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Maintaining quality in lettuces during marketing through ethylene atmosphere control

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Maintaining Quality in Lettuces During Marketing Through Ethylene Atmosphere Control

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HRDC Project VG98068

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This is a final report on research into maintaining quality in lettuces during marketing through ethylene atmosphere control.

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1. MEDIA SUMMARY

A major limitation to the successful marketing of high quality crisphead and fancy lettuces to consumers in Australia and export markets is a rapid rate of deterioration after harvest due mainly to natural metabolism of the produce. The industry has recognised that this deterioration can be greatly reduced by maintaining lettuces at less than 5°C and in an atmosphere of about 5% carbon dioxide and 3% oxygen. Ethylene is well known to also accelerate deterioration but in recent years has been recognised as harmful even at very low concentrations down to 0.005 μL/L. A 3-year study surveyed the quality and environmental conditions inside packages of fancy lettuces in retail outlets and, in laboratory studies, examined the effect of ethylene on loss of quality and evaluated potential technologies to reduce the impact of ethylene.

The survey of fancy lettuces in modified atmosphere packages (MAP) in supermarkets suggested that current handling practices are far from ideal with substantial variation found in temperature and the modified atmosphere between packages. There was also substantial variation in ethylene concentration of 0.16-1.36 μL/L that is in the range where substantial senescence promoting effects will occur. While all lettuce packs were at an acceptable quality at time of purchase, the less than ideal environmental conditions contributed to more than half of the samples failing to meet the stated use-by-date at an acceptable quality.

Fancy lettuces, whether stored in air or a MAP were found to have increased deterioration when any ethylene was present although the magnitude of the benefit varied between different types. Overall a 30-40% increase in postharvest life was achieved when ethylene was reduced from a normal commercial level to <0.005 μL/L. The proportional extension in postharvest life for all types was much less than that previously reported for crisphead lettuce. While storage of fancy lettuces in MAP doubled the postharvest life, it will result in higher ethylene concentrations than in unsealed packages. This reinforces the need to have some control over ethylene accumulation.

The postharvest life of shredded crisphead lettuce was extended by 30-50% when fumigated with the inhibitors of ethylene action, nitric oxide (NO) and 1-methylcyclopropene (MCP). A more detailed study with MCP applied to crisphead lettuce stored in air at 5°C found a 50% increase in postharvest life following fumigation for 1 hr with 0.1 μL/L MCP. Applying the optimum MCP treatment to whole heads of lettuce resulted in about 100% increase in postharvest life. MCP appears to have considerable commercial potential for the crisphead lettuce industry.

Fumigation of shredded fancy lettuces with MCP and NO followed by storage in MAP at 5°C, the standard industry practice, was found to only give a marginal increase in postharvest life unless the oxygen level in the MAP was substantially reduced. When 10 μL/L NO was injected directly into a sealed MAP where the atmosphere was about 5% carbon dioxide and 4% oxygen, there was a 70% increase in postharvest life. Direct addition of NO into a MAP appears to have potential with lettuces marketed in MAP.

2. TECHNICAL SUMMARY

A major limitation to the successful marketing of high quality crisphead and fancy lettuces to consumers in Australia and export markets is due to the rapid rate of physiological deterioration after harvest. The industry has recognised that deterioration can be greatly reduced by holding lettuce at <5°C and in an atmosphere of about 5% carbon dioxide and 3% oxygen. Ethylene is well known to also accelerate deterioration is now recognised as harmful at concentrations down to 0.005 µL/L. A 3-year study surveyed the quality and environmental conditions in packages of fancy lettuces in retail outlets and in laboratory studies, examined the effect of ethylene on loss of quality and evaluated potential technologies to reduce the impact of ethylene.

A survey of the temperature, atmosphere and quality of fancy lettuces packaged in sealed plastic film bags held in supermarket refrigerated cabinets suggested that current handling practices are far from ideal. Substantial variation in temperature existed which ranged from 0.6-11.0°C with only 24% of cabinets operating at <5°C. The modified atmosphere inside packages showed a mean value of 6% carbon dioxide and 3.5% oxygen, which is acceptable, but there was considerable variation between packages of 0.8-12.3% carbon dioxide 0.6-12.0% oxygen. It is considered that these arise from operational deficiencies in supermarkets and processing plants. There was also a substantial range in ethylene of 0.16-1.36 µL/L, concentrations where senescence promoting effects are expected. While all lettuce packs were of acceptable quality at time of purchase, more than half failed to meet the use-by-date at an acceptable quality.

Hydroponic fancy lettuces held in air containing ethylene at <0.005 or 0.1 µL/L had a 30% increase in postharvest life at the lower concentration, but there was considerable variation between the different types ranging from 4%-52%. The proportional extension in postharvest life for all types was much less than previously reported for crisphead lettuce. Fancy lettuces held in a modified atmosphere of 6-7% carbon dioxide and 3-4% oxygen, the average modified atmosphere found in the retail survey, had the postharvest life extended as the ethylene concentration was reduced. While lettuces in MAP had double the postharvest life, there was a higher ethylene concentration, which reinforces the need to have some control over ethylene level. Reducing ethylene to <0.005 µL/L from the average level of 0.66 µL/L found in commercial packages would increase the postharvest life by about 30%.

Fumigation of shredded crisphead lettuce with nitric oxide (NO) and 1-methylcyclopropene (MCP) increased the postharvest life of lettuces by 30-50% while direct addition of NO into MAP resulted in 50% increase in postharvest life. Fumigation with MCP on crisphead lettuce stored in air at 5°C showed an optimal treatment was 1 hr fumigation with 0.1 µL/L, which resulted in 50% increase in postharvest life for shredded lettuce and 100% for whole heads. MCP thus appears to be of considerable commercial potential for the crisphead lettuce industry.

Fumigation of fancy lettuces with MCP and NO followed by storage in MAP at 5°C, the standard industry practice, was found to only give a marginal increase in postharvest life unless the oxygen level in the MAP was substantially reduced. When

10 μL/L NO was injected directly into a sealed MAP where the atmosphere was about 5% carbon dioxide and 4% oxygen there was a 70% increase in postharvest life. Direct addition of NO into MAP thus appears to have potential with the atmospheres that are currently generated in MAP by the industry.

