POLYMERIC PACKAGING AND EDIBLE COATINGS FOR MINIMALLY PROCESSED CARROTS

by

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I declare that the dissertation, which I hereby submit for the MSc degree in Food Science at the University of Pretoria is my own work and has not been previously submitted by me for a degree at any other University or institution of higher education.

29/01/2001

TO MY CREATOR

ABSTRACT

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Minimally processed fruits and vegetables are increasingly demanded by local consumers and for export purposes. However, the marketing potential of these produce is limited because of physiological ageing, biochemical changes and microbiological spoilage that lead to a short shelf life. The use of polymeric packaging films to control microbial and metabolic processes and edible coatings to control the white blush formation respectively, have shown potential in improving the quality of minimally processed carrots. In combination they may form a double barrier to gases and water vapour that could provide an interaction effect to enhance the shelf life of minimally processed carrots. The aim of the study was to determine the effects of the polymeric packaging films of different permeability and edible coatings at different levels of concentration, alone and in combination on the physiological and biochemical, microbiological, and sensory quality of minimally processed carrots.

A factorial experiment of 3 polymeric packaging films x 3 levels of edible coating was conducted. The three polymeric packaging films were P-Plus®, an oriented polypropylene which were fully permeable to gases and water vapour (pi,

control), semi permeable (p160) and least permeable (p90) to gases only. The semi permeable and least permeable packaging had similar water vapour permeability. The coating was Nature Seal®, a cellulose based, at 0% (control), 7.5% and 15% w/w. Carrots were minimally processed into slices, dipped in the edible coating, then packed in the polymeric films and stored for 12 days at 10 ^{0}C. Four packs were analysed for each combination treatment on d4, d8 and d12, and d0 was taken as reference point.

With time, the head space in the semi permeable packaging (p160) showed a decrease to about 11.5-13.6 % oxygen and an increase to about 7.5-9.6 % carbon dioxide. The least permeable pack (p90) showed an oxygen decrease to about 9.8-7.6 % and a carbon dioxide increase to about 12.3-13.5 %. This change showed the creation of a modified atmosphere that will decrease the metabolic activities. As the coating concentration increased, a slight increase in carbon dioxide and a slight decrease in oxygen were recorded in the head space of the packs. This change was unexpected as the coating was supposed to be a gas barrier. Thus, this change questioned the gas permeability properties of the edible coating. The polymeric packaging and the coating interacted to give lower oxygen and higher carbon dioxide levels in the head space atmosphere. However, packaging had a more pronounced effect in the creation of the modified atmospheres than the coating.

A lower white blush formation and a higher retention of chroma values was recorded on the lower surfaces of the carrot slices than on the upper surfaces (upper surfaces refer to those that were facing the packaging material, the lower surfaces was the opposite side of the upper surfaces). This showed that the relative humidity gradient was probably not the same between the surfaces. The coating effectively controlled the white discolouration and maintained higher chroma values on both surfaces of the carrot discs, but packaging did not affect the colour changes of the upper surfaces. An interaction effect was also observed between the packaging and coating showing a better control of the white blush formation of the lower surfaces of the carrot discs.

Yeast and moulds did not prove to be a problem in minimally processed carrots as they were lower than 10^3 cfu/g carrots throughout the storage period. When the carrots were visibly spoiled, the lactic acid bacteria were over 10^6 cfu/g and the psychrotrophs were about 10^7 to 10^8 cfu/g. Initially, a high growth rate of psychrotrophic bacteria occurred followed by a high growth rate of the lactic acid bacteria. This showed a dynamic relationship between the two microbes. Visible rot was observed by brown discolouration, tissue softening and exudate production. The packaging controlled the microbiological growth and spoilage as compared with the coating that enhanced it. A decrease in pH from d4 to d12 corresponded to an increase in the lactic acid bacteria and visible spoilage.

Combination of edible coatings and polymeric packaging films did not show any synergistic or additive effects to enhance the shelf life of minimally processed carrots despite some interactions between these two variables. This was because the polymeric packaging films primarily prevented microbiological growth and spoilage, whereas edible coatings partly controlled white blush formation. White blush formation was the most important shelf life determinant of minimally processed carrots. Research efforts should therefore be focussed on overcoming this defect.

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1. INTRODUCTION

Consumer demand for conveniently prepared fruits and vegetables with superior sensory and nutritional quality compared to processed products has lead to the development of minimal processing technology (King and Bolin, 1989). Such trends together with busy lifestyles, increased purchasing power and health consciousness of the consumers have increased the demand for minimally processed fruits and vegetables (MPFV) (Sloan, 1995). Locally the MPFV is a growing industry, catering for the mine industry, export markets, restaurants and retail outlets (Hunt, 2000)

According to Wiley (1994), minimal processing generally refers to unit operations such as washing, sorting, peeling, coring, slicing; or any procedure short of traditional preservation (heat sterilization and freezing) that adds value. For safety reasons and greatest retention of sensory and nutritional quality, these products are distributed and marketed in the cold chain.

As a result of the unit operations, MPFV have a limited shelf life as compared to the raw material (Ahvenainen, 1996). Minimally processed produce deteriorate because of physiological ageing, biochemical changes and microbiological spoilage, thereby resulting in degradation of colour, texture and flavour (King and Bolin, 1989). The shelf life of MPFV at refrigeration conditions as determined by sensory and microbiological spoilage can be 7-14 days or less depending on the produce, the storage conditions and the associated hurdles (Garcia-Gimeno and Zurera-Cosano, 1997). In addition, the growth of psychrotrophic food borne pathogens can be of concern, especially in low acid MPFV (Francis, Thomas and O'Beirne, 1999).

Methods such as refrigeration, use of chemical additives and irradiation have shown potential to extend the shelf life of MPFV (Huxsoll and Bolin, 1989), but new approaches include creating a modified atmosphere using polymeric packaging films of selective permeability or edible coatings or films (Ahvenainen

1996). Both the polymeric packaging films and edible coatings, as hurdles can act as gaseous barriers and have the potential to limit metabolic reactions and microbial growth of MPFV (Carlin, Nguyen-The, Chambroy and Reich, 1990a; Solomos, 1994; Baldwin, Nisperos-Carriedo and Baker, 1995a; Amanatidou, Smid and Gorris, 1999).

Polymeric packaging films have been shown to modify the atmosphere of the pack by controlling the diffusion of gases and water vapour. Thus, it was found to extend the shelf life of MPFV (Carlin, Nguyen-The, Hilbert and Chambroy, 1990b; Yoo and Lee, 1999). The use of edible coatings to extend the shelf life of fresh as well as MPFV has received considerable attention among researchers. Edible coating was reported to have the potential to reduce moisture loss, restrict oxygen entrance, lower respiration rate, retard ethylene production, seal flavour volatiles and carry additives that retard physiochemical changes (Baldwin *et al.*, 1995a).

The use of more then one hurdle can have an additive or synergistic effect that can further increase the shelf life of the produce (Leistner and Gorris, 1995). Thus, the use of polymeric packaging films and edible coating as hurdles can possibly interact together to further extend the shelf life of the MPFV. This extension of shelf life can provide a better market potential for MPFV.

2. LITERATURE REVIEW

The following chapter reviews current literature to understand the quality problems associated with minimally processed fruits and vegetables (MPFV) as well as ways to control these problems. Emphasis will be placed on the roles of polymeric packaging films and edible coatings for minimally processed carrots.

2.1 Quality aspects of minimally processed fruits and vegetables

Minimally processed fruits and vegetables undergo processes to increase their functionality without greatly changing their fresh-like properties. However, minimal or light processing alters the integrity of these products and they become more perishable (Shewfelt, 1986). The perishability as determined by the shelf life is a combination of the quality changes (physiological, biochemical, microbiological and nutritional) and the factors that influence these changes. These quality changes and factors are interrelated and can be schematically illustrated as in Figure 1. Thus it is important to have a fully integrated approach when evaluating quality of minimally processed fruits and vegetables. Figure 1 will be discussed to show the main problems associated with quality deterioration in minimally processed fruits and vegetables and also how to prevent these quality deterioration.

The 'quality' of MPFV generally refers to the sensory quality as determined by texture, flavour and appearance; microbiological quality in terms of spoilage and safety; and to a certain extent the nutritional quality.

2.1.1 Quality changes of minimally processed fruits and vegetables

As a result of peeling, slicing and cutting during the minimal processing of fruits and vegetables, the living tissue changes from a relatively stable fresh product with a skin (or other protective outer layers) to a more susceptible form for

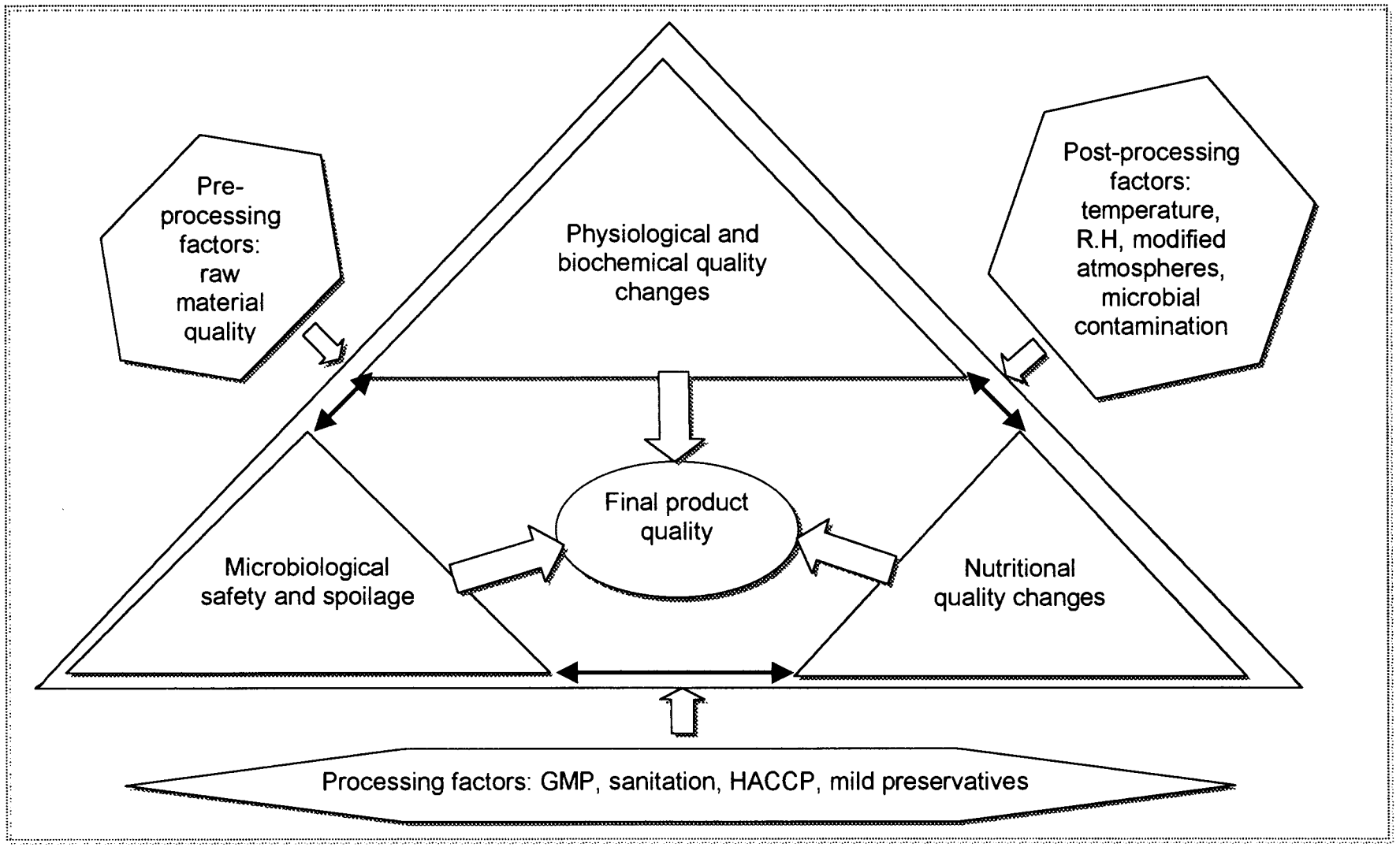

Figure 1: Interrelationship between quality changes and quality factors of minimally processed produce

quality deterioration (King and Bolin, 1989). These operations induce wounding stress, resulting in metabolic activation to cause physiological ageing, biochemical changes and microbiological spoilage. These metabolic processes in turn result in loss of firmness, discolouration and off flavour development that are unacceptable to consumers (Varoquaux and Wiley, 1994).

The deteriorative quality changes that occur in MPFV can be classified as physiological and biochemical; microbiological; and nutritional.

2.1.1.1 Physiological and biochemical changes of minimally processed fruits and vegetables

At the harvest stage, plant tissues start to undergo senescence and there is a shift to total degradative reactions becoming greater than biosynthetic reactions. In addition partial processing that leads to wounding will induce or enhance these deteriorative changes (Watada, Abe and Yamuchi, 1990). These changes include a localised increase in respiration at the site of injury, stress ethylene production, accumulation of secondary metabolites, and cellular disruption leading to mixing of enzymes and substrates (Rolle and Chism, 1987). Consequently, there will be colour, texture and flavour changes that are indicative of quality deterioration.

- Metabolic changes

An increase in respiration rate from intact to cut fruits and vegetables is quite considerable (Table 1) showing an increase in metabolic activities. Similarly, wound ethylene also increases. Ethylene production by kiwi fruit is very low, but slicing caused an increase from 5 nl/kg/h to 40 nl/kg/h after 2 hours and about 80 nl/kg/hr after 4 hours at 20 ^{0}C (Watada *et al.*, 1990). After 24 hours, the average respiration rate was found to be about 1.3 µl/kg/h at 20 ^{0}C compared with the rate being unchanged for the fresh fruit that was not cut.

Table 1: Respiration rate (CO_2 mg/kg/hr) of whole and freshly cut fruits and vegetables at 10 ^{0}C (Watada, Ko and Minott 1996).

Produce	Whole	Cut	% change
Green beans	52.0	78.0	50.0
Cucumbers	6.6	9.7	47.0
Tomatoes	4.7	10.0	112.0
Banana without peel	10.9	21.1	93.6

Moisture loss increases when fresh fruits and vegetables are cut because of transpiration. This is because there is no skin barrier to control transpiration (Wills, McGlasson, Graham and Joyce, 1998). Moisture loss and dry matter loss as a result of transpiration and excessive respiration respectively, can lead to decrease in weight and desiccation. Wilting is very common in leafy produce and shrivelling is more common in bulky tissues. These defects can be easily managed by storing under high relative humidity (85-95%) and low temperatures (Wills *et al.*, 1998).

The effects of minimal processing on the rate of respiration, transpiration and wound ethylene production differ between type of commodity, physiological ripening stage of climacteric produce and extent of tissue damage (Rosen and Kader, 1989; Barry-Ryan and O'Beirne, 1998).

- Colour changes

The most important colour changes occurring in MPFV as a result of wounding are enzymatic browning, destruction of chlorophyll and formation of white translucent tissue on the surface of carrots. The main defect in minimally processed avocados, apples and lettuce is physiological browning. Polyphenol oxidase and phenyl ammonialyase activities induced by wound ethylene were found to increase and this was correlated with visual browning (Couture,

Cantwell, Ke and Saltveit, 1993; Lopez-Galvez, Saltveit and Cantwell, 1996). Similarly ethylene was found to be involved in the destruction of chlorophyll by chlorophyllase (Watada *et al.*, 1990). Moreover as a result of membrane integrity disruption, degradative enzymes and substrate mix to cause colour changes (Rolle and Chism, 1987)

The surface white discolouration, also known as white blush is a major defect in minimally processed carrots. The formation of white blush is attributed to:

- Surface dehydration (Tatsumi, Watada and Wergin,1991; Cisneros-Zevallos, Saltveit and Krochta, 1995) and
- Lignin formation (Bolin and Huxsoll, 1991; Howard and Grifin, 1993)

According to Tatsumi *et al.* (1991), fresh cut carrots examined by scanning electron microscopy revealed that surface cells were either damaged or layers were separated from tissue because of the processing knife. These cells and tissues dehydrated quickly and formed a white tissue.

Cisneros-Zevallos *et al.* (1995) showed that the mechanism for the white discolouration is both a physical and physiological response to wounding. They used Whiteness Index (WI) as an objective measurement to show the extent of the defect. WI was also related to visual grading as shown in Table 2. They found that higher relative humidity conditions were associated with lower white blush incidence. In addition, wetting the white surface significantly decreased the WI, indicating a reversible change (Figure 2). Thus they concluded that this is a physical response reflected in a colour change which is reversible. However, an irreversible change was also noted. Dewetted peeled carrots that were exposed to 75 and 98 % relative humidity and afterwards dipped in water, did not regain their original colour. This irreversible change was explained by a physiological response because of lignin formation (Bolin and Huxsoll, 1991).

Table 2: A visual description of the whiteness index (WI) for peeled carrots (Cisneros-Zevallos *et al.*,1995)

Description	WI[1] (average values with standard deviation)
Non white	32.6 ±2.4
Slightly white	38.4 ±1.3
Moderate white	43.0 ±1.8
Severe white	47.2 ±1.7
Extreme white	50.9 ±3.1

[1]$WI = 100 - [(100-L)^2 + a^2 + b^2]^{1/2}$, L. a and b values were obtained from a colour meter

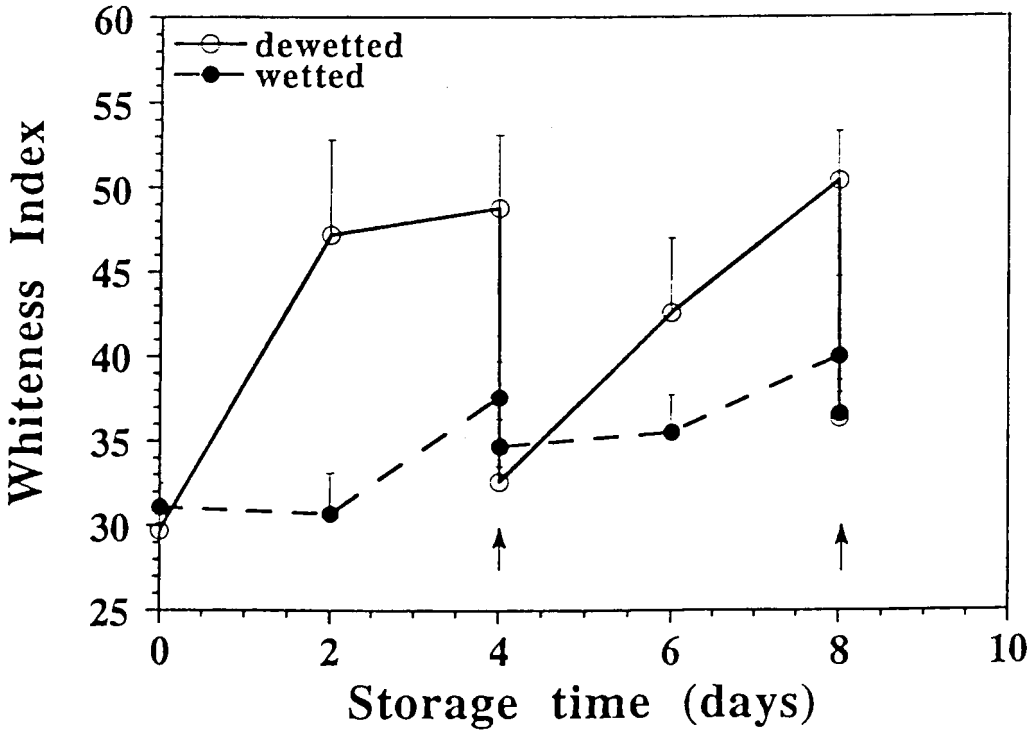

Figure 2: Effect of initial surface moisture and storage time on the whiteness index for peeled carrots stored at 75% relative humidity and 10 ^{0}C. Arrows indicate a ten-minute water dip. Dewetted means centrifuge at start to remove excess water, wetted means no centrifugation (Cisneros-Zevallos *et al.*, 1995)

White tissues from the carrot surface was found to give positive lignin test as compared to the non white part, showing the involvement of lignin formation (Bolin and Huxsoll, 1991). Further research by Howard and Griffin (1993) confirmed the close relationship between development of white discolouration and lignin formation. They also showed that lignin formation was stimulated by wound induced phenyl ammonialyase and peroxidase enzymes and was not affected by ethylene.

- Textural changes

Texture changes in minimally processed carrots showed an increase in firmness in the first few days of storage followed by a decrease afterwards, but the changes were not consistent (Izumi and Watada, 1994). Other minimally processed products such as Kiwi fruit and banana showed significant decreases in firmness with storage time (Watada *et al.*, 1990). The loss as shown in Table 3 was suggested to be a consequence of enzymatic hydrolysis of the cell wall components that provide cell wall integrity. Moreover it was emphasized that ethylene played a role in enhancing loss of firmness.

Table 3: Average firmness (Newton) of sliced kiwi fruit and banana held in an atmosphere of 0, 2 and 20 ppm ethylene in air at 20 ^{0}C (Watada *et al.*, 1990, modified).

Fruit	Ethylene (ppm)	Hours		
		0	24	48
Kiwi fruit	0	3.67	2.78	2.21
	2	3.67	2.28	1.96
	20	3.67	2.0	1.89
Banana	0	4.83	4.61	4.86
	2	4.83	4.24	4.04
	20	4.83	4.03	3.53

- Flavour changes

Flavour changes providing a pleasant aroma typical of the MPFV are desirable. However, off flavour development due to enzymatic peroxidation of the unsaturated fatty acid as well as anaerobic respiration are undesirable (Watada *et al.*, 1990). In minimally processed carrots, ethylene was highly associated with the development of a bitter taste during storage as a result of isocoumarin formation (Lafuente, Cantwell, Yang and Rubatzky, 1989).

2.1.1.2 Microbiological changes

The type of produce, processing (including the hurdles) and storage conditions will generally affect both the spoilage and safety of the MPFV (Brackett, 1987; Zagory, 1999).

- Microbiological spoilage

Microflora responsible for spoilage of MPFV include mainly yeast and moulds, lactic acid bacteria, mesophilic, psychrotrophic and pectinolytic microflora (Nguyen-The and Carlin, 1994). Fresh green salads and vegetables that are generally low acid foods are mainly spoiled by a variety bacteria as compared to high acid foods such apples that are mainly spoiled by yeast and moulds, and lactic acid bacteria (Brackett, 1994).

Fresh cut spinach stored at 10 ^{0}C for 12 days was dominated by mesophiles, psychrotrophs and the pseudomonaceae reaching levels of 10^{10} cfu/g (Babic, Roy, Watada and Wergin, 1996). Using low temperature scanning microscopy these researchers found that the microbes were mainly in areas where the cuticle was broken, and the microbes infected the internal palisade parenchyma. Similarly, Nguyen-The and Prunier (1989) found that fluorescent pectinolytic pseudomonads, namely *Pseudomonas marginalis* caused soft rot in ready to eat endive packaged into polypropylene bag. However, a modelling approach by Garcia-Gimeno and Zurera-Cosana (1997) showed that lactic acid

bacteria at levels 10^6 cfu/g appeared to be related to both spoilage and could theoretically be used to predict shelf life of a mixed salad (red cabbage, lettuce and carrots) packed in a semi permeable polypropylene film. The difference in spoilage microorganism might be because of the commodity factor. Many authors suggested that there is no simple correlation between microbial load and chemical markers such as pH, lactic acid, acetic acid, carbon dioxide levels and sensory quality (Guerzoni, Gianotti, Corbo, Sinigaglia, 1996; Zagory, 1999). In fact, spoilage depends on several processing factors and quality changes

Minimally processed carrots were found to have a typical lactic acid fermentation type of spoilage by the lactic acid bacteria (Carlin, Nguyen-The, Cudennec and Reich, 1989). Principal component analysis showed that head space composition of high carbon dioxide (over 30%) and low oxygen (below 1.5%), ethanol and lactic acid bacteria were the most discriminating variables between spoiled and well preserved packs regardless of storage time. The identified lactic acid bacteria were *Leuconostoc mesenteroides*. The pectinolytic pseudomonads present in the spoiled carrots were variable and they concluded that there was no relationship between rate of deterioration and these microbes. Research to show the involvement of yeasts revealed that even if the yeasts increased with time, neither the number nor the composition was related to the spoilage of minimally processed carrots (Babic, Hilbert, Nguyen-The and Guiraud, 1992).

