CHAPTER 1

INTRODUCTION

Avocados are one of the major fruit crops in the world because of their dietary value and taste (Lewis, 1978; Bower & Cuttings, 1988). They are indigenous to tropical America, but are cultivated in nearly all tropical and subtropical countries, including South Africa (Bower & Cuttings, 1988; Wiley, 1994).

South Africa's avocado export industries have a dilemma of long transport and storage times of up to ± 30 days at temperature of about 5.5°C. The extended storage period and fluctuating temperatures during transport often result in poor fruit quality, particularly due to physiological disorders (e.g. discolouration). There appears to be a seasonal trend with poor fruit quality both early and towards the end of the marketing seasons, with seasonal variation (Cutting & Wolstenholme, 1992b; Eksteen, Truter & Vorster, 1992). Despite of this, there is an economical benefit for South African producers to export avocados to other counties.

One way of marketing avocados is through minimal or light processing (MPR). Minimal or light processing refers to trimming, peeling, sectioning, slicing, and coring of fruits (Güntensperger, 1994; Ohlsson, 1994; Wiley, 1994; Ahvenainen, 1996). Recently, the demand for MPR fruits has grown due to consumers with busy lifestyles as well as, health and diet conscious consumers with a high purchasing power. Catering industries, which mainly use MPR fruits for the preparation of salads, also require MPR avocados due to reduced expenses, labour and hygienic reasons (Huxsoll & Bolin, 1989; Baldwin, Nisperos-Carriedo & Baker, 1995; Ahvenainen, 1996).

Hans Merensky Fruit Processing (HMFP) Pty (Ltd) is involved in avocado processing. They have been approached by catering concerns in Europe (i.e.

United Kingdom) to export minimally processed avocados. A shelf life of at least 7 days is required: two days for transport to the UK and at least 5 days for retailing and consuming purposes when stored at less than 5°C (Fouche, Technical Manager, Hans Merensky Fruit Processing Pty (Ltd), 1999 - personal communication).

As a results of minimal processing, avocados change from a relatively stable product with a shelf life of several weeks to months to a perishable one that has only a very short shelf life, even as short as 1-3 days at chilling temperatures (Huxsoll & Bolin, 1989; Güntensperger, 1994). This is due to physiological and biochemical changes (transpiration, respiration, effects of ethylene and cell wall degradation) as well as microbial spoilage which may result in degradation of the colour, texture and flavour of the produce (Ohlsson, 1994; Wiley, 1994; Ahvenainen, 1996).

One possible method of extending the shelf life of MPR avocados is by the use of edible coatings. Edible coatings are made of edible materials that are used to enrobe fresh product, providing a semipermeable barrier to gases and water vapour, thereby reducing respiration and water loss. Other known functions of using edible coatings are retarding solute transport; improving mechanical-handling properties of foods and imparting added structural integrity to foods (Kester & Fennema, 1986; Ahvenainen, 1996; Krochta & De Mulder-Johnston, 1997).

CHAPTER 2

LITERATURE REVIEW

Avocado is a fruit, which is mostly consumed fresh. Botanically, fruits are those portions of a plant that house seeds (Salunkhe & Kadam, 1995; Wills, McGlasson, Graham & Joyce, 1998). An important feature of fruits is that they are "living" structures, with continued metabolic reactions and sustained physiological processes for a considerable time during their postharvest period (Eksteen, 1996; Mitra, 1997).

2.1 Morphology and composition of avocados

Avocado fruits are mainly pear-shaped. The colour of the peel ranges from green to purple during ripening. The pulp is firm, but on ripening it acquires a smooth, buttery texture. The large, round to egg-shaped central seed may be loose or closely apprised to the pulp (Seymour & Tucker, 1993; Eksteen, 1996). The mature fruit of avocado is referred to as a berry and is comprised of soft pericarp and a single large seed. The pericarp consists of the exocarp, mesocarp and endocarp as the inner layer (Fig 1) (Nagalingam, 1993; Eksteen, 1996).

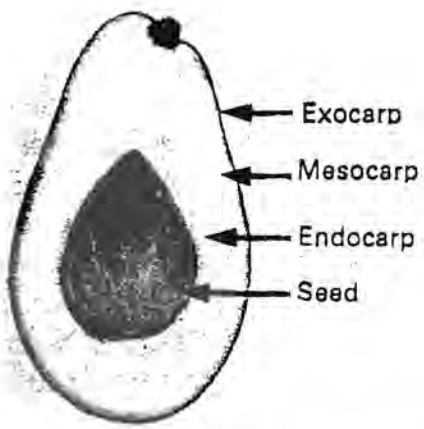

Fig. 1: Longitudinal section through the avocado fruit (Eksteen, 1996)

The avocado is an oleaginous fruit and the lipid level in the mesocarp varies from 10-30% in the mature stage (Salunkhe & Kadam, 1995). Unlike other fruits, the avocado is high in fat, proteins and minerals, but low in carbohydrates. The fruit therefore serves as an important role in diet as a source of energy, vitamins, minerals and unsaturated lipids, especially monounsaturated fat (Bower & Cuttings, 1988; Cutting & Wolstenholme, 1992a; Anonymous, 1994).

2.2 Physiology and biochemistry of avocados

Avocado is a living, biological entity when it is attached to the growing parent plant in its agricultural environment. Even after harvest, the avocado is still living as it continues to perform metabolic reactions and maintain the physiological system which was present when it was attached to the plant (Holdsworth, 1983; Mitra, 1997; Wills *et al.*, 1998). An important feature of plants, and therefore of fruits and vegetables, is that they respire by taking up O_2 and giving off CO_2 and heat. They also transpire, that is, lose water (Wills, Lee, Graham, McGlasson & Hall, 1981; Wills *et al.*, 1998).

2.2.1 Physiology of respiration

Respiration can be described as the oxidative breakdown of the more complex materials normally present in cells, such as starch, sugars, and organic acids, into simpler molecules, such as CO_2 and water (Wills *et al.*, 1981; Bower & Cutting, 1988; Eskeen, 1996). Most of the energy required by fruit and vegetables is supplied by aerobic respiration, which involves the oxidative breakdown of certain organic substances stored in the tissue. The normal substrate for respiration is glucose, and if it is completely oxidized, the overall reaction is:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{Energy}$$

Respiration rate of produce is an excellent indicator of metabolic activity of the tissue and thus is a useful guide to the potential storage life of the produce (Potter & Hotchkiss, 1995; Eksteen, 1996; Wills *et al.*, 1998).

2.2.2 Physiology of ethylene

Ethylene is a plant hormone, which is produced, in large amounts in climacteric fruits during ripening (Adato & Gazit, 1974; Holdsworth, 1983). In avocados there is a small rise in internally produced ethylene concentration preceding the commencement of the respiratory climacteric. Once ripening has commenced the large amount of ethylene synthesized by climacteric fruits is thought necessary to promote all of the aspects of ripening (Wills *et al.*, 1981; Watanda, 1986; Mitra, 1997).

2.2.3 Physiology of transpiration

Water is lost from fruits and vegetables as they grow on a tree or a vine; they may decrease in volume during the warm and dry part of the day, but regain their moisture at night (Salunkhe & Kadam, 1995). After harvesting, the process of transpiration continues but there is no way to replenish it. The moisture content of most fruits and vegetables is high and weight loss during transpiration and storage can be a serious economic factor, especially if fruits are sold by weight. In most fruits and vegetables with 5-10% loss in moisture, the products are visibly shriveled as a result of cellular plasmolysis (Cutting & Wolstenholwe, 1992a; Anonymous, 1994). The weight loss of fruits and vegetables in storage depends upon size, maturity, composition and structure, air surrounding them, storage temperature and relative humidity (Mitra, 1997; Wills *et al.*, 1998). Preventive loss of water from fruits and vegetables can be attempted both by reducing respiration as well as transpiration. The possible way of reducing the rate of transpiration is by altering relative humidity (RH) and temperature. This is due to the water loss which is rapid at low RH and slower at high RH because

the air in the room contains less water vapour than it can hold at low temperature (Wills *et al.*, 1981; Holdsworth, 1983).

2.3 Physiological stages of avocado fruit and quality of the raw material

The lives of fruits and vegetables can be conveniently divided into three major physiological stages following initiation or germination. These are growth, maturation, and senescence. However, clear distinction between the various stages is not easily made (Wills *et al* 1981; Salunkhe & Kadam, 1995). The growth involves cell division and subsequent cell enlargement, maturity includes different activities in different commodities and it commences before growth and is the period when anabolic (synthetic) biochemical processes give way to catabolic processes, leading to ageing and finally death of the tissue (Salunkhe, Bolin & Reddy, 1991; Mitra, 1997).

Quality of fruits is also important and cannot be improved, but it can be preserved (Potter & Hotchkiss, 1995; Mitra, 1997). Lewis, 1978 stated that fruit quality is "the sum total of all those attributes which combine to make fruit acceptable, desirable and nutritionally valuable as human food and would include factors such as taste, colour, texture, appearance, aroma, cleanliness, size, maturity and ripeness". Good quality is obtained when harvesting is done at the proper stage of maturity. Immature fruit when harvested will give poor quality and erratic ripening. On the other hand, delayed harvesting of fruits and vegetables may increase their susceptibility to decay, resulting again in poor quality and lower market value leading to postharvest losses (Salunkhe *et al.*, 1991).

