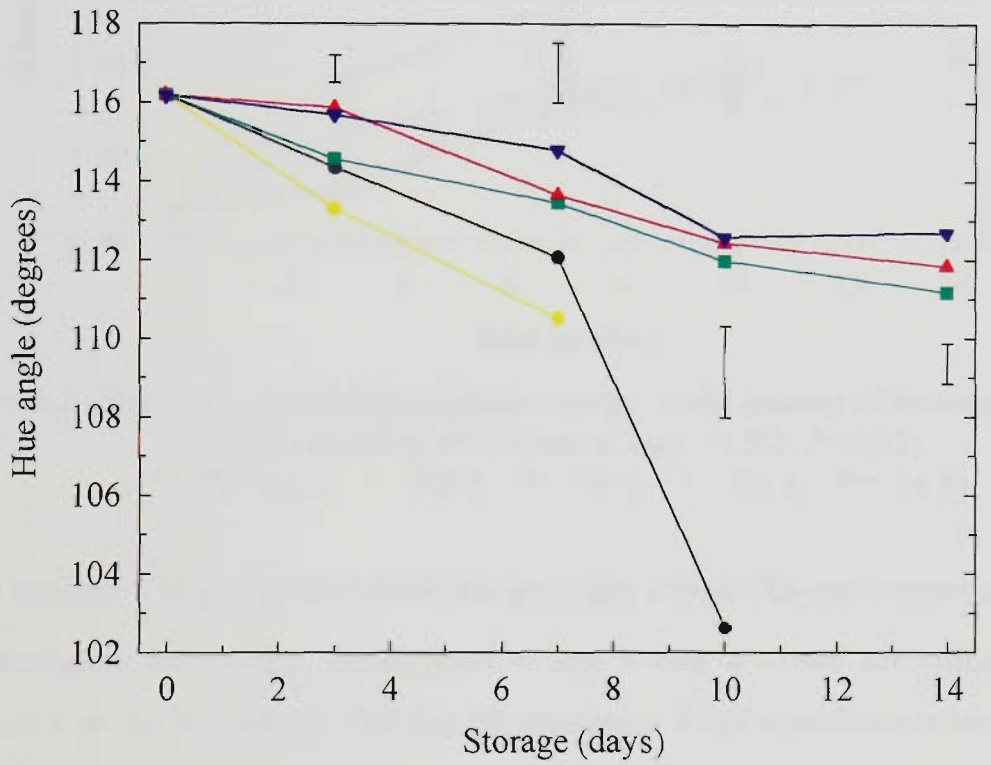


Figure 6.2 Effect of controlled atmosphere storage on the rate of colour loss in shredded lettuce stored at 4°C. Vertical bars = LSD (P=0.05)
(Trt 1; Trt 2; Trt 3: Trt 4; Trt 5)

A high CO_2 environment appeared to inhibit colour loss from shredded lettuce. After 3 days storage, treatments 4 and 5 were significantly greener than treatment 3. After 14 days, treatment 5 was significantly greener than treatment 3.

6.3.1.3 Browning intensity

When compared to the control, CAS significantly reduced the amount of browning present on lettuce tissue. This was evident throughout the storage period (Figure 6.3). Treatment 2 browned extremely fast and had a browning intensity approximately 57% and 60% higher than the control on days 3 and 7 respectively.

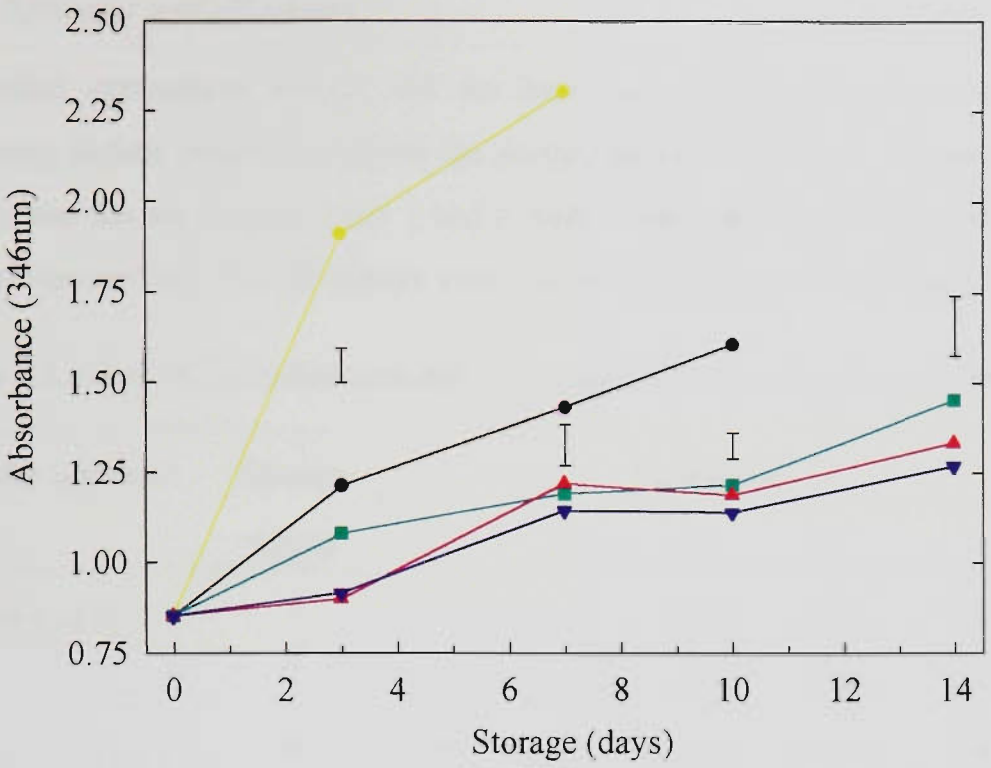


Figure 6.3 Effect of controlled atmosphere storage on the amount of browning on shredded lettuce stored at 4°C. Vertical bars = LSD (P=0.05)
(Trt 1; Trt 2; Trt 3: Trt 4; Trt 5)

Results between CA treatments varied, but generally a high CO_2 environment was associated with decreased browning. Treatments 4 and 5 had a lower browning intensity than treatment 3 on days 3 and 14. On day 10, treatment 5 had significantly less browning than treatment 3.

6.3.1.4 Amount of decay

Decay on shredded lettuce did not pose a major problem during storage, except in treatment 1 after 7 and 10 days (Table 6.2). Treatment 2 only had slight amounts of decay after 3 and 7 days storage. The CA treatments appeared to inhibit decay-forming organisms responsible for the slimy appearance of some tissue samples.

6.3.1.5 Amount of wilting

Lettuce stored in treatment 2 (65% RH) wilted rapidly and lost visual appeal within 3 days as evidenced by high wilting and visual scores (Table 6.2). The high RH (>95% RH) within other treatments generally prevented excess water loss. The control, however, showed some wilting after 10 days storage.

6.3.1.6 Flavour and off-odours

Controlled atmosphere storage did not have any adverse effects on taste, with flavour remaining slightly sweet throughout the storage period (Table 6.2). Because of their inferior quality, the flavour of treatments 1 and 2 were considered objectionable after 10 and 3 days storage respectively. No off-odours were noticed in any treatment throughout the experiment.

Table 6.2 Effect of controlled atmosphere storage on sensory attributes of shredded lettuce

Quality attribute	***Storage***	***Treatment***					***LSD***
	(days)	1	2	3	4	5	$P = 0.05$
Visual quality	3	1.67	4.00	1.00	1.00	1.00	0.49
	7	3.67	5.00	2.00	2.00	1.67	0.73
	10	5.00	-	3.00	2.00	2.00	*
	14	-	-	3.67	3.00	2.67	0.76
Amount of decay	3	1.00	1.33	1.00	1.00	1.00	0.49
	7	1.67	1.33	1.00	1.00	1.00	0.64
	10	3.33	-	1.00	1.00	1.00	0.58
	14	-	-	1.00	1.00	1.00	*
Amount of wilting	3	1.00	4.00	1.00	1.00	1.00	*
	7	2.00	5.00	1.00	1.00	1.00	*
	10	3.00	-	2.00	2.00	2.00	*
	14	-	-	2.00	2.00	2.00	*
Flavour	3	1.00	4.00	1.00	1.00	1.00	*
	7	2.00	5.00	1.00	1.00	1.00	*
	10	4.00	-	1.00	1.00	1.00	*
	14	-	-	2.00	2.00	2.00	*

* LSD not determined because variation within treatments was zero.

6.3.1.7 Visual quality

The overall visual quality was significantly better in CA treatments than lettuce stored in air (Table 6.2; Plates 6.1-6.4). Although treatment 1 prolonged shelf-life over treatment 2, its marketable life was still less than 7 days at 4°C, as evidenced by an average visual score of 3.67 on day 7. In fact, its maximum shelf-life for acceptable visual quality was probably closer to 4 days. On the other hand, all CA treatments were still considered marketable after 10 days, with treatments 4 and 5 still acceptable after 14 days.

6.3.2 Effect of the degree of processing on the respiration rate of lettuce at 4°C

The effect of the degree of processing on the respiration rate of lettuce is presented in Figure 6.4. Whole lettuce heads respired the slowest, equilibrating at 4 mg CO_2 kg^{-1} hr^{-1}. Cutting whole lettuce heads into quarters significantly increased the respiration rate on days 0, 1 and 2, resulting in an equilibrium rate of 5.2 mg CO_2 kg^{-1} hr^{-1}. Differences between whole heads and lettuce quarters were not significant after 6 days storage at 4°C.

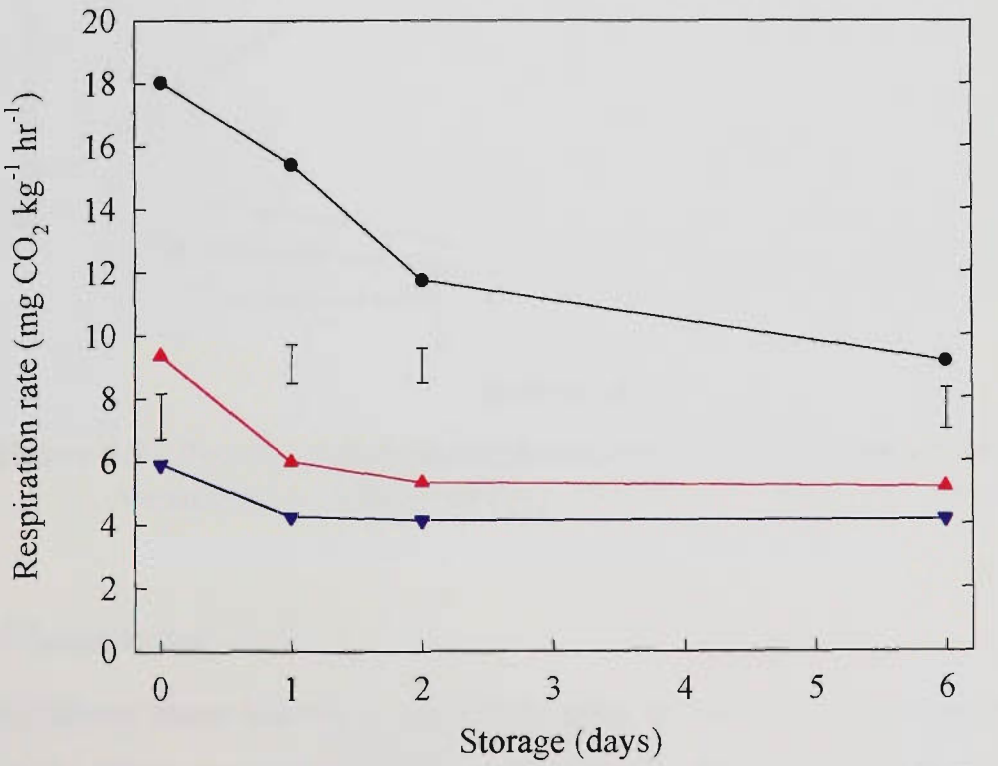


Figure 6.4 Effect of processing on the respiration rate of shredded lettuce stored in air at 4°C. Vertical bars = LSD (P=0.05) (Whole lettuce; Lettuce quarters; Shredded lettuce)

A marked effect on respiration rate occurred when lettuce heads were shredded. Such action caused an immediate increase in metabolic activity, after which the respiration rate decreased to approximately 10 mg CO_2 kg^{-1} hr^{-1} by day 6, significantly higher than whole heads and quarters.

6.3.3 Effect of temperature on the shelf-life of shredded lettuce

6.3.3.1 Respiration rate

Storage temperature had a dramatic effect on the respiration rate of shredded lettuce (Figure 6.5). Respiration rate increased as storage temperature increased, the effect being most pronounced at 20°C. At this temperature, the respiration rate after 3 days was 206 mg CO_2 kg^{-1} hr^{-1}, almost 10 times that of shredded lettuce stored at 10°C. Lettuce stored at 10°C had a respiration rate significantly higher than that at 4°C throughout the experimental period.

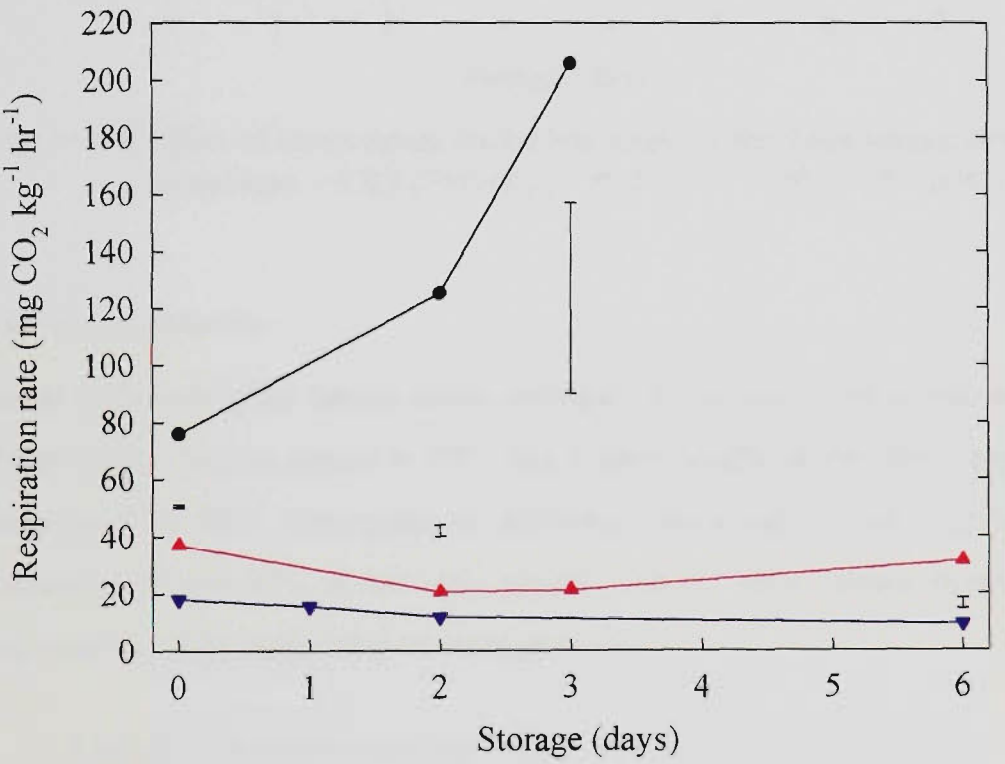


Figure 6.5 Effect of temperature on the respiration rate of shredded lettuce in air Vertical bars = LSD (P=0.05) (4°C; 10°C; 20°C)

6.3.3.2 Change in leaf colour

Shredded lettuce tissue became progressively more yellow as storage temperature increased (Figure 6.6). The most dramatic effect was observed after 3 days storage at 20°C, when the hue angle was significantly lower than lettuce tissue stored at 4° or 10°C. There was no significant difference between the two latter treatments after 3 days storage. However, after 7 days storage, lettuce stored at 4°C was significantly greener than lettuce stored at 10°C.