♦ Microbiological safety

Fruits and vegetables have a very low risk of causing food borne illnesses as compared to other carriers such as meat (Bryan, 1988). Brackett (1994) mentioned several reasons why MPFV are relatively safer as compared to other food. These reasons are:

- Refrigeration conditions of fresh produce are generally unfavourable for the growth of most pathogens except the psychrotrophs
- High acid food also eliminate the possible growth of most pathogens

- Normal spoilage microorganism generally have an antagonistic effect on the growth of most pathogens

On the other hand, Francis *et al.* (1999) suggested that mild preservation systems such as refrigeration and modified atmosphere packaging could not ensure the safety of a product. Psychrotrophic pathogens such as *Listeria monocytogenes* and *Aeromonas hydrophila and the* pathogen *Clostridium botulinum* are capable of growing to infectious levels under these mild preservation regimes.

In assessing the microbiological quality of ready to use vegetables for health care food services, it was found that only the level of *Listeria monocytogenes* increased among the pathogens. This increase was associated with temperature abuse from 4 ^{0}C to 10 ^{0}C (Odumeru, Mitchell, Alves, Lynch, Yee, Wang, Styliadis and Farber, 1997). However, *Listeria monocytogenes* was not found to be a threat in minimally processed carrots because of an inhibitory effect. They suggested that temperature is a critical factor and it should be kept below 5 ^{0}C, but this is difficult to achieve during cold chain distribution. They also recommended that hazard analysis critical control point and quality assurance programmes to check for *Listeria monocytogenes* randomly is critical to ensure safety of MPFV.

2.1.1.3 Nutritional changes

Most of the reviews on MPFV are mainly concerned with the market quality as extensively determined by colour, flavour and texture measurements. However, limited information on the nutritive value is available. Only vitamin A and ascorbic acid losses were reported (McCarty and Matthews, 1994). A decrease in vitamin A and ascorbic acid was reported for leeks stored under a normal cold storage at 0 ^{0}C and 95% R.H (Kurki, 1979). Similarly a decrease in the total carotenoid content was found in minimally processed carrots stored at 2 ^{0}C for 14 days and transferred to 10 ^{0}C for an additional three days as shown in

Table 4 (Howard and Dewi, 1996). This decrease was explained by the oxidation of the carotenoids. The carotenoids losses also resulted in a decrease in the orange intensity of the carrots as determined by the chroma values (Chervin and Boisseau, 1994).

Table 4: Carotene content of mini peeled carrots as affected by minimal processing and storage at 2 ^{0}C for 14 days and transferred to 10 ^{0}C for an additional 3 days (Howard and Dewi, 1996)

Time	Total carotenoids (ppm fresh weight)
0	94.1
3	80.3
7	79.3
14	80.5
17	74.8

2.1.2 Factors affecting quality of minimally processed fruits and vegetables

The factors affecting the quality changes of MPFV can be differentiated into pre-processing, processing and post-processing factors.

2.1.2.1 Pre-processing factors

The pre-processing factors include growing conditions and cultural factors, cultivar, maturity at harvest, harvesting and handling methods, inspection standards and duration of storage (Shewfelt, 1987). As such these pre-processing factors will affect the raw material quality. For example, Varoquaux, Mazollier and Albagnac (1996) found differences in the physiological status in fresh cut butter head lettuce due to the different cultivar and this lead to different quality changes. This shows that cultivar choice and raw material quality is an important point of departure for quality purposes.

2.1.2.2 Processing factors

The processing factors can influence the stresses that will lead to physiological ageing and microbiological deterioration. Barry-Ryan and O'Beirne (1998) clearly showed that the sharpness of the slicing blade affected the quality and storage life of modified atmosphere packaged sliced carrots as shown in Table 5. Slicing was found to cause physical damage, physiological stress and enhanced microbial growth. These damages and stresses were enhanced by a blunt machine blade compared with a sharp machine blade and by the sharp blade machine compared with a razor blade.

Table 5: Quality of carrots as affected by slicing methods (Barry-Ryan and O'Beirne, 1998)

Measured changes	Time	Slicing method		
		Razor	Sharp machine	Blunt machine
Total aerobic count	d1	5.77	5.80	6.27
log_{10}/g	d6	7.02	7.95	8.16
Lactic acid bacteria	d1	4.70	4.93	5.66
log_{10}/g	d6	7.20	7.84	7.94

Ahvenainen (1996) identified the following key requirements during processing to improve quality of MPFV:

- Strict hygiene, good manufacturing practices and hazard analysis critical control point.
- Low temperature processing
- Careful cleaning and/ or washing before and after peeling
- Good quality of water (sensory, microbiological and pH) for washing
- Use of mild additives
- Gentle spin drying following washing
- Gentle peeling, slicing and shredding
- Correct packaging material and methods

2.1.2.3 Post-processing factors

The post-processing factors as stated by Watada (1997) and Watada *et al.* (1996) include temperature of storage, relative humidity, modified atmospheres and post-processing microbial contamination. These post-processing factors together with some processing factors are generally manipulated to improve the quality and shelf life of MPFV and are discussed in section 2.2 below.

2.2 Ways to improve quality of minimally processed fruits and vegetables

Minimally processed fruits and vegetables are very susceptible to deteriorative changes that limit their shelf life. Therefore ways to prevent or delay these changes will extend the shelf life. The most common practice to enhance the shelf life is the use of low temperature processing and distribution under the cold chain (Wiley, 1994). Low temperature preservation can only prevent the deteriorative changes to a certain extent and frequently the cold chain is abused. So, it is important to consider other hurdles that will enhance the quality without changing the fresh like properties. Hurdles that are of interest are the modification of the atmospheres surrounding the MPFV with partially permeable polymeric packaging films and edible coatings.

2.2.1 Modifying atmospheres by polymeric packaging film

Modified atmosphere packaging (MAP) is the manipulation of environmental conditions. It involves the alteration of the atmosphere by increasing the carbon dioxide and decreasing the oxygen in the pack (Shewfelt, 1986). MAP can be of two types, passive or active (Zagory and Kader, 1988). Passive MAP involves the packaging of produce in semi permeable (gas and water vapour) bags, sealing the package and allowing the fresh produce to decrease the level of oxygen and increase the carbon dioxide concentration in the pack by respiration until an equilibrium is reached. This equilibrium should provide the desired effects without creating any detrimental or anaerobic conditions. Active MAP also involve the use of a gas permeable packages, but before sealing, the gas

in the package is evacuated and replaced by a pre-selected mixture of oxygen and carbon dioxide and nitrogen gases. This is followed by rapid sealing of the package. Active MAP also includes the utilisation of adsorbers and/or absorbers to generally scavenge oxygen, carbon dioxide or ethylene (Kader, Zagory and Kerbel, 1989).

There are several types of polymeric packaging films that can create a modified atmosphere passively and this influences the metabolic and microbiological status of MPFV. Thus polymeric packaging films have the potential to enhance the quality of MPFV.

2.2.1.1 Polymeric packaging films

The utilisation of polymeric films or plastic materials (such as rigid trays) of appropriate permeability is very desirable. It creates an internal atmosphere of oxygen and carbon dioxide that results in substantial reduction of the respiration rate without inducing significant produce anaerobiosis (Schlimme and Rooney, 1994). Polymeric films as packaging have numerous functions that include control of gas transfer, control of moisture transfer, protection from external physical or mechanical damage and biological contamination.

The design and selection of the polymeric packaging film for passive MAP is of importance as it has to provide a permeability that will establish an equilibrium atmosphere of gases that will extend the product shelf life without any detrimental effects. This achievements depends on the following parameters (Schlimme and Rooney, 1994):

- Product factors that involve the respiration rate and respiration quotient at selected storage temperature; quantity of product in the pack; the oxygen and carbon dioxide levels to achieve optimum reduction of aerobic respiration;
- Packaging film factors that include permeability (O_2, CO_2 and H_2O) of the polymeric film at a specific relative humidity and temperature,

thickness of the pack, total surface area, abuse resistance of the packaging film;

- Other factors such as free volume inside the pack and air velocity and relative humidity around the package.

Polymeric packaging films are generally thermoplastic polymers such as polypropylene, low density and high density polyethylene, polybutylene, polystyrene, ethylene vinylacetate polymers and Pliofilm® as rubber hydrochloride. Among these polymers, polypropylene may be the most preferred as it has a relatively low permeability ratio of oxygen and carbon dioxide. The high activation energy of polypropylene provides a low Q_{10} and thus it can prevent anaerobic respiration during temperature abuse (Artes and Martinez, 1996). P-Plus® is an oriented polypropylene that is microperforated to give desirable gas permeability and is used for packaging of MPFV for the local and export market (Anonymous, 1996).

2.2.1.2 Metabolic effects of modified atmosphere

The effect of reduced oxygen and elevated carbon dioxide on reducing respiration rate has been assumed to be the primary reason for the beneficial effects on fruits and vegetables. However modified atmospheres can also provide the following benefits (Kader, 1986; Powrie and Skura, 1991)

- Lowering ethylene production
- Inhibition of the initiation of ripening
- Decrease rate of ripening and senescence
- Reduction in tissue water loss and maintenance of cell turgidity
- Minimization of nutrient loss and decomposition
- Reduction of specific physiological disorder such as chilling injury

The scientific reasons underlying the beneficial effects of low oxygen and high carbon dioxide on the shelf life are not well elucidated (Solomos, 1994). apparently hypoxia (ca 1.5% oxygen) induces the expression of anoxic genes,

while suppressing those that are involved in plant development and eventually plant senescence.

Most research has been carried out on fresh fruits and vegetables rather than on minimally processed fruits and vegetables and conclusions were mainly drawn under a controlled atmosphere rather than a modified one. Watada and Qi (1998) experimented on the effects of low oxygen and high carbon dioxide on the quality of fresh cut products. They found that low oxygen storage was beneficial in maintaining the quality of the cut produce as reported in Table 6. The effects differed among commodities, with carrots and spinach having better effects than other produce as they are more tolerant to low oxygen atmosphere.

The physiological response of low oxygen on carrots' respiratory pattern is known. When carrots were stored in an atmosphere of 0.5% oxygen, it was found to sustain itself for a short period of time without any anaerobic respiration. This was because of a shift in respiratory pattern. At 0.5% oxygen, fructose 2,6 biphosphate (a carbohydrate regulatory metabolite) in the glycolytic pathway was found to accumulate sufficiently to increase the activity of phosphate dependent phosphofructokinase and to inhibit fructose 1,6 biphosphatase (Kato-Noguchi and Watada, 1996a). An increase in the activity of phosphate dependent phosphofructokinase, which catalysed the reaction between fructose 6-phosphate and fructose 1,6 biphosphate towards glycolysis, led to the accumulation thus fructose 1,6 biphosphate (Kato-Noguchi and Watada, 1996b). The accumulation of fructose 1,6 biphosphate in turn increased the rate of glycolysis and generated enough ATP to sustain metabolic activity of sliced carrots. However, a continuous increase in glycolysis could lead to the accumulation of ethanol and acetaldehyde. Under similar conditions, where carrot tissues were stored under a continuous flow of 0.5% oxygen, ethanolic fermentation was favoured by an increase in the activity of alcohol dehydrogenase and ethanol concentration (Kato-Noguchi, 1998).

Table 6: Quality condition and respiration rate of fresh cut fruits and vegetables after storage in air or low oxygen atmosphere at 5 ^{0}C (Watada and Qi, 1998).

Commodity	Atmosphere	Days in storage				Respiration rate (ml CO_2/kg/hr)
		3	7	10	14	
Spinach	Air	-	Excellent to good	-	Good	11.3
	0.8% O_2	-	Excellent	-	Excellent to good	6.8
Broccoli	Air	-	Fair	-	Poor	13.4
	1% O_2	-	Good	-	Fair	9.1
Carrots	Air	-	Good	-	Fair	15.3
	0.5% O_2	-	Excellent to good	-	good	10.6
Zucchini	Air	-	Poor	-	-	10.8
	1% O_2	-	Fair	-	-	5.7
Peach	Air	Good	Fair	Poor (browning)	-	2.9
	1% O_2, 5% CO_2	Good	Good to fair	Poor (browning	-	2.5
Honeydew	Air	Good	Fair	Poor	-	1.5
	2% O_2, 10% CO_2	Good	Good	Fair	-	0.8
Strawberry	Air	Good	Good	Fair to poor	-	4.9
	1% O_2, 10% CO_2	Good	Good	fair	-	2.9

- no observation

For minimally processed carrots stored under controlled atmosphere, an equilibrium of about 5% oxygen was found to be critical as respiration rate was highly dependent on the oxygen concentration as shown by applying the Michaelis-Menten model (Sode and Kuhn, 1998). However in a passive modified atmosphere Carlin *et al.* (1989) found a critical value of 1.5% for oxygen. No dependency of carbon dioxide was found by Sode and Kuhn (1998), but a carbon dioxide level greater than 30% was not recommended by Carlin *et al.* (1989) as it favoured lactic acid bacteria.

2.2.1.3 Microbiological effects of modified atmospheres

The microbiological effects of modified atmospheres can be of two dimensions: spoilage and pathogenic. Generally it is agreed that elevated carbon dioxide might exert a biostatic effect on bacteria and it can extend the lag phase of bacterial growth (Farber, 1991). However, the effects were found to be quite variable and it depends on the initial numbers and type of the microorganism; ability of food to support the microbial growth (acidity, nutritional composition and presence of antimicrobial factors); water activity, temperature and the concentration of gases.

Low oxygen and high carbon dioxide atmospheres were found to decrease total plate count and yeast and moulds on minimally processed cut fruits as shown in Table 7. However, low oxygen can favour microaerophillic microbes such as lactic acid bacteria. Buick and Damoglou (1987) found a shift from the growth of *Erwinia spp.* to *Leuconostoc spp.* when an anaerobic environment was created. Similarly Carlin *et al.* (1989) showed that under a modified atmosphere, lactic acid bacteria, namely *Leuconostoc mesenteroides* were favoured as compared to aerobic mesophiles and pectinolytic bacteria.

Under modified atmosphere packaging of MPFV stored at refrigeration conditions, food borne pathogens that are anaerobic or microaerophilic can be of concern (Farber, 1991). *Listeria monocytogenes* and *Clostridium botulinum*

were the main pathogens under study. A study conducted by Amanatidou *et al.* (1999) showed that *Enterobacter agglomerans*, *Salmonella typhimurium*, *Salmonella enteritidis* and *Escherichia coli* were significantly decreased under modified atmospheres in a agar surface model, but *Listeria monocytogenes* were found to increase. Similarly, *Listeria innocua* (indicator microorganism for *Listeria monocytogenes*) were found to increase on minimally processed cabbage (Omary, Testin, Barefoot and Rusting, 1993). However, on minimally processed carrots *Listeria innocua* were found to have one log decrease when stored under modified atmosphere (Finn and Upton, 1997). This decrease might be due to the inhibitory activity of carrots towards *Listeria spp.* (Nguyen-The and Lund, 1991).

Table 7: Microbial population (log_{10} cfu/g) at day 10 on fresh cut fruits stored under low oxygen atmosphere at 5 ^{0}C (Watada and Qi, 1998)

Fruits	Atmosphere	Total plate count	Yeast and moulds
Peach	Air	8.0	5.3
	1% O_2, 5% CO_2	6.5	4.8
Honeydew	Air	9.7	6.2
	2% O_2, 10% CO_2	8.4	4.4
Strawberry	Air	4.3	4.2
	1% O_2, 10% CO_2	2.9	2.6

Clostridium botulinum, producing botulinal toxin can also be a threat in low acid MPFV. Botulinal toxin was detected in broccoli packaged in bags of low oxygen permeability. However all the toxic samples were already visibly spoiled at this stage (Hao, Brackett, Beuchat and Doyle, 1999)

2.2.1.4 Benefits of using polymeric packaging films

Polymeric films are commercially used to create passive modified atmospheres and to extend the shelf life of MPFV. Carrots stored in polymeric packaging

films were found to maintain a good quality. The permeability of these films was critical in extension of shelf life. A high permeability polymeric packaging of about 10,000 to 20,000 $cm^3/m^2/day/atm$ at 25 ^0C was recommended for minimally processed carrots because of high respiration rates (Carlin *et al.*, 1990b). This suggested that permeability of polymeric packaging films should relate to the respiratory activities of the produce. Modelling approaches could be used to ensure this dynamic system between the respiring produce and the film permeability to achieve an internal gas concentration at a steady state (Solomos, 1994).

Other potential benefits of using polymeric packaging films on fresh and minimally processes fruits and vegetables are:

- Prevention of russet spotting and pink rib of salinas lettuce packed in a polypropylene packaging film (Artes and Martinez, 1996)
- Better retention of sugars, organic acids and soluble proteins in asparagus spears packed in a commercial polymeric film (Baxter and Waters, 1991)
- Chlorophyll preservation, petiole firmness and decreased vitamin C loss in parsley packed in a ceramic film (Park, Kang, Yang and Jung, 1999)
- Decreased anthocyanin degradation in shredded onions packed in a perforated polypropylene films (Ferreres, Gil, Thomas-Barberan, 1996)

2.2.2 Edible coatings

There has been considerable interest in the development of biopolymer films and coatings from protein, polysaccharide and lipid based materials in recent years and research is still at an infancy stage. The qualities such as renewability, degradability, nutritional composition and edibility of the films make them desirable in packaging and coating applications (Buffo, Weller and Gennadios, 1997).

According to Gennadios and Weller (1990), edible coatings and films can be defined as thin layers applied on (or even within) foods by wrapping, immersing, brushing, or spraying in order to offer a selective barrier against transmission of water vapour, gases, solutes together with mechanical protection. Coatings are directly applied on the surface of products, while films are generally referred to as thin sheets that are formed separately and wrapped around the products. However both terms are used interchangeably. Edible coatings can be of different types, have different properties and applications.

2.2.2.1 Types of edible coatings

Edible coatings can be polysaccharide, protein, lipid based or a composite with added plasticizers such as polyhydric alcohols, waxes and oils to improve the flexibility and practicability of the polymeric substances.

- Polysaccharide based

A variety of polysaccharides and their derivatives that have shown potential as edible coatings include alginate, pectin, carrageenan, starch, starch hydrolysate, chitosan, gum and cellulose and its derivatives. These coatings are generally good gas barriers, but their hydrophilic nature makes them poor moisture barriers (Kester and Fenemma, 1988). The poor moisture barrier properties can be enhanced by certain polysaccharides when used in the form of a gelatinous coating. The gel then acts as a sacrificing agent rather than a moisture barrier.

Among the above mentioned polysaccharide based coating, cellulose and chitosan have shown some promise in extending the shelf life quality of MPFV as they adhere well to cut surfaces (Baldwin, Nisperos-Carriedo and Baker, 1995b). Nature Seal®, a cellulose based coating was formulated by the USDA and patented by the federal government in USA. The cellulose in Nature Seal® has been modified to provide a good gas and moisture barrier. The future of

polysaccharide based coatings depend on further research to tailor the coating characteristics to specific fruits and vegetables to allow proper permeability to enhance quality without allowing anaerobic respiration (Nisperos-Carriedo, 1994).

- Protein based

Proteins are good film formers and it can be derived from maize zein, wheat gluten, soy protein, egg albumen, collagen (Baldwin *et al.*, 1995b) and sorghum kafirin (Buffo *et al.*, 1997). Protein based coatings adhere well to hydrophilic surfaces, have good gas barrier properties, but do not resist water vapour diffusion (Gennadios and Weller, 1990). As an advantage, protein can add a critical nutritional component, but the use of protein from animal origin may raise some concerns among certain religious and vegetarian consumers, and some proteins have known allergenic effects. Edible coatings such are collagen is used as casings for hard sausages. Other protein coatings are used as casings for pharmaceutical tablets, confectionery products and have shown potential for fruits and vegetables (Gennadios, McHugh, Weller and Krochta, 1994).

- Lipid based

Lipid based coatings for food products have been practised for centuries as wax and oil coatings, but nowadays they are often used with solvents, emulsifiers, surfactants and plasticizers to improve their properties (Hernandez, 1994). Lipid based coatings are made up of bee wax, carnauba wax, candelilla wax, mineral oil, vegetable oil, acetylated monoglycerides, stearic acid, lauric acid and sucrose esters of fatty acids. These coatings are generally effective barriers to moisture, but have low gas permeability characteristics (Hagenmaier and Shaw, 1990; Hagenmaier and Shaw, 1992).

♦ Composite type

In the light of the advantages and disadvantages of various coating components, many formulations comprising the different components in the form of emulsions or bilayers have been investigated to improve the functional properties. Bilayer film of methyl cellulose and beewax as bilayer was found to have better moisture properties than the methyl cellulose alone (Greener and Fenemma, 1989).

2.2.2.2 Functional properties of edible coating

The possible functional properties of edible coatings as quoted by Kester and Fenemma (1988) are:

- Retards moisture, oil and fat migration
- Retards gas and solute transport
- Imparts added structural integrity to food
- Retains volatile flavour compounds
- Carriers of food additives

Ideally, for MPFV, edible coatings should create a barrier that can retard loss of flavour volatiles and water vapour vapour together with restricting exchange of gases. The exchange of gases will ultimately create an internal modified atmosphere without any anaerobiosis (Baldwin *et al.*, 1995a). Thus, concerning MPFV, the gas and moisture barrier properties received most attention.

Most of the permeability properties of edible coatings/films were done in model systems. Generally, films are formed and are kept under controlled environmental conditions (temperature and relative humidity) and the vapour and gas permeability is determined (Gennadios, Weller and Testin, 1993; McHugh, Huxsoll and Krochta, 1996). No such literature was found where the permeability was determined on a MPFV system and Park (1999) suggested that in future, prediction of internal gas composition of fruit and vegetables is important to verify the actual permeability properties of edible coatings.

The functional properties of edible coatings are affected by many factors. Increasing temperature and relative humidity increase the gas permeability properties (Gennadios *et al.*, 1993; McHugh *et al.*, 1996). This increase in permeability followed the Arrhenius activation energy model for the temperature and exponential function for the relative humidity. Other factors that affect the coating functionality as stated by Avena-Bustillos and Krochta (1994) are the commodity species and cultivar, uniformity and thickness of coating, respiration rate, the physiological state of the produce, source of produce and storage conditions.

Among future research on edible coating, Baldwin *et al.* (1995a) suggested that it is important to establish whether there is an interaction between the use of coatings and semi permeable plastic packaging as together they potentially provide double semi permeable boundary to gases and water vapour around the coated and wrapped product. This interaction could be synergistic and could lead to a better enhancement of the product quality. In addition it might also help for a better design in edible coating experiments to remove the potential influence of packaging during storage.

2.2.2.3 Benefits of using edible coatings on minimally processed fruits and vegetables

The use of edible coatings for minimally processed fresh produce is at the experimental stage. Most of the published articles on the application of edible coating are mainly on fresh fruits and vegetables. In addition, most researchers have concentrated on the physiochemical rather than microbiological quality.