3. INTRODUCTION

Lettuces, in common with all leafy vegetables, are highly perishable and therefore have a short postharvest life. Deterioration arises firstly from the high rate of metabolism that results in rapid loss of essential metabolites and cell integrity producing general senescence and increasing susceptibility to microbial attack. The high surface area of leafy vegetables leads to high rates of water loss resulting in shrivelling and wilting. In addition, lettuces that are, minimally processed into salad mixes have an enhanced rate of perishability through deliberate cutting or breaking, of leaves and incidental damage incurred through the various handling processing operations.

Attempts to minimise the rate of deterioration usually involve utilisation of low temperature, high humidity and modified atmosphere storage. For lettuces (with most research having been conducted on crisphead lettuce), the optimum storage temperature is 0°C and a relative humidity of 95% (Ryall & Lipton 1979). However, in most marketing systems and retail operations in Australia and airfreight to export markets, it is very difficult to hold produce continuously at 0°C and a temperature of 5°C is often considered a more attainable temperature. Unfortunately, the use of 5°C as a feasible target temperature is often misconstrued as the optimum temperature with little attempt to try and attain lower, more desirable temperatures. Observation suggests that even the attainment of 5°C is not realised in many marketing situations. Attainment of a humidity greater than 95% RH can be achieved relatively easily by packaging produce into a bag of polyethylene or some other plastic film with low water vapour permeability. However, this results in a humidity of close to 100% RH which may lead to condensation and there is a greatly increased susceptibility to bacterial induced rotting.

In a review of published information, Ryall & Lipton (1979) record that the optimum modified atmosphere for crisphead lettuce is 2% carbon dioxide and 5% oxygen; carbon dioxide levels greater than 3% and oxygen levels less than 1% can cause injury while oxygen levels greater than 10% have no beneficial effect. There is much less data available on fancy leafy lettuces but it is clear that the optimum atmosphere varies between the different types with some types such as butter lettuce showing no benefit from modified atmosphere storage (Tataru & Weichman 1974). Reyes (1996) suggested that packaged lettuce should have a shelf life of 11-15 days at 1-4°C in an atmosphere of 10-20% carbon dioxide with 3-5% oxygen, while Ballantyne *et al.* (1988) reported that the use of 5-6% carbon dioxide and 1-3% oxygen at 5°C inhibited off-odours and delayed browning. Some of the fancy lettuces now grown in Australia have no published literature as to the optimum modified atmosphere.

Hydroponic lettuces are generally of the leaf or semi-head types and are quite often minimally processed by washing, cutting in the desired piece size and shape and rapidly cooled before packaging. A market pack commonly contains a mixture of lettuce types and may also include a small proportion of culinary herbs and flowers. The physical damage incurred, during processing enhances the rate of general senescence with major outcomes of increased senescence being loss of green leaf colour and greater susceptibility to rotting. An additional problem in minimally processed produce is the onset of non-enzymic browning particularly on the cut or broken leaf surfaces. While low temperature will delay the onset of non-enzymic browning, the major method used to inhibit non-enzymic browning in minimally processed lettuces is through modified atmosphere packaging (MAP). However, a very high level of carbon dioxide is required and this is achieved by the use of very impermeable plastic films. While such atmospheres may delay non-enzymic browning, they can also result in the production of off-flavours and other browning symptoms associated with abnormal metabolism arising from the excessive atmosphere.

Ethylene is well known to accelerate deterioration in non-climacteric produce such as lettuces. However, it has been universally considered that ethylene is not active at concentrations lower than 1 µL/L in the atmosphere. Recent studies in Australia by Wills & Kim (1995; 1996) on strawberries and green beans and Kim & Wills (1995) on crisphead lettuce has clearly demonstrated that there is no safe level of ethylene. Wills *et al.* (1999) have suggested that the presence of any ethylene will enhance deterioration and thereby decrease the market life of all non-climacteric produce. Conversely, any intervention that reduces the concentration of ethylene around produce after harvest will increase the market life. The above studies have found that the extension in market life increases logarithmically as the ethylene concentration is decreased down to the limit of detection of ethylene at 0.005 µL/L. Since the concentration of ethylene found around cartons of crisphead lettuce in wholesale markets was found to be in the range 0. 1 1-0.85 µL/L, there is considerable scope to extend the postharvest life of crisphead lettuce by reducing ethylene concentrations that currently accumulate in commercial practice.

A range of technologies has been developed over the years to reduce ethylene concentrations in postharvest situations. The technologies were initially developed to retard the ripening, of climacteric fruit and there has been little attention given to their use with non-climacteric produce. Even with climacteric fruit, there has been limited commercial application of these technologies due primarily to the misplaced notion that ethylene levels less than 0.1 µL/L are not physiologically active. A study is required to re-examine these technologies in the light of present knowledge of the absence of a safe ethylene level. This can be particularly important with produce that is marketed in MAP where the sealed conditions should result in the accumulation of much higher levels of ethylene. The use of potassium permanganate, first developed by Scott *et al* (1970) is of prime interest as it offers a relatively cheap disposable method of chemically reducing ethylene levels. The technique was developed to prevent ripening in climacteric fruit and few studies have been conducted with non-climacteric produce. The much newer fumigation techniques utilising 1-methylcyclopropene (Serek *et al.* 1994) and nitric oxide (Leshem & Wills 1998) offer a short postharvest gas treatment that may provide sustained protection against ethylene action during storage or marketing.

The original research proposal was to examine only fancy hydroponic lettuces, but following representation from the HRDC Vegetable Committee, the major part of the project also included examination of crisphead lettuce. This report details a 3-year study commenced in 1999 to investigate the environmental conditions of minimally processed fancy lettuces during marketing and evaluate a range of technologies for reducing ethylene concentrations around fancy and crisphead lettuces and their effect on extending postharvest life. The project was conducted in four phases, namely:

- surveying the temperature and atmosphere of packages of fancy lettuces in supermarkets,
- determining the effect of ethylene on fancy lettuces held in air and MAP,
- evaluation of technologies for reducing ethylene in an air atmosphere around crisphead lettuce,
- evaluation of successful technologies on lettuces held in air and MAP.

