VG224
Packaging of pre-prepared vegetables

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VG224

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Packaging of Pre-prepared Vegetables

2. PROJECT NUMBER

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1. SUMMARY

1.1 INDUSTRY SUMMARY

The Food Research Institute (Department of Agriculture, Victoria) conducted this project to evaluate the effectiveness of modified atmosphere packaging for value-adding and shelf-life extension of shredded lettuce, mixed lettuce, broccoli florets and cauliflower florets. This project was jointly funded by the Horticultural Research and Development Corporation (HRDC) and All States Food Service P/L (NSW).

The project investigated the effects of various packaging films, initial package gas composition, and storage temperature on the shelf-life of pre-prepared vegetables. Sensory appeal and microbial load which served as indicators of product quality were monitored at regular intervals. The package gas composition was also analysed to determine whether beneficial or dangerous gas levels are attained in each experimental package.

The results of this project have shown that modified atmosphere packaging combined with low temperature handling effectively delayed enzymatic browning in shredded lettuce, mixed lettuce, broccoli florets, and cauliflower florets; and extended their shelf-life.

Shredded lettuce and mixed lettuce require control of preparation and storage temperature, and gas flushing for shelf life extension. Gas flushing of pouches/bags to produce an initial atmosphere of 3% O_2/17% CO_2/ 80% N_2 extended the shelf-life of shredded lettuce and mixed lettuce to 9-10 days when stored at 2°C, and 7-8 days when stored initially at 2°C for 3 days and subsequently stored at 4°C. The shelf-life of these products packaged without gas flushing is about 4-5 days. The package developed for shredded and mixed lettuce is a "pillow pack" made from 55 μm LLDPE/LDPE film designed to contain 200 g of product.

The pillow pack developed for broccoli florets and cauliflower florets does not require gas flushing. Results have shown that the natural respiration (CO_2 production/O_2 consumption) of these florets was sufficient to attain acceptable shelf-life. A shelf-life of 20 days was achieved in broccoli florets stored under a simulated handling condition - 3 days at 4°C, 1 day 7°C, and back to 4°C. A shelf-life of 13 days was attained in cauliflower florets stored under similar conditions. The package developed for broccoli florets and cauliflower florets is a pillow pack made from microperforated 30 μm OPP designed to contain about 350 g of florets.

The adoption of the techniques developed in this study would aid processors in the marketing of premium quality, premium price, convenient, "fresh" pre-prepared vegetables in domestic and overseas markets. The greatest impact is expected in the domestic retail outlets where pre-prepared vegetables are starting to gain popularity.

1.2 TECHNICAL SUMMARY

This study showed that modified atmosphere packaging combined with low temperature handling effectively delayed enzymatic browning in shredded lettuce, mixed lettuce, broccoli florets, and cauliflower florets; and extended their shelf-life.

1.2.1 Shredded Lettuce and Mixed Lettuce

The respiration of shredded lettuce and mixed lettuce was found to be of the same magnitude. Therefore, a similar package was developed for both shredded and mixed lettuce.

The "pillow-pack" developed for shredded and mixed lettuce is 210 mm (width) x 250 mm (length) made from 55 μm LLDPE/LDPE film. The package was designed to develop an equilibrium oxygen level of 3% and to contain about 200 g of shredded or mixed lettuce. An initial gas concentration of 3% O_2/17% CO_2/80% N_2, attained by gas flushing maintained visual quality and extended the shelf-life of shredded and mixed lettuce. Shredded lettuce which was mainly Iceberg lettuce had shelf-life of up to 9-10 days when stored at 2°C; and 7-8 days when stored initially at 2°C for 3 days and subsequently stored at 4°C. Enzymatic browning which developed mainly on cut surfaces of the lettuce leaves was the main factor that limited shelf-life.

Mixed lettuce samples which contained diced Iceberg, Cos, and Red Mignonette had similar shelf-life extensions to shredded lettuce. Differences were noted between the visual appearance of Iceberg lettuce, Cos, and Red Mignonette during the storage period. Enzymatic browning occurred first and mainly in Iceberg lettuce. Consequently, shelf-life achieved for mixed lettuce samples were limited by the Iceberg lettuce shelf-life.

1.2.2 Broccoli Florets and Cauliflower Florets

Experimental results for broccoli and cauliflower florets have shown that natural production of modified atmosphere was sufficient to attain acceptable shelf-life for these products.

The pillow pack developed for broccoli florets is 210 mm (width) x 250 mm (length) made from 30 μm OPP (oriented polypropylene) film. The pack which was designed to accommodate about 350 g has one microperforation on both sides of the bag. An equilibrium modified atmosphere of 6-9% O_2/12-16% CO_2 was established within 2 days which enabled the florets to attain an acceptable shelf-life of 20 days when stored using a simulated handling condition - 3 days 4°C, 1 day 7°C, and back to 4°C.

The package developed for cauliflower florets is 210 mm (width) x 240 mm (length) made of the same 35 μm OPP film used for broccoli florets. This package which contained about 350 g, established an equilibrium atmosphere of 8-10% O_2/11-13% CO_2. This modified atmosphere enabled the cauliflower florets to attain an acceptable shelf-life of 13 days under similar simulated handling condition used for broccoli florets.

2. RECOMMENDATIONS

This study has shown the benefits of modified atmosphere packaging for extending the shelf-life of shredded lettuce, mixed lettuce, broccoli florets, and cauliflower florets. The techniques developed in this study can be easily adopted by processors of pre-prepared vegetables. Processors need to equip their existing packaging lines with gas flushing facilities for packaging shredded and mixed lettuce. The success or failure of the system developed in this study would also depend on three (3) basic factors: (i) high quality fresh vegetables, (ii) hygienic and low temperature processing conditions, and (iii) low temperature handling, distribution, storage and display.

For further research, the following are suggested:

1. To evaluate carbon dioxide generators, like solid CO_2 as a means of maintaining CO_2 levels above 10%, or as an alternative method to gas flushing.

2. To evaluate the effectiveness of the techniques used in this study on extending the shelf-life of other pre-prepared vegetables like stir fry mix, dry coleslaw, and other vegetable salads with or without dressing.

3. Suppliers and/or manufacturers of packaging films should be encouraged to supply gas permeability data at low temperature (1-5°C) aside from standard measurements done at 21°C.

3. TECHNICAL REPORT

3.1 INTRODUCTION

The Horticultural Research and Development Corporation (HRDC) and All States Food Service Pty Ltd. (NSW) commissioned this project to evaluate the effectiveness of modified atmosphere packaging in extending the shelf-life of selected pre-prepared vegetables. Industry considers that pre-prepared vegetables are growth areas in both domestic and overseas markets. However, expansion is limited by the relatively short shelf-life of these products. The shelf-life of currently available products is about 2-4 days. All States Food Service P/L believes that a shelf-life of 7 days would be reasonable for domestic retail and food service industry.

Modified atmosphere (MA) packaging involves packaging foods in a gas atmosphere different from that of air, in order to extend the shelf-life of food products. Modified atmospheres can be produced naturally (called "passive" MA) or by flushing with gasses of controlled composition (called "active" MA).

The beneficial and detrimental effects from MA packaging have been extensively reviewed (Zagory, 1990; Day, 1988; Brecht, 1980; Dewey, 1983; Kader *et al.* 1989; Priepke *et al.* 1976). Enriched CO_2 and low levels of O_2 can reduce respiration, delaying ripening, decrease ethylene production and sensitivity, slow down compositional changes associated with ripening, reduce enzymatic browning, preserve vitamins, and alleviate physiological disorders, thereby extending the product shelf-life. However, exposure of the produce to O_2 and CO_2 levels (generally, $<1\%$ O_2 and $>20\%$ CO_2) beyond their limits of tolerance can initiate anaerobic respiration with the production of undesirable flavours and odours, as well as cause other physiological disorders.

In MA packaging of raw and prepared fruit and vegetables, the optimum CO_2 level is normally below 20% (Reyes, 1992). MA and low temperature storage in combination limits microbial enzyme activity, tissue metabolism and respiration, thereby prolonging the time before senescence after which the bacterial population could cause tissue damage (Brocklehurst *et al.* 1987, Barriga *et al.* 1991).

Although most researchers have reported little effect of high CO_2 ($\leq$ 20%) on the general profile and growth of microorganisms, significant reduction in spoilage have been reported in MA packaged prepared vegetables (Chamboy and Nguyen, 1988; Beuchat and Brackett, 1990; Picoche and Denis, 1988; Buick and Damoglou, 1989). These reported positive effects on shelf-life extensions could be due to a direct action on microbial enzyme activity, rather than to reduce microbial growth (Nguyen and Carlin, 1988).

Increased CO_2 and low levels of O_2 could reduce enzymatic browning and thus, extend the shelf-life of lettuce and other cut vegetables (Singh *et al.* 1972; Stringer, 1990). CO_2 is known to prevent browning of damaged plant tissue by blocking production of phenolic compounds, as well as inhibiting polyphenol oxidase activity (Siriphanich and Kader, 1984).

The objective of this project was to evaluate the effectiveness of MA packaging and low temperature storage in extending the shelf-life of shredded lettuce, mixed lettuce, broccoli florets and cauliflower florets. Specific goals were:

(i) to conduct a literature review on modified atmosphere (MA) and controlled atmosphere (CA) packaging/storage of various fresh and pre-prepared vegetables,

(ii) to determine experimentally the optimum gas concentrations of selected pre-prepared vegetables at specific storage conditions,

(iii) to evaluate the "in-storage" shelf-life of selected pre-prepared vegetables packaged using recommended atmospheres in the literature, and

(iv) to determine the shelf-life "after opening" of selected pre-prepared vegetables.

3.2 MATERIALS AND METHODS

3.2.1 Literature Search (Stage 1)

A literature search was conducted on modified atmosphere (MA) and controlled atmosphere (CA) storage/packaging of "minimally processed" (also termed "prepared" or "pre-prepared") vegetables. Emphasis was made on the following produce:

- broccoli florets
- cauliflower florets
- mixed lettuce
- shredded lettuce
- diced onions
- grated carrots
- whole and sliced mushrooms
- other leafy vegetables

The search included information on optimum gas concentration, storage temperature, and suggested preparation and processing protocol. The literature search has been submitted to HRDC and All States Food Service P/L, and a further copy of the text is appended in this report (Appendix C).

3.2.2 In-Storage Shelf-life Evaluation (Stage 2)

Products were packaged and stored under the following conditions:

- Films	-	2 types (plus control)
- Gas packaging	-	2 types (with and without gas flushing)
- Package format	-	"pillow-pack" bag
	-	350 g/pack for broccoli and cauliflower florets
	-	200 g/pack for shredded and mixed lettuce
- Temperature	-	3 temperatures (1°C, 5°C, and simulated commercial and consumer handling - 3 days at 4°C, 1 day at 7°C, and 4°C until spoilage)

Analyses of the internal package gas composition were carried out on samples at 2-4 day intervals throughout the storage period. Gas analyses were carried out using a gas chromatograph (Section 3.2.8).

Sensory appearance assessments were carried out on duplicate samples at 1-2 day intervals during the storage period (Section 3.2.9).

The following packaging films were used for packaging shredded and mixed lettuce:

Film Code	Description
A	32 μm multilayer polyolefin (PE, PP), oxygen permeability 7000 mL/m^2.day (23°C, 1 bar).
B	55 μm LLDPE/LDPE, oxygen permeability 7000 mL/m^2.day (23°C, 1 bar).
Control	15 μm perforated film, moisture vapour transmission rate (MVTR) 320 g/m^2.day

The oxygen permeability values were taken from the product literature of suppliers/manufacturers. The names and addresses of suppliers/manufacturers are given in Appendix A. Abbreviations are explained in Appendix B.

The following packaging films were evaluated for modified atmosphere packaging of broccoli and cauliflower florets:

Film Code	Description
C	19 μm multilayer polyolefin (PE, PP), oxygen permeability 16500 mL/m^2.day (23°C, 1 bar).
D	30 μm microperforated OPP oxygen permeability 18000 mL/m^2.day. (23°C, 1 bar).
Control	15 μm perforated film, moisture vapour transmission rate (MVTR) 320 g/m^2.day.

3.2.3 Evaluation of Optimum Gas Concentration (Stage 3)

Four (4) controlled atmosphere (CA) chambers at the Institute of Plant Sciences (Department of Agriculture, Knoxfield) were used to evaluate the optimum O_2/CO_2 levels for shredded lettuce, mixed lettuce, broccoli florets, and cauliflower florets. The CA chambers consisted of: (i) a vacuum pump to remove the initial atmosphere in the chambers, (ii) CO_2 scrubber, (iii) supply lines for CO_2, O_2, N_2; (iv) gas mixing equipment; and (v) a gas monitoring system.

The following were the gas combinations used in this CA storage trial:

Treatment 1	:	Air (21% O_2/0% CO_2)
Treatment 2	:	2-3% O_2/5% CO_2 (± 1)
Treatment 3	:	2-3% O_2/10% CO_2 (± 1)
Treatment 4	:	2-3% O_2/15% CO_2 (± 1)

All the products were packaged in perforated bags. These bags contained adequate perforations to allow gas exchange between the products and the chamber atmosphere. Four (4) packages of each product were placed randomly in CA chambers. The storage temperature used in this storage experiment was 1-2°C.

Visual observations were conducted at days 0, 5, 8 and 15. On the 15th day, all the storage chambers were opened and products assessed for off-odours, texture, off-taste, and general visual appearance.

3.2.4 Shelf-life After Opening (Stage 4)

Using a selected preparation, packaging and storage protocol (based on Stage 2 and 3), the shelf-life of each product after-opening was evaluated. Packages were opened at day 5, 7, 9, 11 (after packaging), and the quality was monitored until the product was obviously spoiled.

Twenty-four (24) packages of each product were produced and stored until day 5, 7, 9 and 11. On days 5, 7, 9 and 11, 6 packages were opened. The quality (microbial load and general sensory) of the product were evaluated (i) immediately after opening, (ii) a day after opening, and (iii) 2 days after opening (2 packs per occasion).

Shredded lettuce and mixed lettuce samples were stored under the following conditions: 3 days at 2°C then transferred at 4°C until the termination of shelf-life. Broccoli florets and cauliflower florets were stored under the following simulated temperature conditions: 3 days at 4°C, 1 day at 7°C, and back to 4°C until the end of shelf-life.

The results from this stage would (i) confirm the in-storage shelf-life established in Stage 2, and (ii) indicate the shelf-life after opening and the factors limiting shelf-life of shredded lettuce, mixed lettuce, broccoli florets, and cauliflower florets.

3.2.5 Preparation of Products

<u>Shredded lettuce</u>

Iceberg lettuce was obtained locally in Werribee through Choice Harvest Company (Vic.), a company associated with All States Food Service P/L. Shredded lettuce was prepared using the following procedures:

(i) Lettuce heads were stored at 4°C prior to processing,
(ii) Outer and damaged leaves, and core were removed,
(iii) Remaining leaves were sliced into 5 mm strips with a knife,

(iv) 200 g portions of shredded lettuce was dipped for 2 min in a 4 ppm sodium hypochlorite solution at about 8-10°C.
(v) Washed lettuce was centrifuged at 500 rpm in a 20 cm diameter basket for 2 min to remove surface water, and
(vi) The material was then put into pouches/bags and heat sealed.

Mixed lettuce

Whole lettuce was also obtained locally in Werribee by Choice Harvest Company. The following recipe of mixed lettuce was prepared prior to packaging: 50% Iceberg, 25% Cos, and 25% Red Mignonette.

The preparation procedure followed for mixed lettuce was similar to that for shredded lettuce. The only exception was the dicing of the leaves to about 25-30 mm instead of slicing the leaves into 5 mm strips.

Broccoli and cauliflower florets

Broccoli and cauliflower heads used in this study were grown locally in Werribee and supplied through Choice Harvest Company (Vic). All produce was stored at 4°C cool room prior to processing. Heads of broccoli and cauliflower were cut into florets weighing up to 50 g. Samples of 350 g were weighed into pillow-pack bags and heat sealed with or without gas-flushing.

3.2.6 Bag Preparation

The packaging film was cut into strips and heat sealed into bags 220 mm (width) x 280 x 280 mm (length). The effective dimension of the bags, discounting the outer heat seal area, was 210 x 250 for shredded, mixed lettuce and broccoli florets and 210 mm x 240 mm for cauliflower florets. The surface area for gas exchange for both sides of the bags was 0.105 m^2 for shredded lettuce, mixed lettuce, and broccoli florets; and 0.101 m^2 for cauliflower florets.

3.2.7 Sealing and Gas Flushing of Packages

"Pillow-pack" bags were heat sealed on either a Webomatic Model E50G (Germany) or Freshpac AVS Series Gas Flush Model (Freshpac, NSW). The Webomatic machine is a chamber type vacuum packaging machine with a facility for gas flushing. This machine was used in "In-Storage Shelf-life Evaluation" (Stage 2).

The Freshpac machine is a semi-automatic machine for vacuum sealing and gas flushing products into pouches or bags. The machine has a retractable snorkel that carries out vacuum and back flush operations which are microprocessor controlled.

Mixture of gases was introduced into the packaging machine using a Witt-Gasetechnik gas mixer unit KM100-2M (Germany).

Air packed samples were heat sealed without the vacuum and gas flush facility.

3.2.8 Gas Analysis

A gas chromatograph, Shimadzu GC-8A with Porapak Q column was used to measure the concentration of O_2, N_2 and CO_2 in gas mixtures (gas sample volume 5 mL). Percentage composition was calculated from the integrated areas using standard graphs of reference gas mixtures accurate to $\pm$ 0.1%. Standard premixtures containing 1.0% O_2/ 99.0%CO_2; 3.0% O_2/ 4.9% CO_2/ 92.1% N_2; 5.3% O_2/ 9.7%CO_2/ 85.0% N_2; 10.4% O_2/ 48.7%CO_2/ 40.9% N_2; 10.2% O_2/ 9.6%CO_2/ 80.2% N_2 (CIG Gases Ltd. Vic.) were used to generate a calibration graph from which the sample gas compositions were determined. Gas samples were taken from the packs by piercing the test film with a fine needle and withdrawing the sample volume with a syringe.