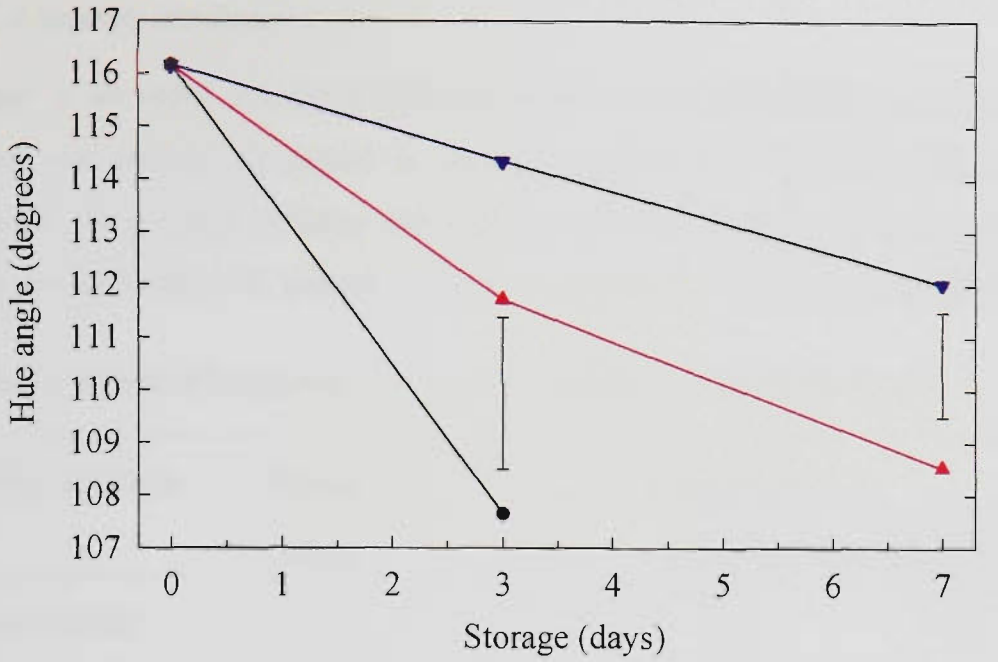


Figure 6.6 Effect of temperature on the hue angle of shredded lettuce stored in air Vertical bars = LSD (P=0.05) (4°C; 10°C; 20°C)

6.3.3.3 Browning intensity

The amount of browning on lettuce tissue increased as storage temperature increased (Figure 6.7). After 3 days, lettuce stored at 20°C had a significantly higher browning intensity than tissue stored at 4° or 10°C. No significant difference was noted in browning intensity between lettuce stored at 4° and 10°C at this time period. After 7 days, lettuce stored at 10°C had a browning intensity 50% higher that tissue kept at 4°C.

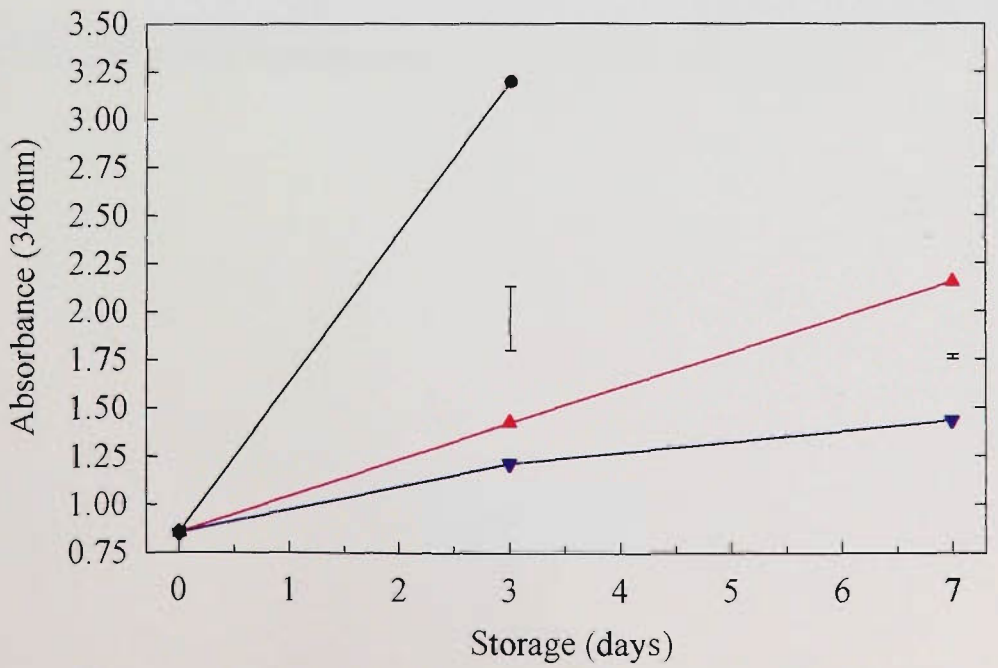


Figure 6.7 Effect of temperature on the amount of browning on shredded lettuce stored in air. Vertical bars = LSD (P=0.05) (4°C; 10°C; 20°C)

6.3.3.4 Sensory attributes

Storage of shredded lettuce at high temperatures was deleterious to product quality. Visual quality and flavour decreased as storage temperature increased (Table 6.3; Plate 6.5). Likewise, the amount of decay and wilting increased as temperature increased. Of the three temperatures tested, 4°C proved to be the most effective in maintaining product quality.

Table 6.3 Effect of temperature on sensory attributes of shredded lettuce

Quality attribute	***Storage***	***Treatment***			***LSD***
	(days)	Air 4°C	Air 10°C	Air 20°C	$P = 0.05$
Visual quality	3	1.67	3.33	5.00	0.94
	7	3.67	5.00	-	0.92
Amount of decay	3	1.00	2.00	4.67	0.67
	7	1.67	4.67	-	1.31
Amount of wilting	3	1.00	2.67	4.00	0.67
	7	2.00	4.00	-	*
Flavour	3	1.00	1.67	5.00	0.67
	7	2.00	5.00	-	*

* LSD not determined because variation within treatments was zero.

Plate 6.1: Quality of shredded lettuce stored in air or controlled atmospheres at 4°C for 3 days

Plate 6.1A Initial lettuce quality on day 0

Plate 6.1B Lettuce stored for 3 days in air in high RH (95%) at 4°C

Plate 6.1C Lettuce stored for 3 days in air in low RH (65%) at 4°C.

Plate 6.1D Lettuce stored for 3 days in 5% O_2/5% CO_2 in high RH (95%) at 4°C

Plate 6.1E Lettuce stored for 3 days in 5% O_2/10% CO_2 in high RH (95%) at 4°C

Plate 6.1F Lettuce stored for 3 days in 5% O_2/15% CO_2 in high RH (95%) at 4°C

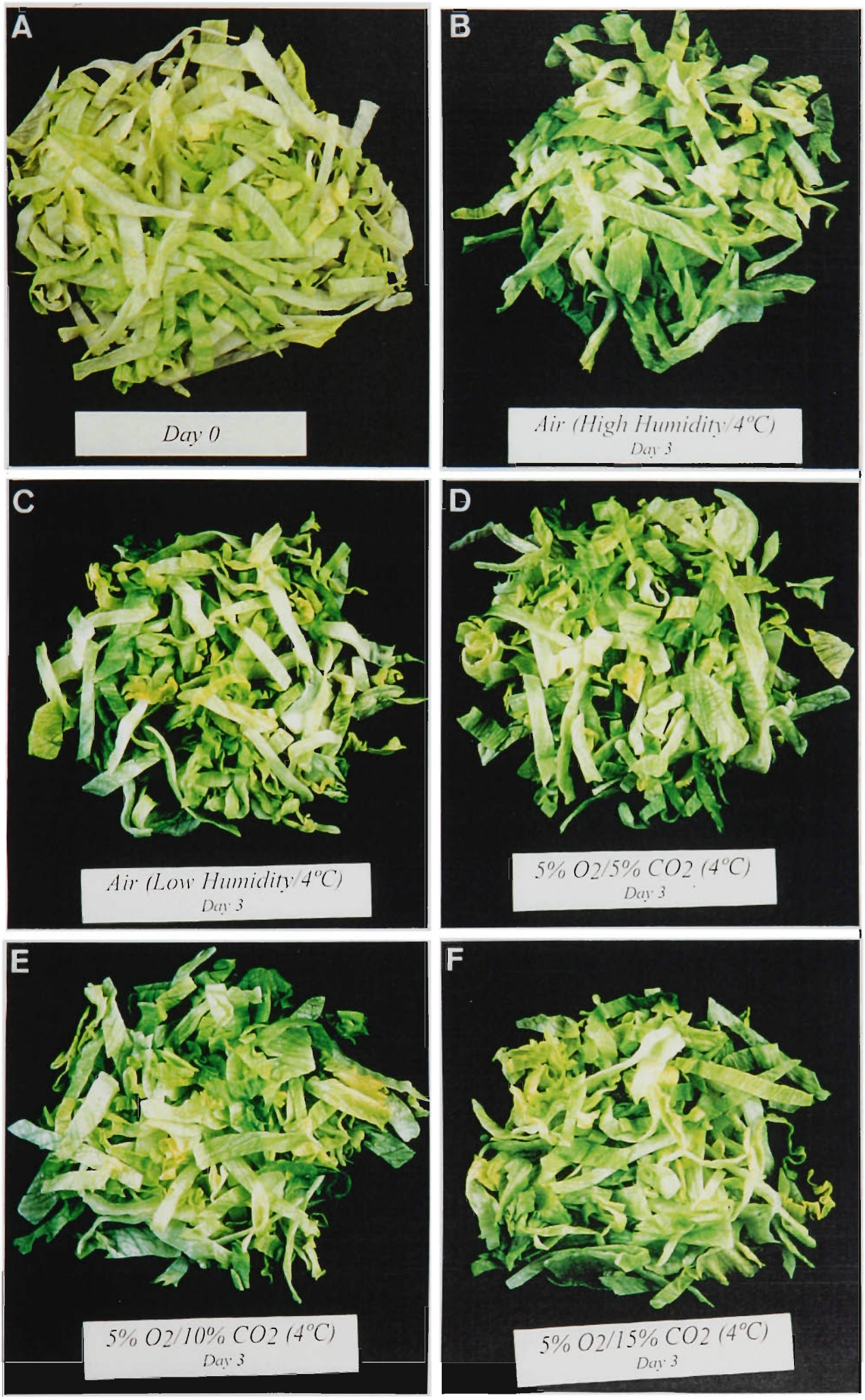

A
Day 0
B
Air (High Humidity/4°C)
Day 3
C
Air (Low Humidity/4°C)
Day 3
D
5% O2/5% CO2 (4°C)
Day 3
E
5% O2/10% CO2 (4°C)
Day 3
F
5% O2/15% CO2 (4°C)
Day 3

Plate 6.2: Quality of shredded lettuce stored in air or controlled atmospheres at 4°C for 7 days

Plate 6.2A Lettuce stored for 7 days in air in high RH (95%) at 4°C. Note slight browning on cut midribs

Plate 6.2B Lettuce stored for 7 days in air in low RH (65%) at 4°C. Note severe wilting of tissue

Plate 6.2C Lettuce stored for 7 days in 5% O_2/5% CO_2 in high RH (95%) at 4°C

Plate 6.2D Lettuce stored for 7 days in 5% O_2/10% CO_2 in high RH (95%) at 4°C

Plate 6.2E Lettuce stored for 7 days in 5% O_2/15% CO_2 in high RH (95%) at 4°C

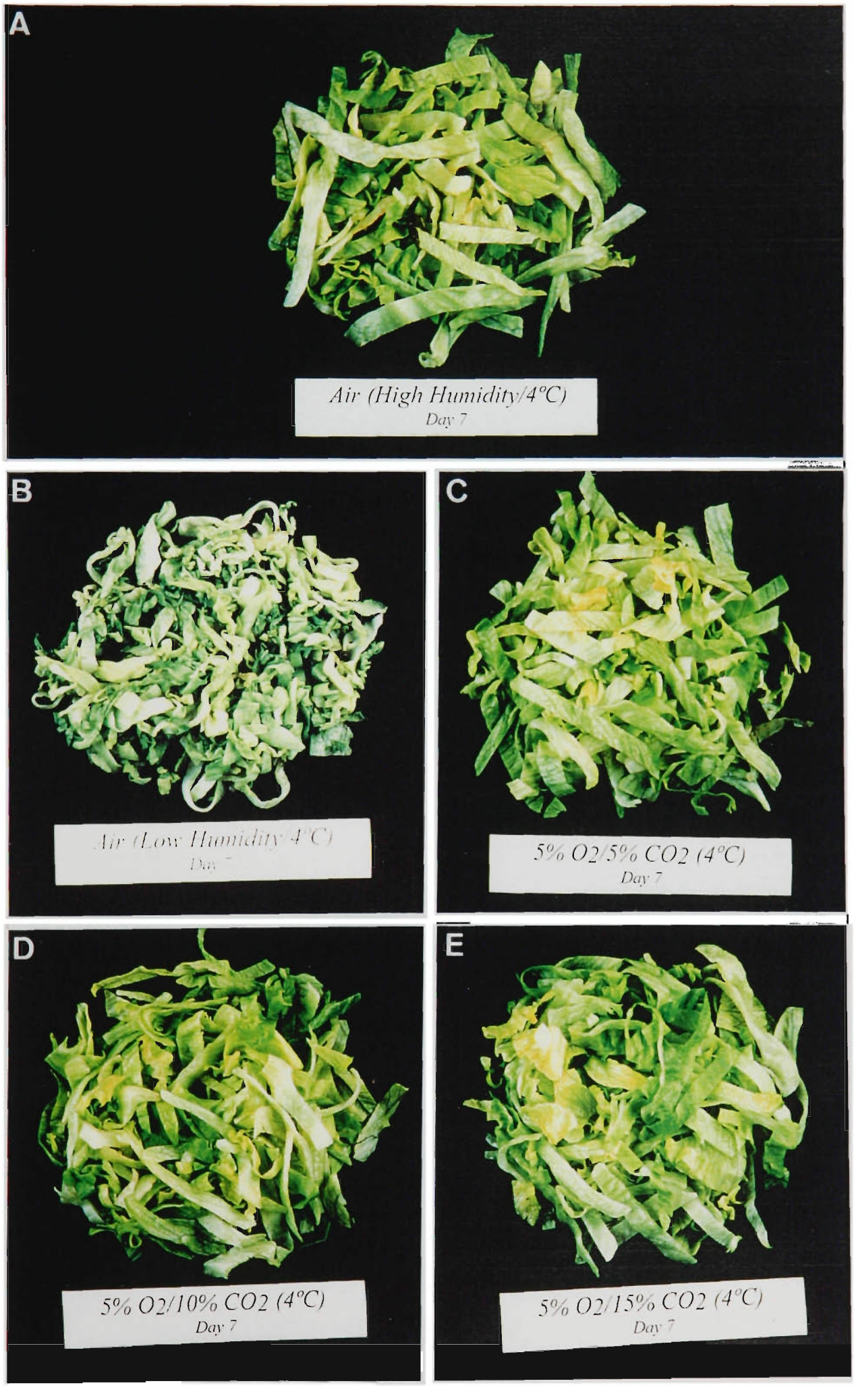

A
Air (High Humidity/4°C)
Day 7
B
Air (Low Humidity/4°C)
Day 7
C
5% O2/5% CO2 (4°C)
Day 7
D
5% O2/10% CO2 (4°C)
Day 7
E
5% O2/15% CO2 (4°C)
Day 7