The main defect of minimally processed carrots known as white blush can be controlled effectively by edible coatings (Avena-Bustillos, Cisneros-Zevallos, Krochta and Saltveit, 1994; Chen, Campbell, Grant, Li and Barth; 1996; Howard and Dewi, 1996; Cisneros-Zevallos, Krochta and Saltveit, 1997; Li and Barth, 1998). In addition to the control of white blush in minimally processed carrots as

shown in Figure 3, edible coatings were found to control the lost of carotenoids as shown in Figure 4, suggesting a better control in nutritional quality. Treated carrots with Nature Seal® showed greater sensory quality in terms of orange colour intensity, fresh carrot aroma, fresh carrot flavour and overall quality. The coating did not affect the microbiological counts in terms of yeast and moulds, lactobacilli and total plate count after 3 weeks as compared to the control (Howard and Dewi, 1995). These carrots were stored in a semi permeable pack at 1.7 ^{0}C. The microbiological consequence might also be a result of the modified atmosphere created by the semi permeable pack. The storage temperature does not reflect the real market situation where temperature can be about 10 ^{0}C. Thus it cannot be concluded that the coating did not affect the microbial growth.

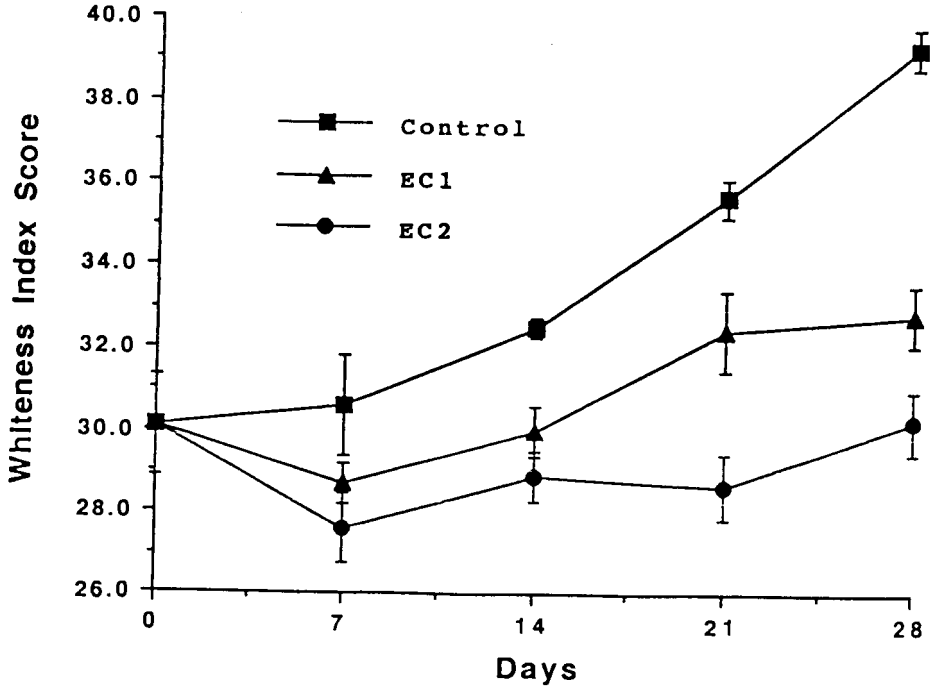

Figure 3: Whiteness Index scores of lightly processed carrots treated with Nature Seal® edible coating (EC1: pH 2.7, EC2: pH 4.6) and stored at 1 ^{0}C at 92% relative humidity in semi permeable polymeric bag (Li and Barth, 1998).

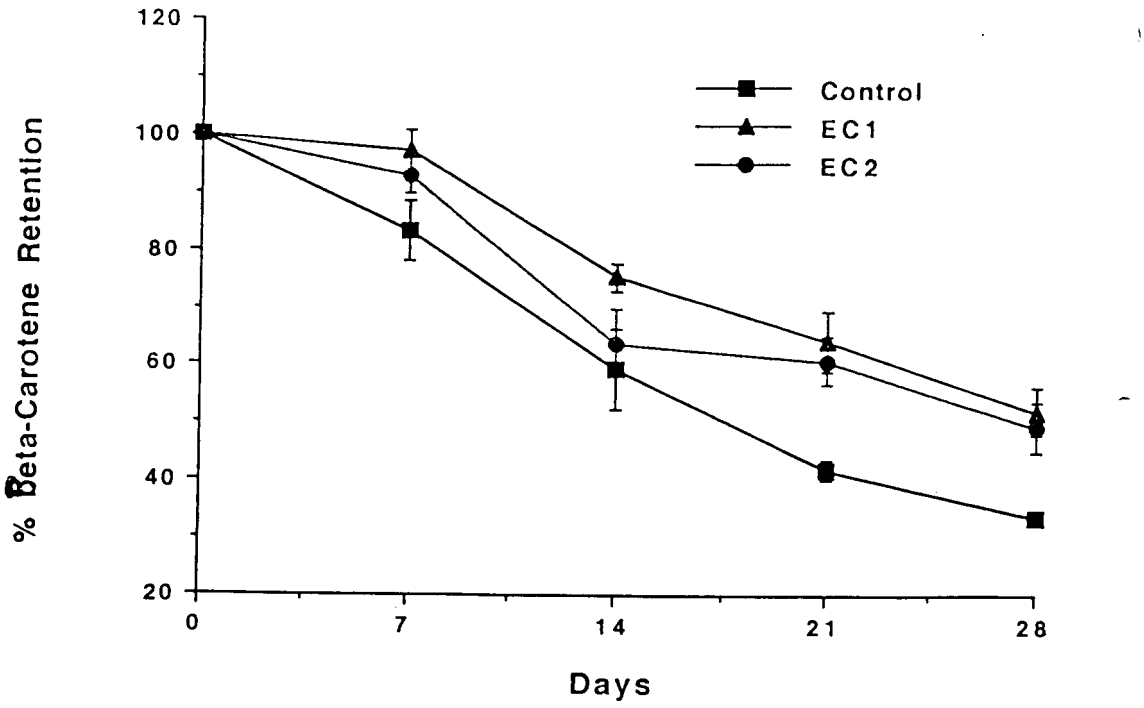

Figure 4: Beta carotene retention of lightly processed carrots treated with Nature Seal® edible coating (EC1:pH 2.7, EC2: pH 4.6) and stored at 1 ^{0}C at 92% relative humidity in semipermeable polymeric bag (Li and Barth, 1998).

Other applications of edible coatings on fresh and minimally processed fruits and vegetables can be summarized as follows:

- Reduction of weight loss by Nature Seal® with soy protein coated on cut potato and apple (Baldwin, Nisperos-Carriedo, Chen and Hagenmaier, 1996)
- Better browning and microbial control by Nature Seal® adjusted to a pH of 2.5-3.0 with an acidulant, together with preservatives and antioxidant on coated fresh cut potato and apple (Baldwin *et al.*, 1996)
- Firmer fruit with high titratable acidity, better colour retention and decrease in respiration rate and fruit decay by the chitosan coating on fresh strawberries (Ghaouth, Arul, Pannampalam and Boulet, 1991)

- Significant decrease in innoculated *Salmonella montevideo* by 2 log cycles on fresh tomato skin by cellulose based coating with acetic and citric acid (Zuang, Beuchat, Chinnan, Shewfelt and Huang, 1996)
- Delay in ripening of tomatoes by application of corn zein film (Park, Chinnan and Shewfelt, 1994)
- Lower respiration rate and better colour firmness retention in green ball peppers with mineral oil based coating (Lerdthanangkul and Krochta, 1996)
- Semperfresh® was found to delay changes in firmness, vitamin C and weight loss (Sumuard and Bayindirh, 1995) and control of the physiological disorder, scald (Chellew and Little, 1995) in coated apples.

2.2.3 Other potential hurdles

During minimal processing of fruits and vegetables, sanitizers such as sodium hypochlorite and hydrogen peroxide are often used to reduce initial microbial load. Microbial load was found to reduce by 1-2 log cycles compared with water when chicory was disinfected with 10% hydrogen peroxide for 2 minutes (Bennik, Peppelenbos, Nguyen-The, Carlin, Smid and Gorris, 1996). Similarly, application of active chlorine at 250 ppm produced a one log decrease in washed salads (Adams, Hartley and Cox, 1989).

Irradiation has the potential to improve the quality of minimally processed carrots. Irradiated samples of carrots that were minimally processed had higher quality, better carotene retention, better orange colour retention, inhibited aerobic mesophilic and lactic acid microflora compared with non irradiated ones (Chervin and Boisseau, 1994). After 9 days of storage, irradiated minimally processed carrots had a microbial load of 3.1 log cfu/g in contrast to 4.9 log cfu/g for the non irradiated shredded carrots (Hagenmaier and Baker, 1998).

The white surface discolouration can be controlled by steam treatment. When carrot sticks were steam treated on both sides for 45 seconds at 99 ^{0}C, control of the white discolouration was achieved and appeared to be reduced due to the retardation of phenylpropanoid metabolism for lignin formation (Howard, Griffin and Lee, 1994). In addition, acidic or basic solution have shown potential to control surface white discolouration of minimally processed carrots (Bolin and Huxsoll, 1991).

Combination of hurdles can have an additive or synergistic effect that can further increase the shelf life of the produce (Leistner and Gorris, 1995). Table 8 below summarise and compare the benefits of edible coatings and the polymeric packaging films as reported previously for minimally processed carrots. It can be observed that the packaging and the edible coatings have specific benefits and some effects still need some clarification. The coatings seem to control the white discolouration of carrots whereas polymeric packaging films are capable of reducing the microbial load. Thus in combination they could provide a beneficial effect in extending the shelf life of minimally processed carrots

Table 8: Comparison between the effects of edible coatings and polymeric packaging films on the metabolic reactions and microbial growth for minimally processed carrots.

	Polymeric packaging films	Edible coatings
Metabolic reactions	Decrease respiration rate and other metabolic reactions	Decrease metabolic reactions
Microbiology	Decrease microbial load, but can alter microflora	No effects reported in available literature
White discolouration	No research reported the effects on the white blush formation	Clearly shown to control the defect

2.3 Summary

- MPFV are very susceptible to deteriorative changes and pre-processing, processing and post-processing factors influence these changes.
- Moisture loss, increased respiration rate, wound ethylene production, colour changes, loss in firmness and off flavour development are the most important changes that occur in MPFV.
- The main microbial spoilage concerns are the pseudomonads and lactic acid bacteria; and for microbiological safety, *Listeria monocytogenes* is of concern in low acid foods.
- White blush is a visual defect in minimally processed carrots and microbial spoilage of this produce is mainly through fermentation by the lactic acid bacteria.
- Modified atmosphere using polymeric packaging films of specific permeabilities can be very desirable as it reduces metabolic activities to an optimum level without resulting in anaerobiosis.
- Modified atmosphere packaging generally decreases the microbial load, but can alter the microbial flora to anaerobes or microaerophilic bacteria such as lactic acid bacteria.
- A high permeability polymeric packaging film of about 10,000 to 20,000 $cm^3/m^2/day/atm$ at 25 ^0C is generally recommended for MPFV because of high respiration rates.
- Edible coatings have the potential to enhance the quality of MPFV. It can control the surface white discolouration of minimally processed carrots.
- No literature was found to show whether there is an interaction between the effects of edible coating and polymeric packaging films to enhance the quality of MPFV.

3. OBJECTIVE AND HYPOTHESIS

3.1 Objective

The objective of the research project was to determine the effects of polymeric packaging films and edible coating alone and in combination on the quality of minimally processed carrots in terms of the following:

- Physiological and biochemical quality as measured by the head space gas composition and pH
- Microbiological quality as measured by rotting index, lactic acid bacteria, psychrotrophs and yeast and moulds.
- Sensory quality as measured by the whiteness index and chroma values.

3.2 Hypothesis

Combination of polymeric packaging films and edible coatings can provide a double (coating and polymeric film) semi permeable barrier to gases and water vapour as compared with a single barrier (either polymeric packaging film or edible coating) when used alone. This double barrier system (Figure 5) may have additive or synergistic effects in extending the shelf life of minimally processed carrots by slowing down the metabolic reactions and microbiological spoilage and growth.

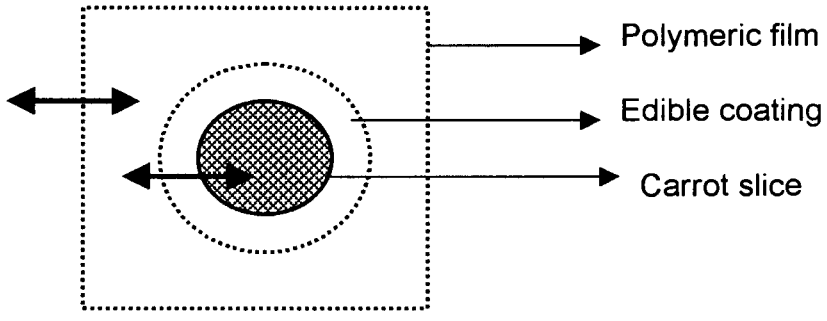

Figure 5: Schematic representation of carrot slice, coated and packaged in a polymeric film, ⟷ indicates the movement of gases and water vapour.

4. MATERIALS AND METHODS

4.1 Minimal processing of carrots

4.1.1 Raw material

Carrots (*Daucus carota*), cultivar Nantes were used for the experiments. Nantes cultivar is generally of a uniform shape and high quality. They are slender, cylindrical and possess a rich internal colour. The raw materials were from the same harvest batch and field. Thus, the raw materials used were uniform since any potential biotic (cultivar) and abiotic (environment and harvest date) factors that could affect the physiological status of carrot storage roots (Suojala, 2000) were eliminated.

The raw materials were supplied by Rosaly Boerdery. After harvesting, the carrots were washed in cold tap water, the leaves discarded, packed into 1kg fully permeable plastic bags and transported to Pretoria market under refrigerated conditions before sunrise. The carrots were collected early in the morning from the Pretoria market to the University of Pretoria and they were immediately separated into two batches (A and B) and stored in a cold room (2-3 ^{0}C) on arrival at the university. Batch A was processed on the same day whereas batch B was stored in the cold room (2-3 ^{0}C) for 48 hours before being processed.

4.1.2 Experimental layout

The experimental layout was a randomised block design with a three-way factorial experiment. Each batch was taken as a block effect to eliminate any potential differences between the two batches.

To address the research objectives, a factorial experiment of 3 Coatings x 3 Polymeric packaging films x 4 Sampling days were investigated. The edible

coatings and the polymeric packaging films were Nature Seal CA1® at three concentrations and P-Plus® of three different gas permeability respectively.

Nature Seal® is a cellulose based edible coating developed and patented by the United State Department of Agriculture. The specific Nature Seal CA1® was designed to treat cut carrots at a commercial scale in England. According to the manufacturer, Nature Seal CA1® improved the quality of minimally processed carrots. Nature Seal CA1® is not used locally.

The P-Plus® packaging material was supplied by Celluphane Distributors Africa (local agent of Danisco Flexible packaging, UK). P-Plus® is an oriented polypropylene flexible film that is microperforated to give desirable gas and water vapour permeability. The P-Plus® is used locally to pack fresh and minimally processed fruits and vegetables.

The coatings and polymeric packaging films treatments were as follows:

- Coatings
    - c0 as control, coating at 0% or no coating (only a water dip)
    - c7.5, Nature Seal CA1® coating at 7.5% (w/w) of the concentrate in water.
    - c15, Nature Seal CA1® coating at 15% (w/w) of the concentrate in water
- Polymeric packaging films of 200 x 270 mm bags
    - pi, fully permeable P-Plus® packaging film to water vapour and gases achieved by making 16 equally spaced holes of 3mm in diameter of the P-Plus® packaging film p160
    - p160, semi-permeable P-Plus® packaging film with oxygen and carbon dioxide permeability of about 14,000 cm^3/linear meter/day/atm or about 35,000 cm^3/m^2/day/atm at 25 ^{0}C. The water vapour permeability is about 0.9g H_2O/m^2/day at 25 ^{0}C and 75% R.H

- p90, semi-permeable P-Plus® packaging film with oxygen and carbon dioxide permeability of about 5200 cm^3/linear meter/day/atm or about 13,000 cm^3/m^2/day/atm at 25 ^{0}C. the water vapour permeability is about 0.9g H_2O/m^2/day at 25 ^{0}C and 75% R.H

The treated carrots were sampled on d0 (as reference values), d4, d8 and d12 to evaluate the changes with time.

The same experimental layout was used for both batch A and B. Each batch was replicated twice. Therefore, statistically there were two blocks and each block had two replicates or each individual combination for the factorial experiment had four replicates.

4.1.3 Processing protocol

The protocol used to produce the minimally processed carrots is illustrated in Figure 6. Strict hygiene was observed in the pilot plant and the temperature was kept at 10 ^{0}C during processing. The Nature Seal CA1® coating was prepared to 7.5 and 15% (w/w) in water according to the manufacturer's instruction. The Nature Seal CA1® concentrate was added to tap water and stirred on a warm plate at 30 ^{0}C. Then the coating solution was left in a fridge at 7 ^{0}C overnight prior to dipping of the carrot slices.

After sealing, the packed carrots were stored at 10 ^{0}C for 12 days. This storage temperature was selected, as it was more representatives of the commercial conditions in South Africa where the cold chain is frequently abused. Moreover, in an African perspective, where maintenance of cold chain is very expensive, most minimally processed fruits and vegetables in the store are at about 10 ^{0}C.

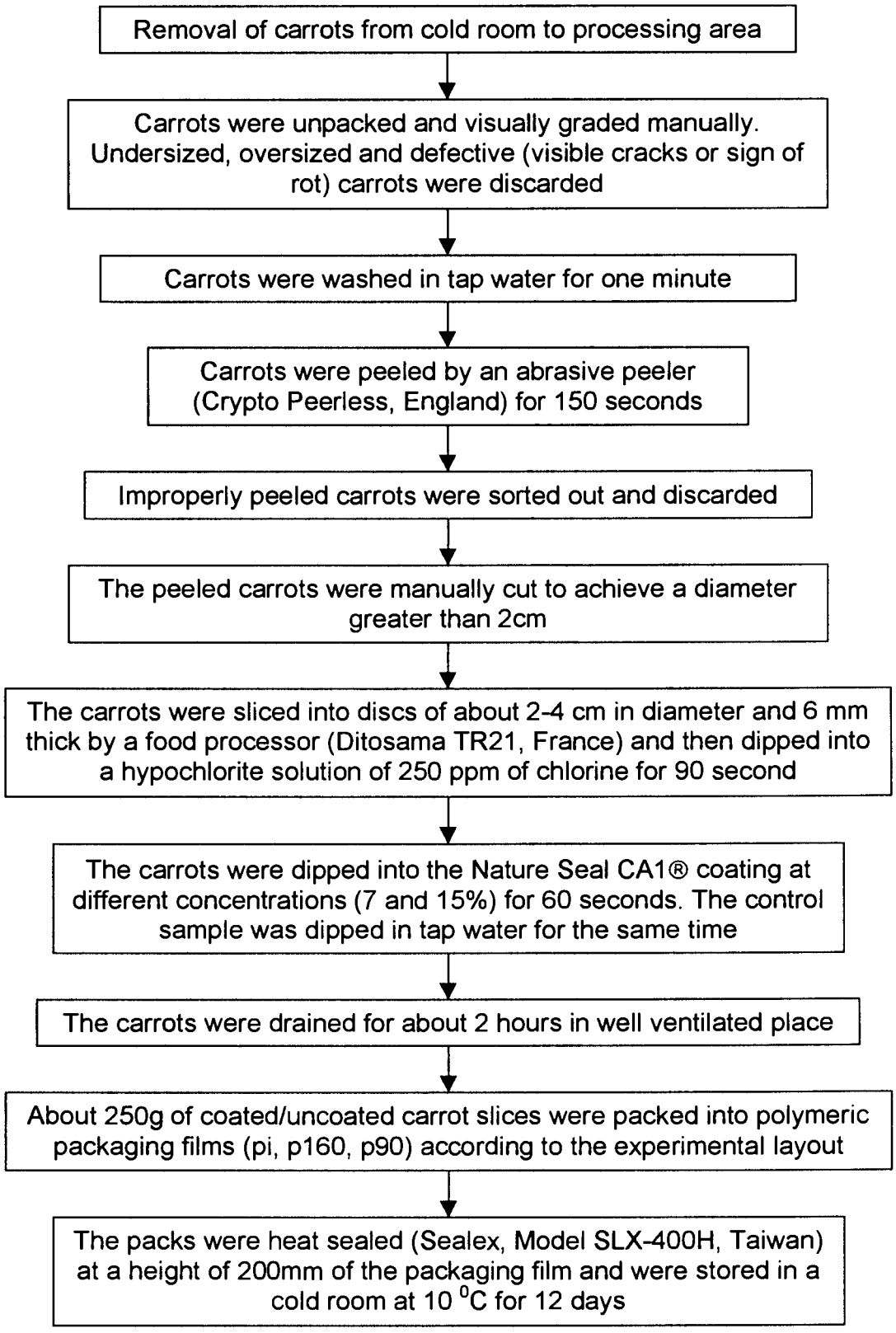

Figure 6: Flow chart showing the preparation of minimally processed carrot

4.2 Quality changes during storage

4.2.1 Sampling and analyses

The carrots were sampled for analyses on d0, d4, d8 and d12 to indicate the quality changes during storage. The analysis performed on the pack or carrot samples and its sequence is shown in Figure 7. The analysis of every pack/sample was done in duplicate except for the colour measurements that were taken on 12 slices of carrots. The sampling on d0 was taken as reference point. Carrots slices were sampled before and after coating dip for the analyses and were taken as d0 (as reference points). On d4, d8 and d12 sampling were done on individual packs that were treated and stored in the cold room.

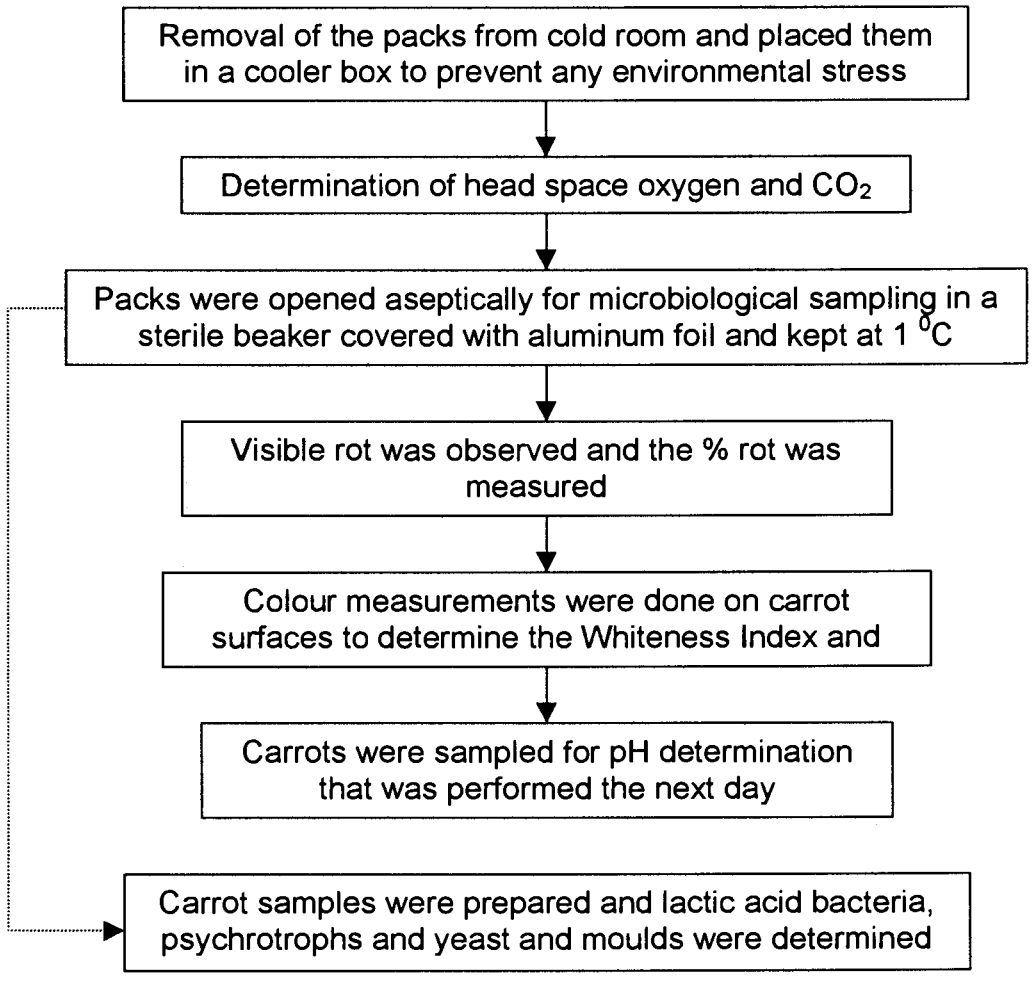

Figure 7: Flow of sequence of carrot sampling and analyses

The above sequence from Figure 7 was followed on all the sampling days to ensure no stress to the carrots, uniformity of sampling procedure and no cross contamination for the microbiological analysis.