3.2.9 Sensory Evaluation

Samples were rated visually by two experimenters according to the following scoring system developed by Kader *et al.* (1973): 9 - excellent, free of defects; 7 - good, minor defects but not objectionable; 5 - fair, slightly to moderately objectionable. Score of 5 was taken as the lower limit of sales appeal or shelf-life. Presence or absence of slime and undesirable odour were also recorded.

3.2.10 Microbiological Analysis

The standard plate count was performed according to Australian Standards (AS) 1766.1.3 (1991a) - "General procedures and techniques - Colony count - Pour plate method".

The yeast and mould count was performed according to AS1766.2.2 (1991b) - "Colony count of yeasts and moulds".

The Listeria spp. examination was carried out using a modification of AS1766.2.15 (Int. 1991c) - "Examination for specific organisms - *Listeria monocytogenes* in dairy products" and the method described in "Selective microbiology for food and dairy laboratories", "*Listeria* selective medium (Oxford Formulation)", and "*Listeria* selective enrichment medium" (Unipath, 1990a).

The examination for *Yersinia* species was performed using the method described in "Selective microbiology for food and dairy laboratories" - *Yersinia* selective medium (Unipath, 1990b).

The examination for *Aeromonas* species was performed using the methods of Palumbo *et al.* (1985) and Oxoid (1992).

3.3 RESULTS AND DISCUSSION

3.3.1 In-Storage Shelf-life of Shredded and Mixed Lettuce (Stage 2)

Shredded lettuce

A summary of gas composition in packages of shredded lettuce is given in Table 1. The results of visual observations are illustrated in Figures 1 to 3. Shelf-life was considered terminated when visual score is 5 or less. Typical profiles of air and product temperatures during storage are given in Figs. A.1 and A.2 (Appendix).

The results of gas analysis indicate that it was only the package made of Film B and gas flushed to give an initial gas concentration of 3% O_2/5% CO_2 was able to maintain desirable levels of O_2 (1-3%) up to the 7th day of storage (Tests numbered 2, 7, 12). These results demonstrate that gas flushing is essential for MA packaging of shredded lettuce since low levels of (<3%) were not attained in any air packaged samples. Results of test No. 12 (Film B) indicate that care should be taken not to expose shredded lettuce for more than a day at 7°C to avoid the development of anaerobic atmosphere (<1% O_2).

High levels of O_2 (4-6%) found in packages made of Film A, suggest that Film A is more permeable to O_2 than Film B. Film A is a potential film for packaging shredded lettuce because it possesses high clarity and good mechanical property. However, its permeability to oxygen should be reduced either by changing its formulation or increasing its thickness.

As expected, all the packages did not develop CO_2 levels above 5%. This is due to the fact that commercially available films are more permeable to CO_2 than O_2 in the order of 3 to 5 times (Mannaperruma and Singh, 1990). Hence, CO_2 level above 5% can be attained only at the expense of developing anaerobic condition. In order to overcome this limitation, higher levels of CO_2 (>5%) could be injected into the package. Recently, Barriga *et al.* (1991) suggested that 3% O_2/10% CO_2 is applicable for CA storage of lettuce. This CO_2 level is higher than those suggested by some researchers (Ballantyne *et al.* 1988a; Gill, 1988).

TABLE 1. MODIFIED ATMOSPHERES OF SHREDDED LETTUCE UNDER VARIOUS CONDITIONS

TEST NO.	FILM	TEMP (°C)	INITIAL ATMOSPHERE		DAY 5		DAY 7	
			% O_2	% CO_2	% O_2	% CO_2	% O_2	% CO_2
1	A	1	3	5	5.3	6.6	6.6	2.3
2	B	1	3	5	1.1	2.0	2.5	3.1
3	A	1	Air	-	9.5	3.1	8.6	2.7
4	B	1	Air	-	11.1	3.4	11.8	3.0
5	Control	1	Air	-			-	-
6	A	5	3	5	4.2	3.1	3.6	3.5
7	B	5	3	5	1.8	4.0	1.2	4.4
8	A	5	Air	-	12.5	2.5	-	-
9	B	5	Air	-	7.8	4.1	-	-
10	Control	5	Air	-			-	-
11	A	Simulated +	3	5	4.1	3.1	-	-
12	B	Simulated +	3	5	0.6	4.9	-	-
13	A	Simulated +	Air	-	6.8	3.6	-	-
14	B	Simulated +	Air	-	11.3	3.4	-	-
15	Control	Simulated +	Air	-			-	-

+ Simulated handling: 3 days 4°C, 1 day 7°C, 4°C until end of shelf-life.

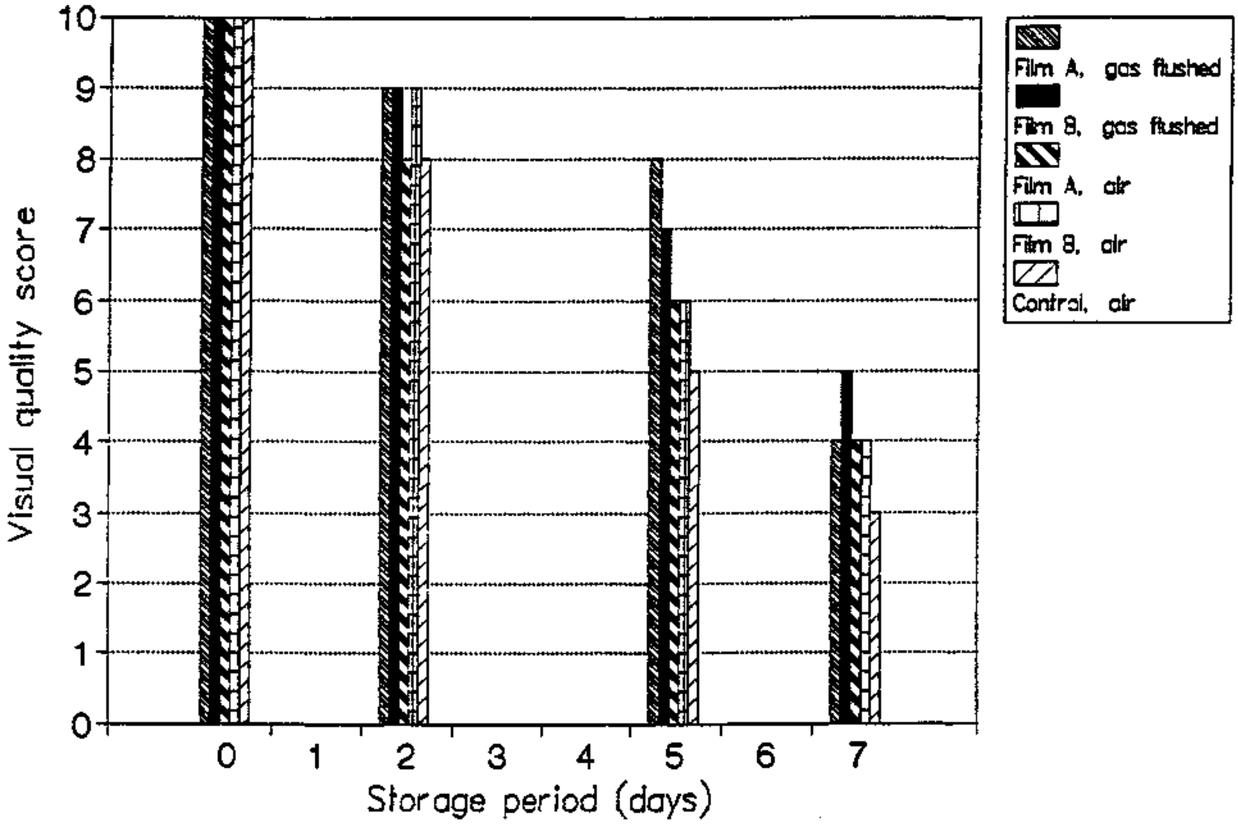

Fig. 1. Visual quality of shredded lettuce stored at 1°C

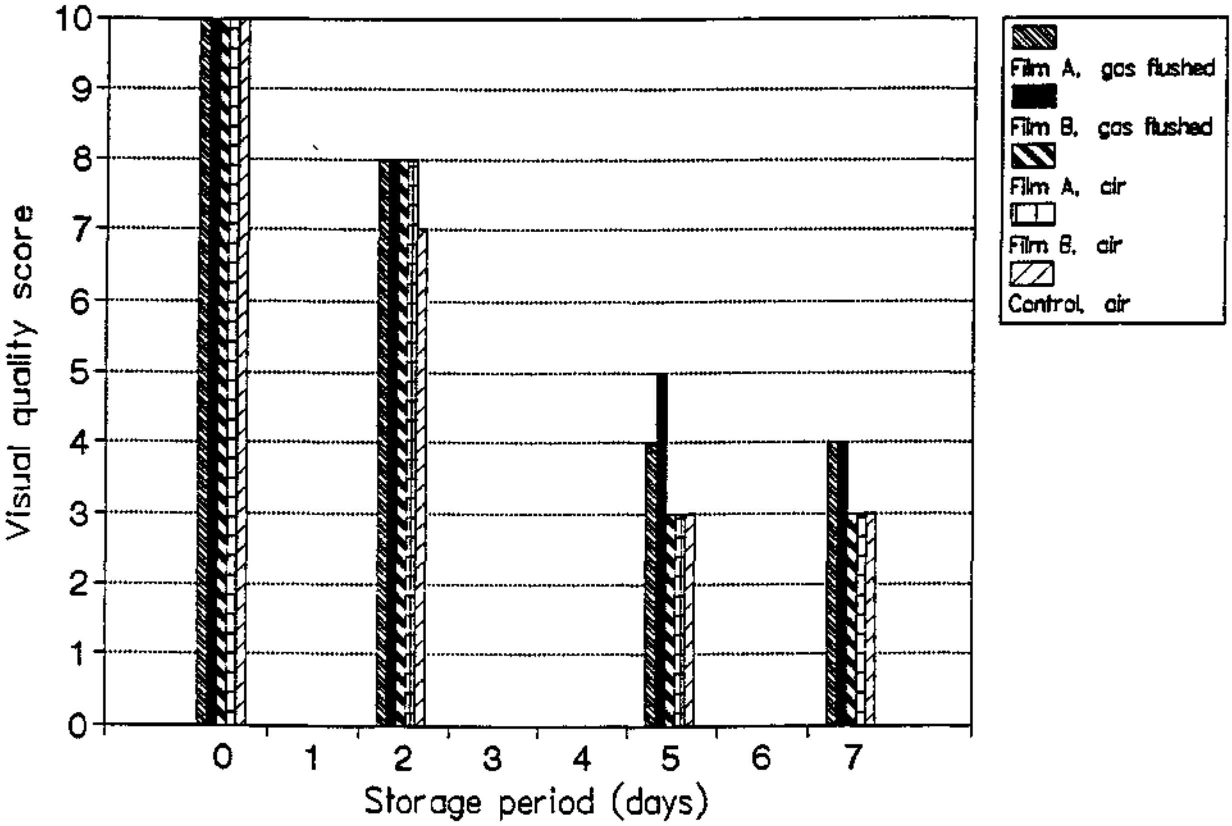

Fig. 2. Visual quality of shredded lettuce stored at 5°C

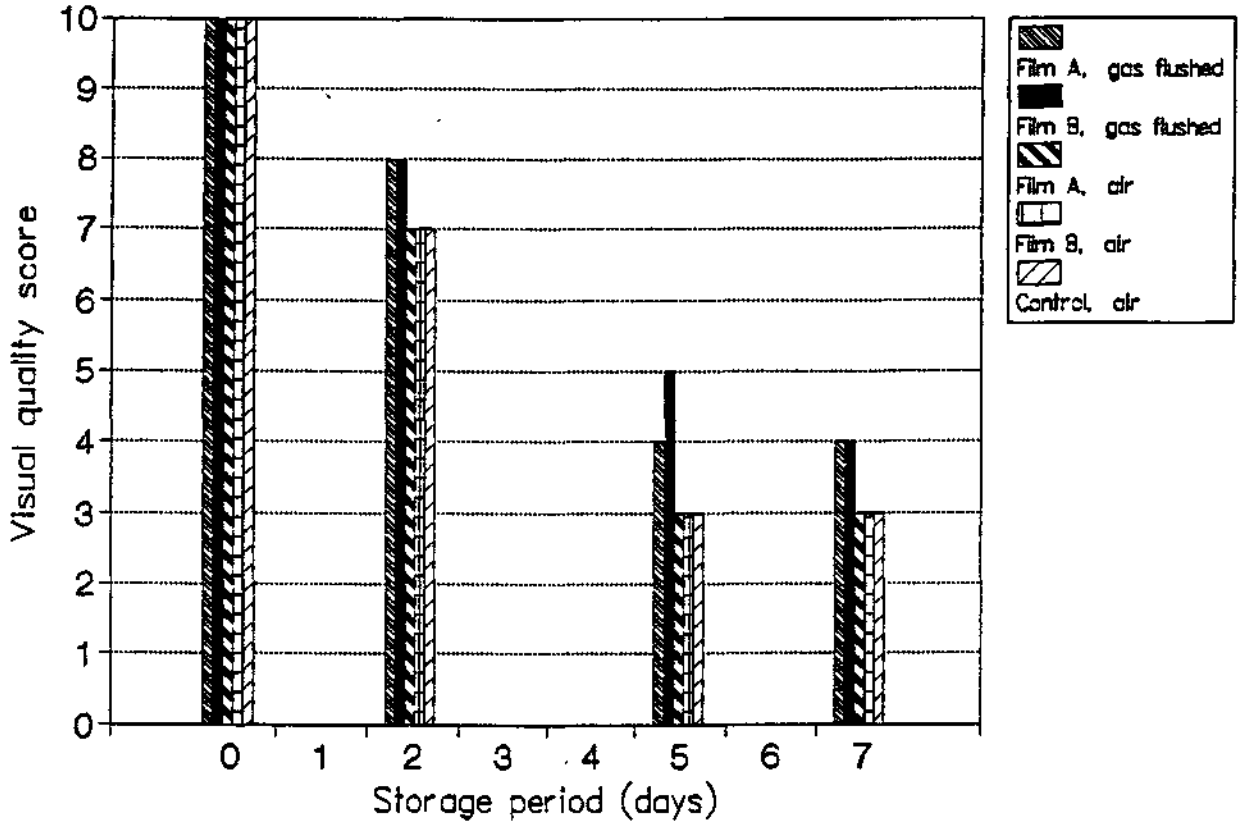

Fig. 3. **Visual quality of shredded lettuce stored under simulated handling (3 days at 4°C, 1 day at 7°C, 4°C until end of shelf life).**

The sensory ratings illustrated in Figs. 1 to 3, clearly demonstrate the benefits of gas flushing shredded lettuce with 3% O_2/5% CO_2 compared to packaging in air. Enzymatic browning which was found to be the main limiting factor in shelf-life extension, was visible at a later storage time in all gas flushed samples when compared to similar air packed samples. This observation is more obvious at higher storage temperature (Figs. 2 and 3). Similar findings were reported by Ballantyne *et al.* (1988a) and Day (1989) who attributed the extension in shelf-life of shredded and mixed lettuce to reduced O_2 levels.

Figs. 1-3 also illustrate that low temperature storage is the single most important factor in preserving the quality of shredded lettuce in modified atmosphere packages. Generally, all samples stored at 1°C were found to be acceptable until day 5. However, gas flushed samples were visually more appealing than air pack samples. One set of samples gas flushed in film B were found to be marginally acceptable up to day 7. In contrast, the control sample was marginally acceptable at day 5.

Of all the samples stored under 5°C and simulated temperature (3 days at 4°C, 1 day at 7°C, back to 4°C), it was only the gas flushed treatment with Film B that remained marginally acceptable at day 5. The main factor that limited their shelf-life was the development of slime or signs of decay on pieces of lettuce with plenty of water condensation. Since water condensation is known to be more severe when the temperature of the product is significantly higher than the storage temperature, a subsequent test was conducted to evaluate the effects of different processing temperatures on the storage quality of shredded lettuce.

The shelf-life attained in this study would be a conservative estimate because of the following:

(i) the preparation and packaging were done at ambient temperature (20°C), and the rinsing water used had a temperature of about 8-10°C. These could be the main reason for water condensation inside the package.

(ii) the Iceberg lettuce used in this study were less than premium quality. Pinkish discolouration was found in most heads prior to preparation. Investigation revealed that the lettuce heads were not properly cooled immediately after harvesting.

The populations of aerobic microorganism for samples that were visually acceptable at day 7 are given in Table 2. The populations of aerobic microorganisms ranged from 1×10^6 to 3.3×10^7 (cfu/g). For prepared vegetables, French legislation (there is no current Australian legislation) allows a maximum of 5×10^7 (cfu/g) at consumption stage (Anonymous, 1988). The microbial quality of shredded lettuce stored at 1°C could thus be considered satisfactory until day 7.

TABLE 2. MICROBIAL COUNTS ON SHREDDED LETTUCE AFTER 7 DAYS OF STORAGE AT 1°C

TEST NO	FILM	INITIAL ATMOSPHERE		SPC* (CFU/g)
		% 0_2	% CO_2	
1	A	3	5	1.2×10^6
2	B	3	5	9.0×10^6
3	A	Air	-	3.3×10^7
4	B	Air	-	1.2×10^6

* Standard plate count (aerobic plate count)

Mixed lettuce

Results of the gas composition analysis on mixed lettuce samples are given in Table 3. The corresponding sensory appearance assessment are illustrated in Figs. 4 to 6.

As expected, the general observations with mixed lettuce samples were similar to that of shredded lettuce. This could be because mixed lettuce contains 50% diced Iceberg which is the sole ingredient of shredded lettuce. All mixed lettuce samples packaged with 3% O_2/5% CO_2 were generally superior than samples packaged in air (Figs. 4 to 6). Enzymatic browning was found to be the main limiting factor for determining shelf-life followed by slime formation. Among the lettuces used, the Iceberg lettuce was the first to show signs of enzymatic browning. Consequently, the overall shelf-life achieved for mixed lettuce samples was determined by the Iceberg lettuce's shelf-life.