Plate 6.3: Quality of shredded lettuce stored in air or controlled atmospheres at 4°C for 10 days

Plate 6.3A Lettuce stored for 10 days in air in high RH (95%) at 4°C. Note objectionable browning on tissue

Plate 6.3B Lettuce stored for 10 days in 5% O_2/5% CO_2 in high RH (95%) at 4°C

Plate 6.3C Lettuce stored for 10 days in 5% O_2/10% CO_2 in high RH (95%) at 4°C

Plate 6.3D Lettuce stored for 10 days in 5% O_2/15% CO_2 in high RH (95%) at 4°C

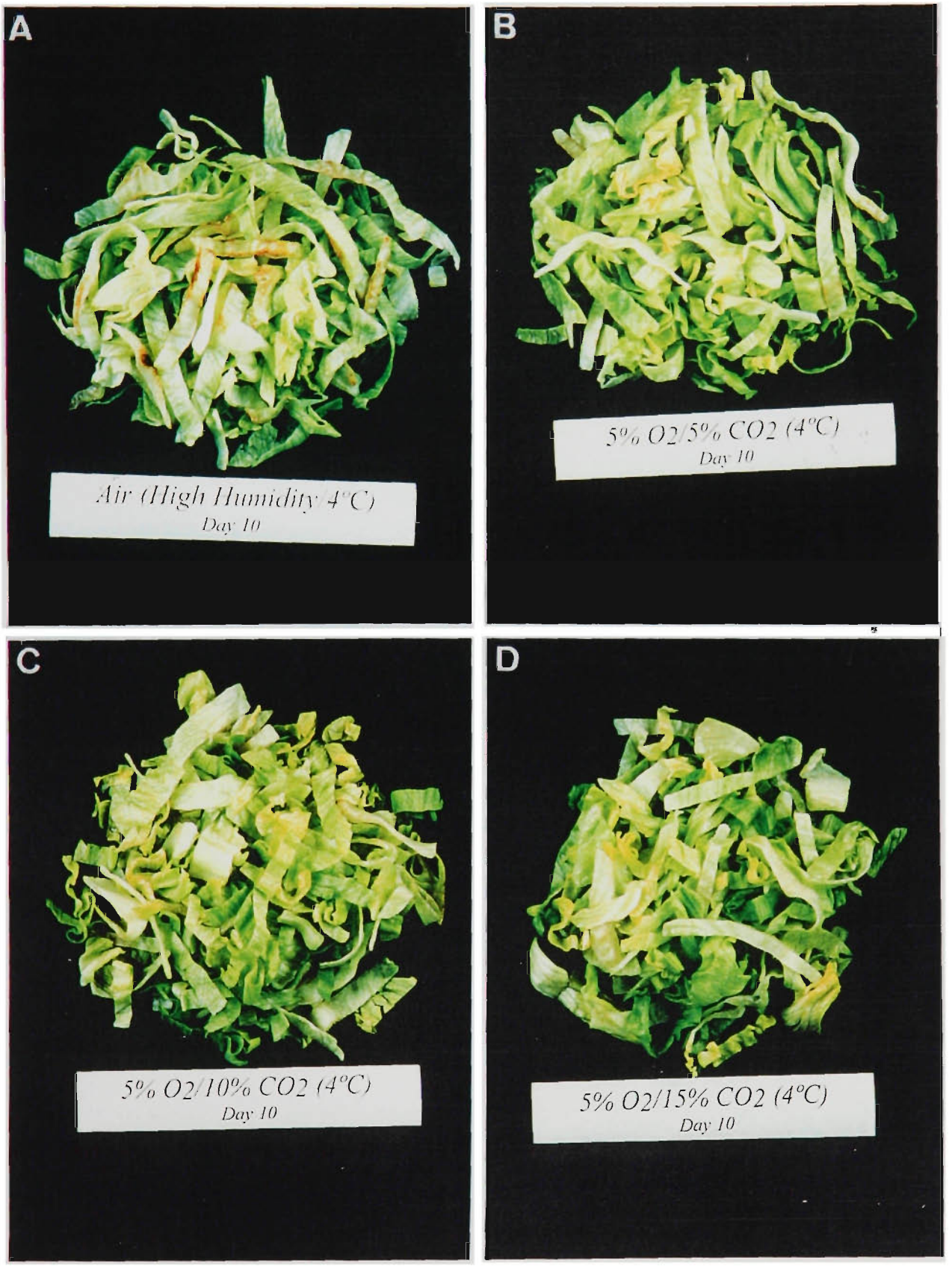
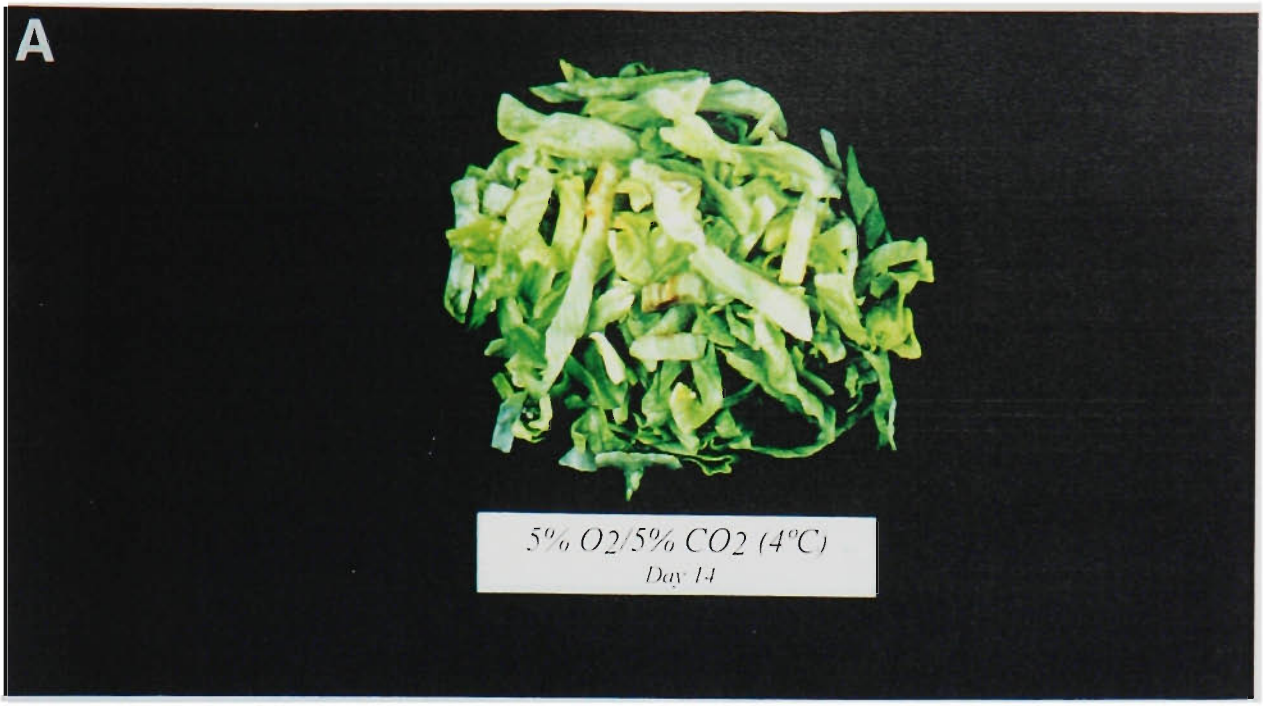

A
Air (High Humidity 4°C)
Day 10
B
5% O2/5% CO2 (4°C)
Day 10
C
5% O2/10% CO2 (4°C)
Day 10
D
5% O2/15% CO2 (4°C)
Day 10

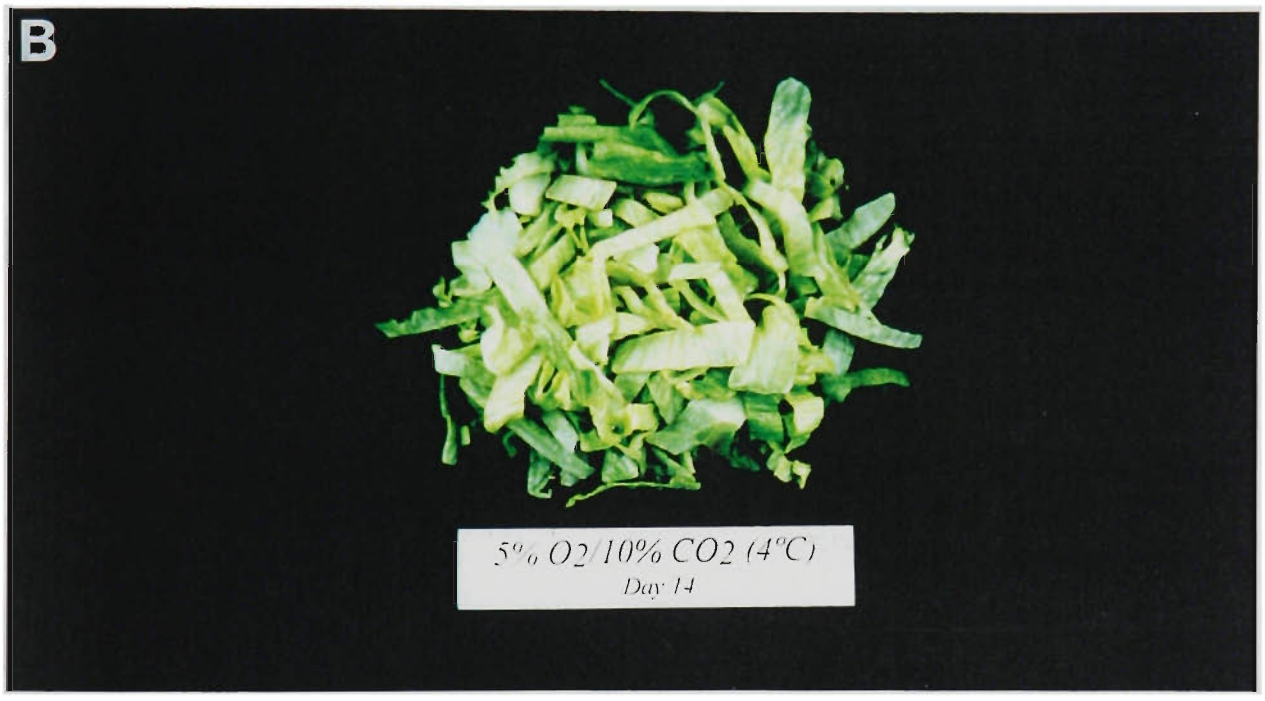
Plate 6.4: Quality of shredded lettuce stored in controlled atmospheres at 4°C for 14 days

Plate 6.4A Lettuce stored for 14 days in 5% O_2/5% CO_2 in high RH (95%) at 4°C. Browning apparent on cut surfaces.

Plate 6.4B Lettuce stored for 14 days in 5% O_2/10% CO_2 in high RH (95%) at 4°C

Plate 6.4C Lettuce stored for 14 days in 5% O_2/15% CO_2 in high RH (95%) at 4°C


A
5% O2/5% CO2 (4°C)
Day 14


B
5% O2/10% CO2 (4°C)
Day 14

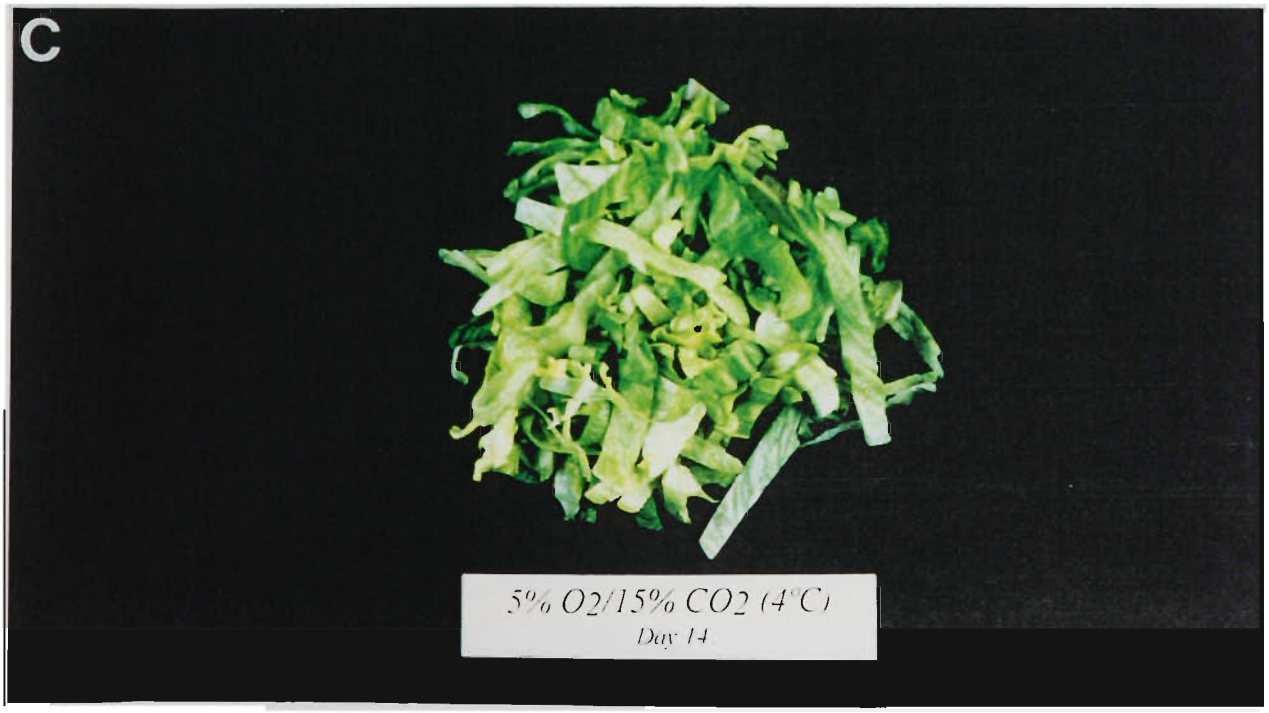

C
5% O2/15% CO2 (4°C)
Day 14

Plate 6.5: Quality of shredded lettuce stored in air at 4°C, 10°C or 20°C

Plate 6.5A Initial quality of lettuce on day 0

Plate 6.5B Quality of lettuce stored for 3 days at 4°C

Plate 6.5C Quality of lettuce stored for 3 days at 10°C. Note moderate browning on midribs

Plate 6.5D Quality of lettuce stored for 3 days at 20°C. Note severe browning

Plate 6.5E Quality of lettuce stored for 7 days at 4°C. Browning beginning to appear on midribs

Plate 6.5F Quality of lettuce stored for 7 days at 10°C. Note severe browning on midribs


A
Initial Quality
Day 0
B
Air (High Humidity/4°C)
Day 3
C
Air (High Humidity/10°C)
Day 3
D
Air (High Humidity/20°C)
Day 3
E
Air (High Humidity/4°C)
Day 7
F
Air (High Humidity/10°C)
Day 7

6.4 Discussion

Minimally processed (MP) vegetables differ from their entire counterparts in that processing ruptures cells and initiates wound-induced physiological and biochemical reactions that shorten shelf-life (Rolle and Chism, 1987; O'Connor *et al.*, 1992; Couture *et al.*, 1993).

In this study, mechanical wounding such as shredding dramatically increased the respiration rate of lettuce compared to intact tissue. Laties (1978) stated that slicing induces a three-to-fivefold rise in respiration over that of the parent-plant organ, consistent with results reported here. These findings are similar to those reported by Priepke *et al.* (1976), who found that after two days storage, total respiration of cut lettuce was twice that of intact produce. A significant increase in respiration rate of produce following cutting has been reported by other authors (McGlasson and Pratt, 1964; Rushing, 1990; Bastrash *et al.*, 1993).