4. TEMPERATURE, ATMOSPHERE AND QUALITY OF LETTUCE SALAD PACKS IN SUPERMARKETS

This study comprised a survey conducted in 29 supermarket outlets in New South Wales during 1999 that determined the range of temperatures and levels of carbon dioxide, oxygen and ethylene in MAP of minimally processed lettuce salad mixes displayed for sale to consumers.

Materials and Methods

Visits were made to 29 retail outlets located in the Central Coast and Sydney regions of New South Wales that were operated by five supermarket companies. The temperature of the cold cabinet in which packaged minimally processed lettuce was held in each store was measured with a digital thermometer inserted between packages located within the stack. Measurements were made at three positions in each cabinet.

Various types of packaged lettuce were purchased from three supermarkets with four packages obtained from each outlet on two different occasions. Immediately after purchase, a sample of the headspace gas in each of the 24 packages was collected in a 1 mL gas-tight syringe. The gas samples were transported to the laboratory and analysed for carbon dioxide and oxygen by thermal conductivity gas chromatography, and for ethylene by flame ionisation gas chromatography.

On arrival at the laboratory, each package was opened and the lettuce rated visually for overall acceptability using a 5-point scoring scale where 5 = excellent fresh-like quality, 3= just acceptable and 1 = very unacceptable. The main criteria for loss of quality were the presence of browning, wilting and off-odours.

A total of 14 other packages of lettuce were purchased from three supermarkets and assessed for visual quality on arrival at the laboratory. The contents were then placed back in the opened bags and held in air at 5°C to simulate refrigerator storage by consumers. The quality score of the lettuce in each package was assessed daily until it became visually unacceptable (i.e. score less than 3). The number of days the lettuces remained in an acceptable condition was designated as the shelf life and the date this was attained was compared to the stated use-by date on the package.

Results and Discussion

Temperature at point of purchase

Temperatures in the refrigerated cabinets in the 29 supermarket stores (Table 1) ranged from 0.6-11.0°C, ignoring the cabinet at 20°C that was not operating but still contained lettuce packs. Only seven of the 29 cabinets were operating at the recommended temperature of 5°C or lower. All cabinets in the nine stores operated by Company A were above 5°C with 90% greater than 7°C and the highest at 11°C. The most acceptable temperatures were in stores operated by Company B and Company C but they still had half of cabinets at greater than 5°C. The need for maintenance of a temperature less than 5°C is reinforced by the labels on the lettuce packs which state to store at or below 4°C, a condition clearly not met in the majority of supermarket stores.

Headspace gas composition and quality at time of purchase

Headspace gas composition of the lettuce packages at the time of purchase showed a mean value of 6% carbon dioxide and 3.5% oxygen over all the packages (Table 2), which is close to the recommended atmosphere of Ballantyne *et al.* (1988) for a storage temperature of 5°C. However, there was considerable variation in composition between packages, with carbon dioxide ranging from 0.8-12.3% and oxygen from 0.6-12.0%. The highest carbon dioxide value observed, 12.5%, was still within the recommendations of Reyes (1996), although they specified a lower temperature range of 1-4°C where higher levels of carbon dioxide can be tolerated. Oxygen levels less than 1% produce off flavours (Ballantyne *et al.* 1988) but only two of the 24 packages were in this range. There were also only two packages where the carbon dioxide was less than 1% or the oxygen was greater than 7%, levels that will not exert a beneficial effect.

The mean level of ethylene inside the packages was 0.66 μL/L with a range of 0.16-1.25 μL/L. These levels will substantially reduce the shelf life of lettuces. A reduction greater than 30% in the shelf life of crisphead lettuce has been calculated to occur in an atmosphere of 0.66 μL/L ethylene compared to a level of 0.005 μL/L (Wills *et al.* 1999, 2000).

The visual quality of the lettuces at the time of purchase was acceptable for all packages although the quality scores ranged from excellent (5.0) to just acceptable (3.0). While browning was not observed on the lettuces in any package on opening, the level of atmosphere variation between packages was great enough to affect the extension in shelf life achieved by a modified atmosphere. The target atmosphere of the manufacturers is not known, but the considerable variation observed would seem to warrant attention by manufacturers and retailers to ensure the desired atmosphere range is sustained during marketing.

Consumer shelf life

The data in Table 3 give the consumer shelf life of lettuce in the 14 packages that were held in air at 5°C in the laboratory after purchase. Six of the packages (about 40%) had no consumer shelf life, that is, they were only in an acceptable condition on the day of purchase. Five of these packages were purchased from the same supermarket. Comparison of the stated use-by date with the date when the lettuces were actually acceptable shows that 60% of packages failed to meet the use-by date.

It is recognised that the use-by date is probably based on maintaining the lettuce in a modified atmosphere but the trial does simulate consumer usage where unused lettuce would be placed back into a refrigerator for later consumption. However, the findings are not incompatible with the higher than recommended temperatures in the supermarket cabinets and the considerable variation in atmosphere that suggest greater than expected loss of quality before purchase by the consumer.

Conclusions

That many lettuce packages did not retain acceptable quality beyond the day of purchase, despite being well within the stated use-by date, must be considered unacceptable from a consumer perspective. Temperature management would appear to be an important issue. In supermarkets it was generally higher than that anticipated by the lettuce processors and could be anticipated to also vary in other parts of the transport and storage chain before products reach the supermarket store.

The substantial variation in the levels of carbon dioxide, oxygen inside the packages would also be a mitigating factor in a higher rate of loss of quality than that expected by the manufacturer. Of particular relevance for this project was the substantial range in ethylene concentration of 0.16-1.36 μL/L that is in the range where substantial senescence promoting effects can be expected. The findings suggest that processors should improve packaging or sealing systems to achieve a more uniform modified atmosphere and take steps to minimise ethylene accumulation.