All the mixed lettuce samples stored at 1°C, achieved a shelf-life of 7 days (Fig. 4). This demonstrates that temperature is the most important factor in extending the quality shelf-life of lettuce followed by maintenance of desirable gas atmosphere. However, gas flushed samples were found to be generally superior in appearance than air packed samples. Samples stored 5°C and simulated temperature conditions (3 days 4°C, 1 day 7°C, back to 4°C) were found to be marginally acceptable on the 5th day of storage.

In summary, gas flushed samples were superior to air packed samples in all storage conditions. The best sensory result was obtained with gas flushed samples in Film B. This could be mainly due to the ability of the Film B package to maintain O_2 levels below 4% (Table 3). However, the levels of CO_2 developed in all the packages was found to be low ($<5\%$).

TABLE 3. MODIFIED ATMOSPHERES OF MIXED LETTUCE UNDER VARIOUS CONDITIONS

TEST NO.	FILM	TEMP (°C)	INITIAL ATMOSPHERE		DAY 4		DAY 7	
			% O_2	% CO_2	% O_2	% CO_2	% O_2	% CO_2
1	A	1	3	5	4.7	2.8	5.8	2.4
2	B	1	3	5	1.6	3.8	1.6	3.2
3	A	1	Air	-	12.3	2.8	10.5	2.4
4	B	1	Air	-	10.0	4.4	-	-
5	Control	1	Air	-			-	-
6	A	5	3	5	5.4	2.8	5.8	2.8
7	B	5	3	5	3.4	4.5	0.2	4.9
8	A	5	Air	-	9.2	3.4	-	-
9	B	5	Air	-	7.8	4.9	9.0	3.1
10	Control	5	Air	-	-	-	-	-
11	A	Simulated +	3	5	3.6	3.4	1.1	4.5
12	B	Simulated +	3	5	3.7	3.5	-	-
13	A	Simulated +	Air	-	9.0	3.8	4.6	4.2
14	B	Simulated +	Air	-	7.3	5.2	-	-
15	Control	Simulated +	Air	-	-	-	-	-

+ Simulated handling: 3 days 4°C, 1 day 7°C, 4°C until end of shelf-life.

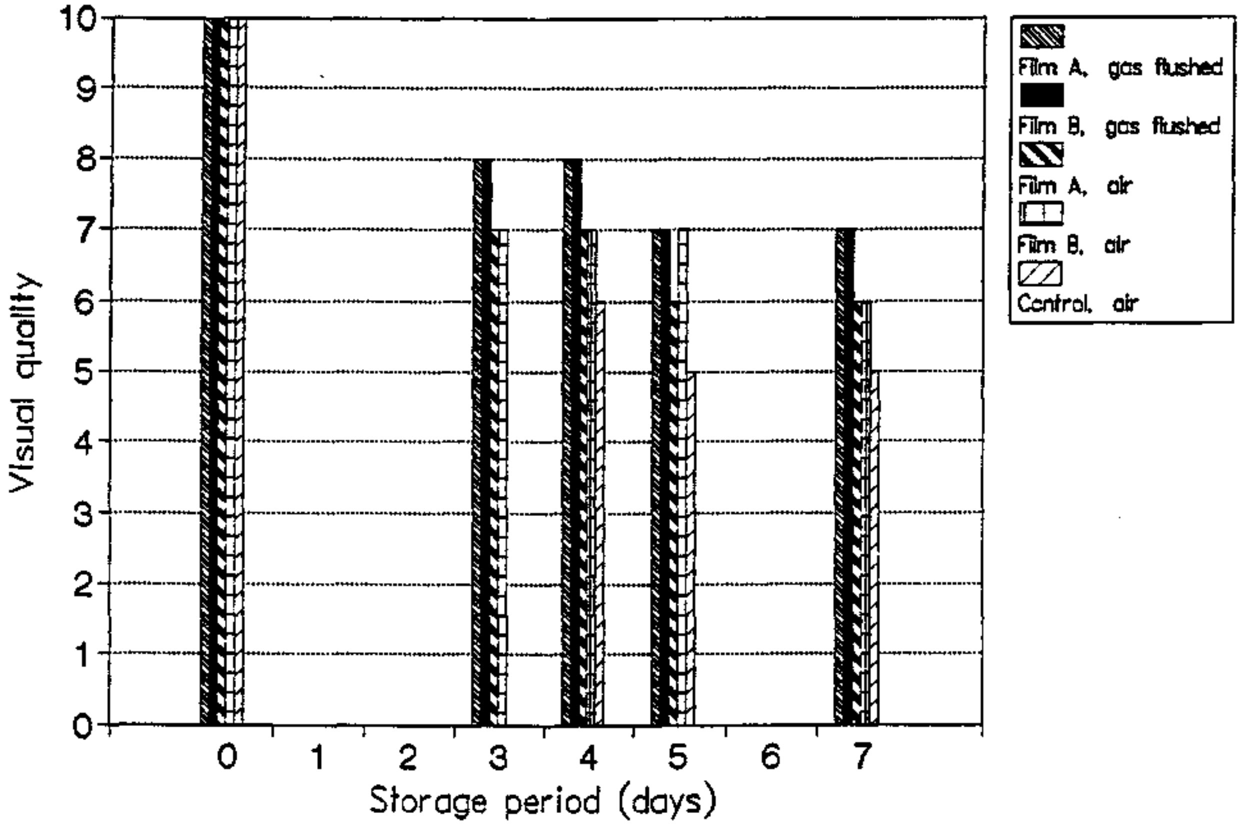

Fig. 4. **Visual quality of mixed lettuce stored at 1°C**

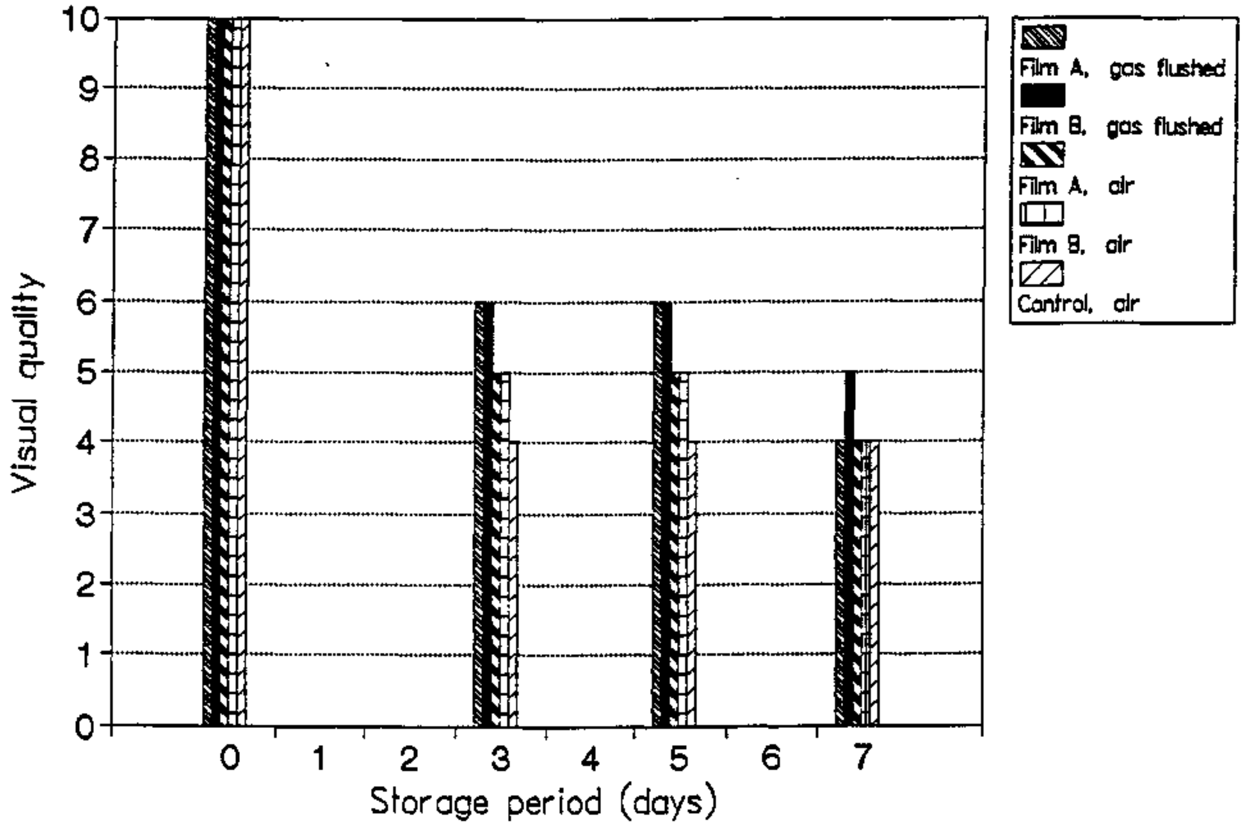

Fig. 5. **Visual quality of mixed lettuce stored at 5°C**

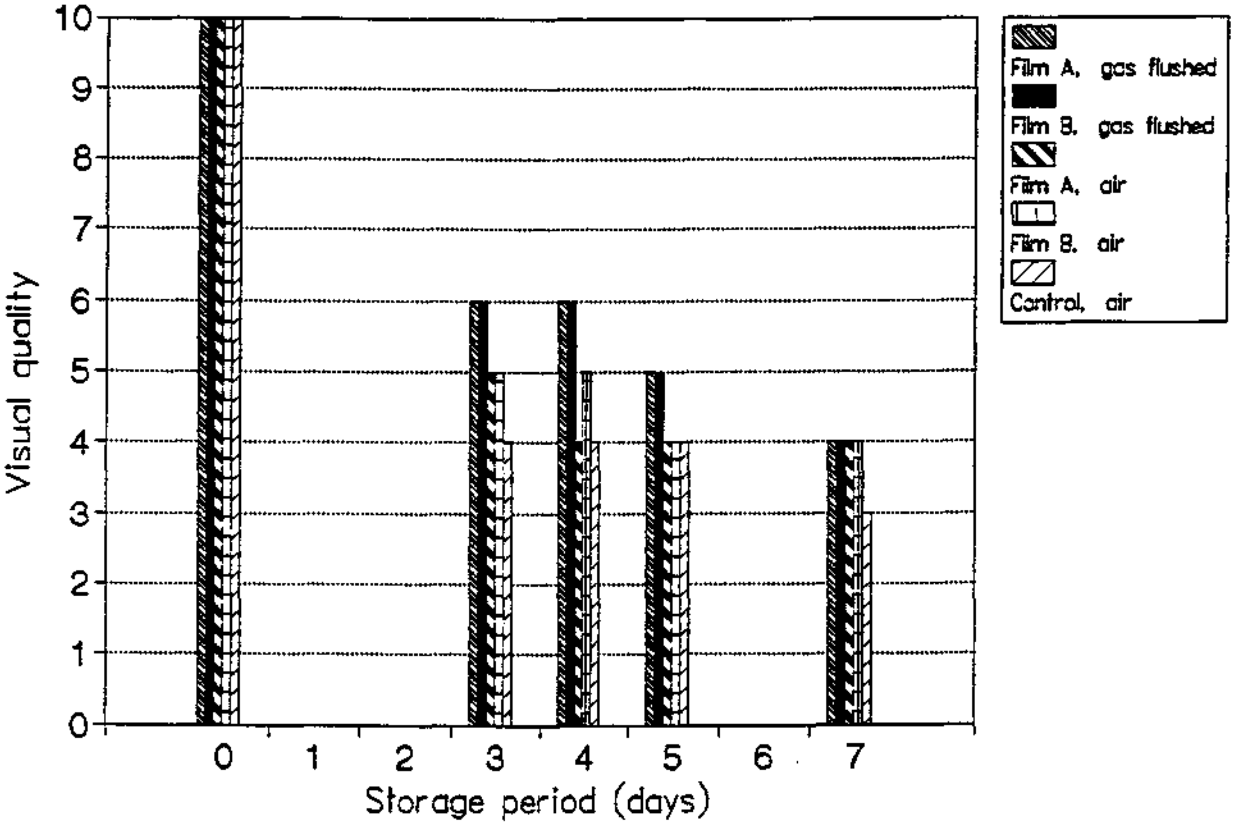

Fig. 6. **Visual quality of mixed lettuce stored under simulated handling (3 days at 4°C, 1 day at 7°C, 4°C until end of shelf life).**

3.3.2 Effects of Processing Temperature on Lettuce Samples

Previous results indicated that slime formation was a significant factor in the termination of shelf-life of shredded and mixed lettuce (section 3.3.2). And the presence of excessive water condensation on the surface of the lettuce could have acted as a catalyst for slime growth. It was hypothesised that reducing water condensation by packaging a cool product could reduce the incidence of slime growth. To evaluate this hypothesis, a processing and storage trial was conducted with mixed lettuce samples. Mixed lettuce was selected because this lettuce was found to have a relatively shorter shelf-life than shredded lettuce.

Four (4) packages of mixed lettuce were prepared in a manner described in section 3.2.5. Another 4 samples were prepared using the same procedure, however, the samples were rinsed in "iced-water" (1-2°C). All samples were gas-flushed to contain 3% O_2/5% CO_2 and heat sealed using the Webomatic machine. Samples were stored at 4°C for visual assessment and shelf-life determination.

As expected, mixed lettuce samples washed in iced-water did not show any signs of slime formation (Table 4). The shelf-life of these samples (5 days) were terminated mainly due to enzymatic browning. In contrast, lettuce samples rinsed in warmer water (8-10°C) developed excessive condensation within the first day of storage. Likewise, these samples had a relatively shorter shelf-life due to slime formation and discolouration. Based on this result, all subsequent lettuce samples were washed/rinsed in iced-water to prevent excessive water condensation during storage.

TABLE 4 VISUAL QUALITY OF MIXED LETTUCE SAMPLES STORED AT 4°C

ITEMS	RINSING WATER TEMPERATURE	
	8-10°C	1-2°C
Shelf-life (days)	4	5
Defects	Slime formation browning	browning

The above results also suggest that the processing, packaging and storage protocol used here is not sufficient to attain a shelf-life of 7 days for mixed lettuce. A higher level of CO_2 may be needed to block the formation of browning compounds (Barriga *et al.* 1991) and/or a storage temperature below 4°C would be required to achieve a 7 day shelf-life for mixed and shredded lettuce.

3.3.3 Optimum Gas Concentration for Shredded and Mixed Lettuce

The visual quality of shredded lettuce and mixed lettuce samples stored under 3% O_2/5% CO_2 and air decreased throughout storage (Tables 5 and 6). However, visual quality was significantly preserved by high CO_2 levels (10% and 15%) up to at least 15 days. Storage test was terminated on the 15th day of storage.

The results in Tables 5 and 6 demonstrate that CO_2 levels equal or above 10% are required to give significant browning inhibition in conjunction with low O_2 level and low temperature storage. This could be the reason why the lettuce samples in previous trials did not have a shelf-life of more than 7 days. Previous MA packages developed CO_2 levels less than 5% after only 5 days of storage at 1°C and 5°C (Tables 2 and 3). Carbon dioxide was reported in the literature to block formation of phenolic compounds, as well as inhibiting polyphenol oxidase activity (Siriphanick and Kader, 1984).

TABLE 5 VISUAL QUALITY OF SHREDDED LETTUCE SAMPLES UNDER CONTROLLED ATMOSPHERE STORAGE AT 1-2°C

ATMOSPHERE	STORAGE DAYS				DEFECTS
	0	5	8	15	
Air	10	8-9	7	4	Severe browning, Dry
2.4% O_2/4.5% CO_2	10	9-10	8	5-6	Browning
2.2% O_2/9.6% CO_2	10	10	10	9-10	None
2.0% O_2/15% CO_2	10	10	10	10	None

TABLE 6 VISUAL QUALITY OF MIXED LETTUCE SAMPLES UNDER CONTROLLED ATMOSPHERE STORAGE AT 1-2°C

ATMOSPHERE	STORAGE DAYS				DEFECTS
	0	5	8	15	
Air	10	9	4-5	4	Severe browning, Dry
2.4% O_2/4.5% CO_2	10	10	6-7	5-6	Browning
2.2% O_2/9.6% CO_2	10	10	9-10	9-10	None
2.0% O_2/15% CO_2	10	10	10	9-10	None

The above results indicate that MA packaging would succeed in preventing enzymatic browning in prepared lettuces if the CO_2 level is equal or above 10% and the O_2 level is about 2-3%. Shelf-life of shredded and mixed lettuce could be at least doubled if this condition can be achieved in MA packages.

Mixed and shredded lettuce samples behaved similarly, except that mixed lettuce discoloured faster than shredded lettuce. This is probably due to the leaching out of more active enzymes out of the smaller strips of shredded lettuce (5 mm) than diced mixed lettuce (20-30 min).

3.3.4 Comparison of Gas Packaging Equipment

After the "In-Storage" trials, the Freshpac machine was made available to FRI. Therefore, it was decided to compare the performance of it with the Webomatic. For comparison purposes, the reproducibility/repeatability of residual oxygen levels achieved in each machine was evaluated. Table 7 gives a summary of residual O_2 levels attained in each machine at various vacuum levels.

Based on oxygen values, both machines were found to give repeatable levels of O_2 since the values of coefficient of variation (COV) were less 15%. The Freshpac machine when set at the maximum level (100) gave the most repeatable data or the least COV value (1.9%). However, in order to use the Freshpac machine for packaging shredded and mixed lettuce, the residual O_2 level should be in the range of 2-3%. This was achieved using the "2 cycle option" (double gas flushing) when the vacuum was set at 100, with a gas flushing time of 3 seconds. This setting gave an average of 2.8% residual O_2.

TABLE 7 COMPARISON OF FRESHPAC AND WEBOMATIC PACKAGING EQUIPMENT

ITEM	WEBOMATIC	FRESHPAC			
		VACUUM SETTING			
		25	50	100	2 cycle+
Average O_2 %	3.10	7.2	5.8	5.2	2.8
Standard deviation (%)	0.29	1.0	0.7	0.1	0.2
COV@ (%)	9.10	14.0	12.1	1.9	7.5

+ "double gas flushing"

@ coefficient of variation: (average/standard deviation) * 100%

The volume of the "pillow-packs" produced from both machines were also measured and compared. The pillow-packs from the Freshpac machine were found to be more inflated than the collapsed packs from the Webomatic. This is mainly due to the efficient snorkel system of the Freshpac machine which evacuates and back-flushes gas directly into the package. On the average, the packs from the Freshpac machine gave 60% more volume than those from the Webomatic. The average total volume of the packs from the Freshpac machine was about 1.5 litres (including the product). The inflated pillow-pack looks more appealing than the somewhat collapsed bag from the Webomatic because the former makes the product free-flowing. Therefore, it was decided to use the Freshpac machine for subsequent packaging and storage trials.