2.3.1 Fruit maturity

Avocado is a climacteric fruit (Mitra, 1997; Will *et al.*, 1998). Climacteric can be defined as the period in the development of certain fruits, during which a series of biochemical changes is initiated by the autocatalytic production of ethylene,

making the changes from growth to senescence. Involving an increase in respiration and leading to ripening (Holdsworth, 1983; Mitra, 1997). Also the respiration rate of immature fruit is higher at first and then steadily declines with age (Wills *et al.*, 1998).

As the fruit approaches the end of the growth phase it enters the period of maturation (Fig. 2). Physiological changes continue in the fruit during maturation but the active physical growth phase is ending for most fruits. Maturation implies that the fruit is ready to propagate the species and probably has a maximum eating potential. The latter is not realised in avocados until it is fully ripened (Tingwa & Young, 1975; Lewis, 1978). As compared to other climacteric fruits avocado has the highest respiration rate (Fig. 3).

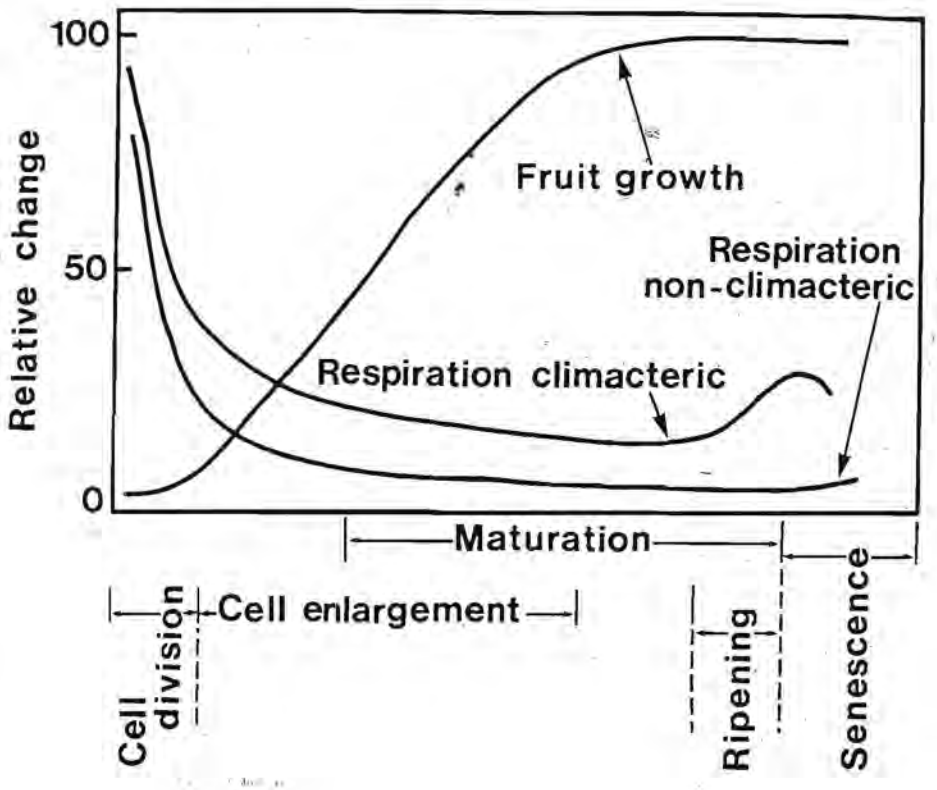

Fig. 2: Respiration rate during ripening of four fruits, highest peak in avocados (Holdsworth, 1983)

Mature avocados fruit does not undergo softening on the tree, but will soften after harvesting. Salunkhe and Kadam (1995) suggested that a possible explanation for this phenomenon could be that as long as the fruit is still on the tree, it receives some factors from the tree, which inhibits its ripening. After harvesting (fruit still immature), the ripening process can be slowed down by low temperature storage (4-6°C). Normal avocado softening with the development of an acceptable taste occurs only when a certain level of maturity has been reached. Picking of avocado at the appropriate maturity stage is essential to ensure maximum palatability (Tingwa & Young, 1975; Wills, *et al.*, 1998). Extended cold storage may however result in chilling injury, which is manifested as discolouration of the mesocarp, improper softening and off-flavour development (Bower & Cuttings, 1988; Cutting & Wolstenholme, 1992b; Eksteen, 1992).

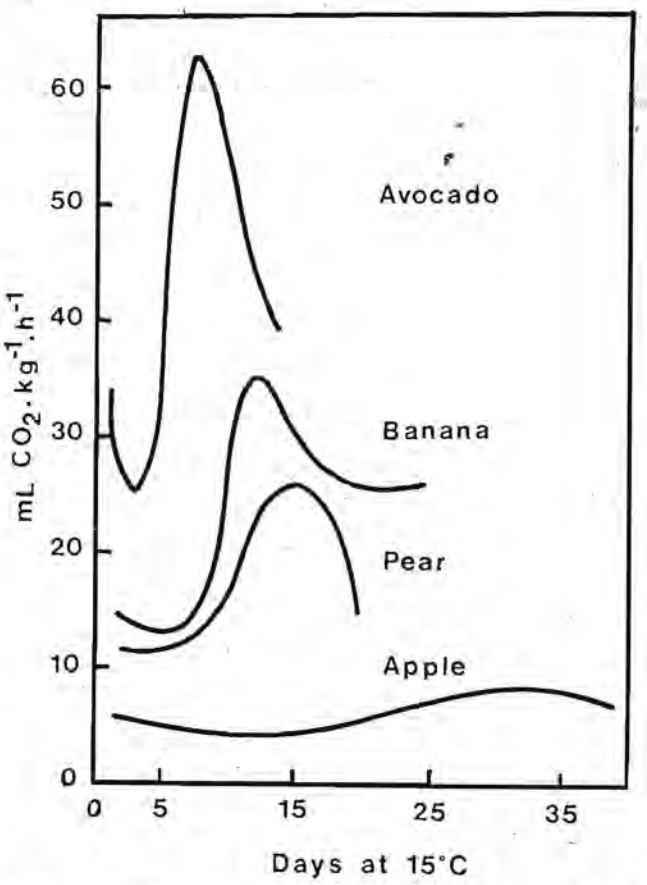

Fig. 3: Growth and respiration pattern of fruit during development (Wills *et al.*, 1998)

2.3.1.1 Changes associated with ripening

Wills *et al.* (1998) defines ripening as "the sequence of changes in colour, flavour and texture which lead to the state which the fruit is acceptable to eat". Ripening fruit undergoes many physico-chemical changes after harvest that determines the quality of the fruit purchased by the consumer. Ripening involves respiratory behavior and the involvement of the gas ethylene.

Mitra (1997), found that during ripening the middle lamella begins to disappear and that pectin removal from the matrix of cell walls occurred. A characteristics of the ripening process, common to most fruit, is an increase in the activity of the cell wall degrading enzymes responsible for fruit softening. Seymour & Tucker (1993) stated that biochemical analysis of avocado have shown large increases in the activities of wall hydrolytic enzymes during ripening. Dissolution of the ordered arrangement of call wall and middle lamella polysaccharides occurs as ripening progresses.

2.4 Minimal processed fruits

The availability of high quality preserved foods has always been important to consumers. The aim of traditional preservation methods is to ensure long shelf life with few changes in nutritional and sensory properties. Usually it is unavoidable that the overall quality is impaired during processing and storage (Güntensperger, 1994; Ohlsson, 1994). One of the newest concepts in food technology is minimal processing, the aim of which is to produce "fresh-like" products with attributes, such as convenience, expected by the consumer (Alzamora, Tapia & Well, 1993). Minimal processing is often regarded as "invisible preservation" and can be applied during postharvest treatment, processing, packaging and storage. MPR fruits are those fruits prepared by a single or any number of appropriate unit operation such as peeling, slicing and shredding, to give a partial but not end-point preservation treatment including use

of minimal heat, a preservative, or radiation (Alzamora *et al.*, 1993; Baldwin *et al.*, 1995).

As a result of peeling, grating and shredding, produce will change from a relatively stable product with a shelf life of several weeks or months to a perishable one that has only a very short shelf life, even as short as 1-3 days at chilled temperatures (Ahvenainen, 1996). MPR fruits deteriorate because of physiological ageing, biochemical changes and microbial spoilage, which results in degradation of the colour, texture and flavour of the produce (Lechowich, 1988; King & Bolin, 1989; Wiley 1994). During peeling and the grating operation, many cells are ruptured, and intracellular products such as oxidizing enzymes are liberated (Alzamora *et al.*, 1993; Wiley, 1994).

2.4.1 Physiological aspects of MPR fruits

Texture loss and changes in appearance, often browning are the most noticeable changes occurring in fruits during prolonged storage of MPR fruits (Rolle & Chism, 1987). These undesirable quality changes are accelerated by the mechanical rupturing of the cell that occurs during cutting, allowing enzymes to intermix with substrate (Fig 4) (King & Bolin, 1989; Wiley, 1994).

Fruits as harvested, usually have a hard protective layer of peel, skin, or rind and wax material on the outer surface. This layer protects the soft inner cells from damage. In minimally processing, this outside layer is usually removed, as is the case with avocados, peaches, apples and other fruits, exposing the fleshy cells (Huxsoll & Bolin, 1989). Inner fleshy material is composed mainly of parenchyma cells about 150-170μm long and large intercellular air space. These contains sugars, organic acids, and other organic substances (Lechowich, 1988; Alzamora *et al.*, 1993).