5. EFFECT OF ETHYLENE ON LETTUCE DETERIORATION

The effect of ethylene concentration on the loss of quality of crisphead lettuce has been quantified down to very low concentrations. Wills & Kim (1996) showed that the postharvest life of crisphead lettuce held at 0, 5 and 20°C declined linearly with increase in ethylene concentration over the range of <0.005 µL/L (the lowest concentration that could be measured by gas chromatography) to 10 µL/L. The postharvest life at all temperatures in 0.005 µL/L ethylene was twice that of lettuce in 0.1 µL/L which was an ethylene concentration commonly found in the commercial marketing of lettuces. Since there is little quantitative information on the relationship between ethylene and fancy lettuces, this study measured the loss of quality of various fancy lettuces at different ethylene concentrations at 5°C. The original research proposal was to also examine the effect of ethylene at other temperatures, particularly at the ambient temperature of 20°C but since the industry recommendation for shredded lettuce mixes is for storage at 5°C or lower, only 5°C was utilised.

Materials and Methods

Fancy lettuces grown commercially under hydroponic conditions were obtained from Pacific Hydroponics on the Central Coast of N.S.W. In the initial trials, cut leaves of 12 types of fancy lettuce were held at 5°C in a continuous flow of humidified air containing ethylene at <0.005 or 0.1 µL/L. The cut leaves were examined daily for change in appearance. Each leaf was scored for browning on a scale of 1-5, where 1=no browning and 5=severe browning (the reverse scale to that used in the supermarket survey). Each sample was stored until the mean score of leaves fell to <2.0; score of 2.0 was considered to be the limit of market acceptability. The time taken to reach a score of <2.0 was taken as the postharvest life of the sample.

In the second set of experiments, cut leaves of butter, mignonette and red coral lettuces were held at 5°C in a continuous flow of humidified air containing ethylene at concentrations of <0.005, 0.01, 0.1, 1.0 and 10.0 µL/L. Each type of lettuce was evaluated in two replicated experiments. The time for browning to reach a score <2.0 and the overall postharvest life of each lettuce type in each ethylene concentration were statistically evaluated by regression analysis to determine the effect of ethylene on lettuce quality.

The effect of ethylene on lettuces held at 5°C in a modified atmosphere was also examined for butter, mignonette and red coral lettuces. The experimental conditions for this phase of the work were the same as in the above paragraph, except that the flowing air stream was replaced by a flowing gas stream containing 6-7% carbon dioxide and 3-4% oxygen, the average modified atmosphere found in the retail survey.

Results and Discussion

The data in Table 4 show that, overall, the holding of the 12 types of fancy lettuce at 5°C in air containing ethylene at <0.005 µL/L resulted in about 30% increase in the postharvest life over that for lettuces held in 0.1 µL/L ethylene. There was, however, considerable variation between the different types of lettuce with the range being a

4% increase for butter asmaralda up to 52% for mignonette lettuce. It was also noted that the proportional increase in postharvest life for all types was much less than the 100-200% previously reported for crisphead lettuce (Kim & Wills 1995).

The postharvest life of butter, mignonette and red coral lettuces held at 5°C in a range of ethylene concentrations in air and a modified atmosphere of 6-7% CO_2 and 3-4% O_2 is given in Table 5. This shows that the postharvest life of all lettuces was extended as the ethylene concentration in the surrounding atmosphere was reduced. Regression analysis showed a significant inverse relationship between postharvest life (y) and $\log_{10}$ of the ethylene concentration (x) for each type of lettuce when held in air and in modified atmosphere ($P<0.01$). The effect of ethylene on fancy lettuces thus exhibits a similar relationship to that reported for other non-climacteric fruit and vegetables (Wills *et al.* 1999).

Maximum postharvest life was attained when the ethylene concentration was maintained at <0.005 µL/L, which is much less than the average level of 0.66 µL/L found in commercial packages of fancy lettuces. A reduction in ethylene to <0.005 µL/L would increase the postharvest life for these lettuces by about 30% which would greatly raise the proportion of commercial lettuce packages that would meet the use-by date.

While there was a similar deleterious effect of increasing ethylene reducing postharvest life of lettuces held in air and modified atmosphere, the actual postharvest life differed greatly between the two atmosphere environments. At comparable ethylene concentrations, the postharvest life of lettuces in the modified atmosphere was about 100% greater than those held in air. This shows the great advantage in using MAP. However, the sealed MAP bag will result in higher ethylene concentrations accumulating than in unsealed packages, which reinforces the need to have some control over ethylene level.

Conclusions

Ethylene has been shown to decrease the postharvest life of fancy lettuces held in air. Overall, reducing the ethylene concentration to <0.005 µL/L increased postharvest life by about 30% although the magnitude of the effect varied between different types and was much lower than that previously reported for crisphead lettuce.

A beneficial effect of reducing the ethylene concentration was also obtained for fancy lettuces stored in a modified atmosphere with the greatest postharvest life. Since the concentration of ethylene present in commercial MAP of fancy lettuces was found in the earlier study to range from 0.16-1.36 µL/L, considerable extension in postharvest life can be achieved if ethylene levels were to be reduced. Reducing the ethylene concentration would seem to assist the industry raise the quality of lettuce salad mixes marketed in MAP to the consumer.

6. TECHNOLOGIES FOR REDUCING THE EFFECTS OF ETHYLENE

Since ethylene around crisphead and fancy lettuces during marketing has been established as being at levels that lead to reduction in postharvest life, this study examined the ability of different technologies to reduce the effect of ethylene on lettuce deterioration. These technologies operate either through ethylene degradation or inhibition of ethylene action. The original project proposal aimed at examining different technologies on the deterioration of lettuces that were held in air and then to examine the technologies on lettuces held in MAP. However, after the project commenced, it was considered more efficient to examine a range of technologies on a single lettuce type held in air and MAP and then specifically evaluate selected successful treatments on a range of fancy lettuces.