3.3.5 In-Storage and After-Opening Shelf-life of Shredded and Mixed Lettuce

In-storage shelf-life under simulated handling

Based on controlled atmosphere storage, a gas concentration of 2-3% O_2 and 15% CO_2 could almost triple the "in-storage" shelf-life of shredded and mixed lettuce samples. Since there are no commercially available packaging film that could produce an equilibrium level of 15% CO_2 while maintaining a O_2 level of 2-3%, it was decided to increase the initial CO_2 level to 17% to compensate for the high permeation of CO_2 out of the package. Previous results also indicated that a 7 day shelf-life is difficult to achieve when the storage temperature is equal or above 4°C. Therefore, it was decided to use the following temperature profile as a compromise with the previous temperature conditions for lettuce:

3 days at 1-2°C, and
4°C until the termination of the test.

All samples were packaged in bags made from Film B and gas flushed using the Freshpac machine.

The results of gas analysis from shredded and mixed lettuce are illustrated in Figs. 7 and 8. The corresponding results of sensory assessment are given in Table 8.

Figures 7 and 8 show that the packages of mixed and shredded lettuce were able to maintain a CO_2 level above 7% until the 5th day. This high level of CO_2 enabled the lettuce samples to maintain their visual appearance as indicated by their visual scores of 9 compared to scores of 5-6 for similar samples packaged in air (Table 8). On the 7th day the level of CO_2 was only about 5%, this was found to be ineffective based on controlled atmosphere storage (section 3.3.3). On the same day, the visual appeal of both lettuces was drastically reduced. Both shredded and mixed lettuce samples were found to be marginally acceptable up to the 8th day of storage. In contrast, samples packed in air were found to be marginally acceptable up to day 6.

In summary, the procedure developed for shredded and mixed lettuce was able to maintain fresh appearance up to day 6-7, and was marginally acceptable up to the 8th day of storage.

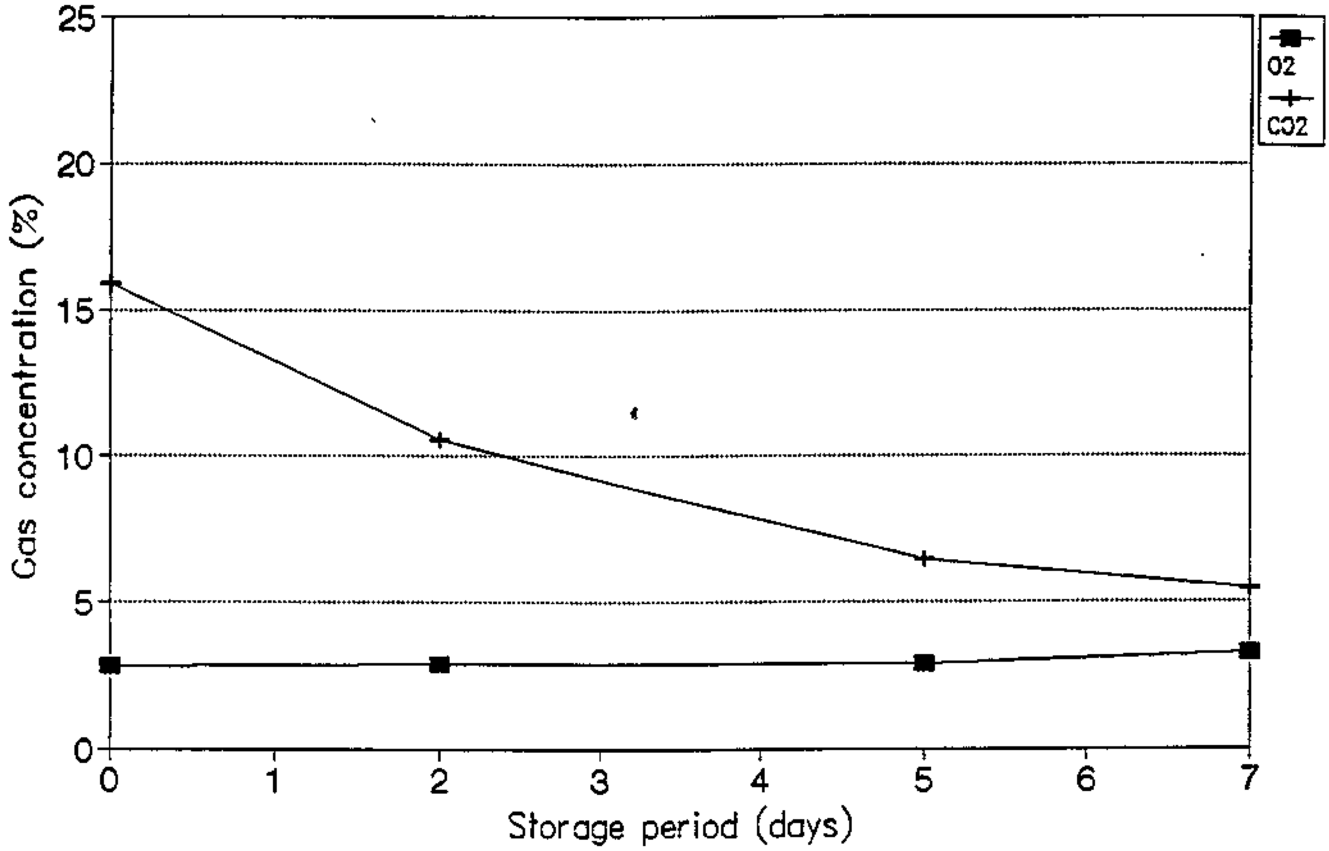

Fig. 7. **Modified atmosphere of shredded lettuce in pillow-pack containing initial atmosphere of 3% O_2/17% CO_2 stored under simulated handling (3 days at 1°C, 4°C until the end of shelf life)**

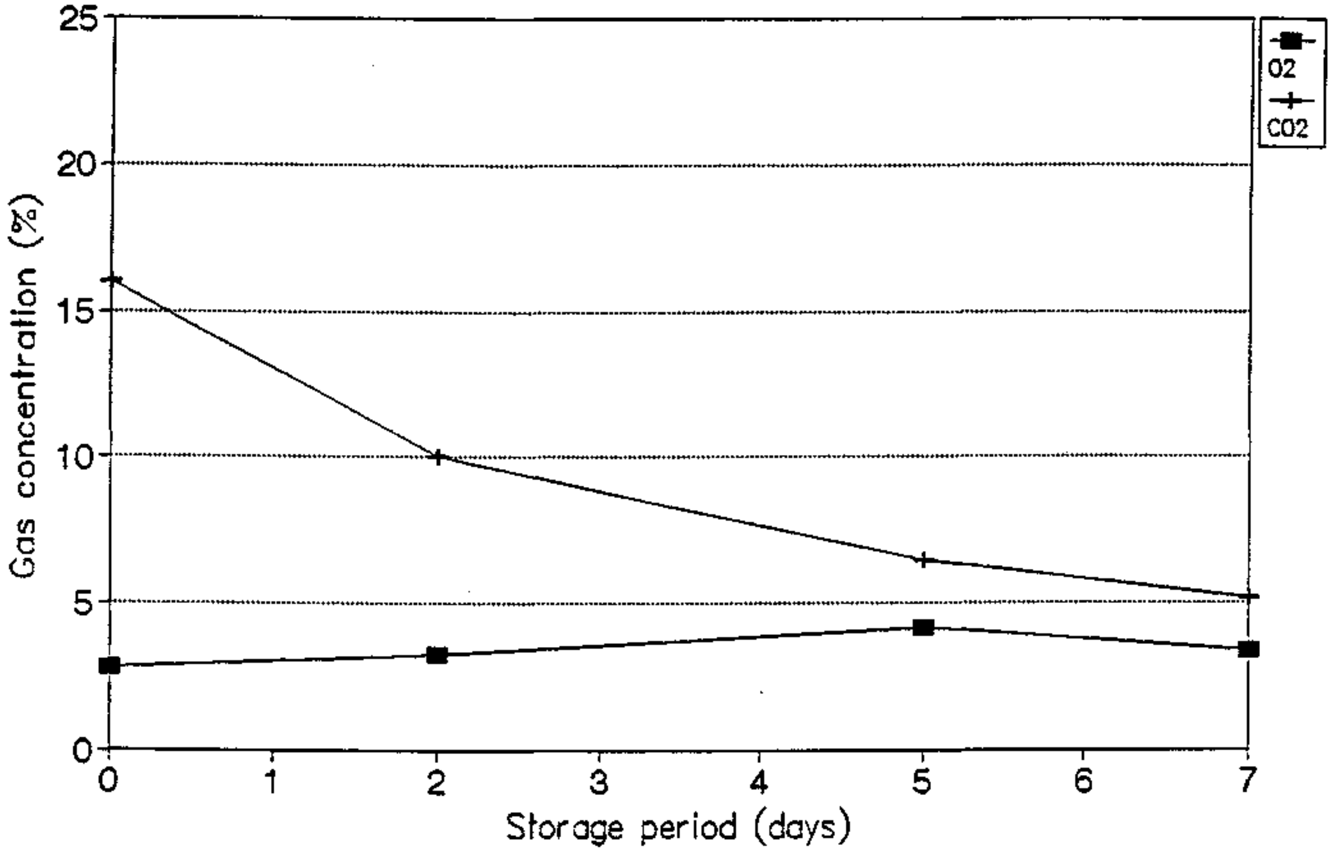

Fig. 8. **Modified atmosphere of mixed lettuce in pillow-pack containing initial atmosphere of 3% O_2/17% CO_2 stored under simulated handling (3 days at 1°C, 4°C until the end of shelf life)**

TABLE 8. VISUAL QUALITY OF SHREDDED AND MIXED LETTUCE SAMPLES UNDER SIMULATED HANDLING+

SHREDDED LETTUCE			MIXED LETTUCE	
DAYS	CONTROL	3% O_2/17% CO_2	CONTROL	3% O_2/17% CO_2
0	10	10	10	10
1	-	-	-	-
2	-	-	-	-
3	8	10	8	9
4	-	-	-	-
5	5-6	9	5-6	9
6	5*	7-8	5*	8
7	4	6-7	4	7
8	-	5-6*	-	5-6*

+ 3 days at 1-2°C, then 4°C until end of shelf-life.
* shelf-life, visual score $\leq$ 5

<u>In-storage shelf-life at 2°C</u>

In order to evaluate the quality of mixed and shredded lettuce stored at 2°C, 4 samples from each treatment of previous trials (simulated storage temperature) were intentionally left at 2°C. The results of visual observation from these samples are given in Table 9.

The gas flushed samples of shredded and mixed lettuce stored at 2°C maintained its fresh-like appearance (score $\geq$ 8, very slight browning) up to day 8. However, they remained marginally acceptable (score 5) until day 10. On the contrary, control samples of shredded and mixed lettuce in perforated bags were marginally acceptable at day 5. This result suggests that modified atmosphere packaging in conjunction with low temperature storage (1-2°C) could effectively double the shelf-life of shredded and mixed lettuce.

TABLE 9 VISUAL QUALITY OF SHREDDED AND MIXED LETTUCE SAMPLES STORED AT 2°C

SHREDDED LETTUCE			MIXED LETTUCE	
DAYS	CONTROL	3% O_2/17% CO_2	CONTROL	3% O_2/17% CO_2
0	10	10	10	10
1	-	-	-	-
2	-	-	-	-
3	8	9-10	7-8	9-10
4	-	-	-	-
5	6	9-10	5-6*	9
6	5*	9-10	4.0	8-9
7	-	9-10	-	7-8
8	-	7-8	-	7
9	-	6-7	-	6
10	-	5*	-	5*

* Shelf-life, visual score $\leq$ 5.0

After-opening shelf-life

The results of visual quality assessment and microbial counts on samples stored under simulated handling condition (3 days at 1-2°C, and 4°C until the end of shelf-life) are given in Tables 10 to 12. The results of microbiological and visual analyses indicated that the discolouration as a result of enzymatic browning was the limiting factor for both shredded and mixed lettuce samples. All the lettuce samples analysed did not exceed the French limit of 5 x 10^7 (cfu/g). Likewise, no human pathogens were detected (*Aeromonas*, *Listeria monocytogenes*, *Yersinia*) - Tables 11 and 12. The in-storage (0 day after opening) and after-opening shelf-life for shredded and mixed lettuce were similar (Table 16). The after- opening shelf-life of lettuce samples opened at day 5 was 2 days. However, the shelf-life of samples opened on day 7 was only 1 day.

TABLE 10. VISUAL QUALITY OF SHREDDED AND MIXED LETTUCE OPENED AT SPECIFIED PERIODS+

STORAGE DAYS	DAYS AFTER OPENING	SHREDDED LETTUCE	MIXED LETTUCE
5	0	9	9
5	1	8	7
5	2	5-6*	5-6*
7	0	6-7	7
7	1	5-6*	5-6*
7	2	4	4
8	0	5*	5*

+Simulated handling: 3 days at 1-2°C, and 4°C until the end of shelf-life.
*Shelf-life, visual score ≤ 5.

TABLE 11. MICROBIAL COUNTS FROM SHREDDED LETTUCE STORED UNDER SIMULATED CONDITIONS+

I.INITIAL ATMOSPHERE 3% O_2/17% CO_2				
STORAGE DAYS	DAYS AFTER OPENING	STANDARD PLATE COUNT (cfu/g)	YEASTS (cfu/g)	MOULDS (cfu/g)
0	0	2.2×10^5	40	10
5	0	8.9×10^4	200	10,000
5	1	2.5×10^5	200	100
5	2	7.3×10^5	1,000	<100
7	0	2.4×10^5	200	<100
7	1	3.6×10^5	200	<10
9	0	8.0×10^5	100	<100
II. AIR PACKED SAMPLES				
7	0	2.2×10^6	11,500	2,300

+Simulated handling: 3 days at 1-2°C, and 4°C until the end of shelf-life.
*Shelf-life, visual score ≤ 5.

TABLE 12 MICROBIAL COUNTS FROM MIXED LETTUCE STORED UNDER SIMULATED HANDLING+

INITIAL ATMOSPHERE 3% O_2/17% CO_2				
STORAGE DAYS	DAYS AFTER OPENING	STANDARD PLATE COUNT (cfu/g)	YEASTS (cfu/g)	MOULDS (cfu/g)
0	0	2.9×10^4	280	10
5	0	9.1×10^6	2500	10,000
5	1	1.0×10^6	300	100
5	2	1.7×10^7	15,300	<100
7	0	9.1×10^6	15,600	<100
7	1	1.8×10^6	43,000	<10
9	0	3.0×10^6	3,800	<100
AIR PACKED SAMPLES				
7	0	3.7×10^7	400	< 100

+Simulated handling: 3 days at 1-2°C, and 4°C until the end of shelf-life.
*Shelf-life, visual score ≤5.

3.3.6 In-Storage Shelf-life of Broccoli and Cauliflower Florets

Broccoli Florets

The gas composition of broccoli florets packaged and stored under different conditions are given in Table 13. The corresponding results of sensory evaluation are illustrated in Figs. 9-11. Storage test was terminated at day 9.

Results of sensory evaluation illustrate that most of the florets were found to be acceptable up to day 9. Best quality florets were obtained from packages stored at 1°C. No significant difference was found in samples stored at 1°C. However, control samples packaged in perforated bags were found to be softer and rubbery in texture than other samples. This could be due to the high water vapour transmission rate of the perforated control bags.

The main factors that reduced the quality of florets stored at 1°C and 5°C were discolouration along the cut sections and the development of rubbery texture, especially in control samples. However, in samples stored at simulated temperature, it was slime formation that limited the shelf-life of the florets. Slime formations were found mainly on the bruised section of the florets.

TABLE 13. MODIFIED ATMOSPHERES OF BROCCOLI FLORETS UNDER VARIOUS CONDITIONS

TEST NO.	FILM	TEMP (°C)	INITIAL ATMOSPHERE		DAY 4		DAY 7	
			% O_2	% CO_2	% O_2	% CO_2	% O_2	% CO_2
1	C	1	3	5	0.7	2.3	0.6	2.9
2	C	1	Air	-	0.5	3.8	0.6	3.3
3	D	1	Air	-	16.0	4.8	15.8	4.5
4	Control	1	Air	-	-	-	-	-
5	C	5	3	5	0.3	3.7	0.2	3.4
6	C	5	Air	-	0.4	3.7	0.3	3.1
7	D	5	Air	-	12.4	8.4	13.6	7.5
8	Control	5	Air	-	-	-	-	-
9	C	Simulated⁺	3	5	0.7	3.6	0.7	3.2
10	C	Simulated⁺	Air	-	0.3	3.8	0.3	3.3
11	D	Simulated⁺	Air	-	12.9	7.9	13.5	7.3
12	Control	Simulated⁺	Air	-	-	-	-	-

⁺Simulated handling: 3 days 4°C, 1 day 7°C, 4°C until end of shelf-life.