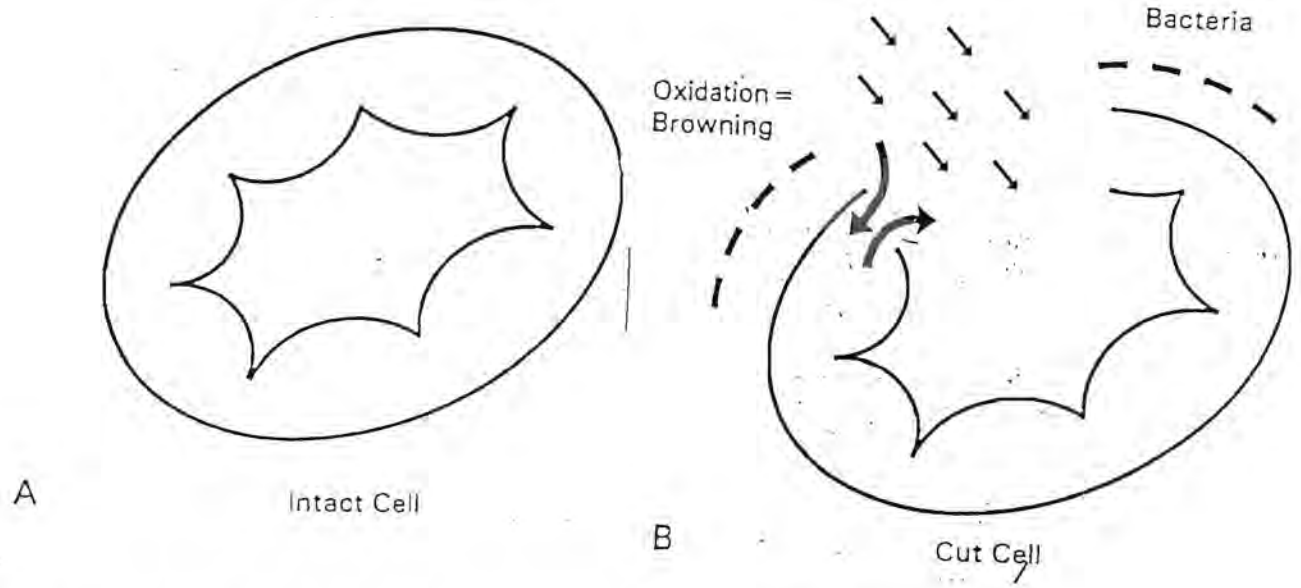

Fig. 4: Diagram of an intact and a processed (cut) fruit cell (Wiley, 1994)

The main physiological manifestations include increased respiration rate and, in some cases, ethylene production. The response depends on the magnitude of the stress. For more damaged plant tissue, respiration averages three to seven times more than that of unpeeled intact tissue (Ronk, Carson & Thompson, 1989).

Cut injury stimulates the biogenesis of ethylene which affect cells expansion, leaf abscission and fruit ripening (Adato & Gazit, 1974; Ronk *et al.*, 1989). Physiological changes indices by elevated ethylene concentration include, (1) increased cell permeability, (2) loss of compartmentation, (3) increased senescence and respiratory activity, and (4) increased activity of enzymes (Wiley, 1994).

2.4.1.1 Cell wall degradation and loss of firmness

The biochemical basis of textural changes in avocado and other fruits is still incompletely understood, but probably involves changes in the structure of the fruits cell walls (Seymour and Tucker, 1990; Wiley, 1994). Enzymes that degrade cell wall polymers have been isolated from avocado fruit, while pectinesterase activity apparently declines during the same period. Also, there appears to be a close relationship between this increase in cell wall degradation activity and the rise in respiration and ethylene production (Bower & Cuttings, 1988; Cutting & Wolstenholme, 1992b).

Slicing plant tissue generally results in loss of firmness. There is substantial variation in cell wall composition among tissue of fruits and even the various botanical groups (Ronk *et al.*, 1989; Wiley, 1994). In addition, there are cellular composition differences within individual products that affect texture. This variation is especially noticeable in fruits such as apricots, where texture readings can vary by more than 30 - 40% from one side of the fruit to the other. When the polymeric network in cells is irreversibly broken, a weakening of the cells occurs, resulting in a loss of cell turgor and texture (Lechowich, 1988; Baldwin *et al*, 1995).

Glucan, galactan, and water-insoluble pectinic acids are the major cells wall components considered to be responsible for textural change (El-Zeftawi, 1978; King & Bolin, 1989). Some of the following changes occur in cells during senescence: conversion of insoluble protopectin to pectin; decrease in cellulose crystallinity; decrease in galacturonic acid; reduction in cell volume; and thinning of cell walls. In addition, during softening, a decrease in wall-bound uronic acid and an increase in soluble uronide occur (Wiley, 1994). Membrane integrity must be maintained and the onset of senescence delayed to maintain the quality of minimally processed fruits (Ohlsson, 1994; Ahvenainen, 1996).

2.4.1.2 Enzymatic browning of avocados

Browning is a major problem in MPR avocados as heat which may lead to bitter taste cannot be used in avocados to inactivate enzymes. Enzymatic browning is the discolouration that results when monophenolic compounds of plants (avocados), in the presence of atmospheric oxygen and polyphenol oxidase (PPO), are hydroxylated to *o*-diphenols, and the latter are oxidized to *o*-quinones (McEvily, Iyenga & Otwell, 1992; Sapers, 1993). A variety of phenolic compounds are oxidised by PPO; the most important substrates are catechins, cinnamic acid esters, 3,4-hydroxyphenylalanine (DOPA), and tyrosine (Fennema, 1986; Sapers, 1993). The optimum pH for PPO activity is between pH 5 and 7. The enzyme is relatively heat labile and can be inhibited by acids, halides, phenolic acids, sulfites, chelating agents, reducing agents such as amino acids, quinone couplers such as cysteine and various substrate-binding compounds Sapers, 1993). Figure 5 shows the reaction mechanisms of enzymatic browning caused by PPO in avocados.

Mechanisms of inactivation of enzymic browning by chemicals

There are many ways in which enzymatic browning can be prevented in food, but only few of them apply to avocados. Examples of antibrowning agents that can be used on avocados are acidulants, e.g. citric acids; reducing agents, e.g. sulphiting agents and ascorbic acid and chelating agents e.g. citric acids and EDTA (Artes, Castener & Gill, 1998). The use of antibrowning agents in the food industry is constrained by considerations such as toxicity, effects on taste, flavour, colour, texture, and cost. A combination antibrowning agent is usually used because of the result in enhancement of activity relative to the use of any single agent individually (McEvily *et al.*, 1992; Artes *et al.*, 1998).

It is postulated that ascorbic acid inhibits the enzyme action primarily by its reducing power. Ascorbic acid is thought to reduce the formed o-quinones to its

phenolic form thus avoiding the formation of coloured pigments. In this reaction amino acids are oxidized to dehydroacids which is believed to bind one or more amino group near or at the active center of the enzyme. Therefore this action is considered as a contributing factor in the inhibition process. The mechanism of browning inhibition by reducing agents (i.e. ascorbic acid) is shown in Figure 6.

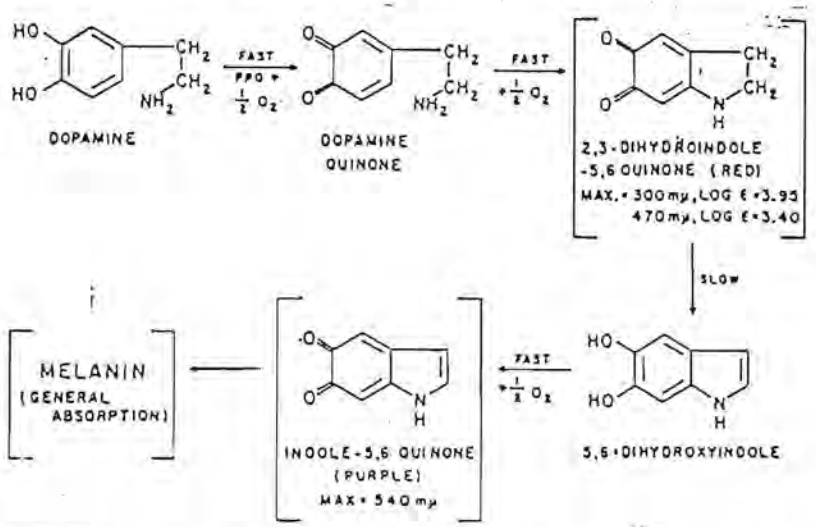

Fig. 5: Proposed reaction mechanisms for the oxidation of dopamine by polyphenol oxidase (McEvily *et al.*, 1992)

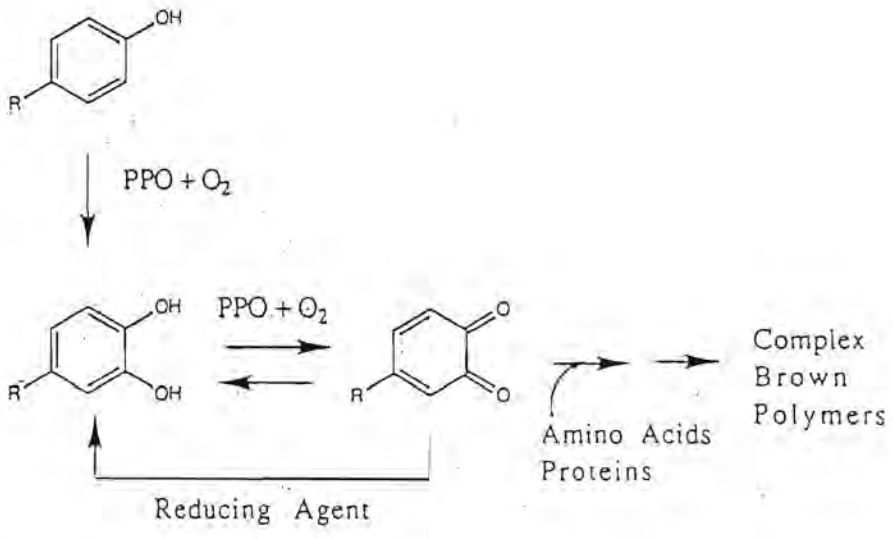

Fig.6: Inhibition of browning by use of reducing agents (Sapers, 1993)

Citric acid is one acid, which is widely used in food industries (Potter & Hotchkiss, 1995). Citric acid may have a dual inhibitory effect on PPO by reducing the pH and by chelating the copper at the enzyme-active site. Mostly this acidulant is used in combination with other antibrowning agents (McEvily *et al.*, 1992).