Compared to whole produce, pre-cut tissue represents a different set of physiological conditions (Kader, 1987). Several researchers have attributed the rise in respiration of pre-cut vegetables to physical damage causing a wound response, and to the larger exposed surface area which allows greater diffusion of O_2 in the tissues (Ballantyne *et al.*, 1988a; Saracino *et al.*, 1991). O'Connor *et al.* (1992) proposes that processing results in the unblocking of steps in the electron transport chain with the consequent induction or enhancement of respiration.

The respiration rate of MP tissue decreased gradually as storage time was increased (Figure 6.1). The respiration rate of pre-cut products is usually elevated after cutting and then often declines (Cameron *et al.*, 1994) due to depletion of respirable substrates, which are typically low in leaves (Kader, 1987).

The degree of processing markedly influenced the respiration rate of lettuce tissue (Figure 6.4). Shredded lettuce respired faster than lettuce quarters which in turn had a higher metabolic rate than whole lettuce heads. These results agree with the findings of Ballantyne *et al.* (1988a), who found that the respiration rate of shredded lettuce was more than double that of lettuce quarters at 10°C.

Controlled atmosphere storage of shredded lettuce resulted in much lower respiration rates than for pre-cut tissue stored in air. The greater extent of respiratory CO_2 production by the air-treated control lettuce indicates the likelihood of more quality loss during storage than the CA-treated lettuce, since total CO_2 output has been shown to be proportional to storage life (Lyons and Rappaport, 1959). King *et al.* (1991) suggest that a significant portion of lettuce spoilage can be attributed to tissue breakdown caused by tissue respiration.

According to Kader (1986), lower O_2 concentrations in the storage atmosphere reduce the reaction rate of enzyme-catalyzed oxidations. This study showed that an atmosphere consisting of 5% O_2/15% CO_2 was the most effective of the three controlled atmospheres in reducing respiration rate (Figure 6.1). Since all three CA treatments contained the same level of O_2, it can be assumed that the respiration rate of shredded lettuce decreases substantially as the CO_2 concentration rises above 10% for up to six days storage. This is because, at CO_2 levels of 5% and higher, commodities have reduced respiration rates (Brecht, 1980; Zagory and Kader, 1988; Kader *et al.*, 1989).

The change in leaf colour over time was monitored on shredded lettuce to determine the effect of CAS on retaining green pigmentation. The analysis of colour is frequently an important consideration when determining the efficacy of a variety of postharvest treatments (McGuire, 1992). Visually, shredded lettuce stored in air changed from green to yellow at a faster rate than tissue held in CAS (Figure 6.2). This result agrees with the findings of Bolin *et al.* (1977) and Bolin and Huxsoll (1991), who reported a dramatic loss in visual green pigmentation in air-stored lettuce. A similar result was obtained by Ballantyne *et al.* (1988a), who optimized colour stability of shredded lettuce in a MA of 1-3% O_2 and 5-6% CO_2.

Loss of green colour appeared to be reduced further as the CO_2 concentration was increased in the storage atmosphere. Several studies have demonstrated that the chlorophyll content of green leaves is maintained by high CO_2 levels (Hruschka and Wang, 1979; Herner, 1987; Aharoni *et al.*, 1989) and that besides preventing chlorophyll loss, CO_2 delays other biochemical changes characteristic to leaf senescence (Philosoph-Hadas *et al.*, 1989). A high RH also appears to be beneficial in retarding loss of visual green pigmentation.

In this study, lettuce held in air exhibited severe enzymatic browning on cut surfaces within a relatively short time (Figure 6.3). Small necrosis and brown discolouration appeared on leaf margins, on cuts and on ribs within a few days of storage and caused marked deterioration in quality of pre-cut salads (Nguyen and Prunier, 1989). Undesirable quality changes such as browning are most likely accelerated by the mechanical rupturing of cells that occurs during cutting, allowing enzymes to intermix with substrate (King and Bolin, 1989; Labuza and Breene, 1989; Watada *et al.*, 1990). The browning reaction is thought to be brought about through the metabolism of phenolic compounds (Luh and Phithakpol, 1972; Vamos-Vigyazo, 1981). The phenolic pathway begins with the deamination of phenylalanine by phenylalanine ammonia lyase (PAL) to cinnamic acid. Cinnamic acid is then sequentially hydroxylated into various phenolic compounds. In the presence of O_2, polyphenol oxidase (PPO) may oxidize these compounds to quinones which polymerize into brown pigments (Rhodes *et al.*, 1981; Rolle and Chism, 1987). This reaction is very rapid, even at refrigerated temperature, and is initiated by any tissue damage (Labuza and Breene, 1989).

The reduction in appearance quality due to browning is an important cause of market losses in affected products, and in many cases may be the primary limiting factor in marketing (Mayer, 1987; Robertson, 1993). Rolle and Chism (1987) state that control of the wounding response is a major hurdle for extending the shelf-life of MP tissues.

In this study, CAS substantially reduced the amount of browning observed on tissue compared to lettuce stored in air (Figure 6.3). Low, but not anaerobic, O_2 levels have been found to reduce browning and senescence for cut lettuce and broccoli florets (Krahn, 1977; Ballantyne *et al.*, 1988 a,b; McDonald *et al.*, 1990). Bolin *et al.* (1977) demonstrated the presence of PPO on the surface of shredded lettuce. PPO is sensitive to O_2 level, decreasing in activity with decreased O_2 level (Labuza and Breene, 1989) because O_2 is a substrate (Murr and Morris, 1974).

The amount of browning generally decreased as CO_2 concentration was increased, supporting the results of Mateos *et al.* (1993b). Carbon dioxide is known to inhibit browning of physically-damaged plant tissues by inhibiting the activity of PPO and lowering the level of phenolic compounds (Buescher and Handerson, 1977; Siriphanich and Kader, 1985a). However, the mechanism by which CO_2 reduces phenolic content is not fully understood; it

may or may not be related to the browning process (Siriphanich and Kader, 1985b). Carbon dioxide may inhibit PPO activity but the direct inhibition of this enzyme has not been fully demonstrated (Murr and Morris, 1974).

Unlike whole lettuce heads, pre-cut tissue was found to be less sensitive to CO_2 damage such as brown stain. These results are consistent with the findings of other researchers (Krahn, 1977; Ballantyne *et al.*, 1988a; McDonald *et al.*, 1990; Mateos *et al.*, 1993b). Due to the removal of the skin diffusion barrier, MP vegetables tolerate higher concentrations of CO_2 and lower O_2 than intact commodities (Kader *et al.*, 1989; O'Connor *et al.*, 1992; Yildez, 1994). Burton (1974) and Kader and Morris (1977) suggested that anatomical differences, leading to differences in diffusion resistance to atmospheric gases, rather than biochemical variations, may be largely responsible for differences in tolerance to low O_2 and high CO_2 among different fruits and vegetables.

The amount of decay increased steadily with time for MP tissue stored in air at a high RH (Table 6.2). Nguyen and Prunier (1989) stated that complete rotting of these lettuce salads never occurs within the average length of storage, but the slight decay which occurs normally is sufficient to hinder the sale of these products. The high humidity in pre-cut salads and the large number of cut surfaces, as a result of shredding, provide ideal conditions for the multiplication of microorganisms (Denis and Picoche, 1986 as stated by Nguyen and Prunier, 1989). The low RH surrounding pre-cut tissue stored in open trays within the coolroom was apparently not conducive to decay development (Table 6.2).

Controlled atmosphere storage appeared to inhibit decay forming organisms. It seems that CAS (3% O_2/10% CO_2) can extend the shelf-life of shredded lettuce by limiting plant and microbial enzyme activity, without appreciably reducing microbial counts (Barriga *et al.*, 1991). Daniels *et al.* (1985) states that CO_2 is effective for extending the shelf-life of perishable foods by increasing the lag phase and generation time of spoilage microorganisms. According to Farber (1991) the inhibitory effects of CO_2 on microorganisms are dependent on the partial pressure of CO_2, temperature, acidity, water activity, the type of microorganism, the microbial growth phase, and the growth medium used. Daniels *et al.* (1985) proposes that for maximum antimicrobial effect, the storage temperature of a MAP product should be kept as

low as possible, because the solubility of CO_2 decreases dramatically with increasing temperature.

Storage of lettuce in air at 4°C in a low RH environment induced severe wilting in tissue samples (Table 6.2). Minimal processing has previously been found to increase the rate of moisture loss via increasing cut surface area (Priepke *et al.*, 1976; King and Bolin, 1989; Day, 1993). A high RH storage environment was effective in reducing the amount of wilting (Table 6.2). Cameron *et al.* (1994) stated that reduction of water loss is one of the most important goals for successful MAP of pre-cut products, despite the emphasis in many studies on modification of O_2 and CO_2. Loss of water not only reduces the saleable weight, but can also induce senescence of the product (Grierson and Wardowski, 1978).

Maintenance of a stable, low temperature would appear to be one of the keys to success for packed MP vegetables (Sugawara *et al.*, 1987; Ohta and Sugawara, 1987; Varoquaux and Wiley, 1994). The trials reported in Section 6.3.3 showed that the most important factor in the storage life of shredded lettuce is proper temperature management, a result which agrees with others (Bolin *et al.*, 1977; Krahn, 1977; Bolin and Huxsoll, 1991). Huxsoll *et al.* (1989) stated that very low refrigerated storage temperatures, just above the freezing point of the products, may provide a high degree of preservation for some forms of MP vegetables and fruits.

The respiration rate of shredded lettuce increased dramatically as storage temperature increased, especially at temperatures above 10°C (Figure 6.5). Krahn (1977) also reported a correlation between storage temperature and respiration rate. He found that the respiration rate of cut lettuce at 5°C was 40% higher than that at 0°C. Lyons *et al.* (1979) found that fruits and vegetables which are tolerant to low temperatures show a decrease in respiration rate with a decrease in temperature, and maintain their respiratory activity in balance with glycolysis at low temperatures.

The amount of browning, decay and wilting on pre-cut tissue all increased as storage temperature increased (Plate 6.5), confirming the results of other studies (Ohta and Sugawara, 1987; Sugawara *et al.*, 1987; Forney and Rij, 1991). Associated with this was a loss in green pigmentation and a reduction in overall visual quality. Krahn (1977) emphasizes that the maximum benefit of CAS results when it is combined with low temperatures around 0°C. McDonald *et al.* (1990) found that chopped lettuce stored at 5°C had a significantly shorter

life, determined by appearance and flavour evaluations, than lettuce held at 1°C. According to Lioutas (1988), for protection from excessive microbial growth and greatest retention of sensory and nutritional quality, pre-cut products should be held during distribution at temperatures ranging from 2° to 4°C.

In summary, the trials reported in Chapter 6 have shown that the shelf-life of pre-cut Crisphead lettuce cv. Greenway is maintained longer, and quality retained better, at 4°C than at higher temperatures. Atmospheres low in O_2 (5%) and high in CO_2 (5-15%) complemented low temperature storage of tissue and maintained quality for approximately 10 days, almost double that achieved in air. Shelf-life was extended to 14 days when lettuce were stored in 5% O_2/10% CO_2 or 5% O_2/15% CO_2. This research has identified that storage of shredded Crisphead lettuce cv. Greenway in 5% O_2/10-15% CO_2 provides an important extension to shelf-life. Because of time constraints, a MA bag for this product could not be designed and tested but these research results provide important base information for development of this product.

Chapter 7

Controlled atmosphere storage and modified atmosphere packaging of pre-cut Romaine lettuce

7.1 Introduction

Pre-cut Romaine (Cos) lettuce is one of the many different types of minimally processed (MP) lettuce that are becoming increasingly popular in the retail and foodservice sectors. Like shredded Crisphead lettuce, chopped Romaine lettuce minimizes waste when compared to the whole product, allows product and price consistency and offers labour savings to the buyer (Dioguardi and Cadman, 1995b).

At present, pre-cut Romaine lettuce is mainly used as the sole ingredient of prepackaged Caesar salads, although its use as a component of other pre-cut salad mixes is increasing (Dunker, 1994). However, physiological problems such as enzymatic browning, wilting and decay have limited its success at the retail level (Hilton, 1994). Any extension of shelf-life over the 5-8 days currently obtained may enable greater penetration of this product into the market (Cook, 1994).

The study reported in this chapter was initiated after market surveys by Vegco Ltd. identified pre-packaged, pre-cut Romaine lettuce as having potential in Australian supermarkets (Roberts per. comm., 1994). To realize its full potential, the shelf-life of chopped Romaine lettuce needs to be extended to allow for orderly distribution and marketing from the processing plant in East Gippsland to supermarkets situated around Melbourne and Sydney metropolitan areas (eight hours by road).

Studies reported in Chapter 6 identified that a key to achieving longer shelf-life of shredded Crisphead lettuce is to ensure the maintenance of low temperatures ($\leq$ 4°C) throughout storage. In addition, MAP would appear to be useful in extending the postharvest life of pre-cut lettuce. Although a limited amount of research has been conducted on MAP of whole Romaine lettuce (Aharoni and Ben-Yehoshua, 1973; Lipton, 1987), there have been no reported studies investigating the use of MAP in extending the shelf-life of pre-cut Romaine lettuce.

The objectives of this study were to develop a MA retail pack for pre-cut Romaine lettuce and to assess the suitability of a commercial processing facility and transport network in maintaining adequate temperature control during and after processing. It was important to trial retail packs of lettuce in a commercial system because any temperature abuse/fluctuations and poor handling practices could be identified and possibly related back to shelf-life problems.

To achieve these objectives, the effects of several variables in MAP needed to be identified and these were addressed in two separate experiments. Experiment 1 used CAS to characterize gas atmospheres that are beneficial/detrimental to the shelf-life of pre-cut Romaine lettuce and the respiration rates of chopped Romaine tissue in the different CA regimes. Experiment 2 utilized data obtained in Experiment 1 to predict and test a film that would establish and maintain a desirable EMA in a commercial situation.

7.2 Materials and Methods

7.2.1 Experiment 1: Controlled atmosphere storage of pre-cut Romaine lettuce

7.2.1.1 Sample preparation

Commercially grown Romaine lettuce (*Lactuca sativa* L. cv. Verdi) were harvested, packed and then transported, unrefrigerated, from Cranbourne, Victoria, to the IHD, Knoxfield on the 5th September, 1994. The journey took approximately 30 minutes after which the lettuce were precooled and stored at 1°C overnight. Cultivar Verdi is the industry standard in Victoria (Knowles per. comm., 1994).

The following day, the Romaine lettuce (average core temperature 2.6°C) was prepared by removing five-to-six wrapper leaves and slicing the head with a sharp stainless steel knife perpendicularly to the midrib into approximately 50 mm × 70 mm segments. The lettuce were then washed, disinfected, rinsed and dried as described for shredded lettuce in Chapter 6.2.1. These preparation procedures were carried out in ≤ 5°C conditions.

7.2.1.2 Controlled atmosphere storage

Four different controlled atmospheres plus an air control (treatment 1) were trialed in the experiment (Table 7.1) using the system described in Section 3.3. Approximately 260g of chopped lettuce was placed in each glass jar as one replicate and four replicates per treatment

were used. To ascertain the effects of relative humidity on storage life, approximately 260g of lettuce was also placed in four open trays in the coolroom (Treatment 2). The RH within the coolroom was approximately 65 ± 5%. To compare the respiration rate of whole and pre-cut tissue, whole Romaine heads (treatment 7) were also stored in air at a RH superior to 95% using the system described in Section 3.5.