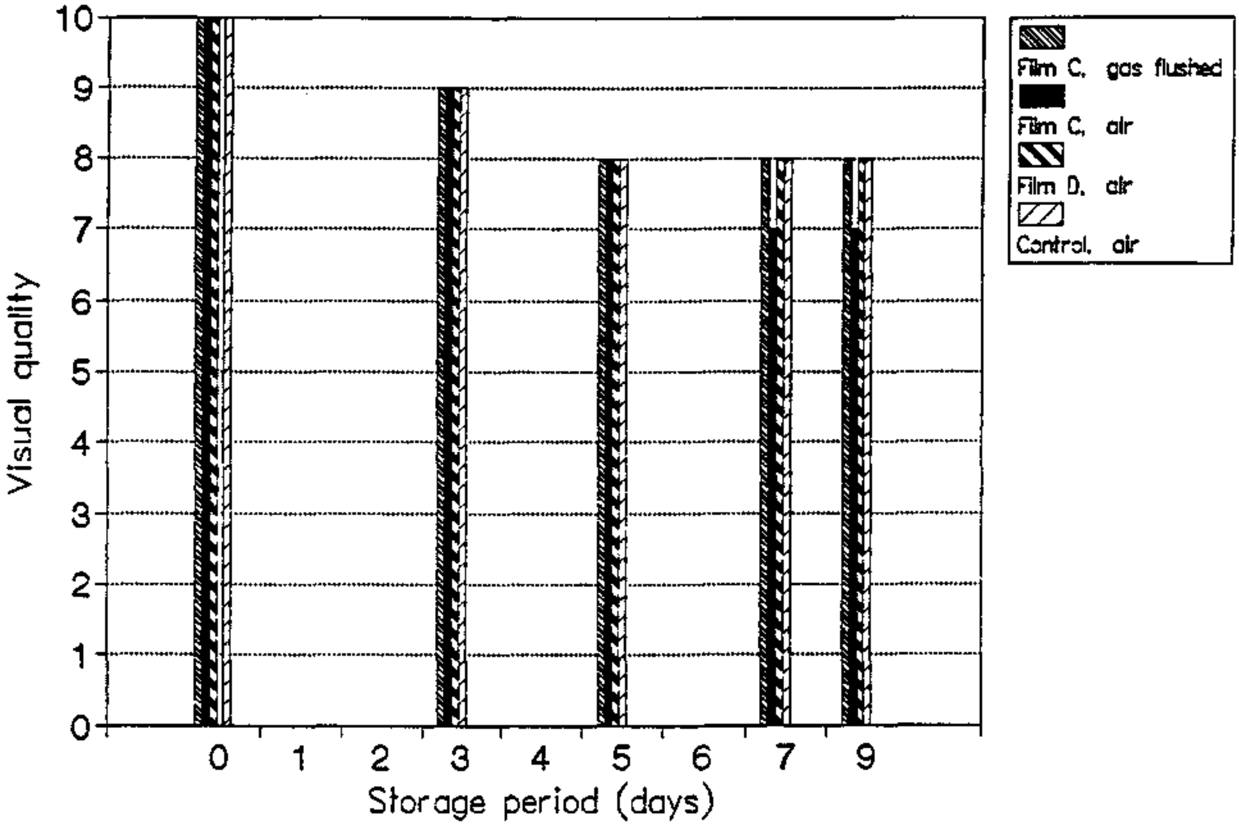

Fig. 9. Visual quality of broccoli florets stored at 1°C

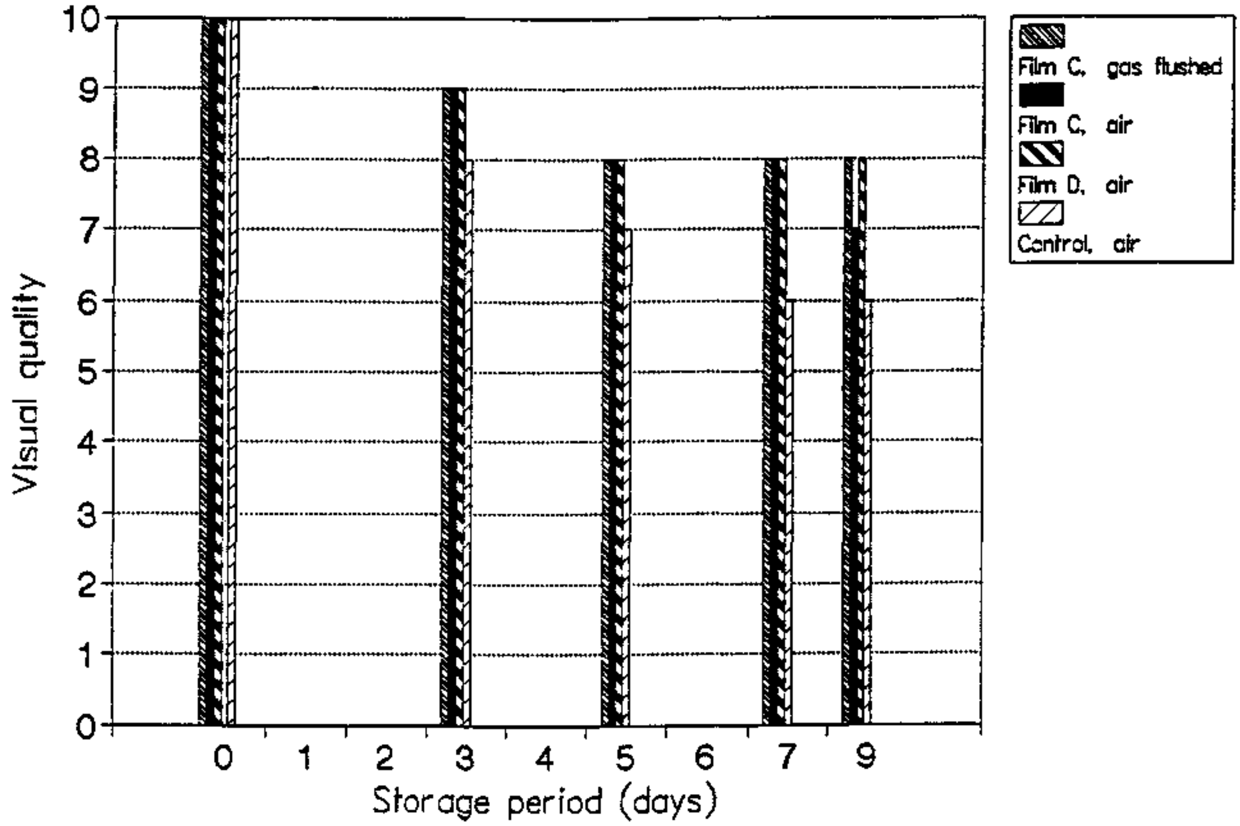

Fig. 10. **Visual quality of broccoli florets at 5°C.**

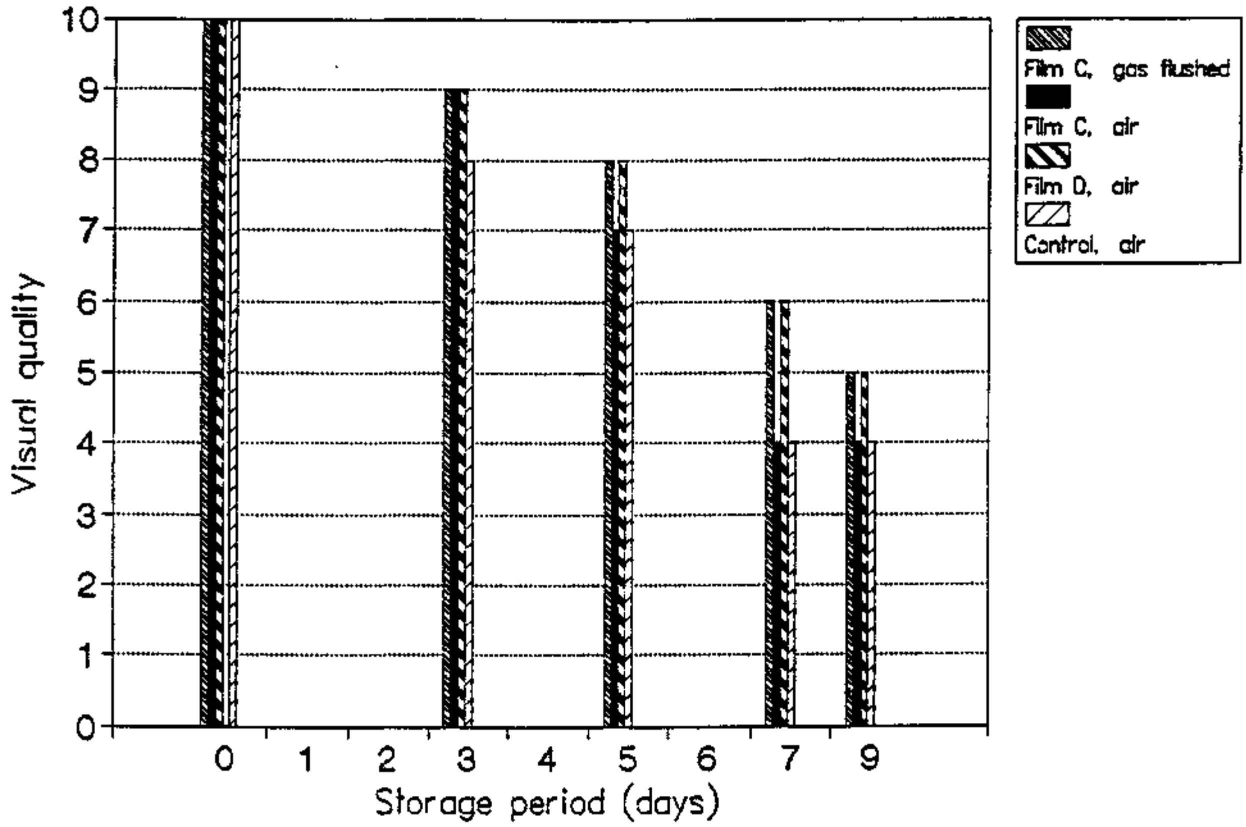

Fig. 11. **Visual quality of broccoli florets stored under simulated handling (3 days at 4°C, 1 day at 7°C, 4°C until end of shelf life).**

The results of gas analysis show that Film C developed dangerous levels of O_2 (<1%) at all storage temperatures (Table 13). These low levels of O_2 were achieved within 4 days indicating that natural production of modified atmosphere could be sufficient for broccoli florets since they are not too sensitive to high levels of O_2 for short duration.

The O_2 levels achieved in Film D (30μm OPP with 2 microperforations on both sides of the bag) were relatively high (12-16%). The levels of CO_2 which is about 5% is near acceptable level. Makhloup *et al.* (1989) recommends gas levels of 2-5% O_2/8-9% CO_2 for broccoli florets. To achieve these gas levels, Emond *et al.* (1991) suggested incorporating silicone membrane in the package, or reducing the size or number of perforations. The later approach was selected for subsequent storage trials because it is more practical.

The total aerobic counts given in Table 14 indicate that most of the samples can be considered satisfactory since their total count did not exceed the 5×10^7 (cfu/g) limit used in this study. Two samples stored under simulated temperature conditions and the control samples at 5°C exceeded the above limit.

TABLE 14 MICROBIAL COUNTS OF BROCCOLI FLORETS AFTER 7 DAYS OF STORAGE

TEST NO.	FILM	TEMP (°C)	% O_2	% CO_2	SPC * (CFU/g)
1	C	1	3	5	6.3×10^6
2	C	1	Air	-	2.9×10^7
3	D	1	Air	-	4.5×10^7
4	C	5	3	5	1.9×10^7
5	C	5	Air	-	4.5×10^7
6	D	5	Air	-	1.6×10^7
7	Control	5	Air	-	1.7×10^8
8	C	Simulated+	3	5	4.5×10^7
9	C	Simulated+	Air	-	1.2×10^8
10	D	Simulated+	Air	-	1.8×10^8

+Simulated handling: 3 days 4°C, 1 day 7°C, 4°C until end of shelf-life.

+ SPC - standard total aerobic count

Cauliflower florets

The results of gas analysis and sensory evaluation are given in Table 15 and Figs. 12-14, respectively. The storage test was terminated on the 7th day.

Figs. 12 to 14 show that all the samples in this study were acceptable up to day 7. Although acceptable, control samples in perforated bags were generally wilted and more rubbery in texture than samples packaged with Films C and D.

The results of gas analysis indicate that the use of pillow-pack made from Film C (with or without gas flushing) is not desirable for cauliflower florets since dangerous levels of O_2 (< 1%) were developed in those packs within 5 days of storage (Table 14). Likewise, the results of gas analysis from Film C confirm similar observation in packages of broccoli florets that natural respiration could be a practical method of developing beneficial atmospheres for these products.

The levels of CO_2 developed in packages made from Film D were found to be within acceptable levels (4-9%). However, the O_2 levels which ranged from 14-16% were relatively high. In order to evaluate whether reducing the number of perforation in Film D could reduce the equilibrium O_2 level, a preliminary trial was conducted (section 3.3.7). Film D was chosen for subsequent trials because of its clarity and mechanical abuse resistance.

The populations of aerobic microorganisms in cauliflower florets after 7 days of storage under various conditions are given in Table 16. The aerobic counts in all samples tested can be considered acceptable because they did not exceed the 5×10^7 cfu/g limit used in this study.

TABLE 15. MODIFIED ATMOSPHERE PRODUCED BY CAULIFLOWER FLORETS STORED UNDER VARIOUS CONDITIONS

TEST NO.	FILM	TEMP (°C)	INITIAL ATMOSPHERE		DAY 5		DAY 7	
			% O_2	% CO_2	% O_2	% CO_2	% O_2	% CO_2
1	C	1	3	5	1.1	3.5	1.9	3.2
2	C	1	Air	-	0.7	3.9	0.9	3.1
3	D	1	Air	-	16.6	4.6	15.9	4.7
4	Control	1	Air		-	-	-	-
5	C	5	3	5	0.8	4.4	1.8	4.0
6	C	5	Air	-	1.6	3.6	1.6	3.6
7	D	5	Air	-	14.4	7.5	13.9	6.3
8	Control	5	Air	-	-	-	-	-
9	C	Simulated+	3	5	1.1	3.9	1.2	3.3
10	C	Simulated+	Air	-	1.4	3.2	2.0	3.2
11	D	Simulated+	Air	-	15.0	8.8	15.0	5.8
12	Control	Simulated+	Air	-	-	-	-	-

+Simulated handling: 3 days 4°C, 1 day 7°C, 4°C until end of shelf-life.

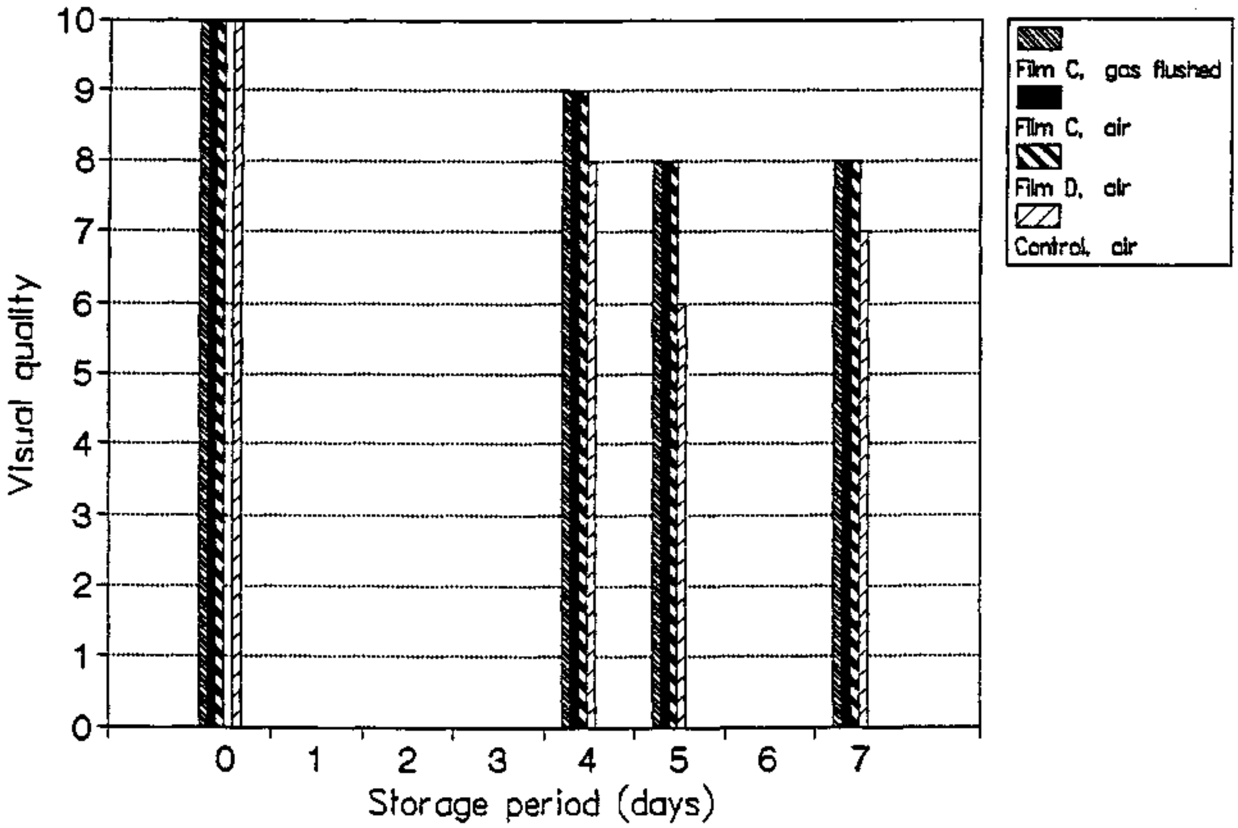

Fig. 12. Visual quality of cauliflower florets stored at 1°C.