Sulphites are highly effective in controlling browning but are subjected to regulatory restrictions because of adverse effects on health. Sodium metabisulphite also act on *o*-benzoquinones and it follows the same mechanism as ascorbic acid (Artes *et al.*, 1998).

Effect of anti-browning compounds on the quality of minimally processed avocados

The prevention of browning of MPR avocados has been widely studied. The most preferred method of preventing browning is by the use of chemicals. Dorantes, Moreno, Pineda, Boix & Cánovas (1996) studied the use of 10 different antibrowning agents on their own and in combination on the quality of MPR avocados (Hass) (Table 1). The following analysis was conducted: sensory analysis for overall flavour and colour acceptance and microbiological analysis such as mesophylic bacteria, moulds and yeasts. They found that the most effective antibrowning agents (other than sodium metabisulphite) are the two mixtures, tetrasodium pyrophosphate and L-cystein. The mixture containing erythorbate, L-cystein, and ethylene diamine tetracetic acid (EDTA) was better (and statistically different at level of 0.05) than the mixture with L-cystein.

Table 1: Antibrowning agents used on minimally processed avocados (Dorantes *et al.*, 1996)

Antibrowning agent	Concentration (%)
Ascorbic acid	1.5
Citric acid	5.0
L-Cystein	0.2
EDTA, disodium salt	0.5
Erythorbic acid	1.0
Erythorbate, sodium salt	1.0
4 Hexyl-resorcinol	0.1
Polyvinylpolypirollidone	1.2
Pyrophosphate, tetrasodium salt	1.0
Zinc chloride	0.4
Mixture	
L-Cystein	0.2
Erythorbate, sodium salt	4.5
EDTA, disodium salt	0.1
Mixture	
Erythorbate, sodium salt	2.25
EDTA, disodium salt	0.05
Metabisulfite, sodium salt	0.2
Blank	—

2.4.2 Microbiological aspects

Microbiology is an important factor in the quality of MPR avocados. Temperature and RH can influence the microbiology of a food. Changes in the microbial ecology can influence the ultimate safety and overall quality of avocados (Brackett, 1987; Lechowich, 1988).

No literature could be found on microbes, which spoil MPR avocados, but information was found on spoilage of fresh avocados. Infectious microbes such as *Furarium salani* and *Furarium sambucicum* are well known microbes that can cause softening of the avocado fruits (Salunkhe & Kadam., 1995). There are also some post-harvest pathogens such as *Colletotrichum gloeosporioides*, *Dothiorella colletotrichum* causing fruit rotting (Bower and Cutting, 1988). According to Cutting & Wolstenholme (1992a) these pathogens are depended on

area as well as time period through the season. If the skin of fresh avocados is infected by the above mentioned pathogens and not properly washed (sanitized) prior to processing, the end product might end up being infected.

2.5 Use of edible coatings

The concept of using edible coatings to extent the shelf life of fresh foods products and protect them from harmful environment effects is not a novel one. Actually the idea originated from the natural protective coating on some foods like the skin of fruits and vegetables (Gennadios & Weller, 1990; Anonymous, 1997; Krochta & De Mulder-Johnston, 1997). During the last thirty years, considerable research work aimed at the development of edible packaging and coatings has been conducted. However, few of these films have been applied commercially (Avena-Bustillos, Krochta, Saltveit, Rojas-Villegas & Sauceda-Pérez, 1994; Anonymous, 1997). Edible coatings generally can be defined as thin layers of edible material applied on (or even within) foods by wrapping, immersion, brushing, or spraying in order to offer a selective barrier against the transmission of gases, vapours, and solutes while also offering mechanical protection (Gennadios & Weller, 1990; Cuq, Gontard & Guilbert, 1995; Baldwin, Burns, Kazokas, Brecht, Hagenmaier, Bender & Pesis, 1999).

2.5.1 Possible functional properties and advantages of edible coatings

Functions of edible coatings in MPR fruits are listed below (Fennema, 1989; Gennadios & Weller, 1990; Avena-Bustillos *et al.*, 1994; Anonymous, 1997; Fishman, 1997).

- Retards moisture migration
- Retards gas transport (O_2, CO_2)
- Retards solute transport
- Retard ethylene production

- Improves mechanical-handling properties of foods
- Imparts added structural integrity to foods

Advantages for using edible coatings over traditional non-edible polymeric packaging materials are listed below (Chen, 1993; Cuq *et al.*, 1995, Anonymous, 1997; Baldwin *et al.*, 1999).

- The coating can be consumed with the packaged product. This is obviously of critical importance since it represents the environmentally ideal package. There is no package to dispose of.
- The films can supplement the nutritional value of the food. This is advantageous for MPR avocados because they can be coated with carbohydrates, which they are lacking.
- The coat can enhance the sensory properties of packaged MPR fruits and provided that various components (flavouring, colouring and sweeteners) are incorporated in them.
- The film can function as carrier for antimicrobial and antioxidant agents (MPR fruits are prone to enzymatic browning).

2.5.2 Different types of edible coatings used on minimally processed fruits and their functional properties

More research has been done on the use of edible coating on MPR vegetables (e.g. cut mushroom, peeled carrots and potatoes) than on MPR fruits. The earlier research done on MPR fruits was mostly on apple snacks (Harman, 1999). This was due to the fact that children, especially young ones, often find a whole apple or orange too large, or hard to peel before eating and also it is easy to prepare for school lunchbox (Harman, 1999). Edible coatings on MPR fruit have the possibility in near future to replace wax as a coating for whole fruit, to extend storage life and prevent mould growth (Fennema, 1989).

No information was found on the use of coatings on MPR avocados, but research have been done on the use of edible coating on fresh avocados e.g. waxes. Formulations for edible films or coatings must include at least one component able to form a suitably cohesive and continuous matrix (Chen, 1995; Baldwin, Nisperos, Chen & Hagenmaier, 1996). The basic materials can be classified into three categories: Polysaccharides, Protein and Lipid based compounds (Kester and Fennema, 1986; Brandenburg, Weller & Testin, 1993; Anonymous, 1997; Krochta and De Mulder-Johnston, 1997).

2.5.2.1 Polysaccharides based coatings

The advantages of Polysaccharide-based coatings are more apparent in the area of gas exchange rather than retardation of water loss. Kester and Fennema (1986) indicated that Polysaccharide films have good oxygen barrier properties when they are not moist and material with oxygen barrier properties are expected to protect fruits from vitamin loss. Polysaccharide films, because of their hydrophilic nature, provide only a minimal moisture barrier. The CO_2 and O_2 permeabilities of the polysaccharide-based coatings, however, result in the creation of a desirable modified atmosphere, thereby increasing shelf life without creating severe anaerobic conditions (Baldwin *et al.*, 1995).

Low D.E. Maize starch hydrolysates (Dextrin)

The low D.E. hydrolysate bridges the property gap between starches and traditional maize syrups. Maize syrup is selected using dextrose equivalency as the basis for differences in functionality (Miller & Krochta, 1997). Dextrose equivalency (D.E.) denotes the total reducing sugar content on a dry basis (Fennema, 1986). As the D.E. increases, the properties of dextrose are approached; as the D.E. decreases, the properties of starch are approached. The sweetness and properties of traditional Maize syrups are significantly affected by a change in D.E. of 5-10 units, but the basis properties of a low D.E.

product, are significantly affected by a shift of only 3-4 units (Anonymous, 1997; Miller & Krochta, 1997).

Figure 7 shows how these properties change as the D.E. is raised or lowered. The direction and increasing width of the arrows indicate an increase in the property. As shown in Figure 6, lower D.E. products contribute more body and cohesiveness to solutions than do higher D.E. products, and the low level of reducing sugars results in less browning, lower hygroscopicity, and lower sweetness. Therefore these low D.E. hydrolysates are soluble, less sweet and form unique coatings.