Table 7.1 Atmosphere treatments during the storage period

Treatment	*Gas concentration*[a]	
	O_2 (%)	**CO_2 (%)**
1 (Control)	20.96[b]	0.03
2	20.96[c]	0.03
3	4.60[b]	4.81
4	5.34[b]	9.91
5	4.97[b]	15.01
6	2.15[b]	15.3
7	20.96 [d]	0.03

[a] Balance N_2; [b] High RH (> 95% RH); [c] Low RH (65% RH)

[d] Whole lettuce in high RH (>95% RH)

On day 0, a random leaf from each jar/tray was used to measure the initial colour of the tissue as described in Section 3.7. After measurement, this designated area was circled with a black marker and subsequent colour measurements were performed on the same area. This method enabled the change in leaf colour over time to be recorded; it assumes the marker had no effect on colour.

A subsample of chopped lettuce from four different heads was used to determine the initial acetaldehyde and ethanol content of tissue according to the method described in Section 3.7. Five labelled pieces of lettuce from each jar/tray were also used to assess the change in chlorophyll content of leaves during storage. On day 0, a 12 mm diameter hole punch was used to remove a tissue disc (1.13 cm^2) from each one of these leaves. These discs were taken from one side of the leaf which was separated by the midrib. The chlorophyll content of each disc was determined by N,N-dimethylformamide extraction according to the method described in Section 3.7. All other sensory attributes of the lettuce segments were assigned a value of 1.0 as detailed in Section 3.7.

The lettuce were stored under the designated conditions for 15 days at 4° ± 0.5°C and except for brief periods when samples were taken for gas analysis and sensory evaluations, the lettuce were kept in the dark. Storage in the dark helped simulate transport within closed cardboard boxes stacked on a pallet.

7.2.1.3 Respiration rate

Respiration rates of whole and pre-cut lettuce were determined on days 0, 1, 6, 8 and 13 as described in Section 3.3 and Section 3.5. The average weight of whole Romaine lettuce was 370g.

7.2.1.4 Sensory and biochemical evaluations

On days 3, 7, 10 and 15, 50g of lettuce was removed from each pre-cut sample and used for sensory and biochemical analysis. Jars were reweighed before being connected back to the CA system. Special care was taken to restore atmospheric conditions immediately after product removal. Treatment 2 was terminated after 7 days because of its very poor quality.

On each sample day, lettuce tissue was evaluated for any off-odours, leaf colour, visual quality, amount of browning, amount of chlorophyll lost in the time period*, amount of wilting, amount of decay and flavour. *The amount of chlorophyll was determined by removing the corresponding leaf disc on the other side of the midrib and subtracting the amount of chlorophyll from the initial values (Refer to Section 7.2.1.2). Preliminary experiments showed that there was no significant difference in chlorophyll content in tissue on either side of the midrib). Acetaldehyde and ethanol content of tissue was measured on days 3, 7 and 15 to determine the levels of O_2 and CO_2 that may induce anaerobiosis.

7.2.2 Experiment 2: Modified atmosphere packaging of pre-cut Romaine lettuce

7.2.2.1 Sample preparation

Commercially grown Romaine lettuce (*Lactuca sativa* L. cv. Verdi) were harvested, packed in polystyrene boxes containing ice and then transported by car from Tyabb, Victoria, to Vegco Ltd., Bairnsdale, Victoria on the 28th February, 1995. The journey took approximately 2.5 hours after which the lettuce (average core temperature 12° ± 0.5°C) were stored at 4°C overnight.

The following day, pre-cut lettuce (average core temperature 5° ± 0.5°C) was prepared by removing five-to-six wrapper leaves and slicing the head with a sharp stainless steel knife perpendicularly to the midrib into approximately 50 mm × 70 mm segments. The pre-cut tissues were dipped for 1 min in tap water (containing 5 mg/L available chlorine) and then disinfected for 2 min in 50 mg/L available chlorine as NaOCl at 5°C. Lettuce segments were further dipped in tap water for 2 min and spun in a kitchen centrifuge to remove surface water. These preparation procedures were carried out in ≤ 7°C conditions (Figure 7.6).

The chopped lettuce (approximately 150g) was then packed into two different films (Table 7.2). This weight of lettuce represents the usual amount marketed in prepackaged bags in supermarkets. Bags for treatment 1 (Control) were purchased as pre-made units (effective surface area of 0.17 m^2) of low density polyethylene film and modified so as to maintain a high RH without establishing any significant MA. This was achieved by perforating the bags with twelve 1.13 cm^2 holes. Treatment 2 was an oriented polypropylene film (OPP) selected on the basis of it having a permeability very similar to that required to maintain an atmosphere consisting of approximately 5% O_2/10% CO_2 (Refer to Section 7.3.1.10)

Table 7.2 Composition, thickness and gas permeability of packaging films used.

Film			Gas permeability ($mL/m^2/day/atm$) at 4°C	
Type	Treatment	Thickness (μm)	O_2	CO_2
Low density polyethylene	1	*	*	*
Biaxially oriented polypropylene[a]	2	35	600-800	2000

* Data not available and not applicable due to macroperforations

[a] Pre-made bags were supplied by Soleco, France and consisted of oriented polypropylene with a 19 cm x 19 cm panel on the front of the bag containing an antifog compound (Unidentified because of proprietary nature of material).

After packing, bags of treatment 1 were closed via a clip lock, whilst bags of treatment 2 (effective surface area of 0.115 m^2) were gas flushed with a premixed gas mixture containing 10% O_2/10% CO_2/balance N_2 (BOC Gases, Melbourne) and then heat sealed with a Rotary Heat Sealer (Ratcliff Model RF). Preliminary experiments showed that if bags were initially flushed with 5% O_2, then the O_2 level approached 1% after 10 days storage. Considering the uncertainty of temperatures throughout distribution, it was decided to flush treatment

2 with 10% O_2. This O_2 level represented a compromise between a higher than optimum O_2 concentration and the fact that bags were likely to become anaerobic if initially flushed with 5% O_2.

Twelve bags were prepared for each treatment. These were stored randomly in aerated cardboard boxes (12 bags per box) and the boxes placed on a wooden pallet. This pallet was transported by truck (Clelands Distribution, Melbourne) from Vegco, Ltd., Bairnsdale to Coles Supermarkets Australia Pty. Ltd. distribution centre (DC), Clayton, Victoria, a journey of approximately 3 hours. The temperature thermostat within the truck was set at 1°C. This journey simulated what usually occurs commercially with pre-cut products; i.e. they are distributed to a central warehouse before being dispatched to individual stores. The lettuce were stored at the DC overnight and then transported in ice-filled chests to the IHD the following morning. At IHD the lettuce were held at 3° ± 0.5°C (Figure 7.6) for nine days to simulate refrigerated display cabinets in retail stores. Except for brief periods when samples were taken for gas analysis and sensory evaluations, the lettuce were kept in the dark. The RH within bags was 96 ± 2% throughout the trial.

Two temperature data loggers (Hobo™ HT 13975) were used to monitor temperatures throughout preparation, transport and storage. During transport, these loggers were placed inside a plastic bag alongside packages of lettuce. The loggers recorded air temperature every 9.6 minutes.

7.2.2.2 O_2 and CO_2 concentration within bags

Gas samples (1 mL) were withdrawn from bags with an air-tight syringe and analysed by gas chromatography (GC) on days 1, 3, 6, 8 and 10 as described in Section 3.6.

7.2.2.3 Acetaldehyde and ethanol within bags

Headspace gas samples from bags were analysed for acetaldehyde and ethanol using a GC on days 6 and 10 (Refer to Section 3.7 for details).

7.2.2.4 Sensory evaluations

On days 3, 7, and 10, four bags from each treatment were opened and the contents of each bag were divided into three subsamples. Each subsample was evaluated for presence of off-odours and flavours, amount of browning, decay and wilting and overall visual quality.

7.3 Results

7.3.1 Experiment 1: Controlled atmosphere storage of pre-cut Romaine lettuce

7.3.1.1 Respiration rate

Chopped Romaine lettuce stored in air had a higher respiration rate than whole lettuce throughout storage (Figure 7.1). After 6 days storage at 4°C, treatment 1 (control) equilibrated at approximately 8.8 mg CO_2 kg^{-1} hr^{-1}, approximately 45% higher than treatment 7.

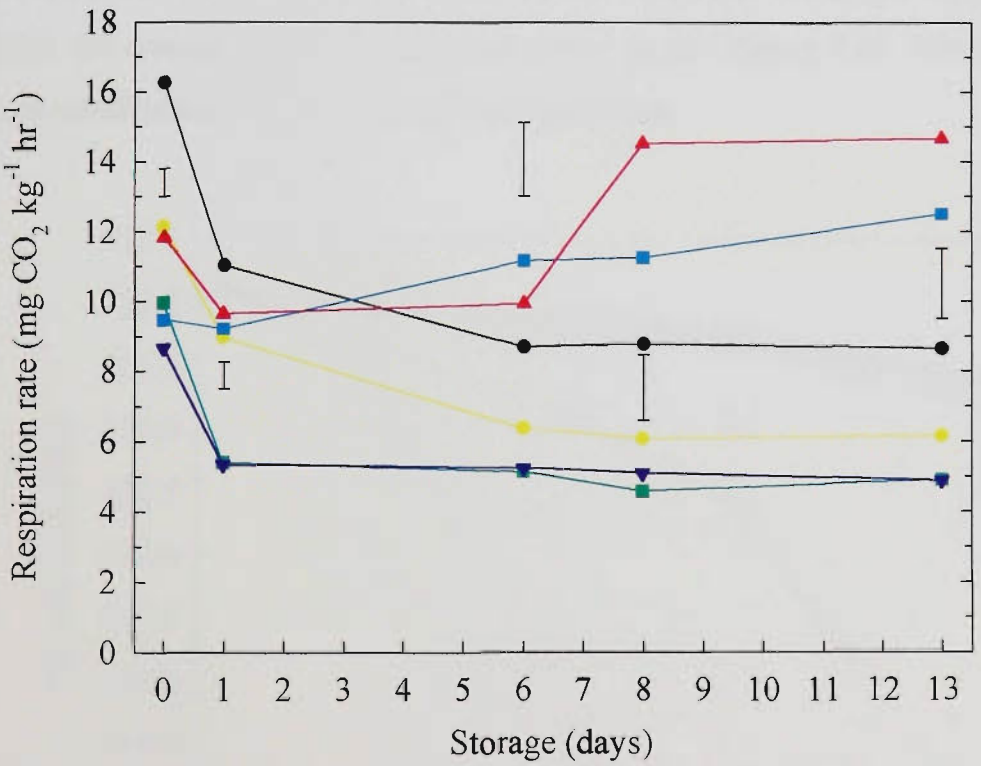


Figure 7.1 Effect of CAS and processing on the respiration rate of Romaine lettuce stored at 4°C. Vertical bars = LSD (P=0.05)

(Trt 1 ; Trt 3: Trt 4; Trt 5; Trt 6; Trt 7)

Trt 1 = Pre-cut tissue in air (>95% RH)	Trt 2 = Pre-cut tissue in air (65% RH)
Trt 3 = Pre-cut tissue in 5% O_2/5% CO_2 (>95% RH)	Trt 4 = Pre-cut tissue in 5% O_2/10% CO_2 (>95% RH)
Trt 5 = Pre-cut tissue in 5% O_2/15% CO_2 (>95% RH)	Trt 6 = Pre-cut tissue in 2% O_2/15% CO_2 (>95% RH)
Trt 7 = Whole lettuce in air (>95% RH)	

The effect of CAS on the respiration rate was variable. Treatments 3 and 4 were effective in reducing the respiration rate of tissue compared to lettuce stored in air. Both these CA treatments equilibrated at approximately 5 mg CO_2 kg^{-1} hr^{-1}, significantly lower than treatment 1. Except for day 0, no significant difference in respiration rate was noted between treatments 3 and 4 throughout the storage period.

Although the respiration rates of treatments 5 and 6 were initially lower than treatment 1, pre-cut tissue stored in these gas concentrations respired faster than the control as storage time increased. This was especially evident after 8 days storage, with treatments 5 and 6 respiring at 11.25 mg and 14.5 mg CO_2 kg^{-1} hr^{-1} respectively. Respiration in treatment 6 was significantly higher than for treatment 5 after 8 and 13 days storage.

7.3.1.2 Change in leaf colour

Pre-cut Romaine lettuce stored in controlled atmospheres remained significantly greener throughout the storage period than lettuce stored in air (Figure 7.2). No difference in hue angle was noted between CA treatments during storage.

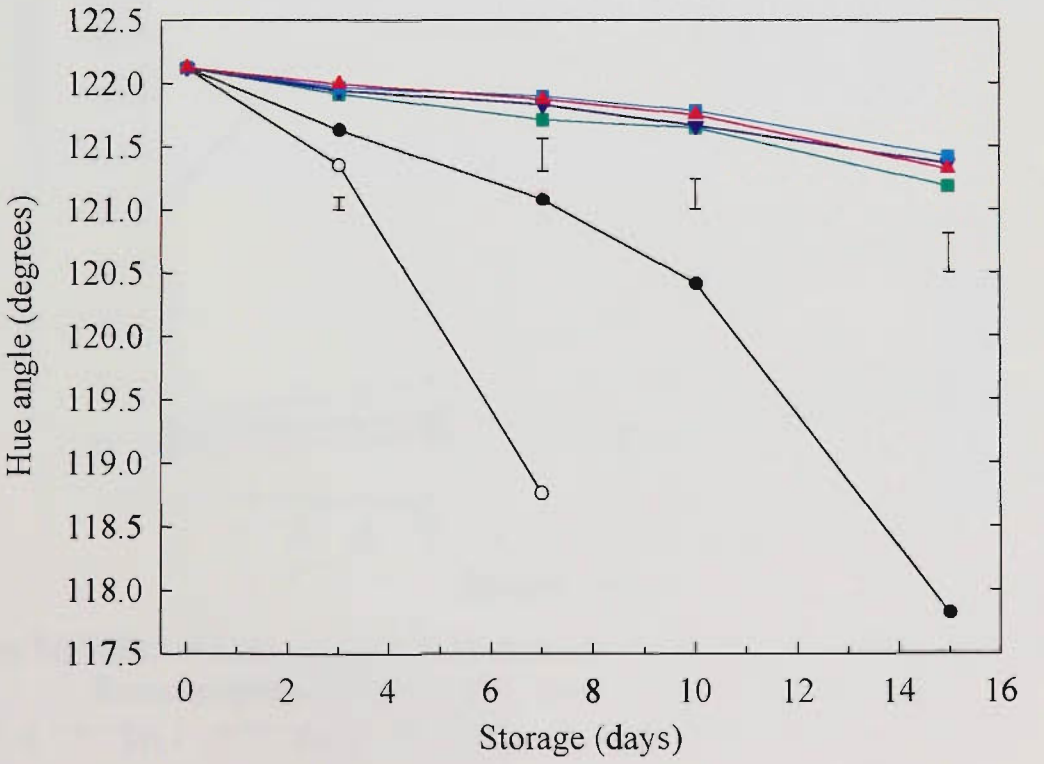


Figure 7.2 Effect of CAS on the hue angle of pre-cut Romaine lettuce stored at 4°C. Values are treatment means adjusted to the mean of the covariate, initial hue angle. Vertical bars = LSD (P=0.05)

(Trt 1 ; Trt 2; Trt 3: Trt 4; Trt 5; Trt 6)

Of the two air treatments, Treatment 2 changed from green to yellow at a faster rate than treatment 1, the result being significant after both 3 and 7 days.

7.3.1.3 Chlorophyll loss

Figure 7.3 shows the effect of different storage treatments on the rate of chlorophyll loss from leaves of pre-cut Romaine lettuce. Treatment 2 was the most deleterious storage environment with approximately 105 mg chlorophyll/100g FW lost after 7 days storage at 4°C. This was over four-fold greater than the amount of chlorophyll lost from treatment 1.