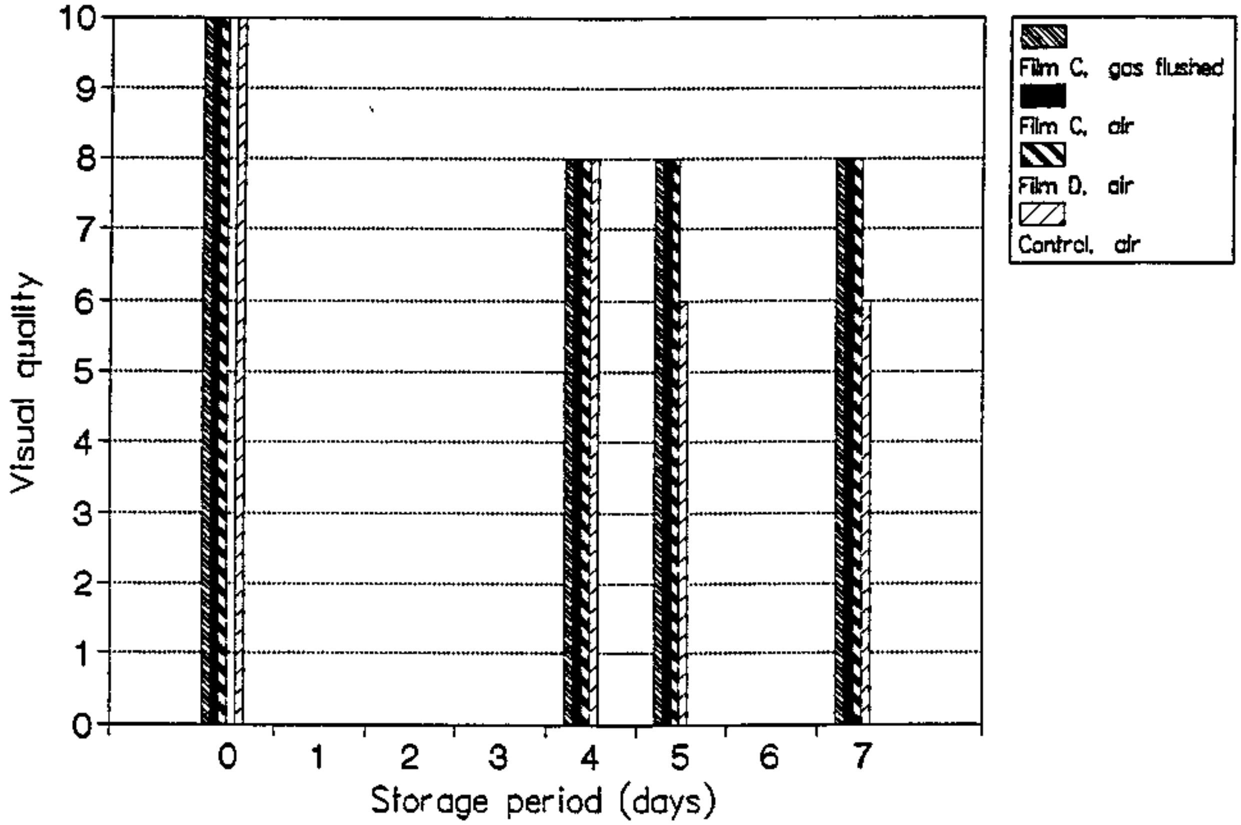

Fig. 13. Visual quality of cauliflower florets stored at 5°C.

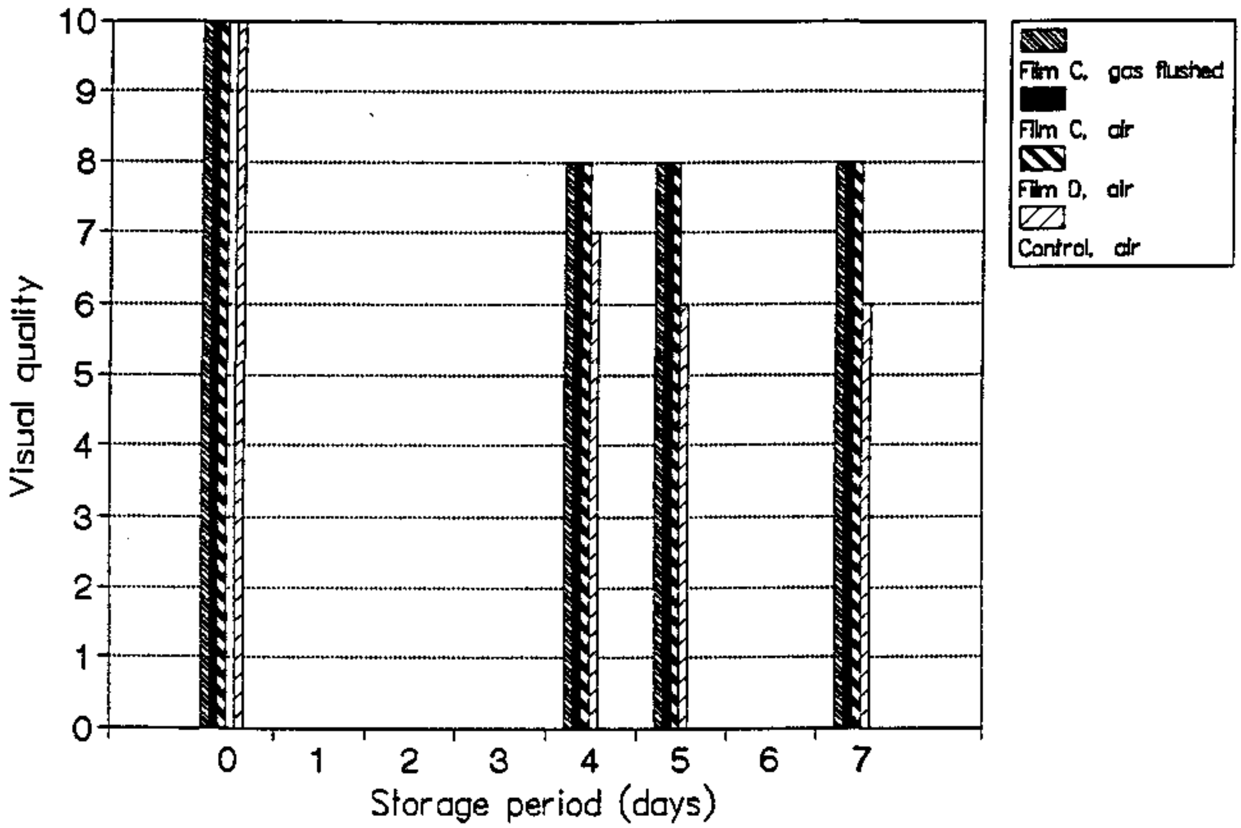

Fig. 14. **Visual quality of cauliflower florets stored under simulated handling (3 days at 4°C, 1 day at 7°C, 4°C until end of shelf life).**

TABLE 16. MICROBIAL COUNTS OF CAULIFLOWER FLORETS AFTER 7 DAYS OF STORAGE UNDER VARIOUS CONDITIONS

TEST NO.	FILM	TEMP (°C)	INITIAL ATMOSPHERE		SPC * (CFU/g)
			% O_2	% CO_2	
1	C	1	3	5	3.0×10^5
2	C	1	Air	-	2.0×10^5
3	D	1	Air	-	5.0×10^5
4	C	5	3	5	3.7×10^6
5	C	5	Air	-	3.2×10^6
6	D	5	Air	-	2.5×10^6
7	Control	5	Air	-	5.0×10^6
8	C	Simulated+	3	5	6.9×10^6
9	C	Simulated+	Air	-	3.2×10^6
10	D	Simulated+	Air	-	1.6×10^6

* SPC - standard total aerobic count
+Simulated handling: 3 days 4°C, 1 day 7°C, 4°C until end of shelf-life.

3.3.7 In-Storage Shelf-life of Broccoli Florets with Modified Film

In order to assess the performance of Film D with only one (1) microperforation on each side of the bag, three (3) 350 g broccoli florets were packaged in air and stored at 5°C. Visual appearance of the samples were assessed every other day and gas analyses was made on the 7th day when equilibrium atmospheres would be expected to occur.

The results of gas analysis indicate that the modified package developed an atmosphere of 10.5% O_2 (± 0.5) and 11.1% CO_2(±0.3). This was a significant improvement since the atmosphere developed in previous package made from Film D was 14% O_2/6% CO_2. The development of 10% O_2/11% CO_2 inhibited formation of brown discolouration, thereby giving the florets an acceptable quality up to 21 days at 5°C. No significant development of water condensation was found in this trial, probably due to the film's high water vapour transmission rate. The result of this storage trial with the modified Film D justifies its use for subsequent trials for broccoli florets and cauliflower florets. Since both florets have similar respiration rates, it is expected that similar results would be achieved for cauliflower florets.

3.3.8 Optimum Gas Concentration for Broccoli and Cauliflower Florets

Tables 17 and 18 give a summary of results for controlled atmosphere storage of broccoli and cauliflower florets at 1-2°C.

Results of visual observation show that all the broccoli and cauliflower florets stored in controlled atmospheres (3% O_2/5% CO_2, 3% O_2/10% CO_2, 3% O_2/15% CO_2,) were acceptable up to the 15th day when all the chambers were opened. No unusual odour was found even in florets exposed to 15% CO_2. This high level of tolerance to CO_2 is significantly higher than those reported for heads of cauliflower and broccoli which ranged from only 4-10% (Romo-Parada, 1989; Makhlouf *et al.* 1989). The tolerance of both florets to high CO_2 levels was found to be beneficial because all samples stored at 10 and 15% CO_2 did not develop any discolouration along the cut sections normally found in samples stored in air. Both broccoli and cauliflower florets were marginally acceptable on the 15th day due to severe discolouration along the perimeter section of the stem and the development of rubbery texture.

In summary, broccoli florets, cauliflower florets, shredded lettuce, and mixed lettuce were able to tolerate CO_2 levels up to 15% and O_2 levels of 2-3%, thus maintaining their "fresh" quality up to 15 days.

TABLE 17 VISUAL QUALITY OF BROCCOLI FLORETS UNDER CONTROLLED ATMOSPHERE STORAGE AT 1-2°C

ATMOSPHERE	STORAGE DAYS				DEFECTS
	0	5	8	15	
Air	10	9	7	5	Browning, rubbery texture
2.4% O_2/4.5% CO_2	10	9	8-9	7-8	Slightly soft
2.2% O_2/9.6% CO_2	10	9	8-9	8	None
2.0% O_2/15% CO_2	10	9	8-9	8	None

TABLE 18 VISUAL QUALITY OF CAULIFLOWER FLORETS UNDER CONTROLLED ATMOSPHERE STORAGE AT 1-2°C

ATMOSPHERE	STORAGE DAYS				DEFECTS
	0	5	8	15	
Air	10	8-9	5	4-5	Severe browning, rubbery texture
2.4% O_2/4.5% CO_2	10	10	9-10	5-6	Browning
2.2% O_2/9.6% CO_2	10	10	9-10	8-9	Slightly dry
2.0% O_2/15% CO_2	10	10	9-10	9-10	None

3.3.9 In-Storage Shelf-life of Broccoli and Cauliflower Florets

In-storage shelf-life

The results of gas analyses on broccoli florets and cauliflower florets are depicted in Figs. 15 and 16. The values of O_2/CO_2 concentrations are average from four packages. The corresponding visual assessment data are given in Table 19. The storage temperature profile used in this study was: 3 days at 4°C, 1 day at 7°C, and 4°C until the end of shelf-life.

The results of gas analyses and visual assessment show that the natural development of modified atmosphere by broccoli florets packaged in Film D (modified) was effective in extending the shelf-life from 11 days (control samples) to 20 days. Therefore, the shelf-life was effectively doubled by the correct use of modified atmosphere (Table 19). This result validated the 3 week shelf-life achieved in the preliminary storage trial (section 3.3.7) An equilibrium atmosphere of about 9% O_2/12% CO_2 was developed in this package with only one microperforation on each side of the bag (Fig. 15). The main factor that limited shelf-life was discolouration on the cut section of the florets, followed by the development of rubbery texture. No slime formation or signs of decay was found in all samples tested.

TABLE 19 VISUAL QUALITY OF BROCCOLI AND CAULIFLOWER FLORETS STORED UNDER SIMULATED HANDLING CONDITIONS+

BROCCOLI FLORETS			CAULIFLOWER FLORETS	
DAYS	CONTROL	MODIFIED PACKAGE	CONTROL	MODIFIED PACKAGE
0	10	10	10	10
5	8	9-10	8	9-10
7	7-8	8-9	7	8-9
8	-	-	5-6*	8-9
9	6-7	8-9	-	8-9
11	5-6*	7-9	-	7-8
12	-	-	-	6-7
13	-	7-9	-	5-6*
19	-	6-8	-	-
20	-	5-6*	-	-

+ 3 days 4°C, 1 day 7°C, and back at 4°C.
* Shelf-life, visual score ≤ 5.

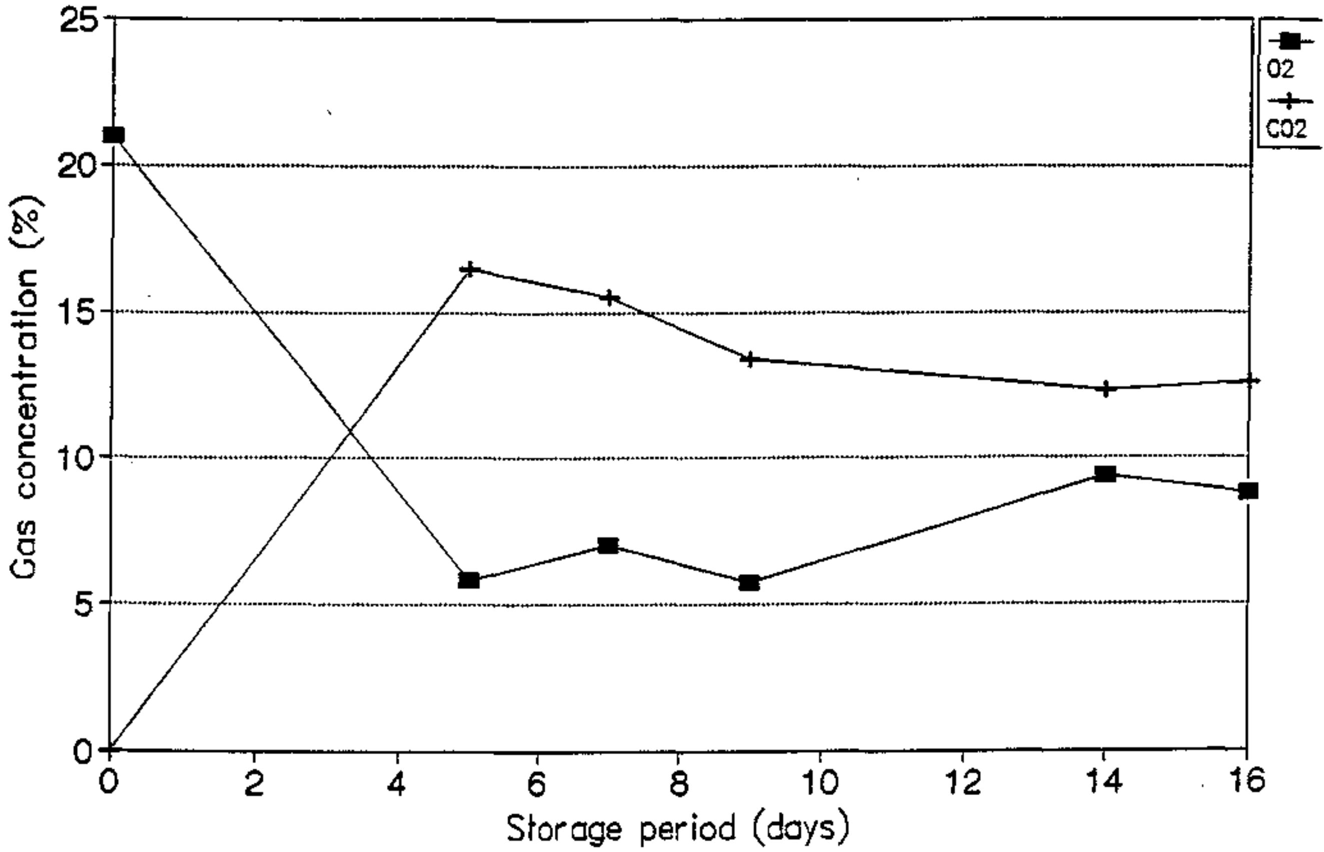

Fig. 15. Modified atmosphere of broccoli florets in pillow-pack stored under simulated handling (3 days at 4°C, 1 day at 7°C, 4°C until the end of shelf life).

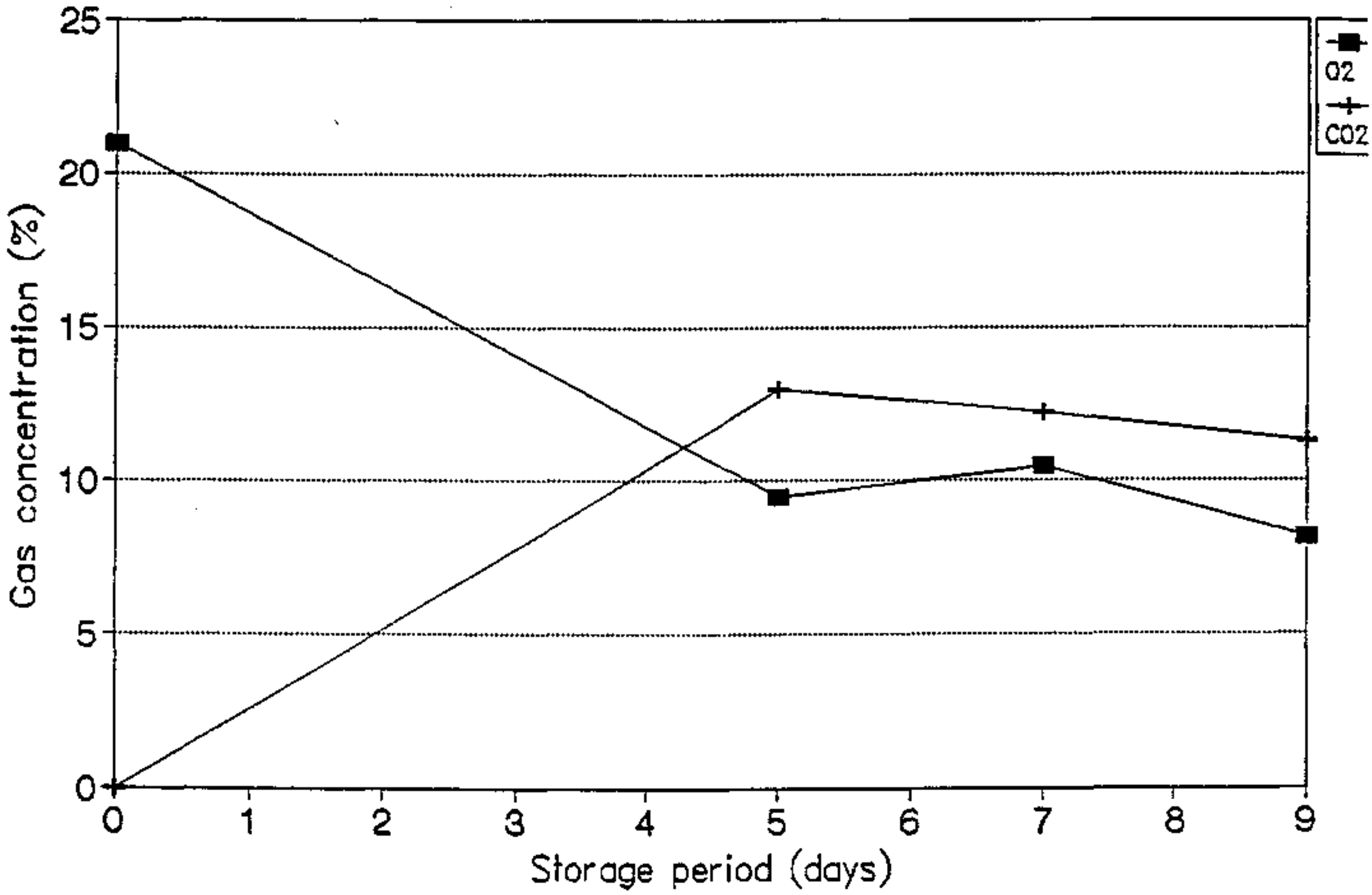

Fig. 16. **Modified atmosphere of cauliflower florets in pillow-pack stored under simulated handling (3 days at 4°C, 1 day at 7°C, 4°C until the end of shelf life).**

Similar levels of O_2 and CO_2 were observed in the headspace of cauliflower packages (Fig. 16). The gas concentrations of O_2 and CO_2 after 5 days ranged from 8-10% and 11-12%, respectively. This modified atmosphere enabled the cauliflower to have an acceptable in-storage shelf-life of 13 days under the storage temperature used in this study - 3 days at 4°C, 1 day at 7°C, and 4°C until the end of shelf-life. The in-storage shelf-life of cauliflower florets was shorter compared to that of broccoli because of pronounced discolouration in the cut stem section of cauliflower florets.