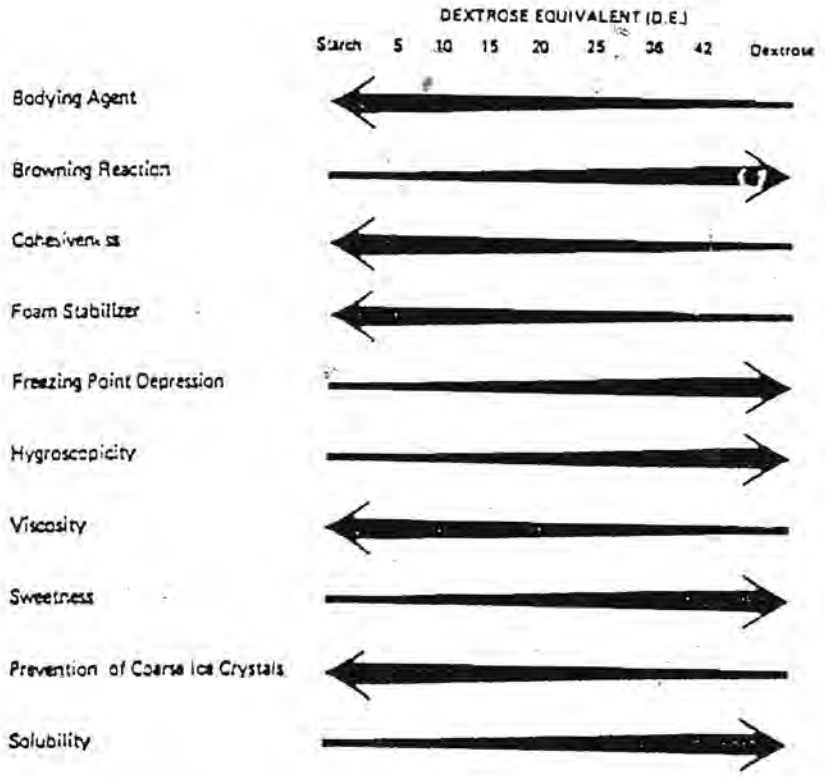

Fig. 7: How properties of maize starch hydrolysates vary with dextrose equivalency (Miller & Krochta, 1997).

The low D.E. hydrolysates showed good barrier properties when coated on (1) MPR apple slices (40% solution of a 15 D.E. hydrolysate), improving colour, texture and flavour; (2) MPR sliced apricots (30% solution of a 15 D.E. hydrolysate) which had natural flavour but browned somewhat; (3) Almonds (50% solution of a 10 D.E. hydrolysate) improving flavour and shelf life (Kester & Fennema, 1986; Miller & Krochta, 1997).

Alginates

Alginate is a polysaccharide, like starch and cellulose. It is composed of several (100-3,000) building units linked together in a partly stiff and partly flexible chain (Coultate, 1989). Long molecules constructed from identical or nearly identical building units are called polymers, while the building units themselves are called monomers. Polymers of natural origin are commonly named biopolymers. Cellulose is composed of glucose molecules, while alginate is built up on the basis of two sugars, which are both uronates, the salts of mannuronic and gluronic acid (Moe, Draget, Skjak-Braek & Smidsrod, 1995).

The viscosity of an alginate solution depends on the length of the alginate molecules i.e. the number of monomer units in the chains (Fennema, 1986). The longer the chains the higher the viscosity at similar concentration. On dissolving alginate in water the molecules hydrate and the solution gains viscosity. The dissolved molecules are not completely flexible; rotation about the glycosidic linkages in the G-block regions is somewhat hindered, resulting in a stiffening of the chain (Moe *et al.*, 1995).

The main property of alginate which is depended on distribution of monomers, is the gelling with calcium (Fennema, 1986; Moe *et al.*, 1995). To form a gel by reaction with calcium, alginates need to contain a sufficient level of guluronate monomer and a certain proportion of these gulurate monomers (G) must occur in a block. The reaction with calcium and the consequent gelling capacity is a direct function of the average length of the G-block (Coultate, 1989).

When a thin layer of alginate gel or alginate solution is dried, a film or coating is formed (Coultate, 1989). Alginate films are utilized in a number of applications as a gas barrier. In MPR apple slices it improved the colour (Fennema, 1989).

2.5.2.2. Lipids based coatings

Lipidic compounds are often used to make moisture and oxygen barrier coatings (Cuq *et al.*, 1995). Water is not very soluble or mobile in lipid-based films because of the low polarity and dense well-structured molecular matrixes that can be formed by these compounds (Fennema, 1989; Cuq *et al.*, 1995). Moisture resistance of lipid coatings is generally inversely related to polarity of the lipids. The moisture barrier capacities of different films can be classified in increasing order of efficiency, as follows: liquid oils < solid fats < waxes (Kester & Fennema, 1986). Materials with suitable barriers properties are required to protect oxidizable foods (to reduce rancidity and vitamin loss), but some permeability to O_2, and especially to CO_2, is essential for MPR and fresh fruits and vegetables coatings because they can cause anaerobic conditions at high storage temperature (Kester & Fennema, 1986; Fennema, 1989).

Olive oil

Olive oil consist predominantly of glycerides formed by a mixture of unsaturated and saturated fatty acids; unsaturation is due primarily to oleic acid (67-83%) and only about 2% is fully unsaturated. The desired colour of the oil is pale yellow and its flavour is sweet and fruity. One of the major indication of olive oil quality is the free fatty acid content (Fennema, 1989).

The best storage conditions for olive oil are at a temperature close to 14°C in tightly closed containers which prevent contact with light, air, water, and harmful metal, such as Fe and Cu (Cuq *et al.*, 1995). Deterioration of the oil during storage will cause (a) an increase in acidity due to the action of lipases and (b)

the development of rancidity due to oxidation involving changes in the aroma and taste of the oil (Fennema 1989). The oil is very expensive in relation to other edible oils. Therefore fresh oil must always be used to prevent the above mentioned problems. Vegetable oils have been used to retard moisture migration of MPR apples and peaches, but the products suffered from flavour stability. Hydrogenated vegetable oils that were resistant to rancidity was used and gave better results (Baldwin *et al.*, 1995).

Lecithin

Lecithin is a phospholipid, which is an emulsifier. There are many lecithin differing in their fatty acid content. Emulsifiers belong to a broader group of chemicals known as surface-active agents, designated as such because they exert their effects largely on the type of food system to be emulsified (Kamper & Fennema, 1985; Guilbert, Gontard & Gorris, 1996). Lecithin was used on MPR peaches and gave a good moisture barrier (Kotze, Sales Representative, Chempure company, 2000 - personal communication).

2.5.2.3 Protein based coatings

Protein coatings provide good barriers for O_2 and CO_2, but not water and are used mostly on fruits and vegetables (Baldwin *et al.*, 1995). Protein coatings are produced from renewable resources and degrade more readily than other types of polymeric material. Such films could add a nutritional component to coated foods (Fennema, 1989). Proteins are good film formers and will adhere to hydrophilic surfaces, but in most cases, they do not resist water vapour diffusion.

Sodium caseinate

Caseinates are produced by treating acid-precipitated caseins with alkali (sodium or calcium hydroxide) at 80-90°C and pH 6.2-6.7. Caseinates are soluble above

pH 5.5. Sodium and potassium caseinates are more soluble and often possess better functional properties than calcium and magnesium derivatives (McHugh & Krochta, 1994). Sodium caseinate is very heat stable, whereas calcium caseinate is only heat stable above 4% at 120°C for 15 minutes. Sodium caseinate easily forms a film from aqueous solutions due to their random-coil nature and ability to form extensive intermolecular hydrogen electrostatic, and hydrophobic bonds (Fennema, 1986; Macrae *et al.*, 1994). The films are attractive for use on food products due to their transparent, flexible, and bland nature (McHugh & Krochta, 1994).

Sodium caseinate was used as an edible coating for MPR carrots slices. It was found that it acted as a good gas barrier but was a poor moisture barrier (Krochta & De Mulder-Johnston, 1997).

2.5.2.4 Composite coatings

Composite coatings can be formulated to combine the advantages of the lipids, proteins and carbohydrates components and lessen the disadvantages of each. When a barrier to water vapour is desired the lipid components can serve this function while the protein and carbohydrate components provide the necessary shelf life (McHugh & Krochta, 1994; Anonymous, 1997; Fishman, 1997).

2.6 Polymeric Packaging

Packaging of fresh produce using polymeric films has been practiced for several decades to contain and protect fruits and vegetables from environmental contaminants (Schlimme & Rooney, 1994). In more current times polymeric films packages have been used to minimize moisture loss and reduce respiration rate of produce commodities as well as for MPR fruits and vegetables (PeiYin & Barth, 1998). Polymers are a class of organic chemicals that have long, high molecular weight (up to 10^6) molecules capable of being synthesized from or

depolymerized into numbers of chemically identifiable simple recurring units termed monomers (Wiley, 1994). Indeed, the use of sealed, unperforated polymeric packaging with carefully selected gaseous permeability characteristics in conjunction with appropriate prepackaging, cooling/preparation and sanitation treatments is a major tool utilized to achieve adequate shelf life for both unprocessed produce and MPR fruits and vegetables.

Therefore, food packaging involves both the art and the science of preparing foodstuffs for storage, transport, and sale. Thus, major requirements of a food packaging material encompass at least several of the following factors: control of moisture transfer, control of gas transfer, protection from external physical or mechanical damage and biologic contamination, tolerance of routine storage environment without undue loss of functionality (Wiley, 1994)

Polymeric films used are: polypropylene, polythene, polystyrene, pliofilm, cellulose and cellulose acetate (Schlimme & Rooney, 1994).

Respiring produce and MPR products utilize considerable oxygen and plastic films that are suitable for use with fresh and MPR produce need to have relatively high oxygen permeability coefficients. This is in order to avoid development of an anoxic atmosphere within the package. The diffusion of gases such as oxygen and carbon dioxide depends on the size, shape and polarity of the penetrating molecule, and crystallinity, degree of crosslinking, and polymer chain segmental motion and film matrix (Wiley, 1994; PeiYin & Barth, 1998).

CHAPTER 3

OBJECTIVES

The primary objective of the project was to investigate the use of edible coatings to extend the shelf life of minimally processed avocados stored at 5°C.