4.2.2 Head space oxygen and carbon dioxide analysis

The head space analysis of oxygen and carbon dioxide of the packs were analysed with a gas analyser (Gas Space 2, System Instrument, UK). The apparatus was first calibrated with air (20.9% oxygen and 0.3%CO_2). Then a syringe was inserted in the package to suck in the head space and the head space O_2 and CO_2 was read as % when stable values appeared.

4.2.3 Colour measurements for Whiteness Index and Chroma

The colour measurements of the surface of the carrot slices were determined by a chromameter (Model CR 200, Minolta, Japan). The apparatus was first calibrated with a standard white tile to L, a and b values corresponding to 97.79, -5.55 and +7.35 respectively. L value refer to the lightness to darkness, a is from green to red and b is from green to yellow.

Then carrot slices were gently removed from the package by holding the sides to prevent any possible hydration of the surface as this could affect the WI (Cisneros-Zevallos *et al.*, 1995). Only carrots that faced the packaging films were sampled. This represented the slices where the consumer will first observe any defects The probe was first placed on the phloem region of the upper surface and then the phloem region of lower surface of the carrot disc and the L, a, and b values were read.

The upper surface refers to the side that faced the packaging film and the lower surface was the opposite of the same slice. Colour measurements were done on 12 slices for both the lower and upper surface per pack.

Using the recorded L, a and b values, the whiteness Index (WI) was calculated according to Bolin and Huxsoll (1991) and chroma was calculated according to Chervin and Boisseau (1994) for minimally processed carrots. The calculation is as follows:

- $WI = 100 - [(100-L)^2 + a^2 + b^2]^{1/2}$
- $Chroma = (a^2 + b^2)^{1/2}$

4.2.4 Microbiological spoilage and analyses

4.2.4.1 Rotting Index (%)

Rotting was indicated by any brown or black spots or surfaces of the carrots. The rotted slices were separated from the non-spoiled ones and both were weighed. The Rotting Index (%) was determined as follows

$$RI\ (\%) = \frac{A}{(A + B)} \times 100$$

Where RI is the rotting index, A is the amount of rotting carrot (g) and B is the amount of non-spoiled carrot (g).

4.2.4.2 Sample preparation for microbiological analysis

20 g of carrots was sampled from the pack under aseptic condition. One pack at a time was opened and carrot slices were taken with a sterile tongue at different locations in the bag in order to have a representative sample. The 20 g sample was homogenised with 200ml sterile peptone water (0.1% w/v) in a stomacher bag using a stomacher (Model Lab Blender 400, Art Medical Equipment, South Africa). Further decimal dilutions were performed using one ml of the homogenate and nine ml of the sterile peptone water (0.1% w/v) and for each dilution inoculation was done in duplicate with 1 ml of the solution. The

microbiological counts, namely lactic acid bacteria, psychrotrophs and yeast and moulds were calculated in $\log_{10}$ cfu/g of carrot.

4.2.4.3 Lactic acid bacteria

Lactic acid bacteria was enumerated using pour plate count method with lactobacillus MRS agar (Merck, 1990). The first layer of agar was covered with another layer, allowed to set, inverted and incubated at 37 ^{0}C for 3 days in a forced air circulation incubator (Garg, Churrey and Splittstoesser, 1990)

4.2.4.4 Psychrotrophic bacteria

Psychrotrophic bacteria were counted using Plate Count Agar (Merck, 1990). The culture medium was inoculated by using the pour plate method, inverted and incubated at 7 ^{0}C for 10 days.

4.2.4.5 Yeast and Moulds

Yeast and moulds counts were carried outon Potato Dextrose Agar using the pour plate method (Merck, 1990). The agar was adjusted to a pH of 3.5 by adding about 14 ml of 10% tartaric acid (w/v) prior to plating. The plates were inverted and incubated at 25 ^{0}C for five days.

4.2.5 pH measurements

50 g of carrots was sampled from a pack and was immediately frozen at -20 ^{0}C for the pH determination the next day. The frozen 50 g of carrots were blended (Warring Commercial Blender, USA) with 100 ml of distilled water at low speed for 30 seconds. The pH of the slurry was then determined with a pH meter (Mettler DL25, Switzerland).

4.3 Statistical analysis

The analysis of the factorial experiment was performed by a computerised Statistical Analysis Software system (SAS/STATS®, 1990) using the PROC GLM (procedure general linear model). A three-way analysis of variance (ANOVA) were done using packaging, coating and time as independent variables and the measured data as an dependent variable to give the main and two and three-way interactions effects between the variables. A blocking effect was also included due to the potential influence between two batches (A and B).

Following a significant ($P<0.05$) main or interaction effects, the data was summarized in a two-way table of means and orthogonal polynomial contrasts were performed on the data to yield linear, quadratic and/or cubic responses of the equally spaced factors only (coating, time and the coating x time interaction). Least significant means were also calculated at $P<0.05$ for the non-equally spaced packaging and its two and three way interaction between coating and time.

All the data was analysed according to the above mentioned procedure unless specified otherwise due to missing observations.

A paired t-test was also performed by SAS system (SAS/STATS®, 1990) according to the PROC means procedure to yield any difference between upper and lower carrot surfaces for the WI and Chroma values at $P<0.05$.

5. RESULTS

Only significant ($P<0.05$) main and interaction effects of the factorial combinations with packaging, coating and time are illustrated in this chapter. The mean values for each treatment combination with standard deviations are provided in Appendix A.

5.1.Head space oxygen and carbon dioxide values

The head space oxygen and carbon dioxide levels of the carrot packs were found to be influenced by packaging, coating and time. This as well as the two-way interactions between the packaging x coating, packaging x time and coating x time are shown in Table 9.

Decreasing the permeability of the packaging and increasing the coating concentration had a significant decrease in oxygen and increase in carbon dioxide as shown in Figure 8. Similarly, with time, the oxygen concentration decreased while the carbon dioxide level increased. In comparison to coating levels that gave a linear relationship, the changes between the time intervals showed a linear, quadratic and cubic contrast at $P<0.001$. There was a substantial change in the head space gas composition from d0 to d4; from d4 to d8 the concentration of oxygen and carbon dioxide was similar followed by a decrease in oxygen and an increase in carbon dioxide for d12 (Figure 8).

The packaging x coating interaction was less significant than the main effects (Table 9). This interaction showed that decreasing the packaging permeability further influenced the head space gas composition with increased coating concentration (Figure 9). It seemed that packaging had a more pronounced effect than coating to increase and decrease the carbon dioxide and oxygen levels respectively.

Table 9: P-values for 3-way ANOVA of head space oxygen and carbon dioxide to illustrate the effects of packaging, coating and time on the sliced carrots packs at 10 ^{0}C

Source of variation	Oxygen		Carbon dioxide	
	P- value	Significant contrast	P- value	Significant contrast
Block effect	P<0.05	-	NS	
Packaging (P)	P<0.001	-	P<0.001	-
Coating (C)	P<0.001	C-l***, C-qd*	P<0.001	C-l***
Time (T)	P<0.001	T-l***, T-qd*** T-cb***	P<0.001	T-l***, T-qd*** T-cb***
P x C	P<0.05	-	P<0.05	-
P x T	P<0.001	-	P<0.001	-
C x T	P<0.001	C-l x T-l** C-l x T-qd***	P<0.05	C-l x T-qd* C-qd x T-cb*
P x C x T	NS	-	NS	-

- not determined as not equally spaced.
l, qd and cb are linear, quadratic and cubic response respectively
*, **, *** show significance at P<0.05, P<0.01 and P<0.001 respectively
NS is not significant at P>0.05

The two-way interaction effect between packaging x time and coating x time showed similar trends (Figures 10 & 11). The change in the head space gas level seemed to be more pronounced from d0 to d4 followed by a plateau from d4 to d12. With time, decreasing the permeability of the packaging film further decreased the oxygen and increased carbon dioxide levels respectively. However, for the coating x time interaction, increasing the coating level from 7.5% to 15% did not significantly affect the head space gas concentrations.

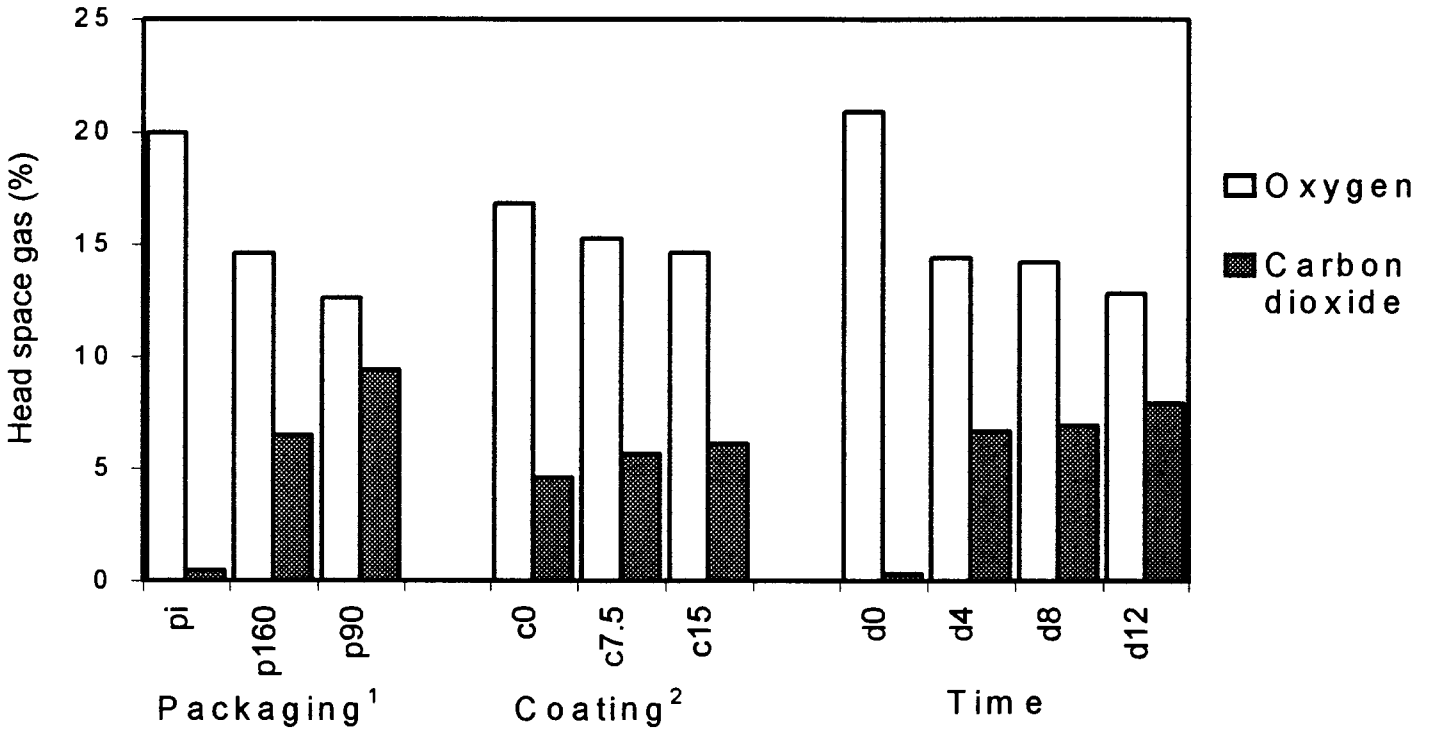

Figure 8: Effect of packaging[1], coating[2] and time on the head space gas composition of lightly processed carrots at 10 ^{0}C

[1] pi, p160 and p90 are fully permeable (control), semi permeable and least permeable P-Plus® packaging respectively

[2] c0, c7.5 and c15 are the Nature Seal® coating levels at 0 (control), 7.5 and 15 % respectively

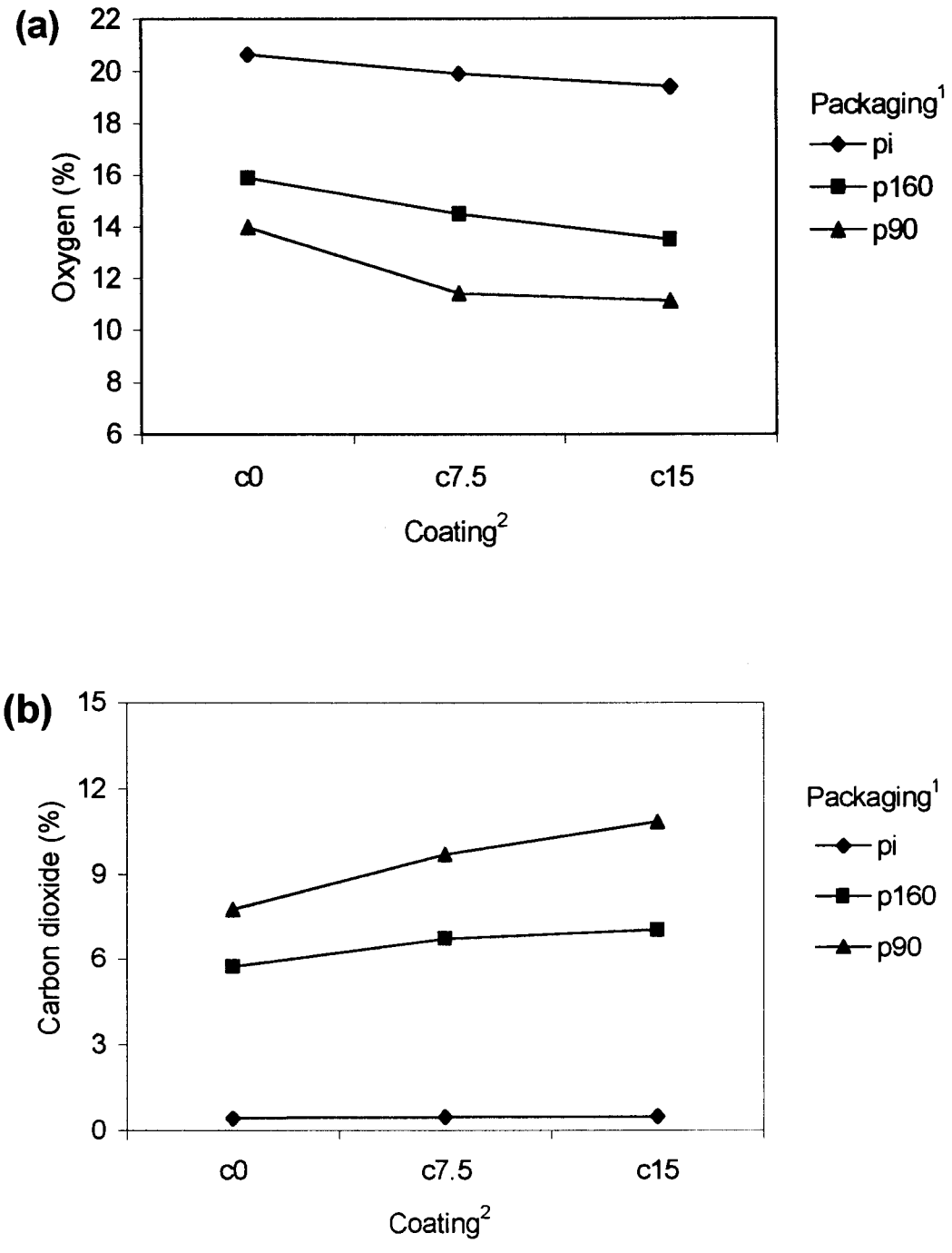

Figure 9: Plot of means of oxygen (a) and carbon dioxide (b) in the head space of the carrot packs showing the two-way interaction between the packaging[1] and coating[2]

[1] pi, p160 and p90 are fully permeable (control), semi permeable and least permeable P-Plus® packaging respectively

[2] c0, c7.5 and c15 are the coating Nature Seal® levels at 0 (control), 7.5 and 15 % respectively

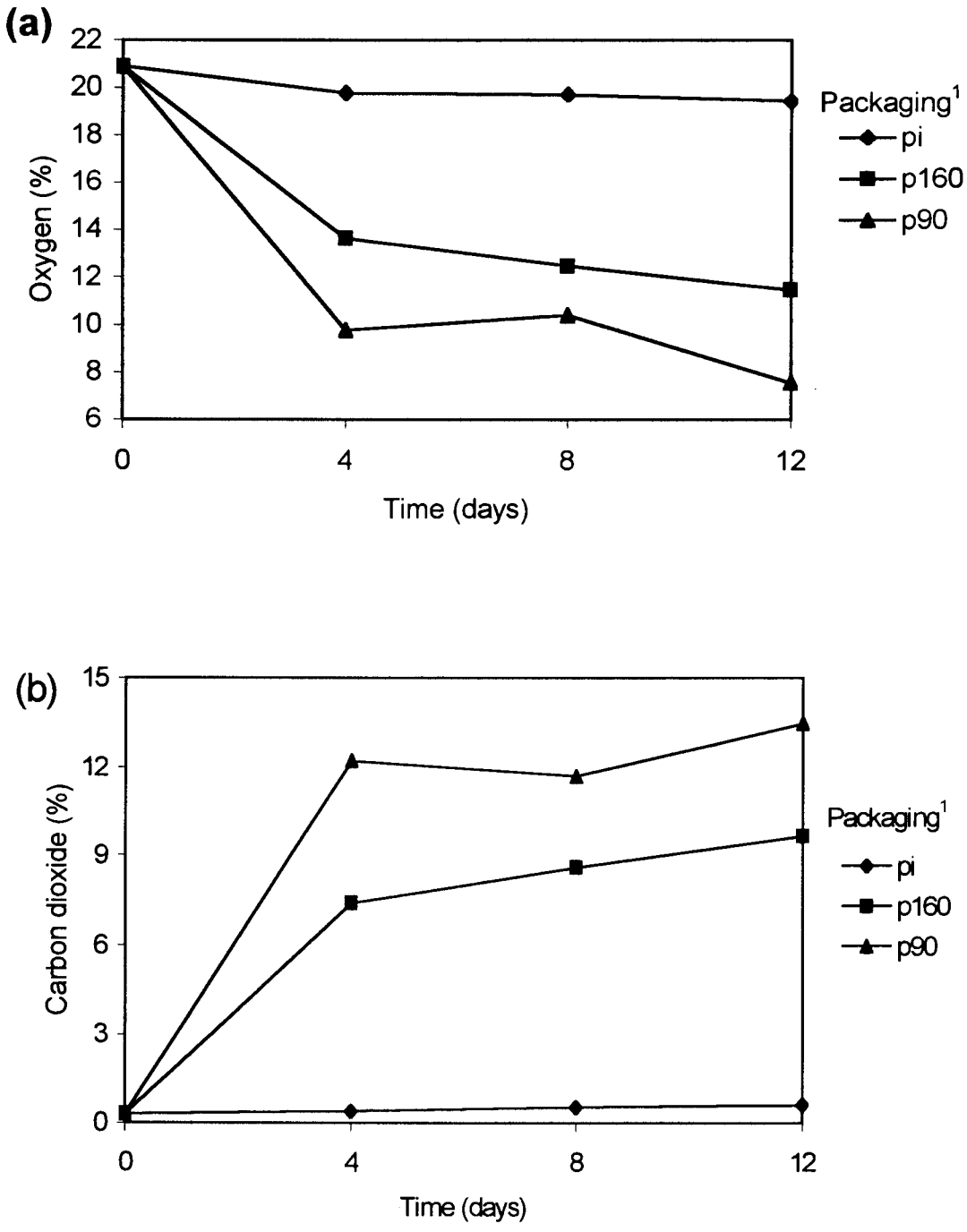

Figure 10: Plot of means of oxygen (a) and carbon dioxide (b) in the head space of the carrot packs showing the two-way interaction between the packaging[1] and time

[1] pi, p160 and p90 are fully permeable (control), semi permeable and least permeable P-Plus® packaging respectively

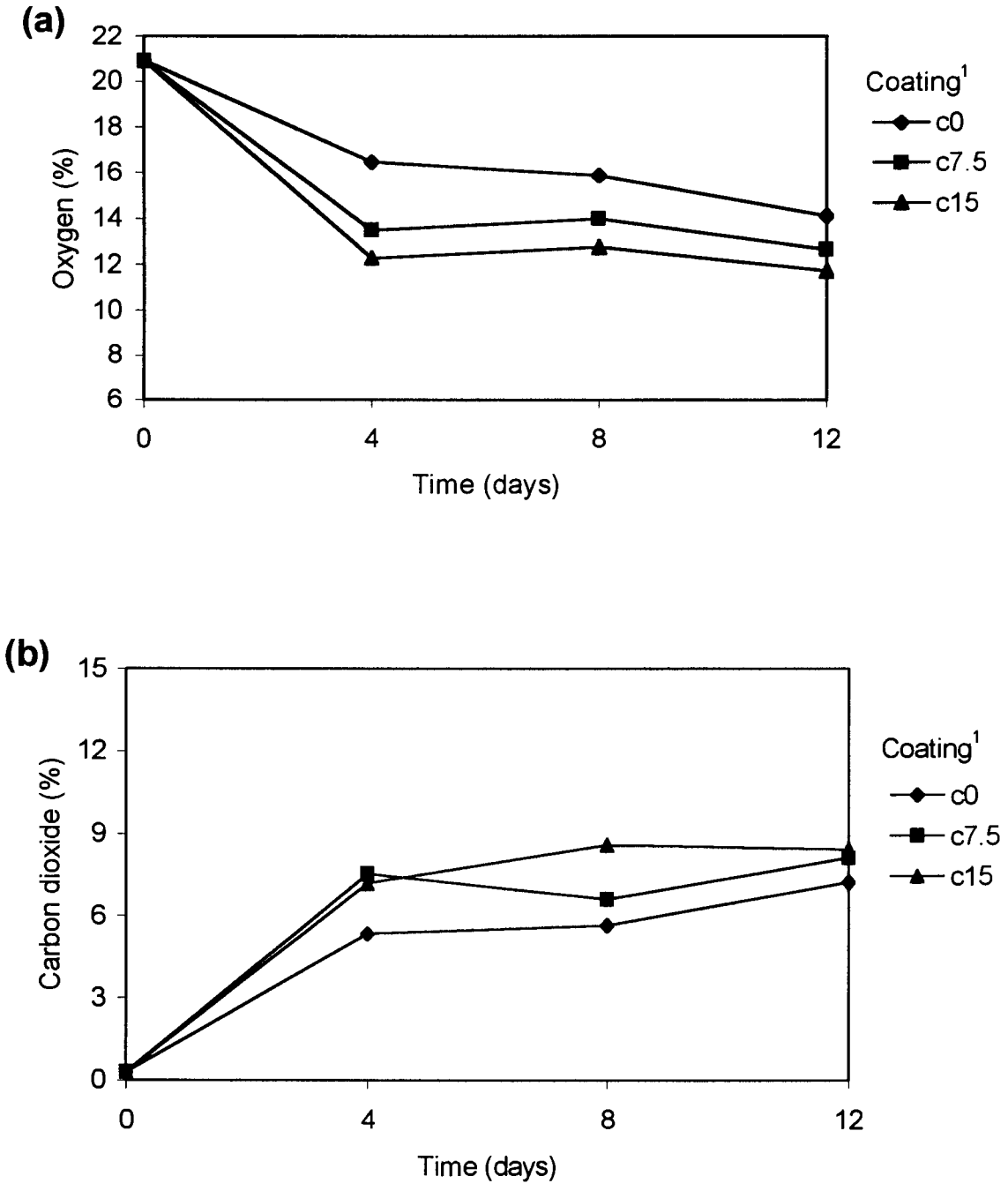

Figure 11: Plot of means of oxygen (a) and carbon dioxide (b) in the head space of the carrot packs showing the two-way interaction between coating[1] and time

[1] c0, c7.5 and c15 are the Nature Seal® coating levels at 0 (control), 7.5 and 15 % respectively

5.2 Colour measurements

5.2.1 Whiteness index scores

From the 3-way ANOVA in Table 10 (data from fully permeable packaging was removed because of spoiled carrots), it can be seen that only coating, time and their interaction significantly affected the white blush for both the upper and lower surfaces of sliced carrots. White blush occurrence is shown in Figure 12.