<u>After-opening shelf-life</u>

As would be expected, the after-opening shelf-life of samples stored for longer duration was relatively shorter than samples stored for a few days (Table 20). The after-opening shelf-life of sample opened at day 5 was 6 days, while those opened at day 7 and 9 was about 5 days. A 5 day shelf-life should enable the consumer to use the all the 350 g florets contained in a pillow-pack.

Cauliflower florets in the developed pillow-pack had a similar shelf-life after-opening although the corresponding visual quality is less compared with that of broccoli florets (Table 20). At longer storage period (9 days or more), the shelf-life of the cauliflower florets was about one day less than that of broccoli florets.

The microbial population before and after opening was not a limiting factor in terminating the shelf-life of broccoli florets and cauliflower florets because the maximum limit of 5×10^7 (cfu/g) was not exceeded by all samples used in this study (Tables 21 and 22). Likewise, all the samples in this study were found to be negative for *Listeria monocytogenes, Yersinia spp.*, and *Aeromonas spp.*

TABLE 20 VISUAL QUALITY OF BROCCOLI AND CAULIFLOWER FLORETS OPENED AT SPECIFIED PERIODS

STORAGE[+] DAYS	DAYS AFTER OPENING	BROCCOLI FLORETS	CAULIFLOWER FLORETS
5	0	9-10	9-10
5	1	9-10	9-10
5	2	9-10	9-10
5	4	6-8	5-7
5	6	5-6*	5*
7	0	8-9	8-9
7	1	7-8	7-8
7	2	7-8	7
7	5	5-6*	5*
9	0	8-9	8-9
9	3	7-8	6-7
9	4	6-8	5-6*
9	5	5-6*	-
11	0	7-9	7-8
11	1	-	7
11	2	7-8	5-6*
11	3	5-6*	-
13	0	7-9	5-6*
13	1	6-7	-
13	2	5-6*	-
19	1	6-8	-
19	1	5-6*	-

[+] 3 days 4°C, 1 day 7°C, and back at 4°C.
* Shelf-life, visual score ≤ 5.

TABLE 21 MICROBIAL COUNTS OF BROCCOLI FLORETS STORED UNDER SIMULATED HANDLING CONDITION[+]

STORAGE DAYS	DAYS AFTER OPENING	STANDARD PLATE COUNT (cfu/g)	YEASTS (cfu/g)	MOULDS (cfu/g)
I. MODIFIED PACKAGE				
0	0	3.7×10^4	180	430
5	0	7.5×10^4	6400	800
5	1	9.8×10^4	3600	1100
5	2	3.0×10^4	5300	700
7	0	4.9×10^4	6900	1100
7	1	1.3×10^4	6100	800
7	2	5.3×10^4	23000	1500
9	0	2.1×10^4	35000	3000
9	1	3.6×10^4	5300	1300
9	2	3.6×10^4	3600	900
11	0	2.9×10^5	440	240
11	1	8.0×10^4	7900	1200
11	2	1.6×10^5	5800	700
II AIR PACKED SAMPLE				
7	0	3.0×10^5	8400	400

[+] 3 days 4°C, 1 day 7°C, and back at 4°C.
[*] Shelf-life, visual score ≤ 5.

TABLE 22 MICROBIAL COUNTS OF BROCCOLI FLORETS STORED UNDER SIMULATED HANDLING CONDITION[+]

I. MODIFIED PACKAGE				
STORAGE DAYS	**DAYS AFTER OPENING**	**STANDARD PLATE COUNT (cfu/g)**	**YEASTS (cfu/g)**	**MOULDS (cfu/g)**
0	0	1.6×10^5	9900	10
5	0	4.2×10^6	2900	50
5	1	7.9×10^6	390	50
5	2	2.7×10^5	1090	20
7	0	3.1×10^5	490	< 10
7	1	2.5×10^5	2500	200
7	2	5.8×10^5	1090	< 10
9	0	4.7×10^5	580	60
9	1	2.5×10^5	1590	50
9	2	5.0×10^6	16400	40
11	0	1.6×10^6	530	40
11	1	1.1×10^6	7600	700
11	2	1.7×10^5	1500	< 100
II AIR PACKED SAMPLE				
7	0	6.2×10^5	9300	< 100

[+] 3 days 4°C, 1 day 7°C, back to 4°C.

4. DISSEMINATION OF FINDINGS

A copy of the final report will be sent to All States P/L (NSW). Extracts of this report will be sent to companies which donated packaging materials for research purposes.

It is anticipated that several extension articles and at least one research article should result from this report. Copies of these articles will be forwarded to HRDC as they are accepted.

The following articles have been published in relation to this study:

Reyes, V. and Gould, I. 1992. New packaging lifts shelf-life. *Good Fruit & Vegetables* 2(9):23.

Reyes, V. and Gould, I. 1992. Modified atmosphere packaging of fresh produce and prepared foods. Paper presented at the Conference on Engineering in Agriculture, Oct. 1992, Albury, NSW.

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APPENDIX A: Details of Packaging Films

Film Code	Description	Supplier/Manufacturer
A	32 μm polyolefin (PE/PP)	-withheld-
B	55 μm LLDPE/LDPE RPP500	Renown & Pearlite Caringbah, NSW
C	19 μm polyolefin (PE/PP)	-withheld-
D	30 μm OPP microperforated	ICI Films (Vic)
Control	15 μm perforated PY30	Cryovac Division W.R. Grace Aust. Ltd.

Fig. A.1 Air and product temperature profiles at 4 °C

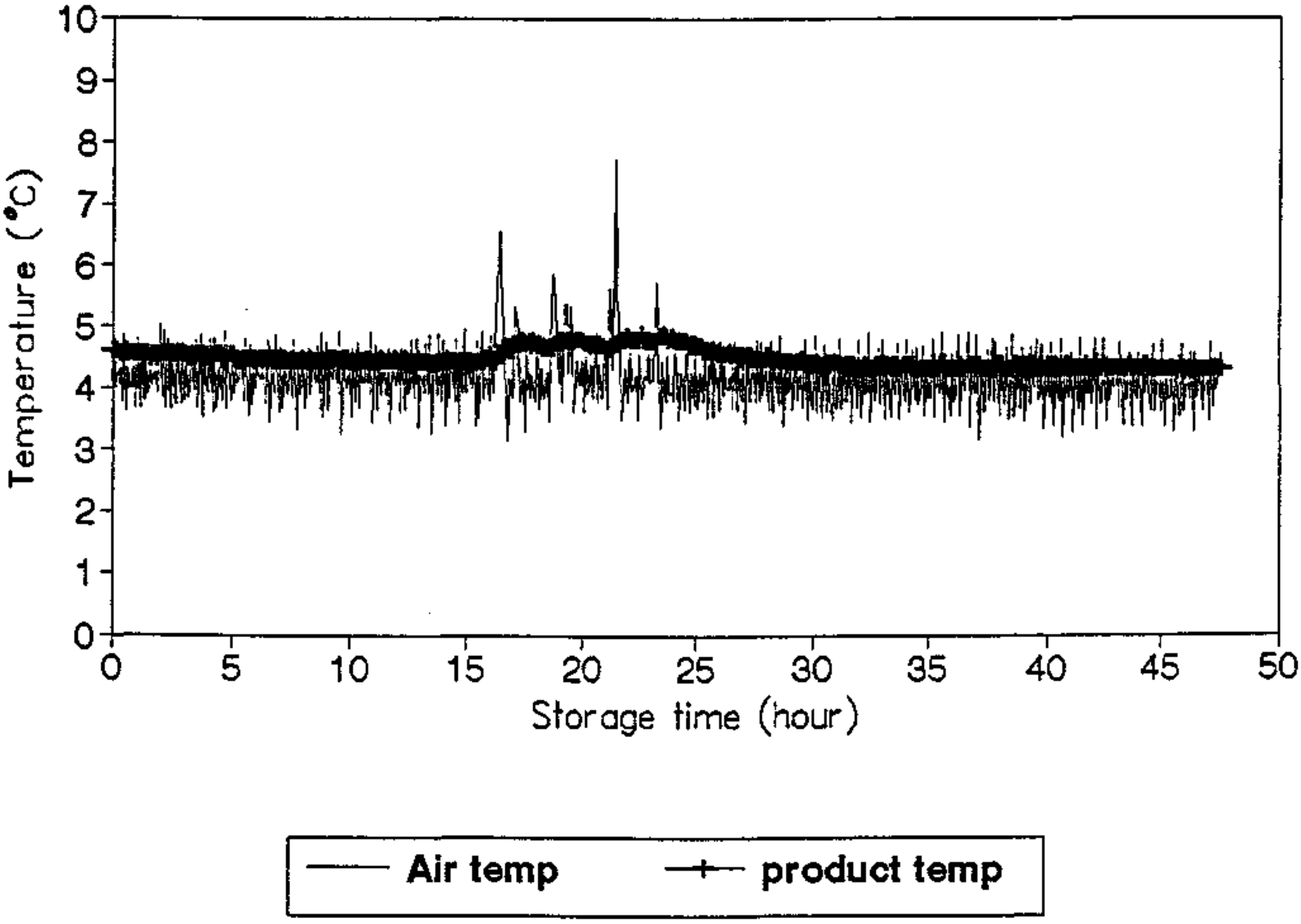
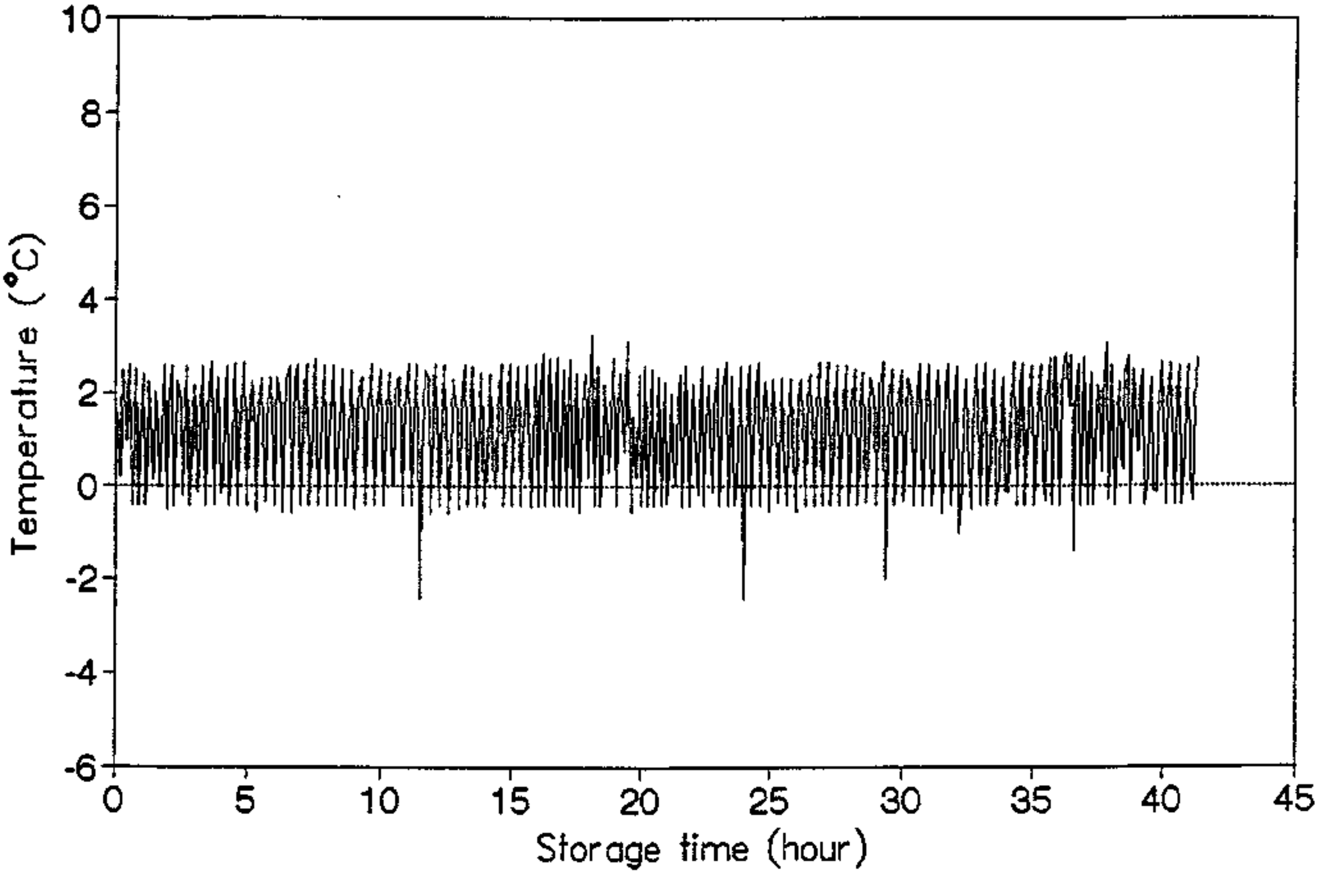


Fig. A.2 Air temperature profile at 1 °C cool room



APPENDIX B: Packaging Film Abbreviations

EVA	Ethyl-vinyl acetate
LLDPE	Linear low density polyethylene
LDPE	Low density polyethylene
OPP	Oriented polypropylene
PE	Polyethylene
PVC	Polyvinyl chloride

APPENDIX C

LITERATURE SEARCH REPORT

PACKAGING OF PREPARED VEGETABLES

DEPARTMENT OF AGRICULTURE

FOOD RESEARCH INSTITUTE, WERRIBEE

LITERATURE SEARCH REPORT

PACKAGING OF PREPARED VEGETABLES

Prepared for: All States Food Services Pty Ltd
Prepared by: Dr V Reyes

October 1992

TABLE OF CONTENTS

LITERATURE SEARCH REPORT
ON
FRESH-CUT VEGETABLES

1.0 INTRODUCTION

Fresh-cut vegetables or prepared vegetables are also termed minimally processed vegetables. By definition, minimal processing encompasses any procedure short of the traditional complete preservation procedures (heat sterilisation, freezing, etc.) that adds value (King and Bolin, 1989). General reviews on this subject have been given by Huxsoll and Bolin (1989), King and Bolin (1989), Ronk et al. (1989), Shewfelt (1987), and Barmore (1987).

There are two types of minimally processed vegetables:

(A) those which undergo no heat treatment, and

(B) those which are heat-treated.

The vegetables in type A are in the raw state and are thus capable of undergoing all storage changes associated with respiring fresh commodities. These changes include vitamin losses and physiological changes associated with reduced product quality. In type B products, the heat treatment is usually sufficient to inactivate respiration associated enzymes and some factors which determine shelf-life.

In both types of minimally processed vegetables, extension of shelf-life (in term of quality and safety) can be achieved by a combination of the following treatments or "hurdles" to spoilage.

- Low temperature storage
- pH modification
- Chemical dips and preservatives
- Mild heat treatment (type B only)
- Modified atmosphere (MA) packaging

Treatments chosen should not cause the product to lose its essential fresh-like appearance, and that combination treatments are mutually compatible. Additive or synergistic effects between treatments are often encountered and can be exploited.

Generally, treatments or processes are aimed to prevent or slow down the two distinct spoilage processes: (i) the digestion of food by enzymes present within the tissues which are released when the cell membranes loose their integrity, and (ii) the invasion by bacteria and fungi.

2.0 LOW-TEMPERATURE STORAGE

The single most important factor controlling shelf-life is the storage temperature. According to the Van't Hoff rule, Q_{10} values for vegetables (i.e. the increase in rate of deteriorative reaction for each 10°C rise in temperature) are in the order of 2 to 3 in the 0 to 25°C temperature range. Thus, many vegetables which will remain at good quality for two weeks at 1°C will only last 3-4 days at normal ambient temperatures (20-30°C).

Application of other processes or treatments to extend refrigerated shelf-life will be irrelevant if temperature control is not practised. Reduction of temperature restricts a range of microbial growths, however, minimally processed vegetables will still be subject to cold-tolerant microorganisms (psychrotrophs) unless further steps are taken to eliminate or minimise these organisms (Brackett, 1987).

It should be noted that certain fruit and vegetables are susceptible to chilling injury by exposure to temperature below 5-10°C. However, some studies have shown that chilling injury manifests only on whole and unprepared produce. For example, research at Campden Food and Drink Research Association (U.K.) has shown that pineapple, pawpaw, and melon segments do not experience chilling injury at 2°C - a temperature at which whole fruit show damage. Chilling injury was probably prevented because the surface skin was removed during preparation and before modified atmosphere packaging (Day, 1992a).