The secondary objectives were as follows:

- To determine the effect of selected carbohydrate, protein and lipid based edible coatings, alone, on the microbiological, physico-chemical and sensory quality of minimally processed avocados.

- To determine the effect of selected edible coatings, in combination, on the quality and shelf-life of minimally processed avocados stored for nine days at 5°C.

CHAPTER 4

MATERIALS AND METHODS

4.1 Raw material

Two types of Hass avocado cultivars supplied by Hans Merensky Fruit Processing Pty (Ltd), were used in this research. *Persea americana* Mill, cultivar Westalia Hass (a non browning cultivar) was used in Phase 1, whereas *Persea americana* Mill, general Hass cultivar (a browning cultivar) was used in Phase 2 of the study. The reason for using both cultivars was due to unavailability of the non-browning cultivar throughout the investigation. The non-browning Hass avocados were ripened at the University of Pretoria at normal room temperature (± 25°C) whereas the browning Hass avocados were supplied ripe. On arrival both the cultivars were stored in a cold room at 5°C and RH of 86% until used or ripened.

The potential benefit of using the non browning cultivar is that some countries in Europe where minimally processed avocados could be exported don't allow antibrowning agents such as sodium metabisulphite, due to its adverse health effects on asthmatic individuals. Sodium metabisulphite control enzymatic browning by reducing the *o*-quinones produced by polyphenol oxidase to the less reactive, colourless diphenols; it is also said to be poisonous to the enzyme polyphenol oxidase (McEvily, Iyenga & Otwell, 1992). Sodium metabisulphite is an extremely effective anti-browning agent; a suitable replacer has not yet been found that is as effective as sodium metabisulphite.

4.2 Experimental design

The primary objective of the project was to investigate the use of edible coatings to extend the shelf-life of minimally processed avocados stored at 5°C. The research was sub-divided into two phases, each with its own objectives.

4.2.1 Phase 1: Screening of potential edible films

The objective of Phase 1 was to determine the effect of selected carbohydrate, protein and lipid based edible coatings, alone, on the microbiological, physico-chemical and sensory quality of minimally processed avocados. The experiment was repeated twice. The following tests were conducted on days 1 and 7 of storage at 5°C (in duplicate):

- Moisture content
- Colour measurements
- Level of oxygen(O_2) and carbon dioxide (CO_2) in the atmosphere surrounding the product in the pack
- Total plate counts
- Anaerobic counts
- Organoleptic evaluation (appearance, colour and taste)

4.2.1.1 Preparation of minimally processed avocados

A flow diagram of the processing of minimally processed avocados stored at 5°C and RH of ± 86% coated with carbohydrate, protein and lipid based coatings is given in Fig 8.

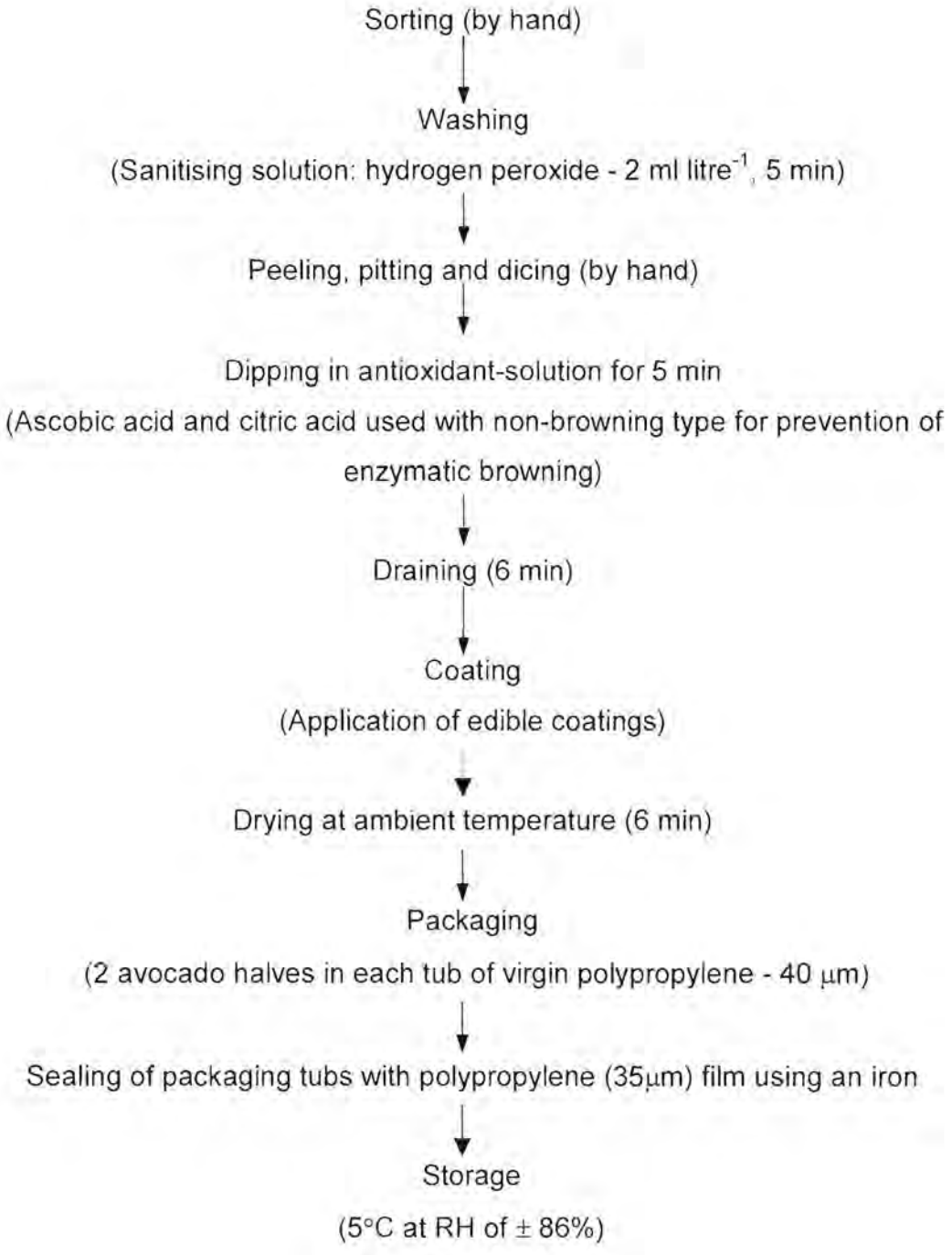

Fig. 8: Preparation of minimally processed avocados coated with edible coatings

Fresh avocados were sorted by hand, then immersed (5 min) in a sanitising solution (Super san: hydrogen peroxide - 2 ml litre^{-1}) to reduce initial microbial counts (Fig. 9). The washed avocados were halved, pitted and peeled by hand (Fig. 10). The avocados were cut into halves along the longitudinal axis and the pips were removed. The opened halves were inspected for spots or areas of discolouration and for any soft or rotten areas. Then the avocado halves were dipped in an antioxidant-solution for 5 min to prevent enzymatic browning. Ascorbic acid and citric acid were used during Phase 1 (with non-browning cultivar) whereas sodium metabisulphite was used in addition to the ascorbic acid and citric acid during Phase 2 for the browning cultivar (Fig. 11). The avocados were removed from the dip and left to drain (Fig. 12) for at least 6 min prior to application of edible coating.

Edible coatings were applied on avocados using spray bottles (Fig. 13). One side of an avocado half was sprayed first and allowed to dry for 6 min. Then the avocados were turned around and sprayed on the other side (Fig. 14).

Two avocado halves were then packed in a virgin polypropylene tub (40 μm) (supplied by Modern Packaging Company) with the hollow end where the pip has been removed facing upwards and sealed with a layer of polypropylene film (35 μm) using an iron (Fig. 15). The packed halves were then stored at 5°C at ± 86% RH.

Fig. 9: Immersion of avocados in a sanitising solution

Fig. 10: Peeling and pitting of fresh avocados

Fig. 11: Dipping of avocados in an antioxidant-solution

Fig. 12: Draining of avocados halves

Fig. 13: Spraying of edible coatings on avocados

Fig. 14: *Drying of edible coating for 6 min on stainless steel trays*

Fig. 15: Sealing of the packaging tub with a layer of polypropylene

Three different categories of 11 commercially available edible coatings were screened during Phase 1 as illustrated in Table 2.

Table 2: Three categories of 11 commercially available edible coatings that were screened during Phase 1

Category	Coating	Concentration (% m/v)	Supplier
Polysaccharide			
• Dextrins	Dextrin 10 DE[1]	50.00	African Products (Isando, S.A.)
	Dextrin 17 DE	40.00	Roquette (Midrand, S.A.)
	Dextrin 20 DE	30.00	African Products (Isando, S.A.)
• Alginates	Protonal SF 40	0.85	African Products (Isando, S.A.)
	Protonal SF 40 + 0.35 Calcium	0.85	African products (Isando, S.A.)
	Protonal 686	0.85	African Products (Isando, S.A.)
	Protonal 686 + 0.35 Calcium	0.85	African Products (Isando, S.A.)
Proteins	Caseinella QN	10.00	African Products (Isando, S.A.)
	Emulac 50	10.00	African Products (Isando, S.A.)
Lipid	Lecithin	5.00	Chempure (Pretoria, S.A.)
	Olive oil	100.00	Bought at Pick 'n Pay (Borges brand)

[1] DE = Dextrose equivalent

After preparation, the coatings were poured into different spray bottles (Fig 16)

Fig. 16: Spray bottles containing edible coatings

4.2.2 Phase 2: Determination of the quality and shelf life of minimally processed avocados

The objective of Phase 2 was to determine the effect of selected edible coatings, alone and in combination, on the quality and shelf-life of minimally processed avocados stored for 9 days at 5°C and at ± 86% RH.