The coated carrot significantly ($P<0.001$) decreased the occurrence of white blush formation and increasing the coating concentration showed a significant linear contrast in controlling the defect (Figure 13). It seemed that the lower surface white discolouration was better controlled than the upper surface. In fact a comparison of the occurrence of white discolouration between the carrot surfaces revealed a significant ($P<0.001$) decrease from the upper to the lower surfaces[1].

The white discolouration of the carrots increased significantly ($P<0.001$) over time (Figure 13). This increase was more pronounced for the upper surface than for the lower one. From d0 to d12, a linear contrast was observed for increase in lower surface white blush compared with linear, quadratic and cubic contrasts for the upper surface (Table 10). The increase in the defect from d0 to d4 seemed to be of a higher magnitude for the upper surface than for the other time intervals.

The coating interacted with time to have a better control over the white blush of the minimally processed carrots (Figure 14). The interaction response was linear for coating and quadratic for time for the upper surface showing that the change in the whiteness index scores was of a higher magnitude from d0 to d4 compared with the other time intervals. However interaction of coating x time of the lower surface showed a linear contrast.

[1] The statistical analysis to compare the upper and lower surfaces are given in Appendix B

Table 10: P-values for the 3-way ANOVA of whiteness index to show the effects of the packaging[1], coating and time on the sliced carrots

Source of variation	Upper surface		Lower surface	
	P- value	Significant contrast	P- value	Significant contrast
Block effect	P<0.05	-	P<0.001	-
Packaging (P)	NS	-	NS	-
Coating (C)	P<0.001	C-l***	P<0.001	C-l***, C-qd**
Time (T)	P<0.001	T-l***, T-qd***, T-cb***	P<0.001	T-l***
P x C	NS	-	NS	-
P x T	NS	NS	NS	-
C x T	P<0.01	C-l x T-l**, C-l x T-qd***	P<0.01	C-l x T-l***, C-qd x T-qd*
P x C x T	NS	-	NS	-

[1]fully permeable pi packaging data was excluded because of spoiled packs
- no analysis as not equally spaced
l, qd and cb are linear, quadratic and cubic response respectively
*, **, *** is significant at P<0.05, P<0.01 and P,0.001 respectively
NS is not significant at P>0.05

Figure 12: Photograph of sliced carrots showing white discolouration for the control (on the right) compared with the coated sample (on the left) for the least permeable packaging (p90) on day 12

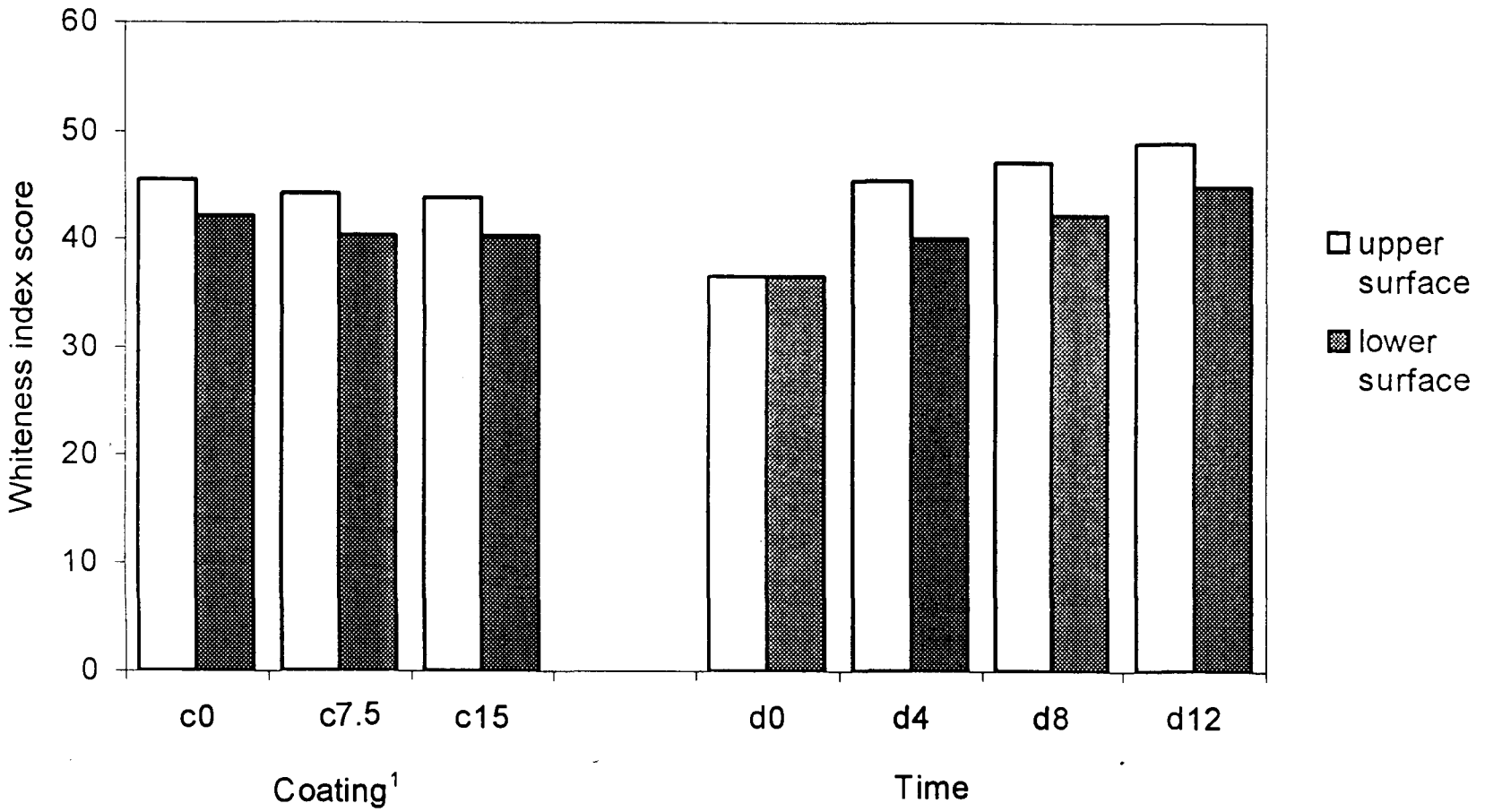

Figure 13: Main effect of coating[1] and time on the whiteness index scores of the sliced carrots

[1] c0, c7.5 and c15 are the Nature Seal® coating levels at 0 (control), 7.5 and 15 % respectively

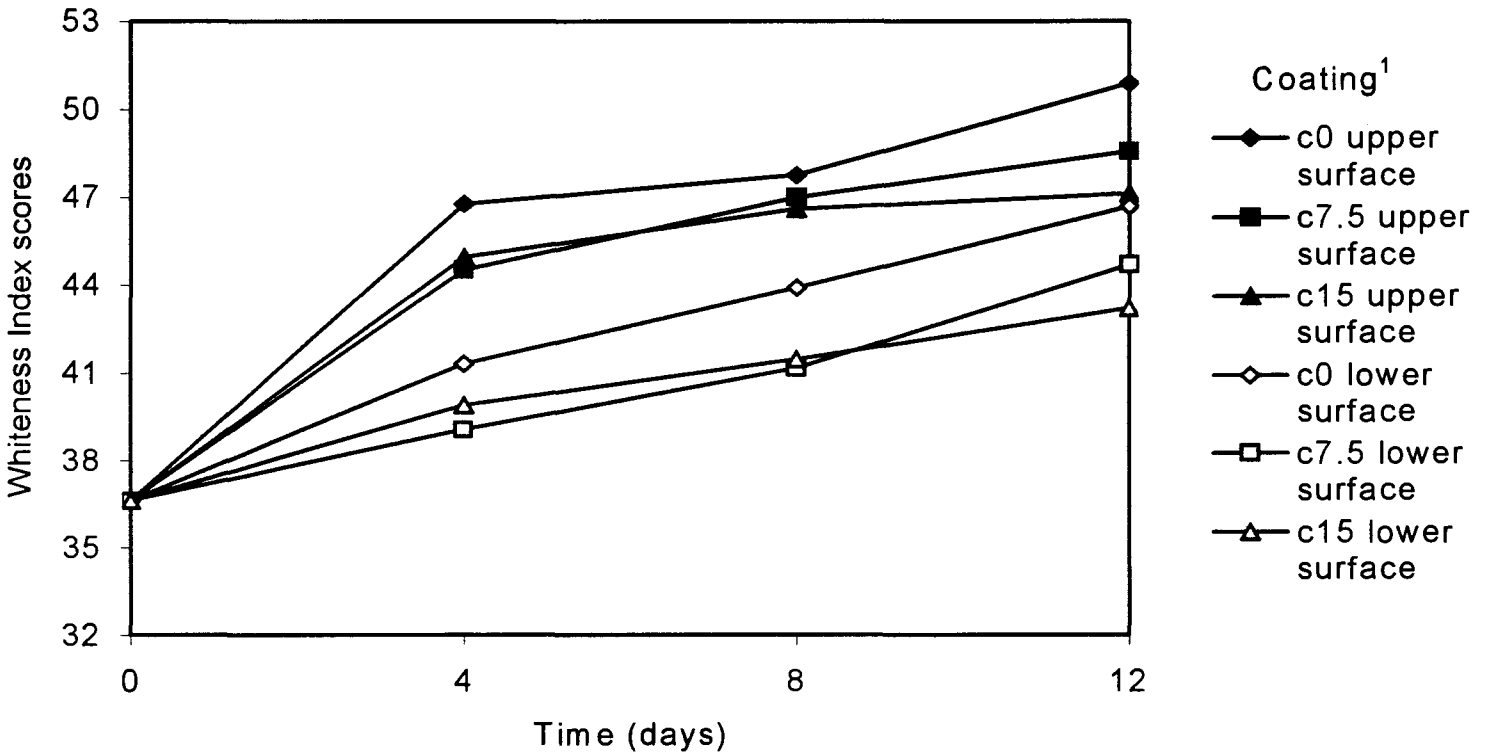

Figure 14: Plot of means of whiteness index scores of the carrot discs (both upper and lower surfaces) showing the two way interaction between coating[1] and time

[1] c0, c7.5 and c15 are the Nature Seal® coating levels at 0 (control), 7.5 and 15 % respectively

To show the influence of the fully permeable packaging (pi) compared with the other packaging films, a two-way ANOVA was performed on data from d4 only. The analysis revealed no significant ($P>0.05$) difference for the upper surface but a significant ($P<0.05$) difference for the lower surface. The fully permeable packaging (pi) enhanced the white blush of the carrot lower surface compared with the other packaging p90 (Table 11). The two-way interaction between packaging and coating for the lower surfaces showed that when decreasing the permeability from the fully permeable films to the less permeable ones, the white discolouration defect was better controlled with increasing coating level compared with the coating and packaging effects alone.

Table 11: The main effects of packaging and coating and their interaction effects on the whiteness index scores of the carrot lower surfaces on d4 stored at 10 ^{0}C

Package (P)[1]	Coating (C)[2]			Packaging main effect
	c0	c7.5	c15	
pi	43.77 d	40.78 bc	39.50 ab	41.35 b
p160	40.48 ab	39.25 ab	40.30 ab	40.01 b
p90	42.21 cd	38.90 a	39.48 ab	40.20 a
Coating main effect	42.16 b	39.64 a	39.76 a	
Coating contrast	C-linear***			

[1] pi, p160 and p90 are fully permeable (control), semi permeable and least permeable P-Plus® packaging respectively

[2] c0, c7.5 and c15 are the Nature Seal® coating levels at 0 (control), 7.5 and 15 % respectively

*** is significant at $P<0.001$

Different letters show significant differences at $P<0.05$ for the interaction data within cells, significant differences at $P<0.05$ for the main effect of packaging within its column and significant $P<0.001$ for the main effect of coating within its row

5.2.2 Chroma

Similar to the whiteness index values, all the data from the fully permeable pi packaging (control) had to be removed to perform a three-way ANOVA because of spoiled packs for d8 and d12. The three-way ANOVA showed that only coating, time and their interaction had a significant influence on the retention of the chroma values of the surfaces of the minimally processed carrot (Table 12). As the coating concentration increased, the chroma values were linearly retained, but a decrease in the chroma values was observed with time for both surfaces (Figure 15). With time, the lower surfaces showed a pronounced linear decrease compared with the upper surfaces. The latter had a higher decrease from d0 to d4 than the other time intervals (Figure 15). The coating and time interaction was of lower significance than the main effect for the upper surfaces, but not for the lower surface of the carrots. This interaction showed that with time, the coated carrots retained the chroma values better than for the non-coated ones (Figure 15).

Table 12: P-values for the 3-way ANOVA of chroma values of packaging[1], coating and time on the chroma values of sliced carrots at 10 ^{0}C

Source of variation	Upper surface		Lower surface	
	P- value	Significant contrast	P- value	Significant contrast
Block effect	NS	-	P<0.05	-
Packaging (P)	NS	-	NS	-
Coating (C)	P<0.001	C-l***, C-qd**	P<0.001	C-l***, C-qd**
Time (**T**)	P<0.001	T-l***, T-qd***, T-cb**	P<0.001	T-l***
P x C	NS	-	NS	-
P x T	NS	-	NS	-
C x T	P<0.05	C-l x T-l** C-l x T-qd***	P<0.001	C-l x T-l** C-qd x T-qd*
P x C x T	NS	-	NS	-

- no analysis as not equally spaced
l, qd and cb are linear, quadratic and cubic response respectively
*, **, *** is significant at P<0.05, P<0.01 and P,0.001 respectively
NS is not significant at P>0.05

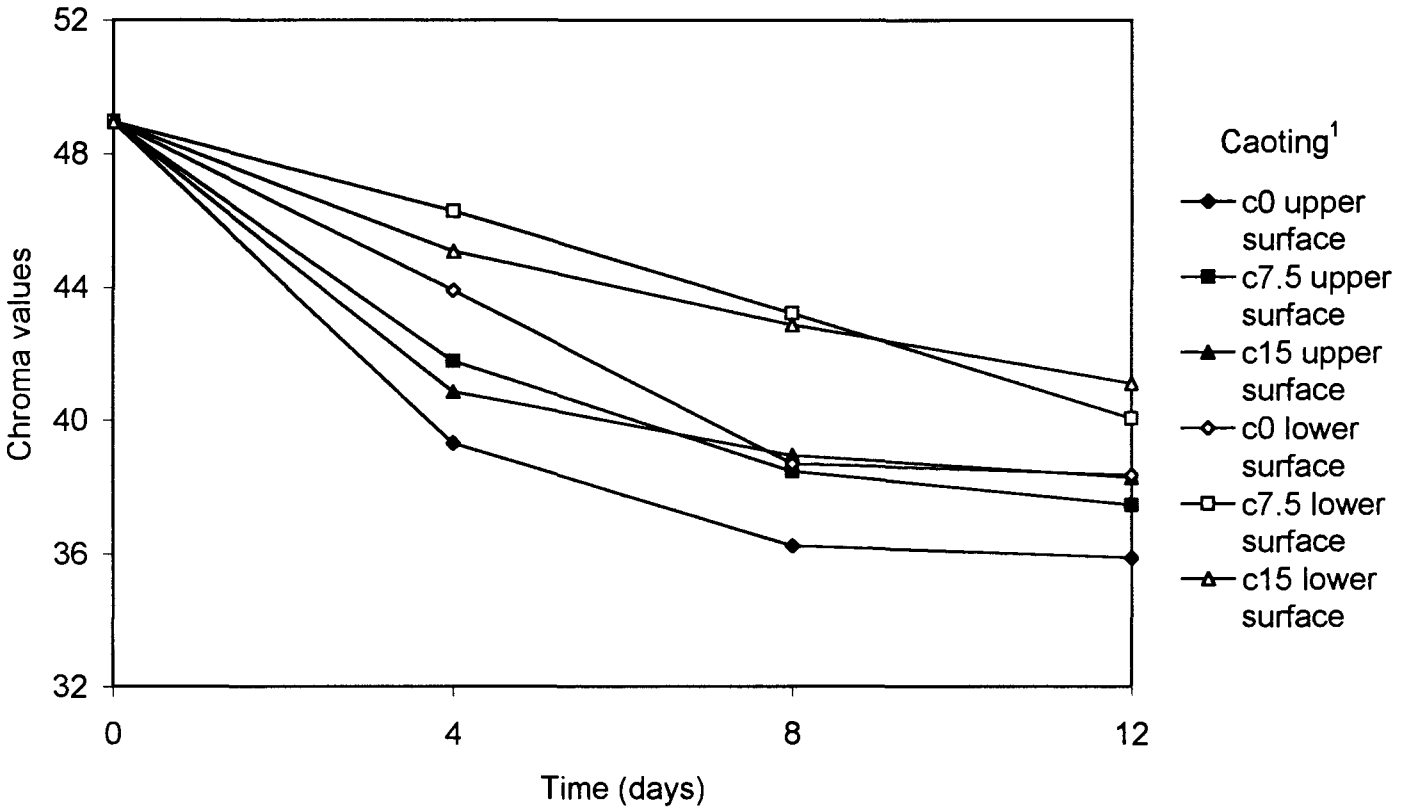

Figure 15: Plot of means of chroma values of the carrot discs (both upper and lower surfaces) showing the two-way interaction between coating[1] and time

[1]c0, c7.5 and c15 are the Nature Seal® coating levels at 0 (control), 7.5 and 15 % respectively

Similar to the whiteness index scores, the chroma values showed a significant ($P<0.001$) difference between the upper and lower surfaces[1]. The lower surfaces maintained chroma values better than the upper surfaces.

To show the influence of the fully permeable pack, a two-way ANOVA was performed between the packaging and coating on d4. The two-way ANOVA showed that the packaging only influenced the chroma values for the lower surface[2]. The semi permeable packaging (p160) and the least permeable packaging (p90) retained the chroma values of the carrot lower surfaces better than the control packaging (pi). However, no significant difference was found between the p160 and p90 packaging.

5.3 Microbiological spoilage

5.3.1 Rot incidence

Black spot, black patches and exudate in the packs were the typical symptoms for the spoiled carrots as shown in Figure16.

Figure 16: Spoiled carrots packed in fully permeable packaging (pi).

[1] The statistical analysis to compare of the upper and lower surfaces are given in Appendix C
[2] The two-way ANOVA can be found in Appendix D

The rot incidence only occurred in the fully permeable packaging (pi) on d8. On d12, the fully permeable packaging (pi) as control had a very high spoilage rate (63-98%) compared with the other packs that had a low (less than 10%) of rot incidence or no spoilage (Table 13). From Table 13, considering only the data from the fully permeable pi packaging, it can be seen that increasing the coating level linearly increased the rot incidence. Similarly, a significant increase in rot incidence was noted from d8 to d12. The time and coating interacted together to spoil the carrots at a faster rate.

Table 13: Effects of coating[1] and packaging[2] on the rot incidence (%)[3] of sliced carrots at 10 ^{0}C and two-way table between coating[1] and time

Time (days)	Packaging (P)[2]	Coating (C)[1]			Time effect[4]
		c0	c7.5	c15	
0	pi	not spoiled	not spoiled	not spoiled	
	p160				
	p90				
4	pi	not spoiled	not spoiled	not spoiled	
	p160				
	p90				
8	pi	31.07 a (±10.33)	65.45 b (±15.60)	84.76 cd (±1.26)	60.43 a
	p160	not spoiled	not spoiled	not spoiled	
	p90	not spoiled	not spoiled	not spoiled	
12	pi	70.80 bc (±11.69)	88.82 d (±4.01)	96.18 d (±2.26)	85.26 b
	p160	not spoiled	2.11 (±1.50)	0.47 (±0.93)	
	p90	not spoiled	2.18 (±1.49)	5.57 (±4.55)	
Coating	main effect	50.94 a	77.14 b	90.47 c	
	contrast		linear***, quadratic*		

[1] c0, c7.5 and c15 are the Nature Seal® coating levels at 0 (control), 7.5 and 15 % respectively

[2] pi, p160 and p90 are fully permeable (control), semi permeable and least permeable P-Plus® packaging respectively

[3] Each value represent a mean of four replicates with standard deviations in bracket

[4] Main effect and contrast is for data from d8 and d12 for the fully permeable packaging pi only

* and *** is significant at P<0.05 and P<0.001 respectively.

Different letters show significant differences at P<0.01 for the interaction data within cells, significant differences at P<0.001 for time within its row; and coating within its column

5.3.1 Lactic acid bacteria

The occurrence of lactic acid bacteria in the carrot packs was significantly affected by the packaging, coating, time and their two-way as well as three-way interactions (Table 14). Decreasing the permeability of the packs from a fully permeable packaging (both p160 and p90) was found to have a beneficial effect in controlling the growth of the lactobacilli. However no significant differences were observed between lactobacilli counts in the semi permeable packaging (p160) and the least permeable packaging (p90) (Figure 17).

Table 14: P-values for the 3-way ANOVA of packaging, coating and time on lactic acid bacteria counts of the carrots packs at 10 ^{0}C.

Source of variation	P- value	Significant contrast
Block effect	NS	-
Packaging (P)	P<0.001	-
Coating (C)	P<0.001	C-l***, C-qd***
Time (T)	P<0.001	T-l***, T-qd***,
P x C	P<0.05	-
P x T	P<0.001	-
C x T	P<0.001	C-l x T-l***, C- qd x T- l **
P x C x T	P<0.01	-

- no analysis as not equally spaced
l, qd and cb are linear, quadratic and cubic response respectively
*, **, *** is significant at P<0.05, P<0.01 and P<0.001 respectively
NS is not significant at P>0.05

From Figure 17, it was found that the coating enhanced the growth of the lactobacilli, but there was no significant difference between the two coating concentrations. With time, the growth of lactobacilli was more pronounced from d4 to d8 than the initial d0 to d4 and time seemed to have a more pronounced effect than packaging and coating.