The initial studies were conducted on shredded crisphead lettuce that was held in air and modified atmosphere and stored at 5°C. The technologies examined for ability to reduce the effects of ethylene were potassium permanganate ($KMnO_4$), nitric oxide (NO) and 1-methylcyclopropene (MCP).

Materials and Methods

Crisphead lettuce grown on the Central Coast was manually shredded in the laboratory and divided into batches of 70 g, which constituted an experimental treatment unit. In each experiment, three units of lettuce were subjected to each treatment then stored at 5°C in a continuous flow of humidified air containing 0.1 µL/L ethylene or in a sealed low-density polyethylene (LDPE) bag. The shredded leaves were examined regularly for change in appearance and scored for browning on a scale of 1-5, where 5=no browning and 1=severe browning (reverse of previous study). The time for each unit to fall to <3.0 (considered to be the limit of market acceptability) was taken as the postharvest life of the sample. Each treatment was replicated on three separate purchases on lettuce.

$KMnO_4$ was applied as a commercial product (Circulaire, Montreal) with $KMnO_4$ absorbed onto alumina pellets. The pellets were placed into a sealed sachet of a gas permeable film (Tyvec, DuPont, Sydney) that was placed in with the package of lettuce. MCP gas was generated from Ethybloc® (Rohm & Hass, Melbourne). Each treatment unit of shredded lettuce was placed in a 4L plastic container with the required amount of Ethybloc® placed in a glass beaker. Immediately after water was added to the beaker to release the 1-MCP gas, the container was sealed and placed at 5°C for the desired fumigation period. NO was applied as a gas from a cylinder (BOC Gases, Sydney) either as a fumigation treatment in nitrogen for 2 hr at 20°C or by injecting NO gas from a syringe needle directly into a sealed LDPE bag.

Results and Discussion

The data in Table 6 show that fumigation of shredded crisphead lettuce with MCP in air for 2 hr extended the postharvest life when the produce was subsequently stored in air containing 0.1 µL/L ethylene or in a MAP with a similar extension in postharvest life at all three fumigation concentrations (0.1, 0.5 and 1.0 µL/L). The control treatment for NO (i.e. fumigation in nitrogen for 2 hr) reduced the postharvest life, presumably due to an adverse effect of the anaerobic conditions. However, the inclusion of NO in with the nitrogen gave an increase in postharvest life over the nitrogen only atmosphere, with the highest concentration of NO (100 μL/L) resulting in the longest postharvest life. There was a similar extension in postharvest life due to MCP and NO of about 30-40% over control produce.

There was a similar postharvest life of the control lettuce stored in air and MAP but this was probably due to the higher ethylene concentration that accumulated in the MAP that negated the positive benefit expected from the MA. Table 7 shows that the ethylene level in the MCP treatments was greatly elevated over the control suggesting that MCP stimulated ethylene production while still protecting the lettuce from ethylene action. A higher concentration of ethylene also accumulated around lettuce that had been fumigated with NO but this was considered due to the anaerobic effect of the nitrogen as the nitrogen-only treatment also had an elevated ethylene concentration. There was no significant difference in carbon dioxide and oxygen levels between treatments.

The need for fumigation with NO in a nitrogen atmosphere arises from the instability of NO in oxygen with reaction to NO_2. While this reaction is considered to be rapid, there is little data to show the rate of reaction at low concentrations of NO and in sub-atmospheric levels of oxygen. It was therefore decided to examine the effect of injecting NO directly into a MAP containing shredded lettuce. The data in Table 8 show that direct addition of NO into the MAP gave a significant extension in postharvest life of the lettuce of about 40%. The findings suggest that NO is relatively stable in the conditions under which it was used, a finding recently confirmed by Soegiarto and Wills (pers. com., University of Newcastle). The addition of $KMnO_4$ also gave a significant extension in postharvest life.

The optimum treatments from the above studies were evaluated on lettuce held in MAP. The data in Table 9 show that the most effective treatment was injection of NO into the bag that gave 50% increase in postharvest life. Fumigation with MCP in air gave a significant extension in postharvest life of about 30%. The use of $KMnO_4$ and fumigation with NO in air also gave a significant extension in postharvest life but at about 20%.

Conclusions

A significant extension in postharvest life of shredded crisphead lettuce can be achieved through the use of an ethylene absorbent ($KMnO_4$), or ethylene inhibitor (NO and MCP). The use of NO in air instead of in nitrogen, as in published studies, provides greater flexibility for NO and increases its effectiveness, particularly in a MAP situation.

7. EFFECT OF MCP ON CRISPHEAD LETTUCE

MCP was shown in the previous study to extend the postharvest life of shredded crisphead lettuce. This study further examines the effect of MCP on shredded and whole head lettuce stored in air at 5°C. The study determined the optimum treatment with MCP that maximises postharvest life.

Materials and Methods

Crisphead lettuce heads were obtained from the Sydney wholesale markets on their day of arrival. For studies with shredded lettuce, each replicate consisted of leaves taken from one head and cut into 40 cm x 40 cm pieces that were distributed into 100 g treatment units. For the study with head lettuce, each replicate comprised lettuce heads selected from the same carton with outer wrapper leaves removed. Lettuces were fumigated at either 20°C or 5C. MCP (as Ethybloc® was applied at a range of concentrations. After fumigation, the lettuce units were stored at 5°C in containers that were flushed continuously with humidified air containing 0.1 μL/L ethylene at 20 L/hr. The quality of shredded lettuce was observed daily by scoring on scale of 5 to 1, where 5 = no browning and 1 = severe browning, and the time for quality to decline to score 3 was determined. For head lettuce, quality assessment was carried out weekly and leaves showing discolouration or rotting were trimmed off and the % trimming loss calculated. Postharvest life was taken as the time for 30% trimming loss to be attained.