The experiment was repeated four times. The following tests were conducted on days 1, 3, 5, 7 and 9 of storage at 5°C in duplicate to investigate the effect of combined coatings on minimally processed avocados:

- Moisture content
- Colour determination
- Level of O_2 and CO_2 in the atmosphere surrounding the product in the pack
- Texture analysis
- Total plate counts
- Coliform counts
- Anaerobic counts

4.2.2.1 Preparation of minimally processed avocados

Individual coatings did not perform optimally, so combined coatings were used during Phase 2. The combined coatings used are given in Table 3.

The preparation of avocados was the same as in Phase 1 (section 4.2.1.1) except that when two different coatings were applied, the first coating was allowed to dry first before applying the second coating.

Table 3: Edible coatings used for the shelf life test on minimally processed avocados

Treatment	Category combination
Control	No coating
30% Dextrin 20 DE	Polysaccharide only
30% Dextrin 20 + 10% Caseinela QN	Polysaccharide + Protein
30% Dextrin 20 + 100% Olive oil	Polysaccharide + Lipid
30% Dextrin 20 + 0.85 Protonal 686 + 0.35 Calcium	Polysaccharide - Dextrin + Polysaccharide - Alginate

4.3 Analysis of samples

4.3.1 Determination of moisture content

Moisture content was determined in duplicate using a modification of the AOAC forced draught oven method 934.06 (AOAC, 1995). Approximately 3 g of samples were weighed, into moisture tins previously dried at 100°C for 1 h. The samples were then dried in an forced draught oven at 100°C for 3 h. Dried samples were cooled down to room temperature in a desiccator and weighed.

The moisture content was calculated as follows:

$$\% \text{ Moisture} = \frac{\text{Mass fresh sample} - \text{Mass dried sample}}{\text{Mass of fresh sample}} \times 100$$

4.3.2 Determination of colour

The colour of the samples was determined using a Minolta Chromometer instrument. The colour measurements were measured as illustrated in Fig 17. L (white/black), a(red/green) and b(yellow/blue) values were recorded.

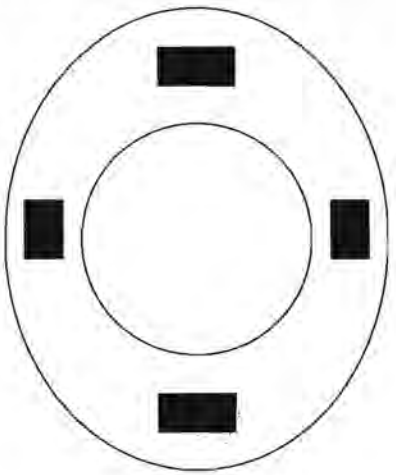

Fig. 17: Illustration of where the colour measurements were taken on the avocado half (Squares show exactly where the colour measurements were taken)

4.3.3 Determination of the level of O_2 and CO_2 in the atmosphere surrounding the product in the pack

Levels of O_2 and CO_2 were determined using a Gaspace 2 Instrument supplied by Set Point Analytics. Two measurements were taken per pack.

4.3.4 Determination of texture

The texture of avocados was determined with a TA-XT2 Texture Analyser using a 6 mm DIA cylinder stainless steal probe. The avocado halves were placed on a stainless steel platform, with the hollow end down. The avocado was penetrated 10 mm with a pre test speed of 5.0 mm/s, test speed of 1.0 mm/s and post test speed of 1.0 mm/s. The force was measured in compression (N) of penetration. The textural values were expressed as partial area (J). Three measurements were carried out on one avocado half as illustrated in Fig. 18.

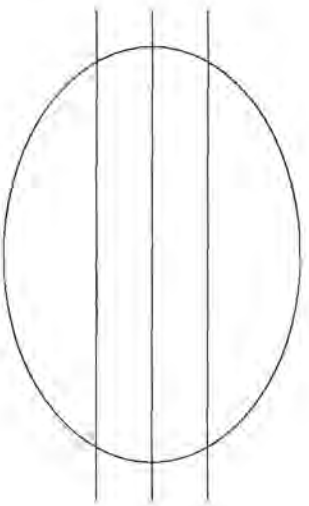

Fig. 18: Illustration of where the texture measurements were taken on the avocado half

4.3.5 Microbiological tests

4.3.5.1 Sampling and preparation of dilutions for microbiological tests

One avocado halve from one pack was aseptically mashed using a sterile small hand held mincer. Then 10 g of product was mixed with 90 ml of sterile peptone water solution and placed in a stomacher for 3 min. One ml of this dilution was then added to 9 ml sterile peptone solution to make consecutive dilutions. The

highest dilution made was 10^{-6}. From each dilution 2 duplicates were plated. The results of total plate count, coliforms and yeasts and moulds were presented in units of cfu/g.

Determination of total plate counts

Total plate counts method was done using Total Plate Count Agar (Merck) according to the method of Refai (1979). The plates were inverted and incubated at 30°C for 72 hours.

Determination of Coliform counts

Coliform test was carried out on a medium of Violet Bile Red Agar (Merck) according to the method of Refai (1979). The plates were inverted and incubated at 30°C for 24 hours.

Determination of yeasts and moulds counts

Yeasts and moulds counts were carried out on a medium of Potato Dextrose Agar (Merck) to which 10% tartaric acid was added to lower the pH to 3.5 ± 0.2 (Refai, 1979). The plates were incubated at 25°C for five days.

Determination of anaerobic sporeformers

Anaerobic sporeformers were carried out according to the method of Speck (1992). Freshly sterilised Reinforcement Clostridium Media (RCM) was cooled to approximately 50°C and inoculated with 1 ml of 5 (10^{0} - 10^{-4}) successive dilutions of the sample; using three tubes for each dilution. For dilution 0, 10 ml of dilution 1 was added to 10 ml of double strength RCM. After inoculation, approximately 2 ml sterile thioglycollate agar was added to each tube so that it formed a seal of at least 10 mm on top of the medium so as to maintain anaerobic conditions in the medium. The tubes were incubated at 37°C for 7 days and inspected daily

for signs of gas production. The results were presented in units of most probable number (MPN/g).

4.3.6 Organoleptic evaluation

A 9-point hedonic scale was used for sensory evaluation by the researcher for each replicate conducted. Samples were evaluated for colour, taste and appearance.

4.4 Statistical analysis

Statistical analyses were performed using Statistical Analyses System (SAS) (Friud & Littell, 1992). One way analysis of variance (ANOVA) followed by Duncan Multiple Range test was done to determine the effect of the treatments at $p < 0.05$.

CHAPTER 5

RESULTS

5.1 Phase 1

In this phase, *Persea americana* Mill cultivar Westalia Hass (a non browning cultivar) was used. This cultivar was ripened at the University of Pretoria at normal room temperature (± 27°C) for three to four days.

5.1.1 Moisture content

The effect of storage for a period of 7 days at 5°C on the moisture content of minimally processed avocados treated with different coatings is presented in Table 4.

Statistical analysis indicated that overall, time of storage had a significant effect on the moisture content. During storage, the moisture content decreased. Overall, only the samples treated with Protonal SF 40 + Ca^{2+} and Protonal 686 + Ca^{2+} had a significantly higher moisture content that the other treatments.

5.1.2 Level of oxygen and carbon dioxide surrounding the avocados in the package

Tables 5 and 6 show the effect of storage for a period of 7 days at 5°C on the level of O_2 and CO_2 carbon dioxide, respectively, in the package of minimally processed avocados treated with different coatings.

Overall, time of storage had a significant effect on the O_2 levels in the air surrounding the avocados in the pack. During storage, the O_2 levels increased. After 7 days of storage, the sample coated with Protonal 686 had a significantly lower O_2 level than those coated with Dextrin 17 DE, Dextrin 20 DE, Emulac 50, Protonal SF 40, Protonal 686 + Ca^{2+} and Lecithin. Overall, no difference in O_2 levels was observed between the treatments.