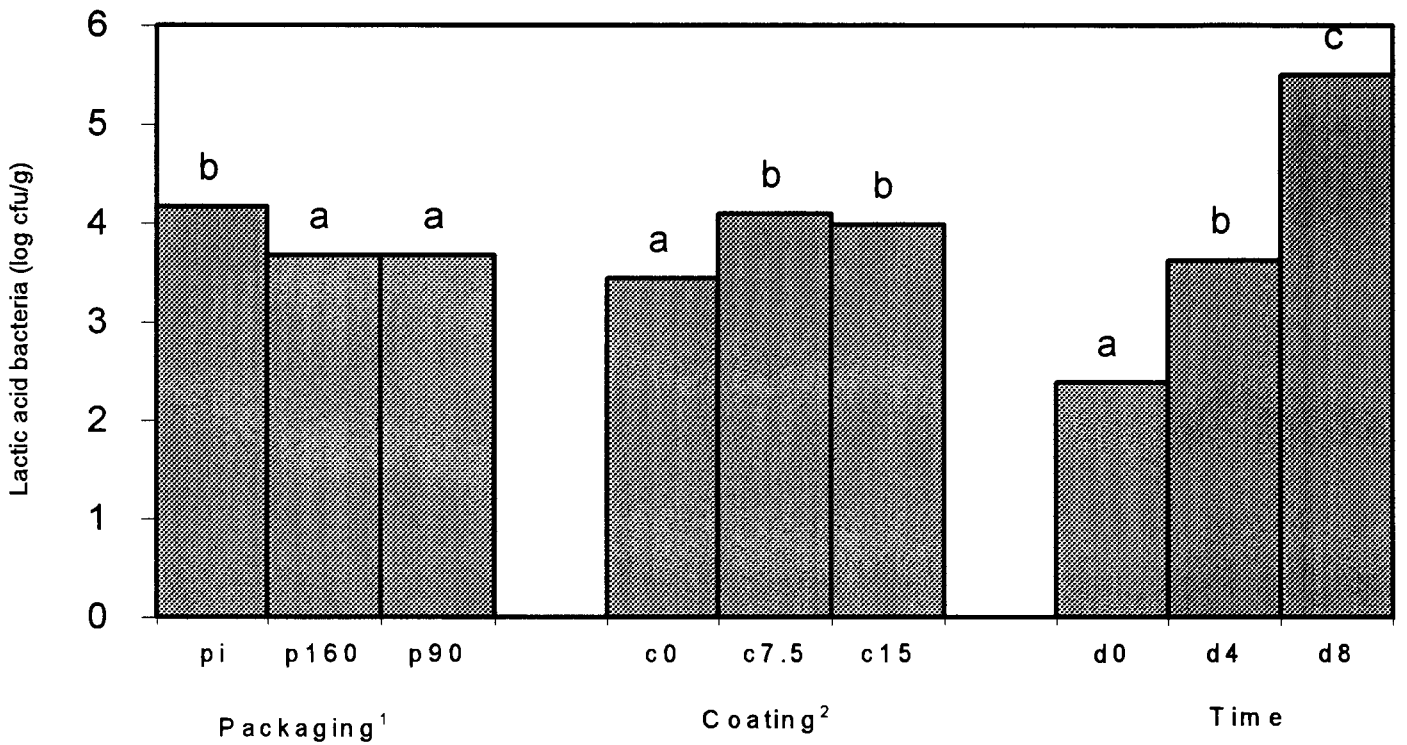

Figure 17: Effect of packaging[1], coating[2] and time on the counts of lactobacilli.

[1] pi, p160 and p90 are fully permeable (control), semi permeable and least permeable P-Plus® packaging respectively

[2] c0, c7.5 and c15 are the Nature Seal® coating levels at 0 (control), 7.5 and 15 % respectively

Different letters show significant differences at $P<0.001$ separately for packaging coating and time

Even though the interaction between coating and packaging was of less significance than that of the main treatments, the combination controlled the growth of lactobacilli more effectively compared with the fully permeable packaging (pi) (Figure 18). Similarly from Figure 19, it is evident that packaging treatments interacted with time to give a better control on the growth of lactobacilli in the carrot packs compared with the fully permeable packaging (pi). With time, the coated carrots showed a linear increase of higher magnitude in the lactic acid bacteria counts in comparison to the uncoated ones (Figure 20).

A three-way interaction as illustrated in Figure 21 showed that packaging, coating and time interacted to influence the lactic acid bacteria count on the carrots. It can be clearly seen that the semi permeable packaging (p90) and least permeable packaging (p160) decreased the counts of lactobacilli compared with the fully permeable (pi) packaging on d8. In addition, the coating effect in enhancing the growth of lactic acid bacteria seemed to be more pronounced on d8 than on d4.

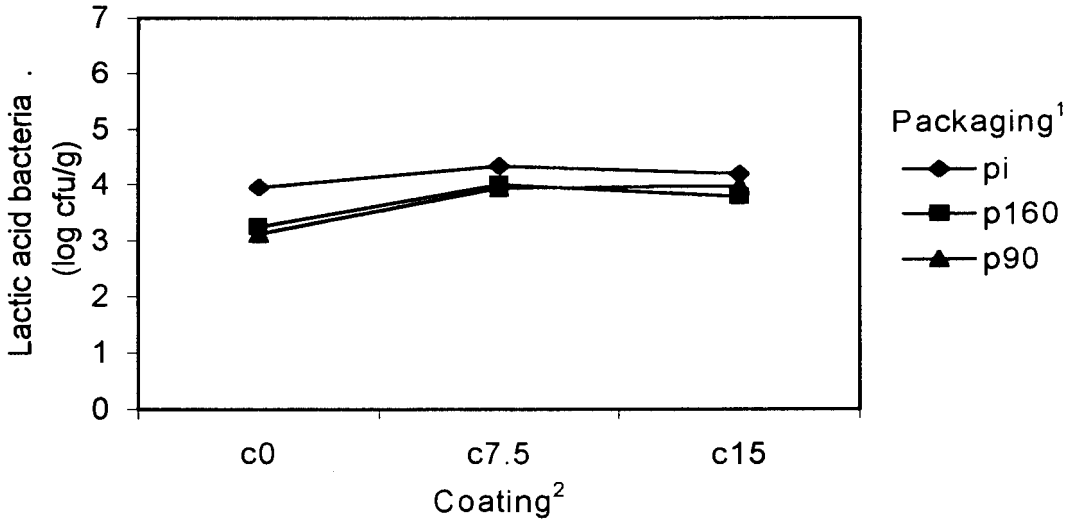

Figure 18: Plot of means of lactic acid bacteria counts showing the two-way interaction between packaging[1] and coating[2]

[1] pi, p160 and p90 are fully permeable (control), semi permeable and least permeable P-Plus® packaging respectively

[2] c0, c7.5 and c15 are the Nature Seal® coating levels at 0 (control), 7.5 and 15 % respectively

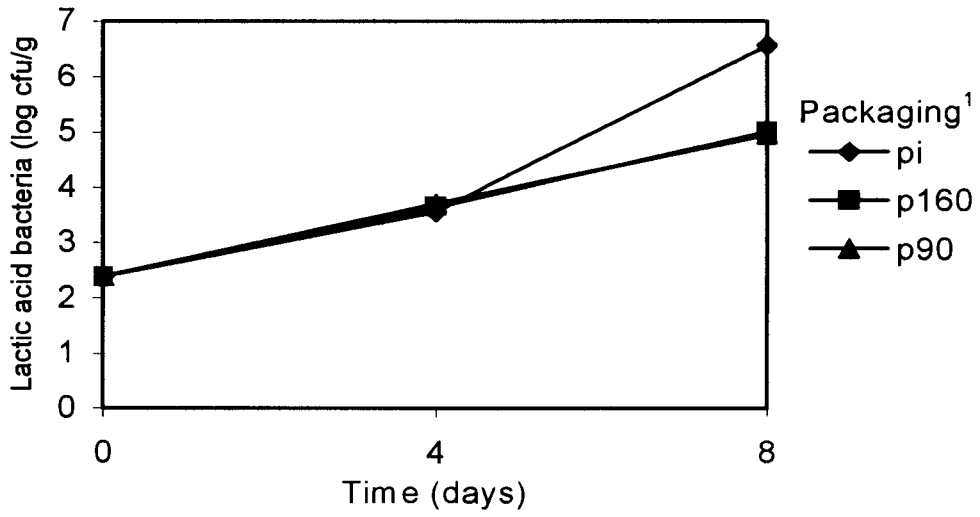

Figure 19: Plot of means of lactic acid bacteria counts showing the two-way interaction between and packaging[1] and time.

[1] pi, p160 and p90 are fully permeable (control), semi permeable and least permeable P-Plus® packaging respectively

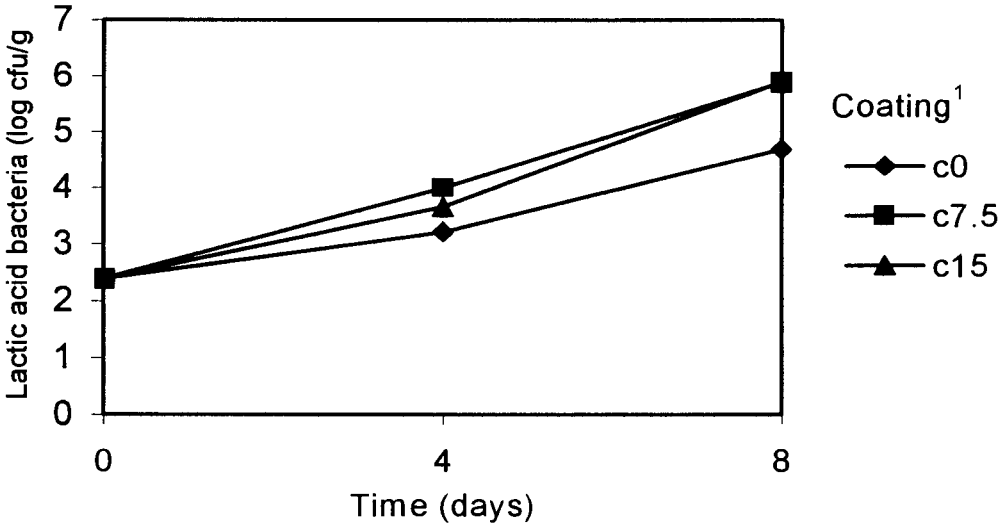

Figure 20: Plot of means of lactic acid bacteria counts showing the two-way interaction between coating[1] and time.

[1] c0, c7.5 and c15 are the Nature Seal® coating levels at 0 (control), 7.5 and 15 % respectively

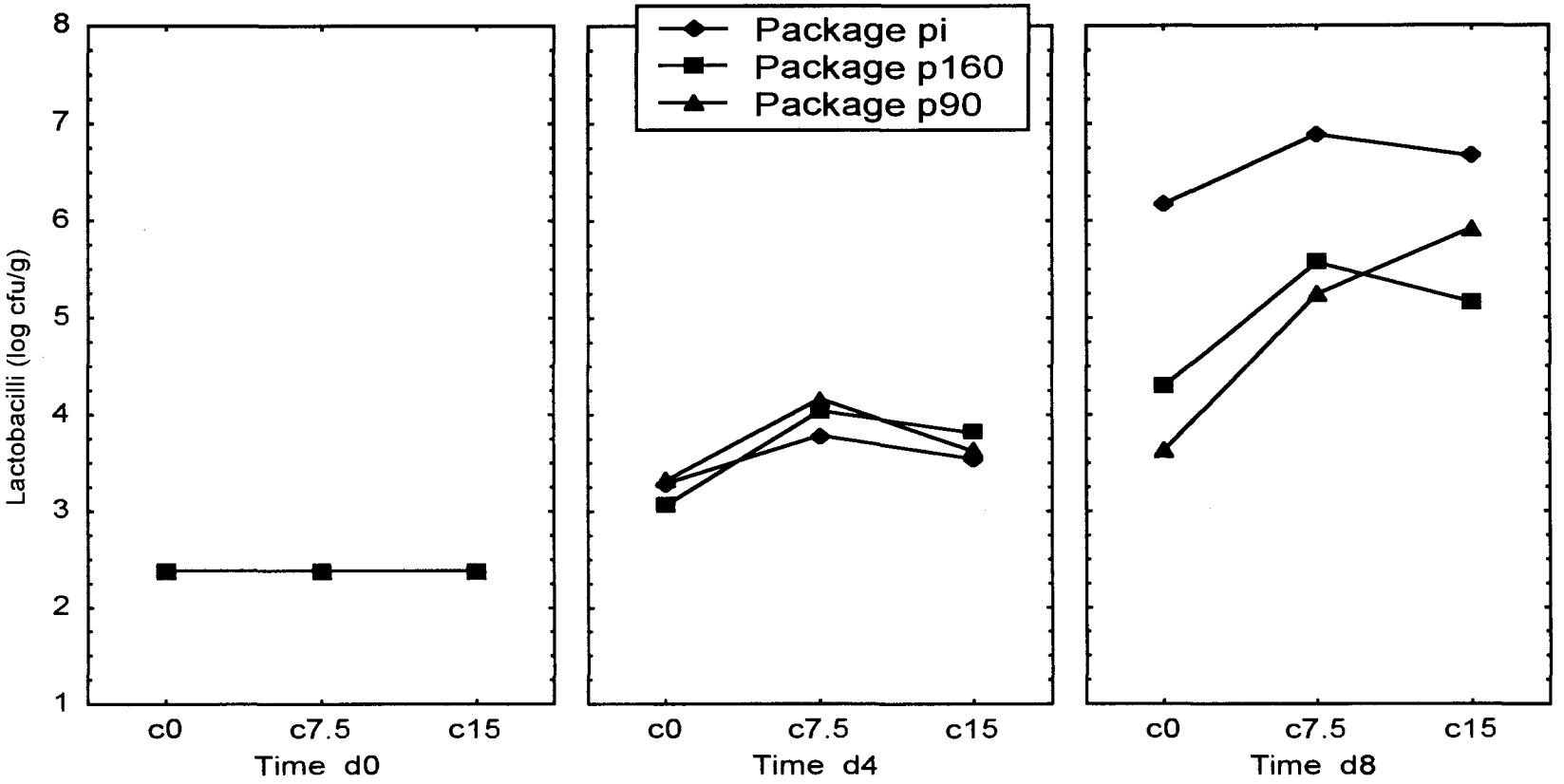

Figure 21: Plot of means of lactobacilli counts showing the three-way interaction between coating, packaging and time

[1] pi, p160 and p90 are fully permeable (control), semi permeable and least permeable P-Plus® packaging respectively

[2] c0, c7.5 and c15 are the Nature Seal® coating levels at 0 (control), 7.5 and 15 % respectively

5.3.3 Psychrotrophic bacteria

Packaging, coating and time and interactions between packaging x time and coating x time (Table 15) influenced the growth of the psychrotrophic bacteria in minimally processed carrots. A decrease in psychrotroph was observed for the semi permeable packaging (p90) and least permeable packaging (p160) in comparison to the fully permeable packaging (pi), but no significant difference was found between p90 and p160 (Figure 22). Increasing the coating concentration, a linear contrast was observed for the psychrotroph counts, but with time, it gave both a linear and a quadratic response. The psychrotroph growth was of higher magnitude from d0 to d4 than from d4 to d8 (Figure 22).

The packaging and time interaction clearly showed that with time, the semi permeable packaging (p90) and least permeable packaging (p160) controlled the psychrotrophs compared with the fully permeable packaging (pi) (Figure 23). However, coating and time interacted to further increase the psychrotroph counts at d8 only and no difference was found on d4 (Figure 24)

Table 15: P-values for the 3-way ANOVA of packaging, coating and time on psychrotroph counts of the carrots packs

Source of variation	P- value	Significant contrast
Block effect	NS	-
Packaging (P)	P<0.001	-
Coating (C)	P<0.01	C-l**
Time (T)	P<0.001	T-l***, T-qd***
P x C	NS	-
P x T	P<0.001	-
C x T	P<0.05	P-l x C-l**
P x C x T	NS	-

- no analysis as not equally spaced
l, qd and cb are linear, quadratic and cubic response respectively
*, **, *** is significant at P<0.05, P<0.01 and P<0.001 respectively
NS is not significant at P>0.05

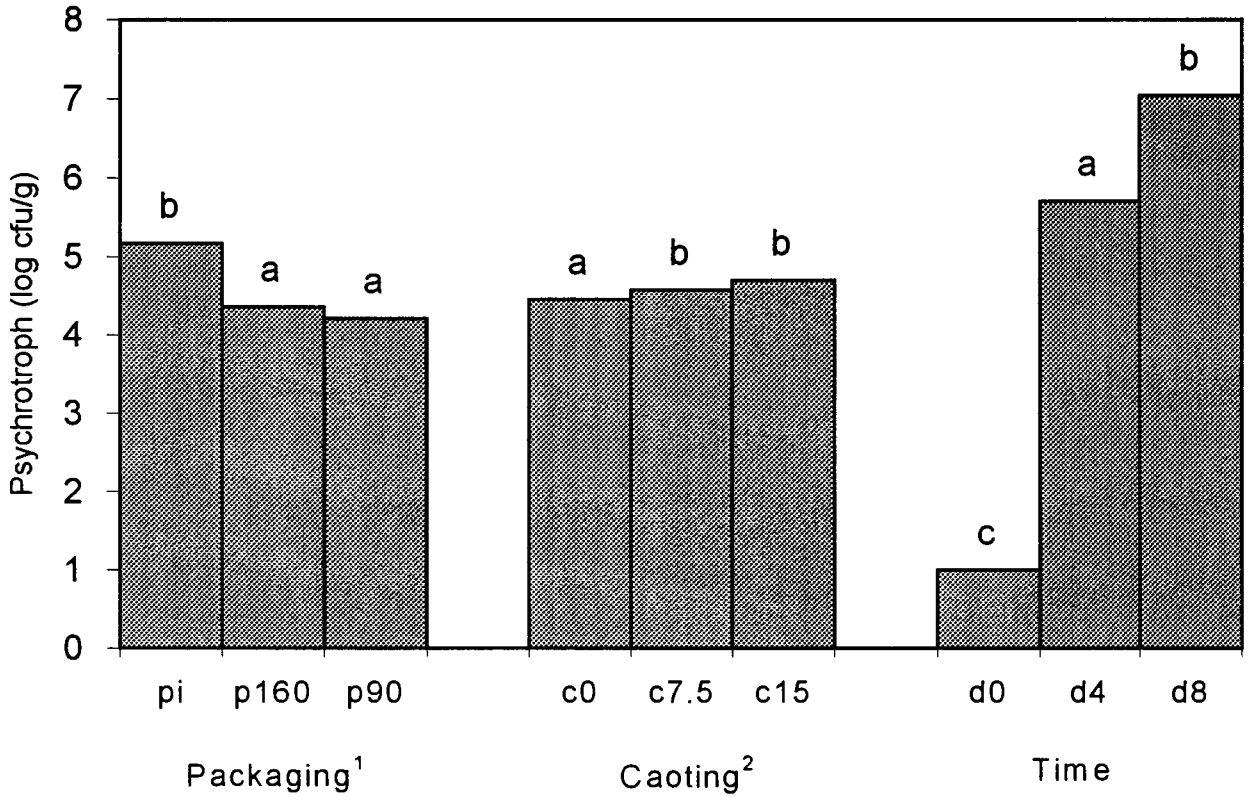

Figure 22: Main effect of Packaging[1], coating[2] and time growth of psychrotroph on sliced carrot.

[1] pi, p160 and p90 are fully permeable (control), semi permeable and least permeable P-Plus® packaging respectively

[2] c0, c7.5 and c15 are the Nature Seal® coating levels at 0 (control), 7.5 and 15 % respectively

Different letters show significant differences at P<0.001 separately for packaging coating and time

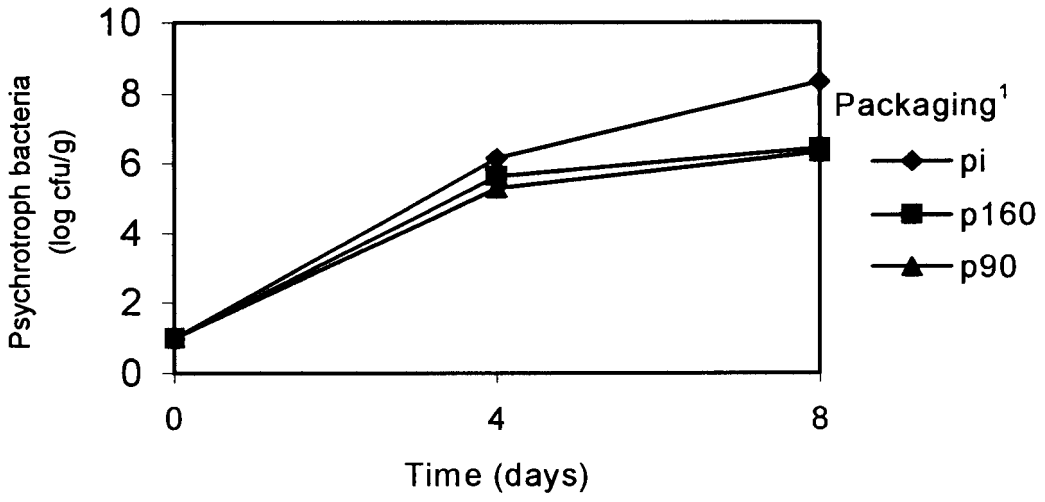

Figure 23: Plot of means of psychrotrophic bacteria counts showing the two-way interaction between and packaging[1] and time

[1] pi, p160 and p90 are fully permeable (control), semi permeable and least permeable P-Plus® packaging respectively

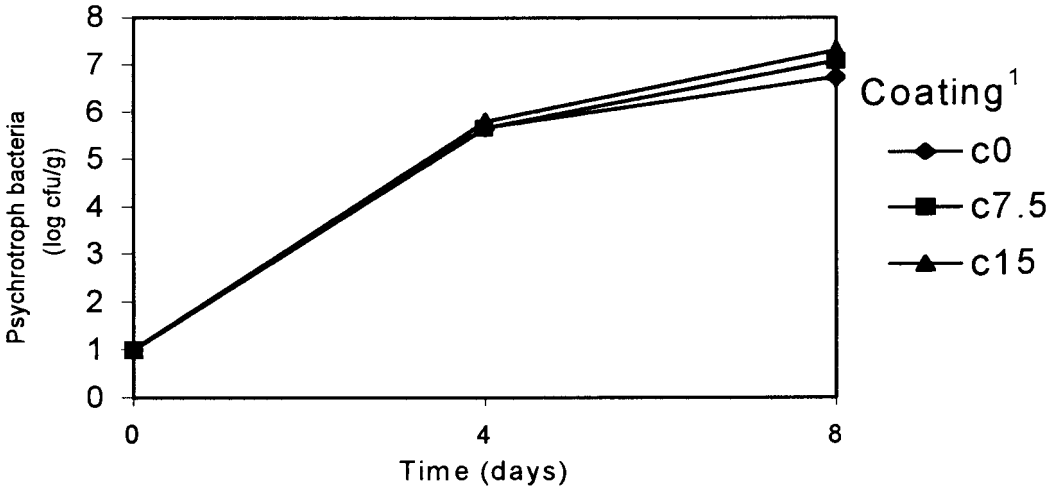

Figure 24: Plot of means of psychrotrophic bacteria counts showing the two-way interaction between coating[1] and time

[1] c0, c7.5 and c15 are the Nature Seal® coating levels at 0 (control), 7.5 and 15 % respectively

5.3.4 Yeast and moulds

The growth of yeast and moulds was not found to be a major problem in the treated carrots. At the end of the 12d period, the counts for all the treatments were below 3 log cfu/g. Analysis of variance showed that only coating and time together with their two-way interaction had a significant effect on the occurrence of yeast and moulds.