Controlled atmospheres were effective in slowing down the rate of chlorophyll loss, the result being more pronounced towards the end of the storage period. After 10 days storage, treatments 4 and 5 had lost significantly less chlorophyll than treatment 1. After 15 days, all CA treatments had lost less chlorophyll from their leaves than treatment 1. No significant differences were noted between CA treatments after 15 days storage.

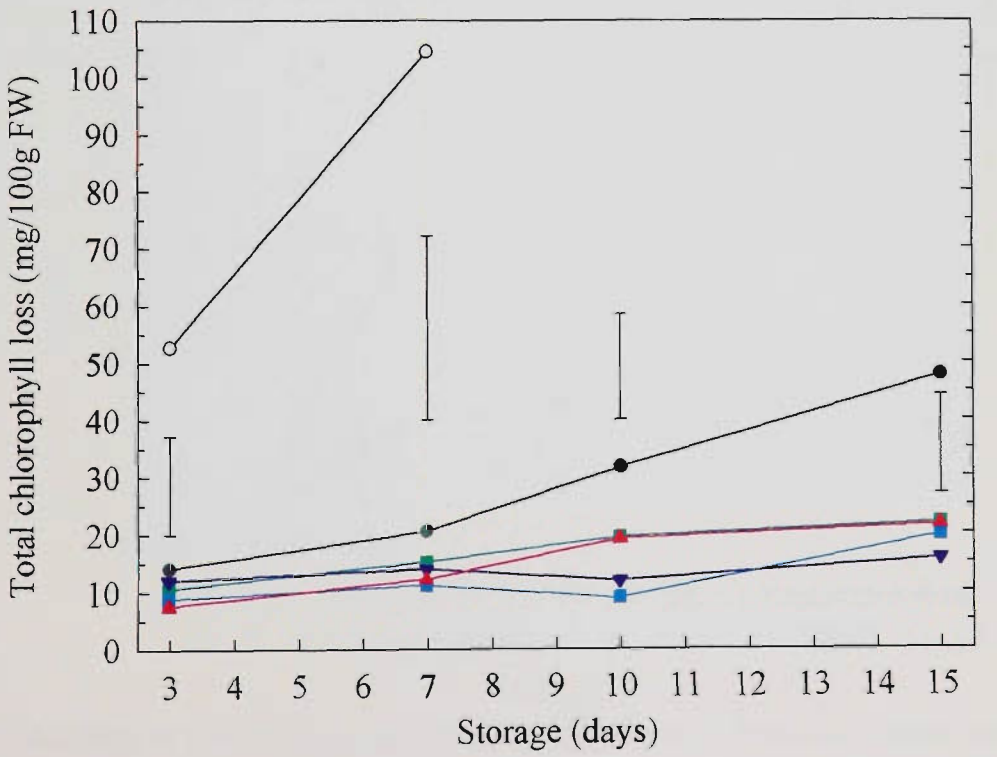


Figure 7.3 Effect of CAS on the rate of chlorophyll loss (Chl a + Chl b) from pre-cut Romaine lettuce stored at 4°C. Vertical bars = LSD (P=0.05)
(Trt 1 ; Trt 2; Trt 3: Trt 4; Trt 5; Trt 6)

7.3.1.4 Acetaldehyde and Ethanol content

Lettuce stored in treatments 5 and 6, contained significantly more acetaldehyde and ethanol than tissue kept in air or any other CA treatment (Figure 7.4). This accumulation of volatiles

increased dramatically from virtually zero on day 0, to over 2200 nmol/100g FW for treatment 6 on day 15. The large concentrations of acetaldehyde and ethanol detected in treatment 6 were significantly higher than the amount found in treatment 5 on all sampling days.

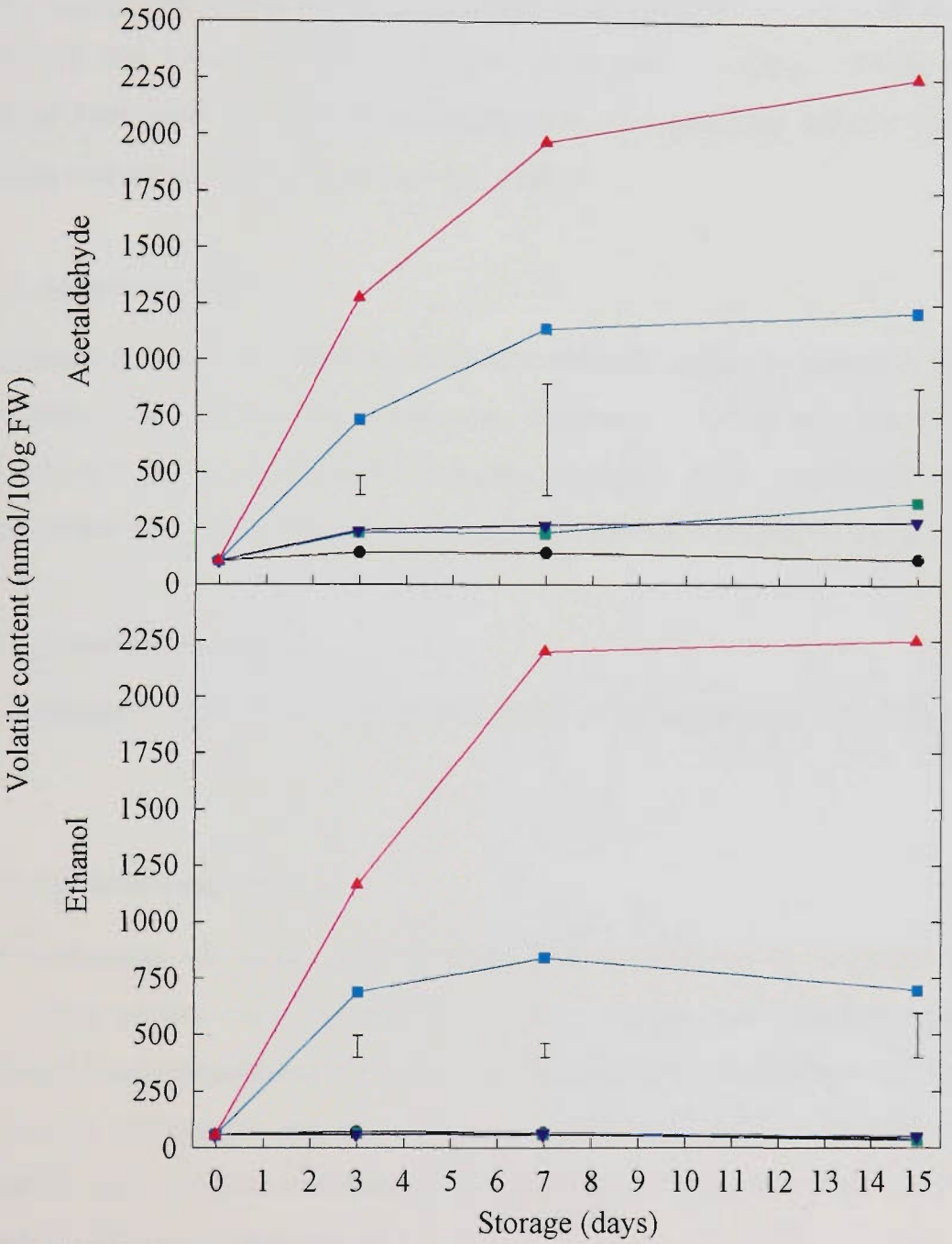


Figure 7.4 Effect of CAS on the volatile content of pre-cut Romaine lettuce stored at 4°C. Vertical bars = LSD (P=0.05) (Trt 1 ; Trt 3: Trt 4; Trt 5; Trt 6)

7.3.1.5 Amount of browning

Treatments 1 and 2 browned very quickly as evidenced by their high browning scores after 7 days storage (Table 7.3). Treatment 2 had the most browning of all treatments during the trial. Browning mainly occurred on the cut midrib which detracted from the visual appearance of the sample. Controlled atmosphere storage appeared to inhibit browning, with all CA treatments significantly less brown than tissue held in air at every sampling. Of the CA treatments, treatment 3 appeared to inhibit browning the least. No significant difference in the amount of browning was noted between treatments 4, 5 and 6.

7.3.1.6 Amount of Decay

The amount of decay was generally low in all treatments, except in treatment 1 after 10 and 15 days (Table 7.3). At the final assessment, treatment 1 had significantly more decay than treatments 4, 5 and 6. Of the CA treatments, treatment 3 had significantly more decay than treatments 4-6 at the end of the trial, although decay levels were still only slight/moderate.

7.3.1.7 Amount of Wilting

Only treatment 2 showed objectionable amounts of wilting during the storage period (Table 7.3)

7.3.1.8 Off-odours and flavour

Slight to moderate off-odours were detected upon opening jars of treatments 5 and 6 (Table 7.3). These odours were present after 3 days storage and increased gradually as time progressed, almost parallelling the increase in fermentative volatiles reported earlier. After 15 days storage, treatment 6 had an average off-odour score of 3.67, significantly higher than treatment 5. Both of these treatments were significantly higher than other CA or air treatments in which no off-odours were detected.

When compared to other CA treatments, the flavour of lettuce in treatments 5 and 6 was somewhat bitter and indicative of anaerobic respiration. Because of this, treatment 6 was considered unpalatable after 15 days storage. Although less bitter than treatment 6, treatment 5 still tasted worse than other CA treatments after 15 days. Lettuce in treatment 1 tended to lose its sweet flavour as storage time increased, with lettuce samples tasting worse than those

in treatments 3 and 4 at the end of the storage period. Due to its inconsumable appearance, treatment 2 was assigned an average flavour score of 5 after 7 days storage.

Table 7.3 Effect of CAS on sensory attributes of Romaine lettuce

Quality attribute	*Storage*	*Treatment*						*LSD*
	(days)	1	2	3	4	5	6	$P = 0.05$
Visual quality	3	2.00	4.00	1.00	1.00	1.00	1.00	*
	7	3.33	5.00	2.00	1.00	1.00	1.00	0.43
	10	4.00	-	2.33	1.33	1.00	1.00	0.60
	15	5.00	-	3.67	3.00	2.33	2.67	0.81
Amount of browning	3	2.00	2.67	1.00	1.00	1.00	1.00	0.43
	7	3.33	4.00	2.00	1.00	1.00	1.00	0.43
	10	4.00	-	2.33	1.33	1.00	1.00	0.60
	15	5.00	-	3.00	2.00	2.00	2.00	*
Amount of decay	3	1.00	1.00	1.00	1.00	1.00	1.00	*
	7	2.00	2.00	1.00	1.00	1.00	1.00	*
	10	3.00	-	1.00	1.00	1.00	1.00	*
	15	3.00	-	2.67	1.00	1.00	1.00	0.49
Amount of wilting	3	1.00	4.00	1.00	1.00	1.00	1.00	*
	7	2.00	5.00	1.00	1.00	1.00	1.00	*
	10	2.00	-	1.00	1.00	1.00	1.00	*
	15	2.00	-	2.00	2.00	2.00	2.00	*
Off-odours	3	1.00	1.00	1.00	1.00	2.00	2.00	*
	7	1.00	1.00	1.00	1.00	2.00	2.00	*
	10	1.00	-	1.00	1.00	2.00	3.00	*
	15	1.00	-	1.00	1.00	2.67	3.67	0.73
Flavour	3	1.00	3.00	1.00	1.00	1.00	1.00	*
	7	2.00	5.00	1.00	1.00	2.00	2.00	*
	10	2.00	-	1.00	1.00	2.00	3.00	*
	15	3.00	-	2.00	2.00	2.67	4.00	0.49

* LSD not determined because variation within treatments was zero.

7.3.1.9 Overall visual quality

The overall visual quality of chopped Romaine lettuce was significantly better when tissue was stored in controlled atmospheres (Table 7.3; Plates 7.1-7.4). Lettuce stored in air deteriorated faster than CA treatments as evidenced by increased browning, decay and other quality factors previously discussed. Lettuce stored in air in a low RH lost visual appeal within 3 days and was totally unmarketable thereafter. Of the CA treatments, 4, 5 and 6 were generally more appealing than treatment 3. Despite this, the off-flavours detected in treatments 5 and 6 generally rendered these treatments unpalatable.

For the reasons outlined above, treatment 4 (5% O_2/10% CO_2) was considered the optimum atmosphere for extended storage of pre-cut tissue. Knowledge of the optimum gas atmosphere and respiration rate of tissue under this storage regime was used to predict the O_2 and CO_2 permeability rates of a polymeric film to maintain this atmosphere around the produce.

7.3.1.10 Required film permeability to maintain 5% O_2/10% CO_2 at 4°C

In theory it should be possible to predict the O_2 and CO_2 permeabilities required to achieve a specific MA under exact storage temperature/storage conditions if the rate of respiration, the fill weight of produce and the surface area of gas exchange is known (Ballantyne, 1987). The calculations proposed by Ballantyne (1987) were used to estimate the O_2 and CO_2 permeabilities of a polymeric film necessary to maintain an atmosphere of 5% O_2/10% CO_2 at 4°C, the optimum gas combination identified in Experiment 1.

$$\text{At equilibrium the } O_2 \text{ permeability required} = \frac{O_2 \text{ consumption for Y}}{\text{surface area x WP}}$$

$$\text{At equilibrium the } CO_2 \text{ permeability required} = \frac{CO_2 \text{ production for Y}}{\text{surface area x WP}}$$

where Y = fill weight (g) and WP = pressure difference across package

Respiration rate of chopped lettuce under 5% O_2/10% CO_2 at 4°C = 2.73 mL CO_2 kg^{-1} hr^{-1}

Fill weight required = 0.150 kg

Surface area for gas exchange = 250 mm (l) x 230 mm (w) = 0.115 m^2

O_2 driving force = 0.16 atm

CO_2 driving force = 0.10 atm

Assuming Respiratory Quotient =1

Required O_2 permeability to achieve 5% O_2:

$$= \frac{(2.73 \text{ mL } O_2 \text{ kg}^{-1} \text{ hr}^{-1} \text{ x } 24 \text{ hr x } 0.15 \text{ kg})}{0.115 \text{ m}^2 \text{ x } 0.16 \text{ atm}}$$

$$= 534 \text{ mL m}^{-2} \text{ day}^{-1} \text{ atm}^{-1} \text{ to achieve } 5\% \text{ } O_2$$

Required CO_2 permeability to achieve 10% CO_2:

$$= \frac{(2.73 \text{ mL } CO_2 \text{ kg}^{-1} \text{ hr}^{-1} \text{ x } 24 \text{ hr x } 0.15 \text{ kg})}{0.115 \text{ m}^2 \text{ x } 0.10 \text{ atm}}$$

$$= 854 \text{ mL m}^{-2} \text{ day}^{-1} \text{ atm}^{-1} \text{ to achieve } 10\% \text{ } CO_2$$

Plate 7.1: Quality of Romaine lettuce stored in air or controlled atmospheres for 7 days at 4°C

Plate 7.1A Initial lettuce quality on day 0. General view

Plate 7.1B Initial lettuce quality on day 0. Close up view emphasizing absence of browning on midribs

Plate 7.1C Romaine lettuce stored for 7 days in air in low RH (65%) at 4°C. Note severe wilting and extremely poor quality of lettuce

Plate 7.1D Lettuce stored for 7 days in air in high RH (95%) at 4°C. Note browning on leaves.