Results and Discussion

The data in Table 10 show that the storage life of shredded lettuce fumigated at 20°C for 2 hr with MCP at concentrations of 0.1-1 μL/L then stored at 5°C was significantly greater than untreated lettuce by about 40% due to the delayed development of browning on cut surfaces. There was, however, no significant difference in the storage life of lettuce fumigated with different concentrations of MCP. The data in Table 11 show that fumigation of shredded lettuce with 0.1 μL/L MCP for 1 hr and 2 hr resulted in a significant extension in the postharvest life but fumigation for 3 hr was not as effective due to development of browning at cut lettuce surfaces. The preferred treatment is thus considered to be fumigation with MCP at 0.1 μL/L for 1 hr, which in this study resulted in an increase of about 50% in the postharvest life over that of untreated lettuce.

Since the practice in many commercial processing companies is to reduce the temperature of lettuce leaves by immersion in chilled water before the shredding operation, a comparison was made of the effectiveness of fumigation with 0.1 μL/L MCP for 1 hr of shredded lettuce at 20°C (to represent ambient temperature) and 5°C (low temperature). The data in Table 12 show that while the storage life was extended by fumigation at both 20°C and 5°C, fumigation of shredded lettuce at 5°C resulted in the longer storage life.

Applying the recommended treatment of fumigation for 1 hr with 0.1 μL/L MCP at low temperature to whole heads of lettuce resulted in a significant increase of about 100% in postharvest life over untreated lettuce due to delayed development of russet spotting. The mean storage life of four replicates of lettuce, with three treatment units per replicate, was 35.5 days for those fumigated with MCP compared to 18.0 days for untreated heads

Conclusions

MCP extended the storage life of shredded crisphead lettuce with the optimum treatment being fumigation with 0.1 μL/L MCP for 1 hr at 5°C which resulted in an extension in postharvest life of about 50% over untreated lettuce. Application of this treatment to whole lettuce heads resulted in a 100% increase in postharvest life. MCP thus appears to be of considerable commercial potential for the lettuce industry.

8. EFFECT OF MCP AND NITRIC OXIDE ON FANCY LETTUCES

MCP and NO were shown in the previous study to extend the postharvest life of shredded crisphead lettuce. This study examines the effect of MCP and NO on shredded fancy lettuces packaged in MAP and stored at 5°C, the standard industry practice.

Materials and Methods

A range of fancy hydroponic lettuces grown on the Central Coast was transported to laboratory immediately after processing at 5°C in a commercial operation and divided into the required number of treatment units. MCP and NO were applied by fumigation in air at 0.1 μL/L and 10 μL/L, respectively, for 1 hr at 5°C then stored in a sealed polyethylene bag (30 x 34 cm) at 5°C. NO was also applied by direct injection of gas at varying concentrations into a sealed bag of lettuce with a syringe needle. The concentration of NO was calculated on the free volume of gas in the bag. In all experiments, three replicates of each type of lettuce were examined with three treatment units in each replicate. The quality of lettuce was generally determined daily. The atmosphere in the sealed bags was analysed for carbon dioxide and oxygen three days after sealing.

Results and Discussion

The initial study on eight types of fancy lettuce (mignonette, butter, red oak, green oak, frizbe, monet, cos and red coral) showed that while there was a trend towards a higher postharvest life in packages fumigated with NO and MCP, any benefit was relatively small being <10% of the postharvest life of untreated lettuces (Table 13). It was noted that the modified atmosphere generated inside the sealed bags was much less than achieved with commercial MAP lettuces with the range of carbon dioxide being 1.3-3.9% and oxygen being 14.9-19.0%.

Fumigation with MCP and NO was repeated with cos, mignonette and fizbe lettuces, and a commercial mixture of fancy lettuces. The data in Table 14 show a similar result with no significant increase in postharvest life for any lettuce. The study also included a treatment in which NO was injected directly into the sealed bag and was therefore present around the lettuces until the NO had decomposed or was absorbed by produce. However, Table 14 shows that this did not result in a significant increase in postharvest life. The mean modified atmosphere in the sealed bags was 3.8% carbon dioxide and 14.8% oxygen.

Since NO is highly reactive to oxygen, it was decided to investigate the addition of NO into an atmosphere of lower oxygen concentration. A lower oxygen level was generated through the use of a film with lower permeability to oxygen. The data in Table 15 show a significant increase in postharvest life due to NO with the most effective initial concentration being 10 μL/L that resulted in about a 70% increase in postharvest life over untreated produce. The mean atmosphere in the sealed bags was 5.1% carbon dioxide and 3.8% oxygen with the oxygen level much lower than in previous trials but similar to the mean level found in the supermarket survey. The lower degree of effectiveness is consistent with the performance of NO in published studies that becomes deleterious as super-optimal concentrations.

While there would be some loss of added NO due to reaction with oxygen in the atmosphere inside the bag, it is speculated that a greater loss of activity in NO could be due to effects of internal oxygen in the lettuces metabolising the NO to pathways that are not influencing an increase in postharvest life. The presence of the lower oxygen level in commercial lettuce mixes could increase the availability of NO inside the lettuce that then leads to a decrease in the rate of deterioration. Regardless of the mode of action, the addition of NO into sealed bags with low oxygen concentration results in a significant increase in postharvest life. No suggestion can be made as to the lack of effectiveness of MCP at higher oxygen levels as it is not reactive with oxygen.

Conclusions

NO was able to substantially increase the postharvest life of fancy lettuces provided it is added into a MAP containing a relatively low oxygen concentration. MCP and NO appear to be much less effective in extending the postharvest life of fancy lettuces in higher oxygen concentrations than both compounds were with crisphead lettuce.

9. TECHNOLOGY TRANSFER

Reports on aspects of the project were given in four presentations entitled:

- "New postharvest treatments – alternatives to STS" to the international conference Flowers 2000 held at the Central Coast, 4 August 2000;
- "Postharvest considerations of greenhouse horticultural products" to the Australian Hydroponic & Greenhouse Conference 2001 held at the Central Coast, 31 July 2001;
- "Nitric oxide: a potential natural chemical to extend the postharvest life of horticultural commodities" presented to the 1999 Australasian Postharvest Horticultural Conference held at Waitangi, New Zealand, 6 October 1999; and
- "A new rating scale for ethylene action on postharvest fruit and vegetables" presented to the IIF-IIR Conference held at Murcia, Spain, 20 October 2000.