Increasing the coating concentration showed an increase in the yeast and moulds counts (Table 16). The growth of yeast and moulds was more pronounced from d0 to d4 rather than from d4 to d8. The interaction effect showed that in combination with time, coated carrot further enhanced the growth of yeast and moulds.

Table 16: Main effect of coating and time together with their two-way interaction for yeast and moulds (log cfu/g) occurrence of minimally processed carrots at 10 ^{0}C

Coating (C)[1]	Time (T)			Coating	
	d0	d4	d8	Main effect	contrast
c0	1.00 a	2.48 cde	2.29 bc	1.92 ab	linear*, quadratic***
c7.5	1.00 a	2.14 bc	2.31 bcd	1.82 a	
c15	1.00 a	2.52 de	2.59 e	2.04 b	
C x T contrast	T-linear x C-linear*, T-quadratic x C-quadratic**				
Time main effect	1.00 a	2.38 b	2.40 b		
Time contrast	linear***, quadratic***				

[1] c0, c7.5 and c15 are the Nature Seal® coating levels at 0 (control), 7.5 and 15 % respectively

*, ** and ***is significant at P<0.05, P<0.01 and P<0.001 respectively.

Different letters show significant differences at P<0.01 for the interaction data in the cells, significant differences at P<0.001 for the main effect of time within its row and of coating within its column

5.4 pH measurements

Only the packaging treatment, time and their interaction significantly affected the pH values of the sliced carrots (Table 17). The semi permeable packaging (p160) and the least permeable packaging (p90) maintained a higher pH compared with the fully permeable packaging (pi). With time, a quadratic response was observed for the pH values. A slight increase in pH was observed from d0 to d4 followed by a decrease from d4 to d12. With time, the packaging treatments maintained a higher pH in comparison to the fully permeable packaging (pi) as control.

Table 17: Main effect of packaging and time together with their two-way interaction for pH values of sliced carrot at 10 ^{0}C

Time (T)	Packaging (P)[1]			Time	
	pi	p160	p90	Main effect	contrast
d0	6.42 d	6.42 d	6.42 d	6.42 b	
d4	6.33 bc	6.58 f	6.53 ef	6.48 c	linear***,
d8	6.21 b	6.50 def	6.45 ed	6.39 b	quadratic***
d12	5.90 a	6.33 c	6.27 bc	6.17 a	
Packaging main effect	6.22 a	6.42 b	6.46 c		

[1] pi, p160 and p90 are fully permeable (control), semi permeable and least permeable p-Plus® packaging respectively

***is significant at P<0.001.

Different letters show significant differences at P<0.01 for the interaction data within the cells, significant differences at P<0.001 for the main effect of time within its column and of packaging within its row

6. DISCUSSION

The results will be discussed in four sections. The first three sections will respectively deal with head space gas concentration, colour changes and microbiological quality and spoilage as affected by the treatments. Lastly the relationship between the packaging and coating will be discussed in terms of the shelf life of minimally processed carrots.

6.1 Head space oxygen and carbon dioxide

The oxygen decrease and carbon dioxide increase in the head space of MPFV could be the result of the respiration rate of the carrots as well as the gas permeability of the coatings and polymeric packaging films. The decrease in oxygen and increase in the carbon dioxide levels followed by a plateau during storage is common in minimally processed carrots packed in polymeric packaging films with a low gas permeability (Rizvi; 1981, Carlin *et al.*, 1990b). This change was obviously because of respiration. The plateau in the study indicated the equilibrium between the use of oxygen and the production of carbon dioxide as well as the creation of a modified atmosphere. However after the equilibration, a further decrease in oxygen and increase in carbon dioxide levels were noted probably because of possible microbial respiration (Hagenmaier and Baker, 1998) since a high microbiological spoilage rate was observed from d8 to d12.

Carlin *et al.* (1990b) found no clear relationship between the respiration rate and head space gas concentrations in packed carrots. They suggested that the diffusion rate of gases across the packaging film was of more importance than the head space. However it can be said that the respiration rate decreased because of the low concentration of oxygen in the pack. Sode and Kuhn (1998) reported that respiration of cut carrots has a high dependency on oxygen concentration. They found that there was a decrease in energy consumption

and a shift to glycolytic metabolism for minimally processed carrots at 5% of oxygen under controlled atmospheres.

The lowest oxygen (ca 8%) and highest carbon dioxide (ca16%) achieved in the head space of the packs during the experiment did not indicate any anaerobic respiration because of the following:

- With similar atmosphere of about 16.5% carbon dioxide and 5.1% oxygen, Carlin *et al.* (1990b) showed an aerobic respiratory quotient of 1.2 for cut carrots.
- Carlin *et al.* (1989) suggested that the critical head space for anaerobic respiration was 1.5% oxygen and 30% carbon dioxide in a passive modified atmosphere for grated carrots. In contrast Sode and Kuhn (1998) recommended at least 5% oxygen in a controlled atmosphere for cut carrots to prevent anaerobic respiration. Both recommended limits were not reached in this experiment.

The gas concentrations indicated that anaerobic condition was not created. It eliminated the risk of potential growth and toxin production of the deadly and anaerobic *Clostridium botulinum*. This is an important issue for the safety of the product.

Comparing the packaging systems, it was found that the least permeable packaging (p90) created a better modified atmosphere than the semi permeable (p160). Thus, it is suggested that p90 should be commercially used instead of p160. The equilibrium of oxygen and carbon dioxide reached in the head space of the packs for p90 suggested that the carrots had a lower respiration rate than that in the p160. Therefore, p90 pack can better maintain the physiological status of the sliced carrots due to lower respiration rate (Watada and Qi, 1998)

The coatings are supposed to act as a barrier to gases (Baldwin *et al.*, 1995a), restricting oxygen to enter in the carrot and carbon dioxide to escape in the pack atmosphere. Therefore, it was expected that as the coating concentration

was increased, the head space oxygen would increase and carbon dioxide would decrease in comparison to the control because of the gas barrier properties of the coatings. However, the opposite was revealed. This decrease in headspace oxygen and increase in headspace carbon dioxide was also reported by Li and Barth (1998) for minimally processed carrots and Howard and Dewi (1995) reported no difference in the head space gas concentration for mini peeled carrots. These researchers did not give any possible explanation for this behaviour and no internal gas atmospheres were determined.

However, in fresh fruits and vegetables, generation of internal modified atmospheres was observed suggesting the gas barrier properties of the coatings (Hagenmaier and Baker, 1995; Bender, Brecht, Sargent, Navarro and Campbell, 1993; Baldwin, Burns, Kazokas, Brecht, Hagenmaier, Bender and Pesis, 1999). Fresh fruits and vegetables still have their peels that act as a barrier to gases and the peel is dry. Minimally processed fruits and vegetables are peeled and they have wet surfaces. This difference might be the cause for the different behaviour of the coatings for fresh and minimally processed produce as discussed below.

The fact that no internal gas atmosphere was measured makes it difficult to assess the barrier properties of the coatings. Avena-Bustillos *et al.* (1994) found that the head space and internal gas concentration was similar for minimally processed carrots. Thus it can be argued that to some extent, the coating created modified atmosphere as compared with the control and equilibrium was reached between internal and external head space of the coating atmospheres during the experiment.

The increase in the carbon dioxide and decrease in oxygen in the head space of the coated carrots could be attributed to the following:

- The coating is cellulose based. The decrease in oxygen could be explained by the fact that cellulose is an oxygen adsorbant (Goldman, Horev and Saguy, 1983).

- The coating as compared to the control enhanced the microbial proliferation. Microbial respiration could have also contributed to this condition (Hagenmaier and Baker, 1998)
- The permeability of the coatings might have allowed oxygen and carbon dioxide to move freely. As relative humidity increased from 40%, the oxygen permeability showed an exponential increase for polysaccharide based films (McHugh *et al.*, 1996). At high relative humidity, Stading, Rindlav-Westling and Gatenholm (2000) showed that there was a structural change in an amylose and amylopectin film. The film became non-homogeneous with a more open structure. This structural change had a direct effect on increasing the oxygen permeability.
- Minimally processed carrots are reported to have a high respiration rate (Carlin *et al.*, 1990b). The high respiration rate might have created a high diffusion gradient to enhance activated diffusion of gases from the head space via the coating to the carrots. The diffusion could have been further promoted by capillary diffusion due to the probability of having a more open structure of the coating as explained above (Greener and Fenemma, 1994). Thus internal and the head space amospheres might have reached an equilibrium

6.2 Colour changes

The white blush formation and decrease in the orange colour intensity are the two most important colour changes in minimally processed carrots. The white blush formation is attributed to surface dehydration and lignification. Peeling and slicing resulted in tissue disruption (Barry-Ryan and O'Beirne, 1998). The surface cells became widespread and this allowed rapid dehydration that caused surface white discolouration as a result of scattering of reflected light. (Cisneros-Zevallos *et al.*, 1995). In addition as a repair mechanism, wounding promote lignification of the cell walls by enzymatic processes (Howard and Griffin, 1993). Occurrence of lignin formation was showed to be a physiological

response that led to surface white discolouration (Cisneros-Zevallos *et al.*, 1995).

Loss of orange colour intensity (as measured by chroma values) is very common in cut carrots. The loss in orange colour was correlated to a decrease in carotenoid content by oxidative reactions (Chervin and Boisseau, 1994). Carotene is degraded by chemical, enzymatic and photo oxidation. The oxidative degradation was found to be influenced by oxygen concentration, temperature, pH, water content and light (Goldman *et al.*, 1983). Wounding in minimally processed carrots also cause ultra structural changes that allows the oxidative enzyme and carotene contact from the chromoplast for degradation (Picchioni, Watada, Roy, Whitaker and Wergin, 1994)

A significantly ($P<0.001$) higher whiteness index and lower chroma retention for the upper surfaces of minimally processed carrota compared with the lower surfaces can be explained by the probable differences in relative humidity. Avena-Bustillos *et al.* (1994) reported that the white blush predominated on carrots facing the polymeric packaging film compared with those in the centre of the pack, but they did not compare both surfaces of one slice. They suggested that the higher relative humidity in the centre caused a lower white blush incidence. Similarly, the lower surfaces of the carrots in this experiment might be in a higher relative humidity region than the upper surfaces. This difference can be schematically presented in Figure 25.

The driving force for moisture loss was reported to be vapour pressure difference between cut carrots and the surrounding atmosphere (Cisneros-Zevallos *et al.*, 1995). According to the supplier of the packaging material for this research, the polymeric packaging film allows water to go through at 0.9 H_2O/m^2/day at 25 ^{0}C and 75% R.H. Thus water will be easily lost from the upper surface of carrot. Carrot Y acts as a water vapour barrier for the lower surface of carrot X (Figure 25). Hence, the water vapour difference between the carrot X and the polymeric packaging film will be higher than that between carrots X and

Y. Therefore, a higher relative humidity will be created between carrots X and Y than between carrot X and the package. This high relative humidity between the carrots probably controlled the surface dehydration rates resulting in lower whiteness index scores for the lower surfaces.

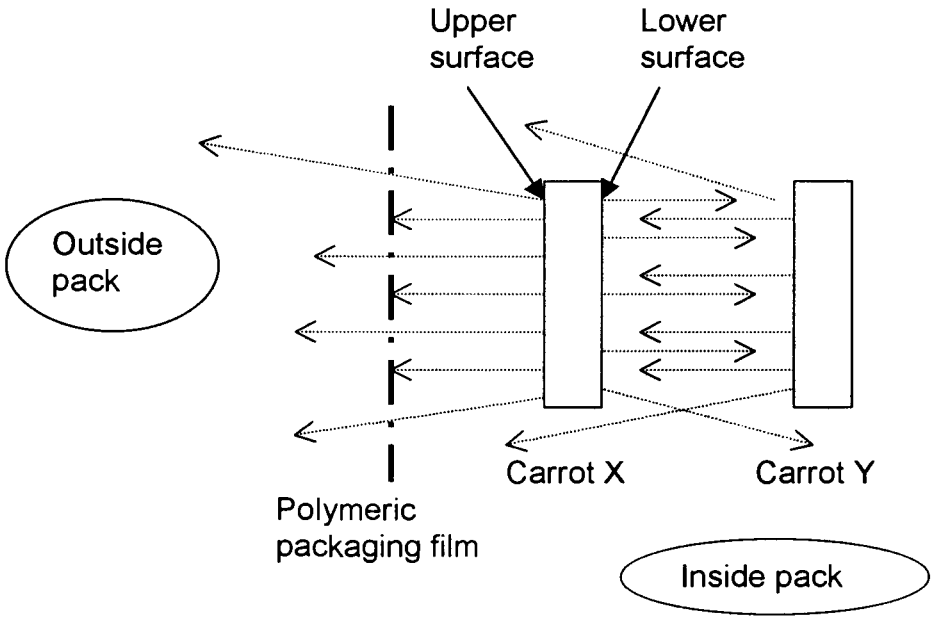

Figure 25: Schematic representation of the lower and upper surface of the carrot slices in a polymeric packaging film (The dashed arrows show the movement of water vapour)

Similarly, the higher orange colour intensity of the lower surfaces can be attributed to a high relative humidity that might induce a higher a_w. High a_w was found to prevent carotene degradation (Goldman *et al.*, 1983). In addition, a high a_w could allow the carbon dioxide created by the modified atmosphere to dissolve in the surface cells to decrease the surface pH. Acidified carrots were found to have a good retention of orange colour of carrots (Juliot, Lindsay and Ridley, 1989).

Coated carrots had a lower incidence of white blush formation and higher chroma values than uncoated samples. Compared with fresh carrots, peeled and sliced carrots generally have a lower water vapour resistance. When edible coatings were applied on the surfaces, increased water vapour resistance was observed and in this respect the coatings acted as a peel (Avena-Bustillos *et al.*, 1994). Polysaccharide coatings can also act as sacrificing agents to water loss (Baldwin et al, 1995a) and can hold water on the surface for a longer period (Chen *et al.*, 1996). Therefore, the coated carrots in the study also were not easily dehydrated and the incidence of white discolouration was lowered. Coated surfaces could also provide a high a_w and an acidified surface as earlier explained to prevent a decrease in chroma values.

Li and Barth (1998) reported that disrupted surfaces of minimally processed carrots contained air and this might promote the peroxidase activity. The increase in the peroxidase activity was related to the stimulation of lignin formation and an increase in whiteness index scores (Howard and Griffin, 1993). Li and Barth (1998) also found that the peroxidase activity was lowered when carrots were coated as the coating might replace all the air in the disrupted surfaces. In addition, the cellulose present in the coating is a good oxygen adsorber (Goldman *et al.*, 1983) and it might reduce the enzymatic activity. The lower enzymatic activity could also be attributed to the fact that oxygen has first to be dissolved in the wet surfaces to reach the cellular carotene. Oxygen dissolution in wet surfaces is lower than in dry surfaces. Thus, in the experiment, the edible coating decreased the white blush formation and retained the orange colour intensity seemingly by reducing the enzymatic activity for lignification and carotene degradation. The lower availability of oxygen could also prevent auto-oxidation of the carotene.

Increasing the coating level from 7.5 to 15% did not show any significant difference in the occurrence of white blush and change in chroma values. This may be because both concentrations exhibited the same extent of water barrier

properties. In fact, Bender *et al.* (1993) reported the same resistance factor for the functional barrier properties of Nature Seal® at two different concentrations.

The whiteness index and the chroma values of the lower surfaces of the carrot slices, independent of the coating, showed a more linear trend compared with a quadratic trend for the upper surfaces. A similar quadratic trend was observed by other researchers (Avena-Bustillos *et al.*, 1994; Cisneros-Zevallos *et al.*, 1995; Howard and Dewi, 1995). The initial high increase in the whiteness index in the study may be attributed to the surface dehydration by the high water vapour difference as explained earlier. In contrast, even though a high relative humidity might prevail on the lower surface the white blush occurred linearly. This may be because of lignification, the physiological response to wounding (Howard and Griffin, 1993).

The two different packaging films (p160 and p90) that created the modified atmosphere did not affect the whiteness index and the chroma values of cut carrots. Similar results were observed by scientists who concluded that modified atmospheres did not affect the whiteness index (Izumi, Watada, Ko and Douglas, 1996; Amanatidou, Slump, Gorris and Smid, 2000). According to the supplier of the packaging material, the two semi permeable packaging films, p90 and p160, had the same water permeability, so they did not affect the water vapour difference and thus the dehydration rates could be similar.

The head space gases as affected by the packaging (p160 and p90) stabilised at 8-14% oxygen and 7-15% carbon dioxide. This head space did not influence the chroma values. Barth, Kerbel, Perry and Schmidt (1993) showed that even with a head space of about 10% oxygen and 8% carbon dioxide, there was no difference in the peroxidase activity of packaged broccoli. Thus it may be proposed that the modified atmosphere created by the packaging films in the experiment did not reach the critical value to influence enzymatic activity for carotene degradation and white discolouration.

Inclusion of fully permeable packaging (pi as control) in the analysis for d4 to compare it with other polymeric packaging films still showed no significant differences between the whiteness index and the chroma values for the upper surfaces. In contrast, the lower surfaces showed significant differences. The possible reason for this difference might be because of different relative humidity gradient between the lower surfaces of carrots in the packaging, but similar ones for the upper surfaces.

An interaction between the packaging and coating to control the whiteness index was only seen for the lower surfaces of the minimally processed carrot slices. This interaction showed that the coating was more effective in controlling the white surface discolouration when packaged in a polymeric packaging film than on its own. The semi permeable packaging film might have provided a lower water vapour difference and thus the coating did not need to lose its water. Hence the coating may have functioned more efficiently by preventing dehydration and enzymatic activities.

6.3 Microbiological quality and spoilage

The brown patches and spots, tissue softening and exudate production as rotting symptoms for the minimally processed carrots resulted from microbiological growth on the minimally processed carrots. The typical brown patches and spots were explained by oxidation of the carrot phenols found to increase during storage (Amanatidou *et al.*, 2000). They found that oxidation and polymerization causing surface browning were catalysed by microbial enzymes (Howard *et al.*, 1994). The tissue softening and the exudate production could be associated with the presence of lactic acid bacteria (Carlin *et al.*, 1989) and the pectinolytic pseudomonads (Nguyen-The and Prunier, 1989).

Carlin *et al.* (1989) showed that spoilage of minimally processed carrots was associated with lactic acid bacteria, mainly *Leuconostoc mesenteroides*. These

heterofermentative lactic acid bacteria convert sugars into acetic acid, lactic acid and ethanol. These researchers did not find any relationship between spoilage and the pseudomonads even if it was present in high numbers in some spoiled packs. However, Nguyen-The and Prunier (1989) found that pectinolytic pseudomonads caused soft rot as they secreted enzymes to dissolve the pectin in the cell wall resulting in tissue softening.

The experimental data showed that both the psychrotrophs and the lactic acid bacteria were involved in the spoilage. The psychrotroph that generally cause spoilage in ready to eat salads were found to be mainly *Pseudomonas spp.* (Garcia-Gimeno and Zurera-Cosana, 1997). The lactic acid bacteria and the psychrotrophs in the study showed high counts in spoiled carrots in comparison to the non-spoiled ones.

The creation of modified atmospheres was found to prevent spoilage as microbial loads (both the lactic acid bacteria and the psychrotrophs) decreased in such atmosphere compared with the control. The coating increased the spoilage rate as it increased the growth of lactic acid bacteria and the psychrotrophs.

The psychrotrophic bacteria, yeast and moulds were initially less than 10^1 cfu/g compared with lactic acid bacteria which were about 10^2 cfu/g. These initial microbial loads were lower than the published data for minimally processed carrots and other fruits and vegetables. Yeast in carrots was reported to be over 10^3 cfu/g for Touchon variety and over 10^4 cfu/g for Karotan variety (Babic *et al.*, 1992). Lactic acid bacteria were reported to be over 10^3 cfu/g (Carlin *et al.*, 1989; Garcia-Gimeno and Zurera-Cosana, 1997) and over 10^5 cfu/g of psychrotrophs. The possible differences might be due to cultivars, different products and more importantly the use of mild antimicrobials during minimal processing. In fact, the carrots used in this research were first washed in disinfectants by the supplier after harvesting. During processing, all the cracks

and visibly spoiled carrots were discarded and before the treatments, the slices were dipped into hypochlorite solution.

Over time, the psychrotrophs and the lactic acid bacteria grew rapidly on the carrots. However, yeast and moulds had a slow growth rate and were less than 10^3 cfu/g after 12 days of storage. The lower oxygen and higher carbon dioxide in the modified atmospheres might have suppressed the growth of yeast and moulds (Garcia-Gimeno and Zurera-Cosana, 1997). The fact that fully permeable packaging treatment (pi) did not promote the yeast and mould growth in the study suggested that other factors can also affect its growth. The other reasons might be that the bacterial growth could be antagonistic to yeast and moulds. Moreover, low acid food such as carrots (pH ca 6), are not generally spoiled by yeast and moulds that prefer low pH for growth (Frazier and Westhoff, 1988)

The growth of lactic acid bacteria and the psychrotrophs in the minimally processed carrots showed a relationship in terms of microbial dynamics in the experiment. There was a high growth rate from d0 to d4 followed by a slower rate from d4 to d8 for the psychrotrophs. The opposite occurred for lactic acid bacteria having a slow growth rate from d0 to d4 and a high rate of growth from d4 to d8. Surface cells are disrupted during slicing and this allows leakage of cellular solutions containing nutrients for microbial growth (Barry-Ryan and O'Beirne, 1998). Initially the psychrotrophs proliferated as the atmosphere was mainly aerobic. Psychrotrophs, which are mainly the *Pseudomonas spp.* (Garcia-Gimeno and Zurera-Cosana, 1997) grew well in the intercellular spaces. They can also increase the carbon dioxide level due to respiration and can increase sugar levels by inducing its transport (Atkinson and Baker, 1987). Filling up the inter cellular spaces, an increase in carbon dioxide and the availability of sugars then probably enhanced the growth of lactic acid bacteria, more specifically the *Leuconostoc spp.* The latter are mostly microaerophilic and need sugars for fermentation (Frazier and Westhoff, 1988) and their growth is favoured at high carbon dioxide levels (Buick and Damoglou, 1987)

The pH change with time reflected the activity of lactic acid bacteria and psychrotrophs. A slight increase from d0 to d4 followed by a decrease was observed. Izumi and Watada (1994) and Amanatidou *et al.* (2000) found a decrease, but Barry-Ryan and O'Beirne (1998) found an increase in pH during storage. The possible increase could be attributed to the use of organic acid such as malic acid in the carrot by the microbes and the decrease could be because of lactic and acetic acid produced by the lactobacilli (Carlin *et al.*, 1989). Only the packaging affected the pH and not the coating. This suggested that the lactobacilli growth enhanced by the coating was not high enough to cause a significant pH decrease.

However, a relationship between spoilage and low pH could be deduced. The lowest pH and the highest spoilage were observed in the fully permeable packaging (pi). This might confirm that spoilage of minimally processed carrot is typically through lactic acid bacteria fermentation producing acetic and lactic acid (Carlin *et al.*, 1989).