Plate 7.1E Lettuce stored for 7 days in 5% O_2/5% CO_2 in high RH (95%) at 4°C

Plate 7.1F Lettuce stored for 7 days in 5% O_2/10% CO_2 in high RH (95%) at 4°C

Plate 7.1G Lettuce stored for 7 days in 5% O_2/15% CO_2 in high RH (95%) at 4°C

Plate 7.1H Lettuce stored for 7 days in 2% O_2/15% CO_2 in high RH (95%) at 4°C


A
Day 0
B
Day 0
C
Air (Low Humidity)/4°C)
Day 7
D
Air (High Humidity)/4°C)
Day 7
E
5% O2/5% CO2 (4°C)
Day 7
F
5% O2/10% CO2 (4°C)
Day 7
G
5% O2/15% CO2 (4°C)
Day 7
H
2% O2/15% CO2 (4°C)
Day 7

Plate 7.2: Quality of Romaine lettuce stored in air or controlled atmospheres for 15 days at 4°C

Plate 7.2A Initial lettuce quality on day 0

Plate 7.2B Lettuce stored for 15 days in air in high RH (95%) at 4°C

Plate 7.2C Lettuce stored for 15 days in 5% O_2/5% CO_2 in high RH (95%) at 4°C

Plate 7.2D Lettuce stored for 15 days in 5% O_2/10% CO_2 in high RH (95%) at 4°C

Plate 7.2E Lettuce stored for 15 days in 5% O_2/15% CO_2 in high RH (95%) at 4°C

Plate 7.2F Lettuce stored for 15 days in 2% O_2/15% CO_2 in high RH (95%) at 4°C


A
Day 0
B
Air (High Humidity) 4°C
Day 15
C
5% O2/5% CO2 (4°C)
Day 15
D
5% O2/10% CO2 (4°C)
Day 15
E
5% O2/15% CO2 (4°C)
Day 15
F
2% O2/15% CO2 (4°C)
Day 15

7.3.2 Experiment 2: Modified atmosphere packaging of pre-cut Romaine lettuce

7.3.2.1 Gas concentration within bags

The gas profiles within the two films during storage is presented in Figure 7.5. As expected, no significant MA was established within treatment 1 (air control consisting of perforated polyethylene bag). On the other hand, treatment 2 established an EMA that was almost exactly that desired (See Section 7.3.1.10). The O_2 concentration of treatment 2, reached approximately 6.5% after 8-10 days, whilst the CO_2 concentration reached a steady state value of 10% after 8 days storage.

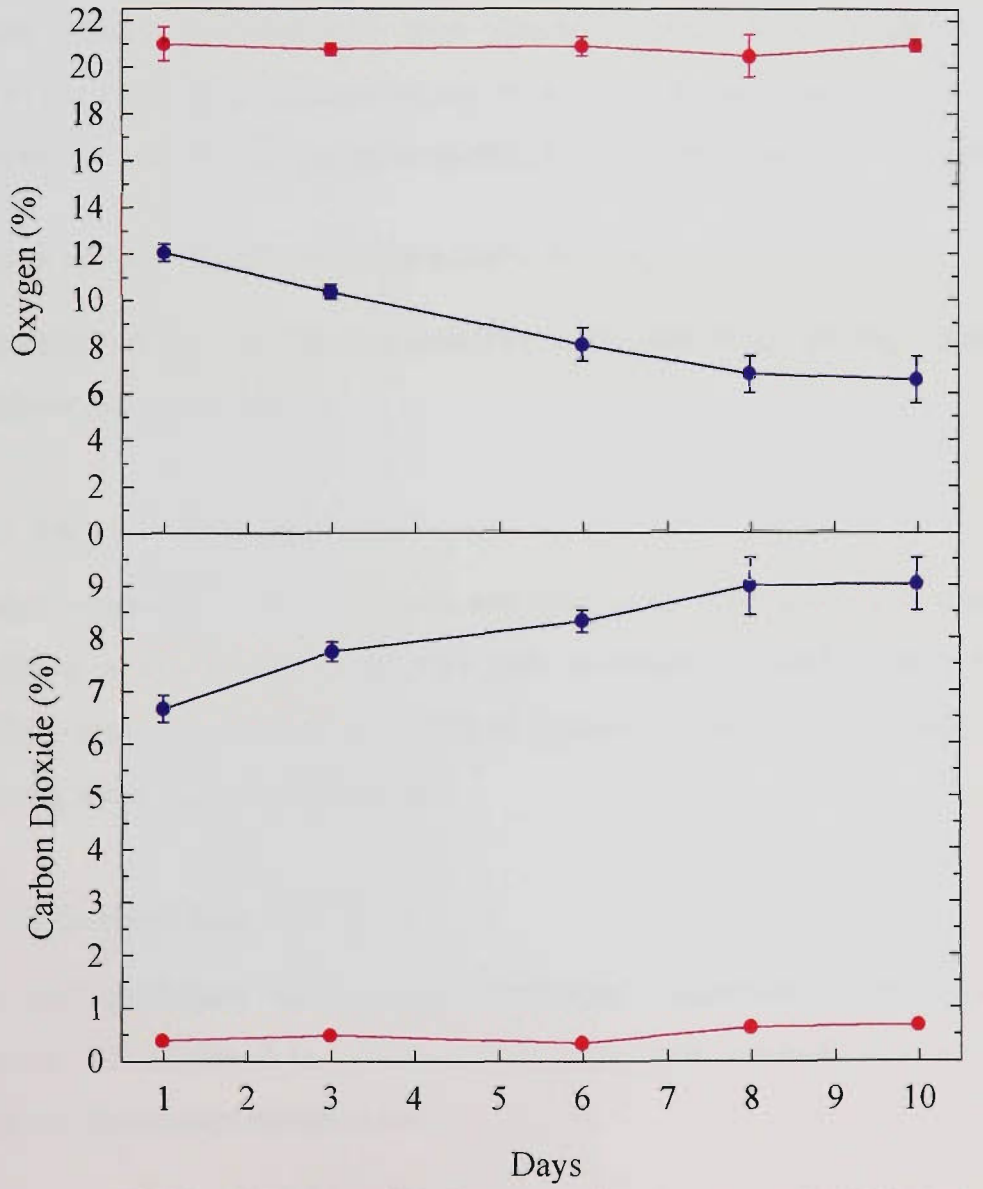


Figure 7.5 Gas concentration within bags over the storage period. Vertical bars represent standard error of the mean. (Trt 1; Trt 2).

7.3.2.2 Temperatures during preparation, distribution and storage

The temperature profile of the lettuce throughout preparation, distribution and storage is shown in Figure 7.6. The temperature during preparation fluctuated between 3° and 7°C, mainly due to a power failure at the processing plant. Despite this, the temperature during preparation was near optimum (4°C) for the majority of the time. The temperature of the lettuce was excellent during transport of the bags from the factory in Bairnsdale to the distribution centre (DC) in Clayton, Victoria. During this time, the temperature of the truck stayed constant at approximately 1.5°C.

Temperature management at the DC was also excellent remaining below 2°C overnight. The dramatic increase in temperature upon transfer of lettuce from the DC occurred in the time taken to put the bagged lettuce in an ice-chest within an air-conditioned car. Lettuce was well maintained around the 4°C preset temperature within the coolroom at the IHD.

7.3.2.3 Acetaldehyde and ethanol content within bags

No acetaldehyde or ethanol was detected in the headspace of bags from either treatment throughout the storage period.

7.3.2.4 Amount of browning

Although treatment 1 did not brown appreciably up until about day three, it subsequently browned at a significantly faster rate than treatment 2 (Table 7.4). The appearance of significant amounts of midrib browning in treatment 1 from day 7 onwards severely affected the overall visual quality of the lettuce.

7.3.2.5 Amount of decay

There was significantly more decay in treatment 1 compared to treatment 2 after 10 days storage at 4°C (Table 7.4). Despite this, decay was generally low and not objectionable throughout the storage period in both treatments.

7.3.2.6 Amount of wilting

Treatment 1 generally wilted at a faster rate than treatment 2, the effect becoming more pronounced from day 3 onwards (Table 7.4). Treatment 1 tended to lose some of its turgor and visual appeal as time progressed.

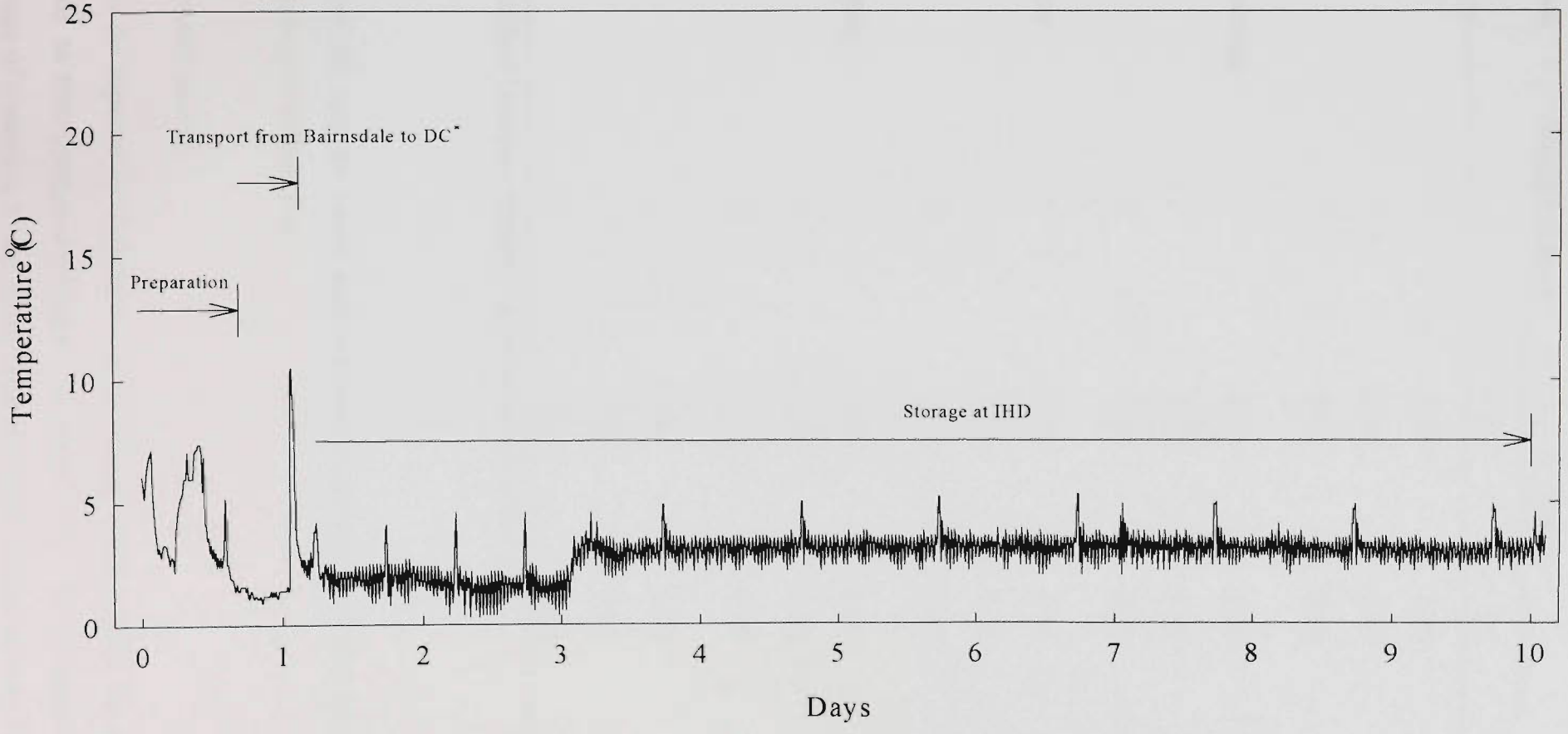


Figure 7.6 Temperature profile of lettuce throughout preparation, distribution and storage. * Distribution Centre

Table 7.4 Effect of modified atmosphere packaging on sensory attributes of Romaine lettuce

Quality attribute	*Storage (Days)*	*Film*		*LSD*
		1	2	$P = 0.05$
Visual quality	3	1.58	1.16	0.38
	7	2.83	1.41	0.38
	10	3.66	2.00	0.32
Amount of browning	3	1.25	1.00	0.27
	7	2.83	1.41	0.38
	10	3.50	2.00	0.33
Amount of decay	3	1.50	1.16	0.38
	7	1.50	1.25	0.41
	10	2.00	1.22	0.36
Amount of wilting	3	1.00	1.00	*
	7	2.25	1.08	0.32
	10	2.25	1.08	0.32
Flavour	3	1.00	1.00	*
	7	2.08	1.00	0.17
	10	3.00	2.00	0.17

* LSD not determined because variation within treatments was zero.

7.3.2.7 Flavour

Treatment 1 generally lost its sweet taste as time progressed and had an inferior flavour compared to treatment 2 (Table 7.4).

7.3.2.8 Overall visual quality

Treatment 2 was far superior to treatment 1 in its overall visual quality, the effect becoming more pronounced as time progressed (Table 7.4; Plate 7.3). As an additional factor, the appearance of bags of treatment 1 was poor, with the front surface of each bag also fogging up. On the other hand, the anti-fog compound on the front of commercial bags (treatment 2) prevented this from happening.

Plate 7.3: Quality of Romaine lettuce stored in air or MAP for 3, 7 and 10 days at ≤4°C

Plate 7.3A Treatment 1 after 10 days storage at 4°C

Plate 7.3B Treatment 2 after 10 days storage at 4°C

Plate 7.3C Treatment 1 after 3 days storage at 4°C

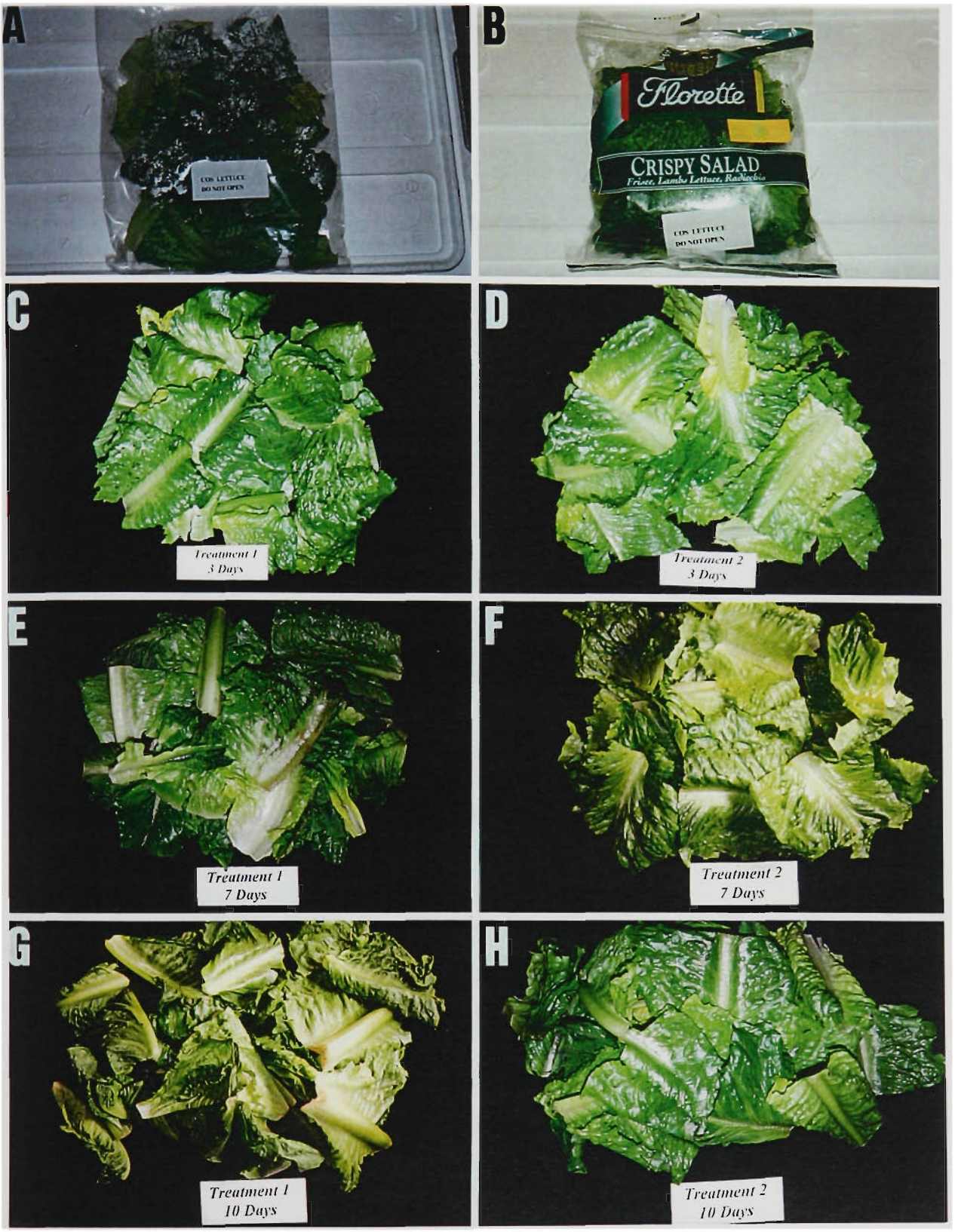
Plate 7.3D Treatment 2 after 3 days storage at 4°C

Plate 7.3E Treatment 1 after 7 days storage at 4°C

Plate 7.3F Treatment 2 after 7 days storage at 4°C

Plate 7.3G Treatment 1 after 10 days storage at 4°C

Plate 7.3H Treatment 2 after 10 days storage at 4°C


A
COS LETTUCE
DO NOT OPEN
B
Florette
CRISPY SALAD
Frisee, Lambs Lettuce, Radicchio
COS LETTUCE
DO NOT OPEN
C
Treatment 1
3 Days
D
Treatment 2
3 Days
E
Treatment 1
7 Days
F
Treatment 2
7 Days
G
Treatment 1
10 Days
H
Treatment 2
10 Days

7.4 Discussion

The studies reported in this chapter demonstrate that CAS and MAP can significantly extend the shelf-life of chopped Romaine lettuce at 4°C compared to tissue held in air.