Three scientific papers have been published or accepted for publication:

- Wills, R.B.H., Ku. V.V.V, Shohet, D. & Kim, G.H. (1999). Importance of low ethylene levels to delay senescence of non-climacteric fruit and vegetables. Aust. J. Expt. Agric. 39: 221-4.
- Warton, M.A. & Wills, R.B.H. (2001). Survey of storage conditions and quality of minimally processed lettuce in supermarkets. Food Aust. in press; and
- Wills, R.B.H., Ku, V.V.V. & Warton, M.A. (2001). Use of 1-methylcyclopropene to extend the postharvest life of lettuce. J. Sci. Food Agric. in press.

10. RECOMMENDATIONS

The survey of fancy lettuces in MAP bags in supermarket refrigerated cabinets suggested that current handling practices are far from ideal due to substantial variation in cabinet temperature and the modified atmosphere inside packages. The higher temperatures and low modified atmospheres result in a shorter than expected use-by date in hands of the consumer and hence erode consumer confidence in the product. It is considered that the industry needs to improve both temperature management throughout the marketing cool chain and packing house operations to generate a more uniform modified atmosphere in packages.

The survey also showed a substantial level of ethylene in MAP bags and that postharvest life would be extended by minimising the effect of this ethylene. Findings from the laboratory study on 12 hydroponically grown fancy lettuces indicate that minimising the ethylene concentration around lettuces would result in about an average 30% increase in the postharvest life, although the magnitude of the benefit varies considerably between different lettuces. The proportional extension in postharvest life was, however, much less than that previously reported for crisphead lettuce. While the use of MAP *per se* is greatly beneficial in extending the postharvest life of lettuces over storage in air, the sealed MAP bag will result in higher ethylene concentrations, which reinforces the need to have some control over ethylene level in order to optimise postharvest life.

Methods to reduce the effects of ethylene on enhancing lettuce deterioration include the use of potassium permanganate ($KMnO_4$), nitric oxide (NO) and 1-methylcyclopropene (MCP). For crisphead lettuces, fumigation with 0.1 μL/L MCP or 100 μL/L NO or direct addition of 10 μL/L NO into the package give a 30-50% increase in the postharvest life of shredded lettuce while MCP fumigation of whole lettuce heads gives rise to 100% increase in postharvest life.

For fancy hydroponic lettuces stored in MAP at 5°C, the standard industry practice, direct addition of 10 μL/L NO into the MAP gives rise to a 70% increase in postharvest life but only if a relatively low oxygen level is present in the bags. Since the mean oxygen level found in the supermarket survey was in the range of effectiveness for NO action, the technology has potential commercial benefits.

Thus, fumigation with MCP and direct addition of NO into a MAP appear to be of considerable commercial potential for the crisphead and fancy lettuce industries to maximise quality through minimising the effect of ethylene action in enhancing deterioration during marketing.

11. ACKNOWLEDGMENTS

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Table 1. Temperature of refrigerated cabinets holding salad mix packages in supermarket stores.

Company	No. of stores	Temperature (°C)		% of Stores		
		Mean	Range	<5°C	5-7°C	>7°C
A	9	8.6	5.7-11.0	0	11	89
B	6	4.5	0.6-8.0	50	17	33
C	6	5.5	2.0-8.2	50	17	33
D	4	9.5	3.7-20	25	25	50
E	4	7.3	6.2-8.8	0	50	50

Table 2. Headspace gas composition and quality of salad mix packages at time of purchase from supermarket stores.

Super-market	Product	N	CO2 (%)	02 (%)	Ethylene (μL/L)	Quality score
A	1	2	3.2 (0.8-5.6)	3.3 (1.8-4.7)	0.75 (0.56-0.94)	3.5
	2	2	7.6 (5.7-9.5)	3.0 (1.9-4.0)	0.54 (0.41-0.66)	5.0
	3	4	4.0 (3.7-4.6)	4.1 (1.1-12.0)	0.36 (0.16-0.66)	3.0
B	1	2	5.9 (5.5-6.2)	1.3 (1.0-1.6)	0.95 (0.54-1.36)	4.5
	2	4	8.8 (7.6-9.5)	3.1 (1.6-5.0)	0.45 (0.25-0.83)	4.0
	3	2	2.8 (2.2-3.3)	4.3 (3.4-5.1)	0.88 (0.65-1.10)	3.5
C	1	2	6.3 (5.8-6.8)	4.9 (4.8-4.9)	0.74 (0.66-0.82)	5.0
	2	2	4.8 (4.7-4.8)	6.9 (6.7-7.0)	0.97 (0.83-1.10)	4.5
	3	2	11 (9.7-12.3)	2.5 (0.6-4.4)	0.53 (0.16-0.90)	5.0
	4	1	3.5	2.1	1.25	4.0
	5	1	12.5	0.6	0.71	5.0

N = number of packages

Table 3. Shelf life (days) of packaged salad mixes after purchase.

Super-market	Product	Shelf life Expected	Shelf life Actual	Difference
A	1	2	0	-2
	2	5	5	0
	3	4	0	-4
	4	2	0	-2
	5	1	0	-1
	6	0	0	0
			Mean	*-1.5*
B	1	2	0	-2
	2	5	6	+1
	3	2	3	+1
	4	2	3	+1
			Mean	*+0.3*
C	1	4	1	-3
	2	4	2	-2
	3	6	4	-2
	4	5	6	+1
			Mean	*-1.5*

Table 4. Postharvest life of fancy lettuces held at 5°C in air containing <0.005 and 0.1 µL/L ethylene.