The modified atmospheres created by the polymeric packaging films were found to reduce the microbial load of lactobacilli and psychrotrophs. Similarly Watada and Qi (1998) and Amanatidou *et al.* (2000) found a decrease in microbial load by modified atmospheres. The modified atmospheres were not found to be bactericidal, but a retardation in senescence and microbial spoilage by reducing the physiological activity of the microbes were found to be the reason for a decrease in microbial growth (Carlin *et al.*, 1990a). Moreover, microbial contamination from the environment could result in a higher microbial growth in packaging (pi) as it had holes to achieve a fully permeable non-modified atmosphere. The two packaging films (p160 and p90) significantly created two different modified atmosphere, but they did not have any effect on the microbial growth. This suggests that the physiological status was not different enough to cause a significant change in the microbial load.

The coating used for this research did not contain any preservatives. The coated carrots showed an increase in microbial growth with regard to lactobacilli and psychrotrophs. Most research on edible coatings has mainly concentrated on physiological and biochemical aspects rather than on microbiological issues. One researcher who did some microbiological determinations on mini-peeled carrots at 1.7 ^{0}C found no significant differences in the coated and the uncoated products probably because of the very low storage temperature (Howard and Dewi, 1995). The coating in the research could have promoted some favourable conditions for the microbes to proliferate by providing some nutrients for their growth. In addition the coating might have provided a higher a_w on the surface of the carrot compared with the uncoated samples. This was shown by relatively higher whiteness index scores for the non-coated carrots. Baldwin *et al.* (1996) found that it was important to incorporate sodium benzoate and potassium sorbate in coatings to effectively control the microbial spoilage of cut apple and potato. However, minimally processed fruits and vegetables are considered to be fresh like and chemical preservatives in the products could result in consumer resistance.

Combination of packaging and coating only affected the growth of lactic acid bacteria. Moreover, the storage time also played a significant role. With time, the lactobacilli increased in the coated carrots, but the created modified atmospheres in turn reduced the growth probably by retarding senescence of the carrots and by decreasing the physiological activity of the microbes (Carlin *et al.*, 1990a)

The three-way interaction effects between, coatings, packaging and time also showed a relationship between the lactobacilli and the spoiled pack. On d8 all the fully permeable packs (pi) had a load of lactic acid bacteria that were over 10^6 cfu/g and all these pack were visibly spoiled. This might confirmed the cut off point of 10^6 cfu/g of lactic acid bacteria for minimally processed salads or at least for carrots as shown by Garcia-Gimeno and Zurera-Cosana (1997).

6.4 Combination of polymeric packaging films and edible coatings on the shelf life of minimally processed carrots

To show the possible combination effects of packaging and coating, whiteness index and lactic acid bacteria were taken as shelf life indicators as shown in Table 18. Cisneros-Zevallos *et al.* (1995) related whiteness index to a visual descriptive scale and showed that an average score of 32.6 was a non white (0% white) and 50.9 was extreme white or 100% white surface as shown in Table 2 of the literature review. The increase in the score as related to the visual description followed a linear change and the cut off point was 50% white. In the experiment, d0 was non white and the score was an average of 36.62. The difference might be in the biotic and abiotic factors. To determine the shelf life of the carrots, in this case an average whiteness index score of 36.62 was used as the non-white (0% white). Assuming a linear change according to the above authors, the cut off point was taken as an average whiteness index score of 45.58. Secondly, in order to calculate the shelf life, it is assumed that the whiteness index change between two-time intervals is linear.

Lactic acid bacteria at the level of 10^6 cfu/g were used as the cut off point and their growth is assumed to be linear in between two time interval

From Table18, it can be seen that the combination of polymeric packaging films and edible coatings did not provide any synergistic or additive effects to enhance the shelf life of minimally processed carrots. The whiteness index was a more limiting factor for shelf life than the lactic acid bacteria counts. Compared with the control (c0 as uncoated and pi as fully permeable packaging), an extension of shelf life from about 3 days to 5-6 days was achieved with the coating when whiteness index is taken as shelf life indicator. This extension however was not enough to have a beneficial effect in combination with the semi permeable polymeric packaging films (p160 and p90) that had an extension of about 7 days to about 12 days when using lactic acid bacteria as shelf life indicator.

Table 18: Shelf life (days) of minimally processed carrots as affected by coatings and packaging treatments in terms of whiteness index (WI) and lactic acid bacteria (LAB) count.

Coating (C)[1]	Shelf life indicators	Packaging (P)[2]		
		pi	p160	p90
c0	WI	3.00	3.60	3.43
	LAB	7.76	[a]>12.00	[a]>12.00
c7.5	WI	4.00< b >8.00	5.37	5.95
	LAB	6.86	12.00	12.00
c15	WI	4.00< b >8.00	5.65	4.39
	LAB	7.15	12.00	12.00

[1] c0, c7.5 and c15 are the Nature Seal® coating levels at 0 (control), 7.5 and 15 % respectively

[2] pi, p160 and p90 are fully permeable (control), semi permeable and least permeable P-Plus® packaging respectively

[a] is taken to be greater than 12 days as these treatments had lactic acid bacteria counts lower than 10^6 cfu/g on d12

b is taken to be greater than 4 days and less than 8 as no measurements were taken due to microbiologically spoiled carrots on day 8.

The combination of packaging and coating did not provide any extra beneficial effect on the white discolouration to promote the shelf life. This might be because the packaging did not perform as a proper moisture barrier to prevent surface dehydration.

Packaging together with coating did not provide any extra beneficial effects in reducing the spoilage as the coating was found to enhance spoilage instead of reducing it. In addition, the combination did not alter the microbial flora of minimally processed carrots when considering the growth profile of lactic acid bacteria, yeasts and moulds and psychrotrophs. This suggested that that the higher modified atmosphere created in the head space by the coating and especially the packaging did not affect the microflora because the edible coating enhanced the microbial growth without affecting the dynamic interaction between the microbes.

7. CONCLUSIONS AND RECOMMENDATIONS

Polymeric packaging films and edible coatings, respectively, impact differently on the quality parameters of minimally processed carrots. The polymeric packaging films exhibit good gas barrier properties, whereas edible coatings primarily function as a good moisture barrier.

Polymeric packaging films have a more pronounced effect than edible coatings on creating modified atmospheres in the head space of the carrot packs. However, to what extent the edible coatings affect the internal oxygen and carbon dioxide levels in the carrots requires further investigation. This will also reveal the actual permeability of the edible coatings to gases when applied to minimally processed carrots.

The polymeric packaging films are able to control the microbiological growth and spoilage in minimally processed carrots because of the generation of modified atmospheres in the packs.

The packaging treatments do not prevent the formation of white blush and do not maintain the desirable orange colour for the upper surfaces of the carrots. This may be attributed to a low relative humidity on these surfaces as a result of water vapour movement through the polymeric packaging films. It is recommended to use packaging films that are able to create a high relative humidity inside the pack without promoting microbial spoilage.

Edible coatings are able to control the white discolouration for about two days longer than uncoated samples because of their moisture barrier properties. To extend the shelf life of minimally processed carrots in this regard, it is essential to improve the functional properties of the edible coating. This can be done by (a) incorporating citric acid in the coating to acidify the surfaces thereby controlling surface white discolouration and (b) using a lipid bilayer coating to enhance moisture barrier properties.

Edible coatings enhance the microbiological growth and spoilage of minimally processed carrots. This is probably because coatings provide nutrients at high water activity levels to spoilage microbes. Thus, the use of chemical preservatives in the coating formulation to control the microbial load should be considered. Nisin, as natural preservative, is recommended because of consumers' negative perception towards chemical preservatives.

Combination of edible coatings and polymeric packaging films does not show any synergistic or additive effects to enhance the shelf life of minimally processed carrots despite some interactions between these variables. This is because the polymeric packaging films primarily prevent microbiological growth and spoilage, whereas edible coatings partly control white blush formation.

White blush formation is the most important shelf life determinant of minimally processed carrots. Research efforts should therefore be focussed on overcoming this defect. However, the use of a combination of improved polymeric packaging films and edible coatings may still be beneficial or even synergistic in extending the shelf life of minimally processed carrots and other fruits and vegetables.

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9. APPENDICES

Appendix A

Table A1: Effect of packaging and coating on the head space oxygen and carbon dioxide values (%)[1] of sliced carrots stored at 10 ^{0}C for 12 days.

Coating (C)[3]	Time (days)	Package (P)[2]					
		pi		p160		p90	
		O_2	CO_2	O_2	CO_2	O_2	CO_2
c0	0#	20.90	0.30	20.90	0.30	20.90	0.30
	4	20.67 (±0.50)	0.44 (±0.025)	15.60 (±1.87)	6.47 (±1.56)	13.08 (±2.59)	9.14 (±1.87)
	8	20.50 (±0.20)	0.41 (±0.063)	13.95 (±2.80)	7.65 (±1.74)	13.15 (±1.69)	8.88 (±1.27)
	12	20.50 (±0.11)	0.50 (±0.06)	13.16 (±1.50)	8.49 (±0.56)	8.70 (±0.88)	12.69 (±1.03)
c7.5	0#	20.90	0.30	20.90	0.30	20.90	0.30
	4	19.54 (±0.10)	0.39 (±0.14)	12.81 (±0.75)	8.09 (±0.66)	8.09 (±2.20)	14.10 (±3.99)
	8	19.76 (±0.17)	0.49 (±0.05)	12.51 (±1.13)	8.82 (±0.54)	9.72 (±1.47)	10.48 (±0.55)
	12	19.36 (±0.26)	0.69 (±0.10)	11.65 (±1.62)	9.74 (±0.10)	6.92 (±0.30)	13.94 (±0.87)
c15	0#	20.90	0.30	20.90	0.30	20.90	0.30
	4	19.15 (±0.19)	0.38 (±0.06)	12.49 (±2.56)	7.66 (±2.18)	8.15 (±2.72)	13.48 (±3.93)
	8	18.94 (±0.08)	0.66 (±0.10)	10.98 (±0.58)	9.36 (±0.19)	8.36 (±1.12)	15.74 (±7.0)
	12	18.51 (±0.32)	0.65 (±0.07)	9.59 (±0.91)	10.76 (±0.69)	7.06 (±0.30)	13.88 (1±.63)

[1] Each value represent a mean of four replicates with standard deviations in bracket

[2] pi, p160 and p90 are fully permeable (control), semi permeable and least permeable packaging respectively

[3] c0, c7.5 and c15 are the coating levels at 0 (control), 7.5 and 15 % respectively

d0 is taken as the amount of oxygen and carbon dioxide in air

Table A2: Effect of packaging and coating on the whiteness index scores[1] on the upper and lower surfaces of sliced carrots stored at 10 ^{0}C for 12 days.

Coating (C)[3]	Time (days)	Package (P) [2]					
		pi		p160		p90	
		Upper surface[4]	Lower surface[4]	Upper surface[4]	Lower surface[4]	Upper surface[4]	Lower surface[4]
c0	0#	36.62 (±0.59)	36.62 (±0.59)	36.62 (±0.59)	36.62 (±0.59)	36.62 (±0.59)	36.62 (±0.59)
	4	48.54 (±0.79)	43.77 (±2.81)	46.56 (±1.55)	40.48 (±1.60)	47.07 (±1.28)	42.21 (±1.92)
	8	nd	nd	47.46 (±2.09)	42.95 (±3.36)	48.08 (±2.42)	44.94 (±1.81)
	12	nd	Nd	51.22 (±0.32)	46.47 (±2.35)	50.60 (±1.36)	46.91 (±1.82)
c7.5	0#	36.62 (±0.59)	36.62 (±0.59)	36.62 (±0.59)	36.62 (±0.59)	36.62 (±0.59)	36.62 (±0.59)
	4	44.87 (±1.27)	40.78 (±1.08)	44.80 (±0.43)	39.25 (±0.73)	44.29 (±2.01)	38.90 (±1.74)
	8	nd	nd	47.08 (±0.640	41.65 (±1.23)	46.93 (±0.60)	40.68 (±0.62)
	12	nd	nd	48.57 (±1.16)	44.66 (±0.76)	48.57 (±0.91)	44.80 (±1.29)
c15	0#	36.62 (±0.59)	36.62 (±0.59)	36.62 (±0.59)	36.62 (±0.59)	36.62 (±0.59)	36.62 (±0.59)
	4	44.89 (±1.56)	39.50 (±1.65)	44.44 (±2.02)	40.30 (±0.85)	45.53 (±1.49)	39.48 (±1.43)
	8	nd	nd	47.21 (±1.46)	42.25 (±2.06)	46.05 (±1.40)	40.72 (±1.58)
	12	nd	nd	46.86 (±1.85)	43.34 (±1.68)	47.42 (±0.76)	43.14 (±1.07)

[1] Each value represent a mean of four replicates with standard deviations in bracket

[2] pi, p160 and p90 are fully permeable (control), semi permeable and least permeable packaging respectively

[3] c0, c7.5 and c15 are the coating levels at 0 (control), 7.5 and 15 % respectively

[4] upper surface mean facing the package, lower surface mean away from the package.

d0 is taken as reference value on fresh carrots prior to treatment

nd is not determined as these packs were rotten

Table A3: Effect of packaging and coating on the chroma values[1] on the upper and lower surfaces of sliced carrots stored at 10 ^{0}C for 12 days.

Coating (C)[3]	Time (days)	Package (P)[2]					
		pi		p160		p90	
		Upper surface[4]	Lower surface[4]	Upper surface[4]	Lower surface[4]	Upper surface[4]	Lower surface[4]
c0	0#	48.96 (±0.88)	48.96 (±0.88)	48.96 (±0.88)	48.96 (±0.88)	48.96 (±0.88)	48.96 (±0.88)
	4	37.79 (±1.68)	41.30 (±3.21)	39.31 (±1.67)	44.55 (±1.30)	39.34 (±1.36)	43.27 (±2.18)
	8	nd	nd	36.73 (±1.56)	39.31 (±1.42)	35.74 (±2.01)	38.11 (±2.32)
	12	nd	nd	35.26 (±0.49)	38.28 (±2.66)	36.48 (±1.20)	38.49 (±2.52)
c7.5	0#	48.96 (±0.88)	48.96 (±0.88)	48.96 (±0.88)	48.96 (±0.88)	48.96 (±0.88)	48.96 (±0.88)
	4	40.49 (±1.38)	43.90 (±1.82)	41.06 (±0.76)	45.74 (±0.26)	42.51 (±1.98)	46.86 (±1.70)
	8	nd	nd	37.95 (±0.60)	42.54 (±1.44)	39.02 (±0.92)	43.90 (±0.68)
	12	nd	nd	37.43 (±0.77)	40.46 (±0.57)	37.52 (±0.76)	39.66 (±1.12)
c15	0#	48.96 (±0.88)	48.96 (±0.88)	48.96 (±0.88)	48.96 (±0.88)	48.96 (±0.88)	48.96 (±0.88)
	4	40.49 (±1.48)	45.22 (±2.05)	41.32 (±1.80)	44.67 (±0.82)	40.42 (±1.71)	45.50 (±1.34)
	8	nd	nd	38.27 (±1.00)	41.75 (±0.92)	39.66 (±1.04)	44.03 (±2.25)
	12	nd	nd	38.44 (±1.63)	40.95 (±1.38)	38.16 (±0.87)	41.29 (±1.28)

[1] Each value represent a mean of four replicates with standard deviations in bracket

[2] pi, p160 and p90 are fully permeable (control), semi permeable and least permeable packaging respectively

[3] c0, c7.5 and c15 are the coating levels at 0 (control), 7.5 and 15 % respectively

[4] upper surface mean facing the package, lower surface mean away from the package.

d0 is taken as reference value on fresh carrots prior to treatment

nd is not determined, as these packs were rotten

Table A4: The effects of coating and packaging on growth of lactic acid bacteria (log cfu/g)[1] of minimally processed carrot packs stored at 10 ^{0}C for 12 days

Coating (C)[3]	Time (days)	Package (P) [2]		
		pi	p160	p90
c0	0#	2.39 (±0.02)	2.39 (±0.02)	2.39 (±0.02)
	4	3.29 (±0.54)	3.06 (±0.32)	3.32 (±0.26)
	8	6.17 (±0.63)	4.29 (±0.59)	3.63 (±0.39)
	12	nd	4.23 (±0.17)	4.32 (±0.56)
c7.5	0#	2.39 (±0.02)	2.39 (±0.02)	2.39 (±0.02)
	4	3.78 (±0.58)	4.05 (±0.30)	4.17 (±0.37)
	8	6.88 (±0.39)	5.57 (±0.31)	5.24 (±0.26)
	12	nd	5.54 (±0.53)	5.07 (±0.60)
c15	0#	2.39 (±0.02)	2.39 (±0.02)	2.39 (±0.02)
	4	3.54 (0.52)	3.81 (±0.14)	3.62 (±0.35)
	8	6.66 (0.65)	5.16 (±0.04)	5.92 (±0.47)
	12	nd	5.41 (±0.13)	5.18 (±0.47)

[1] Each value represent a mean of four replicates with standard deviations in bracket

[2] pi, p160 and p90 are fully permeable (control), semi permeable and least permeable packaging respectively

[3] c0, c7.5 and c15 are the coating levels at 0 (control), 7.5 and 15 % respectively

do is taken as the average of the fresh cut carrots before processing

nd is not determined as too much spoilage

Table A5: The effects of coating and packaging on growth of psychrotroph bacteria (log cfu/g)[1] of minimally processed carrot packs stored at 10 ^{0}C for 12 days

Coating (C)[3]	Time (days)	Package (P) [2]		
		pi	p160	p90
c0	0#	<1.00	<1.00	<1.00
	4	6.19 (±0.40)	5.59 (±0.40)	5.18 (±0.11)
	8	8.04 (±0.16)	6.35 (±0.79)	5.78 (±0.31)
	12	nd	6.10 (±0.40)	6.28 (±0.34)
c7.5	0#	<1.00	<1.00	<1.00
	4	6.12 (±0.36)	5.60 (±0.27)	5.25 (±0.62)
	8	8.46 (±0.04)	6.39 (±0.35)	6.39 (±0.27)
	12	nd	6.95 (±0.15)	6.82 (±0.36)
c15	0#	<1.00	<1.00	<1.00
	4	6.16 (±0.20)	5.73 (±0.24)	5.47 (±0.22)
	8	8.53 (±0.07)	6.57 (±0.49)	6.83 (±0.33)
	12	nd	7.11 (±0.21)	7.06 (±0.15)

[1] Each value represent a mean of four replicates with standard deviations in bracket

[2] pi, p160 and p90 are fully permeable (control), semi permeable and least permeable packaging respectively

[3] c0, c7.5 and c15 are the coating levels at 0 (control), 7.5 and 15 % respectively

do is taken as the average of the fresh cut carrots before processing

nd is not determined as too much spoilage

Table A6: The effects of coating and packaging on growth of yeast and moulds (log cfu/g)[1] of minimally processed carrot packs stored at 10 ^{0}C for 12 days

Coating (C)[3]	Time (days)	Package (P) [2]		
		pi	p160	p90
c0	0#	<1.00	<1.00	<1.00
	4	2.52 (±0.16)	2.61 (±0.23)	2.31 (±0.19)
	8	2.29 (±0.32)	2.23 (±0.28)	2.35 (±0.13)
	12	nd	2.17 (±0.15)	2.03 (±0.05)
c7.5	0#	<1.00	<1.00	<1.00
	4	2.26 (±0.24)	2.03 (±0.05)	2.12 (±0.03)
	8	2.38 (±0.47)	2.47 (±0.06)	2.08 (±0.48)
	12	nd	2.49 (±0.50)	2.36 (±0.27)
c15	0#	<1.00	<1.00	<1.00
	4	2.67 (±0.22)	2.43 (±0.13)	2.48 (±0.33)
	8	2.49 (±0.31)	2.84 (±0.28)	2.44 (±0.45)
	12	nd	2.70 (±0.21)	2.77 (±0.18)

[1] Each value represent a mean of four replicates with standard deviations in bracket

[2] pi, p160 and p90 are fully permeable (control), semi permeable and least permeable packaging respectively

[3] c0, c7.5 and c15 are the coating levels at 0 (control), 7.5 and 15 % respectively

do is taken as the average of the fresh cut carrots before processing

nd mean not determined as too much spoilage

Table A7: The effects of coating and packaging on pH values[1] of minimally processed carrot packs stored at 10 ^{0}C for 12 days

Coating (C)[3]	Time (days)	Package (P) [2]		
		pi	p160	p90
c0	0#	6.42 (±0.032)	6.42 (±0.032)	6.42 (±0.032)
	4	6.25 (±0.038)	6.56 (±0.042)	6.52 (±0.062)
	8	6.20 (±0.068)	6.51 (±0.027)	6.45 (±0.020)
	12	5.94 (±0182)	6.30 (±0.076)	6.25 (±0.101)
c7.5	0#	6.42 (±0.032)	6.42 (±0.032)	6.42 (±0.032)
	4	6.33 (±0.050)	6.57 (±0.047)	6.52 (±0.027)
	8	6.25 (±0.050)	6.50 (±0.030)	6.42 (±0.062)
	12	5.90 (±0.052)	6.34 (±0.114)	6.26 (±0.053)
c15	0#	6.42 (±0.032)	6.42 (±0.032)	6.42 (±0.032)
	4	6.41 (±0.034)	6.61 (±0.053)	6.55 (±0.085)
	8	6.19 (±0.055)	6.51 (±0.056)	6.47 (±0.060)
	12	5.87 (±0.096)	6.34 (±0.084)	6.28 (±0.050)

[1] Each value represent a mean of four replicates with standard deviations in bracket

[2] pi, p160 and p90 are fully permeable (control), semi permeable and least permeable packaging respectively

[3] c0, c7.5 and c15 are the coating levels at 0 (control), 7.5 and 15 % respectively

do is taken as the average of the fresh cut carrots before processing

nd mean not determined as too much spoilage

Appendix B

Table B1: P-value for paired t-test to compare the upper and lower surfaces of the carrot discs

Main treatment		Day 4	Day 8	Day 12
Packaging[1]	pi	P<0.001	nd	nd
	p160	P<0.001	P<0.001	P<0.001
	p90	P<0.001	P<0.001	P<0.001
Coating[2]	c0	P<0.001	P<0.001	P<0.001
	c7.5	P<0.001	P<0.001	P<0.001
	c15	P<0.001	P<0.001	P<0.001

[1] pi, p160 and p90 are fully permeable (control), semi permeable and least permeable packaging respectively

[2] c0, c7.5 and c15 are the coating levels at 0 (control), 7.5 and 15 % respectively

nd is not determined as these packs were rotten

Appendix C

Table C1: P-value for paired t-test to compare the chroma values between the upper and lower surfaces of the carrot discs

Main treatment		Day 4	Day 8	Day 12
Packaging[1]	pi	P<0.001	nd	nd
	p160	P<0.001	P<0.001	P<0.001
	p90	P<0.001	P<0.001	P<0.001
Coating[2]	c0	P<0.001	P<0.001	P<0.001
	c7.5	P<0.001	P<0.001	P<0.001
	c15	P<0.001	P<0.001	P<0.001

[1] pi, p160 and p90 are fully permeable (control), semi permeable and least permeable packaging respectively

[2] c0, c7.5 and c15 are the coating levels at 0 (control), 7.5 and 15 % respectively

nd means not determined as these packs were rotten

Appendix D

Table D1: P-values for the two-way ANOVA to illustrate the effects of packaging and coating on the chroma values of the carrot discs on day 4

Source of variation	Upper surface		Lower surface	
	P-value	contrast	P-value	contrast
Block	P<0.05	-	P<0.001	-
Packaging (P)	NS	NS	P<0.01	P-l**
Coating (C)	P<0.001	C-l**	P<0.001	C-l***
		C-qd**		C-qd**
P x C	NS	NS	NS	NS

- not determined
l and qd mean linear and quadratic response respectively
*, **, *** means significant at P<0.05, P<0.01 and P,0.001 respectively
NS means not significant at P>0.05