Experiment 1 showed that pre-cut Romaine lettuce cv. Verdi will respond favourably to CAS provided the O_2 and CO_2 levels are within limits tolerated by the tissue. The first noticeable effect of CAS on tissue biochemistry was a reduction in respiration rate, confirming the results of previous experiments with shredded Crisphead lettuce (Chapter 6). Although chopping markedly increased the respiration rate of lettuce compared with that of the whole product, atmospheres low in O_2 (5%) and high in CO_2 (5-10%) were effective in reducing the metabolic rate of wounded tissue (Figure 7.1). Presumably, increased CO_2 levels, as in the case of O_2 depletion, inhibit the decarboxylation reactions of normal respiration (Kader, 1987).

Despite this, not all gas atmospheres were beneficial in reducing respiration rate. Treatments containing relatively high levels of CO_2 (15%) combined with low O_2 levels (2-5%) stimulated respiration to levels that were higher than in air stored tissue (Figure 7.1). This increase in CO_2 production was most likely associated with anaerobic respiration confirming the findings of Makhlouf *et al.* (1989a) who noted an increase in CO_2 production of broccoli under an atmosphere containing 2.5% O_2 combined with 10% or more CO_2. Boersig *et al.* (1988) also found that below a minimum of about 1% to 2% O_2, a shift from aerobic to anaerobic respiration occurs with a concomitant increase in CO_2 production. Kader (1987) has reported that, at CO_2 concentrations above 20%, a significant increase in anaerobic respiration occurs (ethanol and acetaldehyde accumulation) which can irreversibly damage the tissue. The extent of damage depends upon CO_2 and O_2 concentrations around the produce, as well as temperature and duration of exposure to these conditions.

Another beneficial response of tissue to CAS in experiment 1 was a slowing down in leaf colour changes and retention of chlorophyll. A significant benefit of CAS is that chlorophyll loss or degradation can be prevented (Brecht, 1980). For green vegetables, chlorophyll content is the primary determinant of greenness (Sweeney and Martin, 1958) and retention of pigments in vegetables after processing is essential for visual acceptability by consumers

(Powrie and Skura, 1991). Yamauchi and Watada (1991) propose that chlorophyll degradation constitutes a good marker of the physiological condition of green plant tissues.

Lettuce stored in air in a high RH changed from green to yellow at an increasing rate as time progressed (Figure 7.2). As chlorophyll is broken down, the carotenoids are exposed, giving rise to a yellowing of the product (Huxsoll *et al.*, 1989). Onset of this effect was hastened when lettuce were held in a low RH environment. This finding supports other research which has shown that many factors affect green colour retention in processed vegetables, such as temperature, RH, and atmospheric composition (Groeschel *et al.*, 1966; Shewfelt *et al.*, 1983; Kader, 1986; Perrin and Gale, 1986; Barth *et al.*, 1992).

In this study, the effect of CAS in slowing down colour changes and preventing chlorophyll degradation in pre-cut tissue were clear (Figure 7.3). Singh *et al.* (1972) found that reduced O_2 (2.5%) in the presence of elevated CO_2 (2.5%) limited chlorophyll loss in Crisphead lettuce. Weichmann (1986) states that high CO_2 and low O_2 levels in the storage atmosphere reduce the rate of colour change, mainly from green to yellow, in vegetables because of reduced breakdown of chlorophyll. A similar reduction in chlorophyll breakdown and/or maintenance of greenness in response to CAS/MAP has been reported in many other vegetables and fruits (Lieberman and Hardenburg, 1954; Wang *et al.*, 1971; Singh *et al.*, 1972; Wang, 1977; Wang, 1979; Isenberg, 1979; Smock, 1979; Wang, 1983; Aharoni *et al.*, 1989; Makhlouf *et al.*, 1989 a,b; Deschene *et al.*, 1991; Geeson *et al.*, 1991; Veierskov and Hansen, 1992; Barth *et al.*, 1993 a,b; Bastrash *et al.*, 1993).

One detrimental effect of storing pre-cut Romaine lettuce in a low O_2 (2-5%)/high CO_2 (15%) environment was the appearance of off-odours and off-flavours (Table 7.3). Low O_2 can induce anaerobiosis, which results in off-flavours and odours (Lougheed, 1987; Schlimme and Rooney, 1994). This odour has been reported in broccoli at O_2 concentrations of 0.25% (Lipton and Harris, 1974), 1% (Kasmire *et al.*, 1974) or even up to 2.5% (Makhlouf *et al.*, 1989a). Off-odours were not detected in pre-cut Romaine lettuce in a 10% CO_2 environment, supporting the results of Larsen and Watkins (1995) but contradicting the results of other researchers (Kasmire *et al.*, 1974; Lipton and Harris, 1974; Makhlouf *et al.*, 1989a; Bastrash *et al.*, 1993). The off-odours and flavours noticed in the treatments reported in Section 7.3.1.8 became progressively worse as storage time

increased. For some products, development of off-flavours may take a few days after the product is exposed to anaerobic conditions, especially at low temperatures (Ke *et al.*, 1991 a,b).

The detection of off-odours and flavours paralleled a dramatic increase in acetaldehyde and ethanol content within these tissues (Figure 7.4). These results support the findings of Ke *et al.* (1991 a,b). According to Kader *et al.* (1989), below O_2 levels of 1 to 2% in the microatmosphere of a commodity, the aerobic respiration shifts over to the anaerobic respiration which involves only glycolysis and the decarboxylation of pyruvic acid to acetaldehyde, which is converted to ethanol. Cut lettuce has been found to ferment when O_2 is at or near 1% (Ballantyne *et al.*, 1988a; McDonald *et al.*, 1990).

Since 5% O_2/10% CO_2 did not induce anaerobiosis, it could be postulated that the high CO_2 (15%) in the 5% O_2/15% CO_2 gas mixture was responsible for tissue becoming anaerobic, supporting the results of other workers (Mateos *et al.*, 1993a). According to Kader (1986), levels of CO_2 above the limits of tolerance of the particular commodity can result in accumulation of acetaldehyde and ethanol within the tissues indicating a shift to anaerobic respiration. These results support the findings of McDonald *et al.* (1990) who noted fermented flavour and off-odour in chopped Crisphead lettuce when CO_2 rose above 20%. Injury from anaerobiosis is also time-dependent, both for the production of its byproducts and development of the resultant injury (Lougheed, 1987).

CAS/MAP were effective in inhibiting enzymatic browning of pre-cut tissue compared to lettuce held in air. One of the main limiting factors on the shelf-life of pre-cut salads is the development of colour variation such as browning on the cut surfaces (Powrie and Skura, 1991; Saracino *et al.*, 1991). The amount of browning observed on midrib tissue tended to decrease as O_2 levels were lowered and CO_2 elevated (Table 7.3). These findings are consistent with the results of previous experiments (Chapter 6). In experiment 2, the initial gas flushing of treatment 2 provided an important extension to storage life by quickly establishing a desirable EMA and therefore decreasing the amount of browning. This result agrees with the findings of other researchers (Ballantyne *et al.*, 1988a).

The choice of film permeability was crucial in determining the success of MAP of pre-cut lettuce, a result which agrees with others (McDonald *et al.*, 1990). As a general rule, packaging of pre-cut items is very important, offering physical protection, reduced desiccation and contamination, as well as facilitating brand identification (Risse *et al.*, 1989). The washing of vegetables following chopping resulted in the production of large quantities of water vapour in the pack (RH 96%; see Section 7.2.2.1), confirming results of Brocklehurst and van Bentem (1990). This high RH combined with temperature swings during the simulated distribution resulted in the condensation of liquid water on the inside surface of LDPE bags, thereby obscuring the product. According to Saracino *et al.* (1991), a high RH, especially at low temperatures, may generate a condensate of water droplets on the internal surface of the film; this favours rotting and diminishes the appearance and attractiveness of the film.

The antifog compound on the OPP film (treatment 2) alleviated this problem and greatly enhanced the appearance of the package. This coating reduces the superficial water tension and stimulates the formation of a thin, continuous and transparent layer of water on the film (Saracino *et al.*, 1991). Many MAP films are treated with coatings or additives to impart antifog properties so as to improve visibility (Day, 1993).

In summary, CAS identified gas atmospheres low in O_2 (5%) and relatively high in CO_2 (10%) as being the most effective treatment in maintaining the quality of pre-cut Romaine lettuce at 4°C. These results were successfully applied to a commercial polymeric film which established a MA very similar to the optimum levels identified in CA trials and significantly extended the storage life of pre-cut tissue over that of air. The results of this study are currently being trialed commercially by Vegco Ltd., and this company hopes to market MA packed pre-cut Romaine lettuce in Australian supermarkets later this year (1995).

Chapter 8

Summary

The quality of lettuce does not improve after harvest, it only declines. It follows that quality can never be better than at harvest and that all effort expended from harvest through final consumption must be devoted to slowing down the rate at which quality declines (Lipton and Ryder, 1989).

In these studies, the most important parameter influencing the rate of quality loss from whole and pre-cut lettuce was temperature. In general, the lower the temperature during preparation, distribution and storage, the longer produce quality was maintained. According to Lioutas (1988), the quality of the raw ingredients (both organoleptic and microbiological), coupled with proper temperature control throughout the food chain, usually account for 50-60% of the total synergy accomplished in a particular CAS/MAP system. This study showed that low storage temperatures slow the respiration rate of lettuce and thus decrease the rate of spoilage. Kader (1992) indicated that the rate of deterioration (perishability) of harvested commodities is generally proportional to the respiration rate, and temperature is the environmental factor that most influences both the respiration rate and overall rate of deterioration.

Wiley (1994) emphasized that, for safety and greatest retention of sensory and nutritional quality, fresh produce must be distributed and marketed in the cold chain. In addition to affecting sensory attributes, increases in temperature during shipping, handling and/or retailing of MA packages could cause a decrease in MA package O_2 levels below safe levels since uptake of O_2 in respiration tends to increase more rapidly than permeation of O_2 through polymers (Kader *et al.*, 1989). Risks include not only loss of product quality through fermentative metabolism but the growth of potential human pathogens which thrive under anaerobic conditions (Hintlian and Hotchkiss, 1986). Use of trucks which are not precooled, improper handling at transfer points and retail display under unrefrigerated conditions contribute to this problem (Cameron *et al.*, 1994). It is therefore vital that whole and pre-cut lettuce be distributed and marketed at low temperatures if product quality is to be maintained and the risk of anaerobic metabolism minimized.

After lowering produce temperatures, MAP (along with CAS) is considered to be the second most effective action in extending the shelf-life of whole and pre-cut produce (Shewfelt, 1986; Hotchkiss, 1988; Schlimme and Rooney, 1994). The studies reported in this thesis have shown that both CAS and MAP provide an important extension to shelf-life of lettuce by reducing respiration rate and by presumably delaying biochemical and other deteriorative processes associated with senescence.

Generally, the effect that reduced O_2 and/or elevated CO_2 have on reducing respiration have been assumed to be the primary reason for the beneficial effects of CAS on fruits and vegetables (Kader, 1986). The reduction of respiration rates has been attributed by Burton (1978) to the suppression of low O_2 affinity enzymes such as ascorbic acid oxidase and glycolic acid oxidase. Kays (1991b) stated that under very low internal O_2 conditions cytochrome oxidase ceases to function which in turn inhibits the tricarboxylic acid cycle. The mode of action of CO_2 on senescence is unclear. It has also been proposed that high concentrations of CO_2 alter the pH of the cytosol, which in turn may affect plant metabolism (Siriphanich and Kader, 1986).

In this study, in addition to reducing respiration rate of lettuce, CAS/MAP also retarded compositional changes such as colour loss and flavour changes and was important in reducing butt discolouration of whole heads and browning of MP tissue.

The objective of MA package design in the current study was to create and maintain an atmosphere best suited for the extended storage of the produce. Despite this, CAS/MAP shortened the shelf-life of the lettuce when O_2 and/or CO_2 levels were outside the optimum concentrations, principally by inducing anaerobic metabolism-m and affecting the odour and flavour of the tissues.

Several differences were noted between intact and pre-cut tissue. Minimally processed lettuce respired faster than its whole counterpart. Kader (1987) has suggested that differences among plant parts in the surface area-to-volume ratio and in the nature of their surface coatings influence their gas-diffusion characteristics and consequently their respiration rates. Such differences are also responsible for genotypic variation in respiratory activity within a given commodity (Kader, 1987). Differences were also found in responses of lettuce to elevated CO_2 atmospheres. Whole Crisphead lettuce developed significant amounts of brown stain

when CO_2 levels exceeded 5% (Chapter 4), whereas CO_2 concentrations greater than 5% effectively inhibited senescent browning on pre-cut tissues (Chapters 6 and 7). Ooraikul (1991) has stated that the headspace atmosphere surrounding a MAP product needs to be chosen for maximum extension of shelf-life while maintaining safety and high product quality.

This thesis has shown that there is considerable potential for maintaining the quality of whole and pre-cut lettuce by CAS/MAP. This requires a detailed understanding of the behaviour of each product (eg respiration rate under particular CA/MA conditions), as well as the optima and tolerance limits for temperature, O_2, and CO_2 and the deleterious physiological effects of storing lettuce outside these conditions. MAP was shown to be an inexpensive way to generate CA conditions within packages if formulated correctly and this effect can be extended throughout the marketing chain. At present, the MA liners developed in this thesis for whole Crisphead lettuce and MA bags for pre-cut Romaine lettuce are being trialed in commercial situations throughout Australia. The use of MAP for whole and pre-cut produce is likely to continue to grow for both food service and retail operations. There remains a great need for research and innovation to determine the most appropriate CA gas levels for extension of shelf-life of individual cultivars, and the best methods for consistently obtaining these levels in MA packages.

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