Lettuce type	Postharvest life (days) 0.1	<0.005	Difference	% increase
Butter Arizona	5.7	6.8	1.1	19
Butter Asmaralda	8.0	8.3	0.3	4
Frillice	6.3	9.3	3.0	48
Green Coral	6.3	8.4	2.1	33
Green Cos	5.9	7.5	1.6	27
Green Oak	5.5	7.8	2.3	42
Mignonette	6.1	9.3	3.2	52
Monet	9.2	12.5	3.3	36
Radiccio	6.3	7.0	0.7	11
Red Coral	6.3	8.4	2.1	33
Red Oak	6.1	8.2	2.1	34
Red Velvet	7.0	7.8	0.8	11
Mean	*6.6*	*8.4*	*1.9*	*29*

Table 5. Effect of ethylene on the quality of fancy lettuces stored at 5°C in air and a modified atmosphere containing ethylene ranging from <0.005 to 10 μL/L.

Atmosphere	Ethylene (μL/L)	Postharvest life (days)		
		Butter	Mignonette	Red Coral
Air	<0.005	2.7	4.0	4.8
	0.01	2.2	3.3	4.3
	0.1	2.3	3.4	3.7
	1	1.8	3.1	3.6
	10	1.3	2.8	3.1
$6\%CO_2$- $3\%O_2$	<0.005	5.2	7.8	10.5
	0.01	5.4	7.0	9.1
	0.1	4.4	6.8	8.2
	1	4.0	6.4	7.9
	10	3.8	5.8	7.5

Table 6: Effect of fumigation for 2 hr with NO in nitrogen and MCP in air on postharvest life of cut crisphead lettuce stored at 5°C in air containing 0.1 μL/L ethylene or a modified atmosphere.

Treatment	Conc (μL/L)	Postharvest life (days) in	
		0.1 μL/L C_2H_4	MA
Control		4.6	3.9
NO	0	3.7	3.0
	1.0	4.8	4.4
	10	5.2	5.3
	100	6.0	6.1
MCP	0	4.2	4.1
	0.1	6.2	6.4
	0.5	5.8	6.3
	1.0	5.9	6.6
LSD (5%)		*±0.9*	

Table 7: Atmosphere in MAP of lettuce fumigated for 2 hr with NO in nitrogen and MCP in air then stored at 5°C.

Treatment	Conc (μL/L)	Atmosphere in MAP CO_2	O_2	C_2H_4
Control		4.1	14.1	0.22
NO	0	4.7	12.7	0.68
	1.0	3.3	16.2	0.48
	10	3.6	14.9	0.53
	100	4.2	13.5	0.63
MCP	0	4.6	13.8	0.23
	0.1	4.3	13.4	0.46
	0.5	4.6	13.5	0.57
	1.0	3.9	14.1	0.55

Table 8: Effect on postharvest life of NO and $KMnO_4$ placed into a MAP with cut crisphead lettuce at 5°C.

Treatment	Conc (μL/L)	Postharv. life (days)	Atmosphere CO_2	O_2	C_2H_4
Sealed bag		4.8	5.7	12.2	0.42
NO	1	5.6	4.8	13.8	0.31
	10	6.3	5.4	12.2	0.46
	100	6.5	5.3	12.1	0.34
$KMnO_4$		5.8	3.8	13.3	<0.005
LSD (5%)		*±0.7*			

Table 9: Effect of fumigation for 2 hr with NO and MCP in air and NO and $KMnO_4$ placed into a MAP on postharvest life of cut crisphead lettuce stored at 5°C.

Treatment	Conc (μL/L)	Postharvest life (days)
Control		6.0
$KMnO_4$	5 g/100 g	7.1
NO in air	10	7.2
NO injected	10	9.2
MCP	0.1	7.6
LSD (5%)		*±0.8*

Table 10. Postharvest life of shredded lettuce fumigated with MCP at 20°C for 2 hr then stored in air containing 0.1 μL/L ethylene at 5°C

MCP concentration (μL/L)	Postharvest life (days)
0	4.2
0.1	6.2
0.5	5.8
1	5.9
LSD (5%)	*±0.7*

Table 11. Postharvest life of shredded lettuce fumigated with 0.1 μL/L-MCP for different periods at 20°C then stored in air containing 0.1 μL/L ethylene at 5°C

Fumigation time (hr)	Postharvest life (days)
0	4.2
1	6.5
2	5.6
3	4.9
LSD (5%)	*±0.9*

Table 12. Postharvest life of shredded lettuce fumigated with 0.1 μL/L MCP for 1 hr at 20° or 5°C then stored in air containing 0.1 μL/L ethylene at 5°C

Fumigation temp.	Postharvest life (days)	
(°C)	1-MCP	Untreated
20	4.7b	4.0c
5	5.4a	4.1c
LSD (5%)	*±0.6*	

Table 13. Postharvest life of eight fancy lettuces fumigated with MCP or NO then stored in a sealed bag at 5°C

Lettuce	Postharvest life (days)		
	MCP	NO	Control
Mignonette	5.3	5.7	5.5
Butter	6.6	7.0	6.4
Red Oak	6.3	6.8	6.0
Green Oak	8.3	8.3	7.8
Monet	13.4	12.9	12.4
Frizbe	9.65	10.2	10.3
Cos	6.7	6.7	6.4
Red Coral	11.7	11.7	11.0
Mean	**8.5**	**8.6**	**8.2**
LSD (5%)			*±0.5*

Table 14. Postharvest life at 5°C of fancy lettuces fumigated with MCP or NO then stored in a sealed bag, or direct injection of NO into a sealed bag.

Lettuce	Postharvest life (days)			
	Fumigation		Injection	Control
	MCP	NO	NO	
Mignonette	5.4	6.0	6.0	5.8
Cos	7.8	7.9	7.4	7.6
Frillice	4.6	4.6	4.4	4.6
Salad mix	7.3	7.2	7.0	6.9
Mean	**6.3**	**6.4**	**6.2**	**6.2**
LSD (5%)				*±0.4*

Table 15. Postharvest life at 5°C of a MAP of fancy lettuce salad mix with NO injected into the bag.

	Postharvest life (days)			
NO added (µL/L)	0	10	50	100
	6.4	11.0	8.7	7.9
LSD (5%)	*±2.4*			