3.0 pH MODIFICATION

Lowering the pH can significantly affect the shelf-life of chilled fruit and vegetables. Most enzymes function effectively at pH 6 and 8. It is exceptional for microorganisms to function less than 4. For example, *Clostridium botulinum* is sensitive to acid conditions and cannot normally grow at pH less than 4.5. However, lowering the pH of foods has limitations in that organoleptic changes produced may be unacceptable for some products. Public tastes now demand less acidic products.

Most vegetables such as lettuce, carrots, potatoes, mushrooms, broccoli, and beansprout have pH values above 4.5 (Holdsworth, 1983), and consequently *Clostridium botulinum* is able to grow when such commodities are stored under anaerobic conditions. The development of these hazardous conditions can be avoided by storing minimally processed products below 3°C (Day, 1992b).

4.0 CHEMICAL DIPS AND PRESERVATIVES

Some cooked or raw prepared vegetables could have significant shelf-life extension by the use of surface dip systems. Chemical dips can have a preservative action, can maintain some sensory attributes (such as firmness), or can prevent surface browning.

Traditionally used preservative dips include organic or inorganic acids, chlorine and sulphur dioxide.

Sulphur dioxide functions both as a preservative and as an anti-browning agent. The recent controversy surrounding allergic reactions and consumer resistance associated with sulphur dioxide on salad vegetables has prompted the investigation of alternatives.

Langdon (1987) reported a chemical treatment of potassium sorbate, ascorbic acid and citric acid combined with vacuum packaging system that provided a 14+ days shelf-life for fresh prepared peeled potatoes.

Rice (1983) reported that a mixture of ascorbic and citric acids gave lettuce a shelf-life of 5 days compared to 7 days for sulphur dioxide.

Goussalt (1985) described a successful method of extending the shelf-life of vegetables by dipping them in weak vinegar, partially drying, spraying with ascorbic acid, chilling and packing in a film permeable to water vapour.

In order to improve the texture of minimally processed fruit and vegetables, calcium chloride dips have been widely used to improve product firmness (Drake and Lambert, 1987).

Benzyladenine dips have also been employed to improve the colour, texture, aroma and flavour of raw and cooked broccoli stored at 2°C and 5°C (Batal et al. 1982).

5.0 HEAT TREATMENT

There are two main objectives of heat treatment: (i) to inactivate enzymes, and (ii) to decrease the initial microbial load. In some cases heat treatment also improves the colour and flavour.

For products when the crunchy texture of the vegetables is a desirable feature, such as in salads, the heat treatment should aim at inactivating the enzymes which cause deterioration, without disturbing the vegetable cell walls excessively.

Some work has been reported on blanching carrot slices followed by acidification, to produce a product for use in salads (Juliot et al, 1989).

Kratky and Vadehra (1989) have demonstrated that at temperatures at or just below 60°C, a processing time of about 3 minutes can decrease the initial vegetative microbial load significantly without affecting the texture and colour of green vegetables detrimentally. The product generally remains green through a 3-4 week shelf-life and the texture firm and crisp. These mild temperatures do not damage the vegetable membranes.

Mildly heat-treated vegetables can still contain heat-resistant vegetative cells and surviving spores which may grow during chilled storage. Therefore, other combination treatments or hurdles may be needed to ensure the required safety and shelf-life.

6.0 RAW MATERIALS, PROCESSING AND STORAGE

Minimal processing must be followed by some mild treatment to minimise deterioration and physiological effects caused by rupturing cells. The treatment can be as simple as a water dip to remove cellular fluids, or multiple dips combined with other treatments (Bolin and Huxsoll, 1991).

Adams et al. (1989) reported that washing chopped lettuce leaves in tap water could reduce the microbial count by an average of 92%. Furthermore, adjusting the pH of sodium hypochlorite solutions (chlorine values 4.5 - 5.0) resulted in a 1.5 - 4.0 fold increase in microbial efficacy. A washing time of 5 minutes was reported to be generally sufficient.

In order to attain satisfactory shelf-life, the following are suggested by various researchers (Ohta and Sugawara, 1987; Rosenfeld, 1985; Baardseth, 1980; Bolin et al, 1977):

(i) high quality raw materials,
(ii) use of sharp blade (preferably slicing action),
(iii) washing in cold and flowing water (5°C),
(iv) water removed in a basket-type centrifuge (1,000 rpm minimum for 30 sec),
(v) temperature less than 5°C during whole processing cycles,
(vi) packaging in containers which can sustain aerobic respiration, and
(vii) storage and distribution at 1-5°C.

Hygiene levels in the manufacture of chilled foods (e.g. machine hygiene, factory fabric hygiene, sanitary practices by operators and factory air quality) generally have to be superior to those applicable to other technologies.

7.0 MODIFIED ATMOSPHERE PACKAGING

The beneficial and detrimental effects from MA packaging have been extensively reviewed (Zagory, 1990; Day, 1988; Brecht, 1980; Dewey, 1983; Kader et al. 1989; Priepke et al. 1976). Enriched CO_2 and low levels of O_2 can reduce respiration, delay ripening, decrease ethylene production and sensitivity, slow down compositional changes associated with ripening, reduce enzymatic browning, preserve vitamins, and alleviate physiological disorders, thereby extending the product shelf-life. However, exposure of the produce to O_2 and CO_2 levels (generally, $<1\%$ O_2 and $>20\%$ CO_2) beyond their limits of tolerance can initiate anaerobic respiration with the production of undesirable flavours and odours, as well as cause other physiological disorders.

In MA packaging of raw and prepared fruit and vegetables, the optimum CO_2 level is normally below 20% (Table 1). MA and low temperature storage in combination limits microbial enzyme activity, tissue metabolism and respiration, thereby prolonging the time before senescence after which the bacterial population could cause tissue damage (Brocklehurst et al. 1987, Barriga et al. 1991).

Although most researchers have reported little effect of high CO_2 ($\leq$20%) on the general profile and growth of microorganisms, significant reduction in spoilage have been reported in MA packaged prepared vegetables (Chamboy and Nguyen, 1988; Beuchat and Brackett, 1990; Picoche and Denis; 1988; Buick and Damoglou, 1989). These reported positive effects on shelf-life extensions could be due to a direct action on microbial enzyme activity, rather than to reduced microbial growth (Nguyen and Carlin, 1988).

Increased CO_2 and low levels of O_2 could also reduce browning or other discolouration in lettuce, brussels sprouts and other cut and raw vegetables (Singh et al, 1972; Stringer, 1990). CO_2 is known to prevent browning of damaged plant tissue by blocking production of phenolic compounds, as well as inhibiting polyphenol oxidase activity (Siriphanich and Kader, 1984).

Carbon monoxide was also reported to be an effective inhibitor of polyphenyl oxidase enzyme in shredded lettuce (Bolin and Huxsoll, 1991). MA packaging of salad-cut lettuce with 5% CO and 5% CO_2 in polyethylene film (50μm thickness) was able to produce good flavour, bright colour and firm texture product up to 2-4 weeks at 2°C.

7.1 Recommended Atmosphere

The "optimum" levels to CO_2 and O_2 of various fruit and vegetables are subject to several variables including type of produce, storage temperature, cultivar, maturity, and physiological condition (Zagory and Kader, 1988). An optimal MA should minimise respiration rate without danger of physiological damage to the produce. Since the effects of low O_2 and high CO_2 levels on respiration are additive, the optimal concentrations of both gases in combinations are difficult to predict without actual measurements in a variety of atmospheres (Zagory and Kader, 1988; Zagory, 1990). For most fruit and vegetables, atmospheres containing 2-5% O_2 and 3-8% CO_2 have been shown to be beneficial in extending their shelf-life.

A summary of recommended atmosphere for various prepared and raw vegetables is given in Table 1.

In this report, "active" MA packaging refers to the once only establishment of a specified gas composition within the package during the packaging operation. In this MA system, gas composition would still change with time depending on the product respiration rate, film type, package design and storage conditions, especially storage temperature.

"Passive" MA refers to the relatively slow natural production of CO_2 and consumption of 0_2 through respiration. In order to achieve and maintain a satisfactory atmosphere within a package, the gas permeability of the selected film must be such that they allow 0_2 to enter the package at a rate offset by the consumption of 0_2 by the commodity. Similarly, CO_2 must be vented from the package to offset the production of CO_2 by the commodity.

8.0 MICROORGANISMS IN RAW AND PREPARED VEGETABLES

There are isolated reports of bacteria of public health significance in fresh and minimally processed vegetables. Their presence even incidental, must not be overlooked. Some organisms have been isolated from raw vegetables and salads- *Staphylococcusaereus, Salmonella spp.* and *Shigella* spp (Saddik et al, 1985). *Yersinia* spp. and *E. coli* have been isolated from vegetable salad mixes (Brocklehurst et al, 1987). *Listeria monocytogenes* has been detected in minimally processed vegetables (Laine and Michaud, 1988).

In order to ensure that potentially hazardous conditions are not created, a minimum level of 2-3% oxygen is recommended (Bernard, 1987). At these oxygen concentrations, pathogens are unlikely to grow on vegetables because normal spoilage flora will usually have a competitive advantage (Brackett, 1987). Maintenance of these oxygen levels can be accomplished by proper design of packages especially the use of materials of relative high permeability. Vacuum packaging using barrier materials should be avoided especially if the storage temperature can not be maintained below 3°C.

9.0 SELECTED LITERATURE ON FRESH-CUT VEGETABLES

Mixed Lettuce. Chopped lettuce leaves (Iceberg, Lollo Rosso, Chinese, Endive) were prepared by dipping whole lettuces in 100 ppm chlorinated water for few seconds, rinsing in chilled water, chopping, 2 minutes dip in 100 ppm chlorinated water, and dewatering in a low-speed centrifuge. Products of acceptable quality were obtained from those packages that used 15μm OPP/30μm PE, gas flushed with 5% O_2/5% CO_2 and stored in light at 5°C. The packages were "pillow pack" bags 25cm x 21cm containing about 200g mixed lettuce. The equilibrium modified atmosphere established in this package was 2-3% O_2 and 7-8% CO_2 which gave the longest shelf-life of about 11 days. The shelf-life of the control sample in perforated film was 2-4 days. In all samples, it was the enzymatic degradation of Iceberg lettuce that limited the shelf-life (Day, 1989).

Shredded Lettuce. Shredded Iceberg lettuce packaged in high barrier tray with a 35μm LDPE lid and gas-flushed with 5% O_2/5% CO_2 exhibited a 10-13 days shelf-life at 5°C. Equilibrium modified atmosphere developed in the package was about 2-3% O_2/5-6% CO_2. Control samples in perforated packages exhibited extensive browning after 6 days (McLachlan, 1984; McLachlan and Stark, 1985; Ballantyne, 1987, Gill, 1988, Ballantyne et al. 1988a).

Gill (1988) reported good results in 35μm OPP film "pillow-pack" bags with an effective surface area of about 0.18m^2. The bags contained 250g shredded Saladin lettuce gas flushed with 5% O_2/5% CO_2 before sealing. Equilibrium modified atmosphere developed in the package was 1-2% O_2/5-6% CO_2. Maximum shelf-life was reported to be 12-14°C days at 1°C. However, samples packaged samples in air had a shelf-life of about 5-6 days.

A recent publication by Bolin and Huxsoll (1991) reported that a shelf-life of up to 2-4 weeks (depending on the initial quality of the product) could be attained with cut Iceberg lettuce by the following procedure: use of sharp knife or tearing; rinsing to remove cellular fluids; centrifuge to remove excess water; gas flushing the product with 5% CO/5% CO_2 (or 5% CO only); and storage at 1-2°C.

Using a controlled atmosphere (CA) experiment, Barriga et al. (1991) found that the optimum atmosphere for shredded lettuce (Iceberg cv Great Lakes) was about 3% O_2 and 10% CO_2 at 4°C. Shredded lettuce samples stored under these conditions had a shelf-life of at least 12 days.

<u>Broccoli Florets</u>. The most favourable results were obtained using 15μm PVC lidding film in a PVC tray (base), 130g florets, and a 5% O_2/5% CO_2 gas flush. Shelf-life of up to 10 days was reported at 10°C which was about double that of control samples packaged in perforated film (Gill, 1988).

<u>Brussels Sprouts</u>. McLachlan (1984), Ballantyne (1986, 1987), and Gill (1988) investigated a large number of lidding films for extending the shelf-life of brussels sprouts in a barrier tray. The best results were attained using 15μm PVC and a 5% O_2/5% CO_2 gas flush. The equilibrium atmosphere established which enabled brussels sprouts to achieve a maximum shelf-life of 15-20 days at 5°C was 2-3% O_2/2-4% CO_2.

<u>Mixed Vegetables</u>. Ballantyne (1986) investigated a number of permeable lidding films. Mixed vegetables were filled into fibreboard or LDPE trays in the following proportion: 90g carrot, 80g leek, and 65g onion, giving a total fill weight of 235g. The best results were obtained using 19μm LDPE or 15μm PVC lidding film in combination with a 5% O_2/5% CO_2 gas flush. An equilibrium modified atmosphere of 2-4% O_2/2-4% CO_2 was established which extended the shelf-life by 2-3 days over the perforated controls to a maximum shelf-life of 6 days. It was moisture loss with resultant silvering of carrots and drying of leek tissues which terminated the shelf-life. Therefore, it was suggested that other packaging material with the same gas permeability properties to 19μm LDPE and 15μm PVC, but lower water vapour transmission rates, could further extend the shelf-life.

Grated Carrots. Carlin et al.(1990b) reported that grated carrots (1.5 x 1.5 mm strips) could be packaged in a pillow pack of 40μm OPP (or other film with an O_2 transmission rate of at least 950 cc/m^2-day at 25°C) and stored for up to 12 days at 2°C. But above 6°C the use of high-permeability films is recommended.

Mushrooms. Some experimental results have been reported by McLachlan (1984), and Ballantyne (1986, 1987). Mushrooms have a very high respiration rate and no commercially available films (among those evaluated) were found to be permeable enough to maintain a low oxygen but aerobic equilibrium modified atmosphere. Accumulation of excessive amounts of water was another problem cited in MA packaging. Such should be expected because the amount of water produced is directly proportional to respiration rate. Their best results were a small 2-3 day shelf-life extension achieved using an experimental film bag system which consisted of a OPP bag in which a window of highly permeable film was inserted. Such system was able to establish 2-8% O_2 and 4-10% CO_2 at 5°C. Further development of microporous, microperforated and other experimental films is necessary to achieve shelf-life extension of mushrooms.

Table 1. Recommended atmosphere for selected minimally processed vegetables

Commodity	Temperature (°C)	O_2 (%)	CO_2 (%)	Shelf-life (Days)	Comments	Reference
Shredded lettuce (Iceberg cv Saladin)	5	1-3	5-6	14-20 (Control- 5)	35μm LDPE lid in barrier tray; gas flush 5% O_2/5% CO_2	Ballantyne et al. (1988a)
Shredded lettuce (Iceberg cv Saladin)	5	1-2	5-6	12-14 (Control-5)	35μm OPP pillow pack, gas flush 5% O_2/5% CO_2	Gill (1988)
Shredded lettuce (Iceberg)	1-2	not reported	not reported	14-28	50μm PE pillow pack, gas flush 5% CO_2/ 5%CO/ 21% O_2	Bolin and Huxsoll (1991)
Shredded lettuce (Iceberg cv Great Lakes)	4	3	10	>12 (Control 4)	CA storage	Barriga et al. (1991)
Mixed lettuce leaves	5	2-3	7-8	12 (control 4)	15μm OPP/ 30μmPE pillow pack, gas flush 5% O_2/5% CO_2	Day (1989)

Table 1. (continued)

Commodity	Temperature (°C)	O_2 (%)	CO_2 (%)	Shelf-life (Days)	Comments	Reference
Broccoli florets (cv Skiff)	5	2-5	3-6	>14, not too different from control	15μm LLDPE lid in barrier tray; gas flush 5% O_2/ 5% CO_2	Ballantyne et al. (1988b)
Broccoli florets (Emperor)	10	not reported	not reported	10 (control 4-5)	15μm PVC lid in barrier tray; gas flush 5% O_2/ 5% CO_2	Gill (1988)
Diced onion	5	-	-	13-14	Passive MA 15μm/30μm PE	Day, 1989
Vegetable salad (mayonnaise-based)	4 12	- -	20 20	54 12	Blanched vegetables	Buick and Damoglou (1989)
Grated carrots	10 or lower	2	25	Test terminated at day 10	CA storage	Carlin et al. (1990a)
Grated carrots	2	not reported	not reported	12	40μmOPP pillow pack	Carlin et al. (1990b)

Table 2 Recommended atmosphere for selected raw vegetables

Commodity	Temperature (°C)	O_2 (%)	CO_2 (%)	Reference
Asparagus	0-5	2-1	5-10	Edwards (1988)
Beans, snap	5-10	2-3	5-10	Kader and Morris (1977)
Brussels sprouts	0-5 5	1-2 2-4	5-7 2-4	Edwards (1988) Gill (1988); Stringer (1990)
Cabbage	0-5 1-5	3-5 2-3	5-7 3-5	Edwards (1988) Gariepy et al. (1984)
Cauliflower	0-5 1	2-5 3	2-5 2.5-5	Edwards (1988) Romo-Parada et al. (1989)
Celery	0-5	2-4	0-2	Edwards (1988)
Corn	0-5	2-4	10-20	Edwards (1988)
Broccoli	1	2-2-5	8.9	Makhlouf et al. (1989)
Cucumber	8-12	3-5	0	Edwards (1989)
Mushrooms	0-5	21	10-15	Edwards (1988)
Peas, green	-	$\geq$7	$\leq$7	Kader et al. (1989)
Pepper, bell	8-12	3-5	0	Kader (1980)
Spinach	0-5 -	21 4	10-20 9	Edwards (1988) McGilbert et al. (1966)

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APPENDIX A: Packaging Film Abbreviations

EVA	Ethyl-vinyl acetate
LDPE	Low density polyethylene
OPP	Oriented polypropylene
PE	Polyethylene
PVC	Polyvinyl chloride