Table 4: The effect of storage (at 5°C) on the moisture content of minimally processed avocados treated with different edible coatings

Treatment	Moisture content (%)		
	Day 1 [1]	Day 7 [1]	Treatment effect [1]
Control	76.00 abc (± 0.19) [2]	75.86 ab (± 0.97)	75.93 a (± 1.41)
Dextrin 10 DE	75.21 ab (± 0.47)	74.71 ab (± 1.07)	74.96 a (± 1.58)
Dextrin 17 DE	77.87 abc (± 1.15)	72.58 a (± 1.62)	75.23 a (± 3.39)
Dextrin 20 DE	76.16 abc (± 4.92)	72.27 a (± 3.44)	74.22 a (± 5.90)
Emulac 50	74.87 a (± 0.28)	73.06 ab (± 0.04)	73.97 a (± 1.77)
Casinella QN	76.50 abc (± 0.54)	73.32 ab (± 1.09)	74.92 a (± 1.86)
Protonal SF 40	76.51 abc (± 2.00)	75.54 ab (± 3.08)	76.02 a (± 2.36)
Protonal SF 40 + Ca^{2+}	79.39 c (± 0.20)	75.99 ab (± 4.18)	77.70 b (± 2.97)
Protonal 686	78.04 bc (± 0.04)	74.71 ab (± 0.99)	76.38 a (± 2.50)
Protonal 686 + Ca^{2+}	78.44 bc (± 0.49)	76.80 b (± 0.27)	77.62 b (± 2.29)
Lecithin	76.17 abc (± 0.37)	71.27 a (± 2.69)	73.73 a (± 3.84)
Olive oil	75.61 ab (± 2.28)	71.72 a (± 2.30)	73.67 a (± 2.75)
Time effect [3]	76.73 a (± 2.42)	73.98 b (± 3.11)	

[1] Means with different letters in a column are significantly different ($p < 0.05$)

[2] Standard deviations are given in parenthesis

[3] Mean values in row with different letters are significantly different ($p < 0.05$)

Table 5: The effect of storage (at 5°C) on the O_2 levels in the package of minimally processed avocados treated with different edible coatings

Treatment	Oxygen		
	Day 1 [1]	Day 7 [1]	Treatment effect [1]
Control	20.37 a (± 8.38) [2]	29.27 ab (± 2.08)	24.82 a (± 3.18)
Dextrin 10 DE	19.30 a (± 11.87)	28.02 ab (± 0.81)	23.66 a (± 1.43)
Dextrin 17 DE	19.12 a (± 5.20)	30.95 bcd (± 1.76)	21.03 a (± 1.27)
Dextrin 20 DE	20.57 a (± 11.42)	31.10 bcd (± 3.46)	25.83 a (± 1.67)
Emulac 50	17.92 a (± 5.83)	30.37 bc (± 2.08)	24.14 a (± 2.92)
Casinella QN	15.73 a (± 1.23)	27.80 ab (± 2.47)	21.76 a (± 0.46)
Protonal SF 40	21.32 a (± 8.16)	30.40 bc (± 2.19)	25.86 a (± 1.53)
Protonal SF 40 + Ca^{2+}	21.42 a (± 13.82)	27.72 ab (± 0.74)	24.57 a (± 1.83)
Protonal 686	20.12 a (± 6.82)	20.25 a (± 5.16)	20.18 a (± 2.59)
Protonal 686 + Ca^{2+}	21.77 a (± 8.80)	31.92 cd (± 1.59)	26.84 a (± 1.17)
Lecithin	21.60 a (± 11.17)	32.97 cd (± 0.74)	27.28 a (± 0.64)
Olive oil	18.07 a (± 9.93)	29.10 ab (± 1.34)	23.58 a (± 0.29)
Time effect [3]	19.10 a (± 3.87)	29.15 b (± 2.61)	

[1] Means with different letters in a column are significantly different ($p < 0.05$)

[2] Standard deviations are given in parenthesis

[3] Mean values in row with different letters are significantly different ($p < 0.05$)

Although time of storage did not affect the CO_2 levels in the air surrounding the avocados in the packaging significantly, there seemed to be a general trend towards a decrease in CO_2 during storage. Overall, avocados treated with Olive oil had a significantly higher CO_2 level than samples treated with Emulac 50, Casinella QN, Protonal 686, Protonal 686 + Ca^{2+} and Lecithin.

5.1.3 Colour

Tables 7, 8 and 9 show the effect of storage for a period of 7 days at 5°C on the L (lightness), a (redness to greenness) and b (yellowness to blueness) values of minimally processed avocados treated with different coatings.

During storage, the L-values decreased significantly. Initially the L-value of the sample treated with Olive oil was significantly lower than samples treated with Protonal SF 40 + Ca^{2+}, Protonal 686 + Ca^{2+} and Lecithin. Also Olive oil differed from all samples except Dextrin 10 DE and Dextrin 17 DE. Overall, the treatments did not differ significantly.

Time of storage did not affect the a-values (redness/greenness) of the avocados significantly. Initially, control was significantly lower than all the treated samples except for the sample treated with Emulac 50. However there seemed to be a trend towards avocados being less green after 7 days of storage. After 7 days of storage, control and Dextrin 10 DE treated samples were significantly less green than samples treated with Dextrin 20 DE, Casinella QN, Protonal SF 40 and Protonal 686 + Ca^{2+}.

Time of storage did not affect the b-values (yellowness/blueness) of the avocados significantly. Overall, avocado samples treated with Dextrin 20 DE had a significantly higher b-value than all the other treatments. On day 7 of storage, the b-values of the control and the samples treated with Dextrin 10 DE and Emulac 50 were significantly lower than the samples treated with Dextrin 17 DE, Dextrin 20 DE, Casinella QN, Protonal SF 40 + Ca^{2+}, Protonal 686, Protonal 686 + Ca^{2+}, Lecithin and Olive oil.

Table 6: The effect of storage (at 5°C) on the CO_2 levels in the package of minimally processed avocados treated with different edible coatings

Treatment	Carbon dioxide		
	Day 1 [1]	Day 7 [1]	Treatment effect [1]
Control	6.20 b (± 2.89) [2]	1.62 ab (± 0.95)	3.91 ab (± 2.94)
Dextrin 10 DE	6.55 b (± 0.21)	3.32 c (± 1.87)	4.93 ab (± 2.02)
Dextrin 17 DE	6.55 b (± 3.32)	1.20 ab (± 0.14)	3.87 ab (± 3.37)
Dextrin 20 DE	5.85 b (± 1.06)	2.15 abc (± 1.06)	4.00 ab (± 2.14)
Emulac 50	2.77 ab (± 1.94)	2.60 bc (± 1.97)	2.68 a (± 1.49)
Casinella QN	1.65 a (± 0.98)	2.14 abc (± 0.22)	1.89 a (± 0.61)
Protonal SF 40	4.02 ab (± 3.35)	2.42 bc (± 0.10)	3.22 ab (± 2.00)
Protonal SF 40 + Ca^{2+}	6.65 ab (± 6.15)	2.88 bc (± 1.16)	4.76 ab (± 3.92)
Protonal 686	3.87 ab (± 0.45)	2.15 abc (± 0.63)	3.01 a (± 1.03)
Protonal 686 + Ca^{2+}	4.12 ab (± 1.59)	0.65 a (± 0.53)	2.38 a (± 2.06)
Lecithin	3.97 ab (± 0.03)	1.30 ab (± 0.21)	2.63 a (± 1.47)
Olive oil	9.80 b (± 1.55)	2.62 bc (± 0.46)	6.21 b (± 3.94)
Time effect [3]	5.17 a (± 2.44)	2.07 a (± 1.88)	

[1] Means with different letters in a column are significantly different ($p < 0.05$)

[2] Standard deviations are given in parenthesis

[3] Mean values in row with different letters are significantly different ($p < 0.05$)

Table 7: The effect of storage (at 5°C) on the L- values of minimally processed avocados treated with different edible coatings

Treatment	L-value [4]		
	Day 1 [1]	Day 7 [1]	Treatment effect [1]
Control	68.09 bc (± 0.08) [2]	63.14a (± 4.34)	65.61 a (± 7.02)
Dextrin 10 DE	65.44 ab (± 3.36)	59.72 a (± 4.03)	62.58 a (± 7.32)
Dextrin 17 DE	66.73 ab (± 1.07)	63.07 a (± 0.92)	64.90 a (± 4.20)
Dextrin 20 DE	68.91 bc (± 3.60)	63.25 a (± 2.68)	66.08 a (± 6.30)
Emulac 50	68.09 bc (± 3.45)	59.85 a (± 0.82)	63.97 a (± 6.81)
Casinella QN	68.42 bc (± 0.10)	58.68 a (± 1.20)	63.55 a (± 8.27)
Protonal SF 40	69.09 bc (± 1.96)	61.79 a (± 1.29)	65.44 a (± 6.96)
Protonal SF 40 + Ca^{2+}	71.69 c (± 0.57)	65.43 a (± 4.21)	68.56 a (± 6.48)
Protonal 686	72.89 c (± 3.27)	65.80 a (± 3.94)	69.34 a (± 8.82)
Protonal 686 + Ca^{2+}	68.38 bc (± 0.71)	65.92 a (± 6.62)	67.15 a (± 5.03)
Lecithin	70.96 c (± 1.83)	65.18 a (± 0.98)	68.07 a (± 4.77)
Olive oil	61.44 a (± 2.24)	64.56 a (± 4.41)	63.00 a (± 7.01)
Time effect [3]	67.80 a (± 2.78)	62.97 b (± 3.83)	

[1] Means with different letters in a column are significantly different ($p < 0.05$)

[2] Standard deviations are given in parenthesis

[3] Mean values in row with different letter are significantly different ($p < 0.05$)

[4] L = 0 indicates blackness; L = 100 indicates whiteness

Table 8: The effect of storage (at 5°C) on the a-values (redness/ greenness) of minimally processed avocados treated with different coatings

Treatment	a-value [4]		
	Day 1 [1]	**Day 7** [1]	**Treatment effect** [1]
Control	- 2.76 a (± 1.17) [2]	0.21 a (± 0.02)	- 1.27 a (± 2.34)
Dextrin 10 DE	- 5.46 b (± 2.28)	1.82 a (± 0.31)	- 1.82 a (± 4.88)
Dextrin 17 DE	- 4.83 b (± 1.44)	- 2.70 abc (± 0.70)	- 3.76 a (± 2.24)
Dextrin 20 DE	- 6.57 b (± 0.39)	- 3.25 bc (± 0.75)	- 4.91 a (± 3.04)
Emulac 50	- 4.26 ab (± 1.36)	- 1.48 ab (± 0.53)	- 2.87 a (± 2.08)
Casinella QN	- 4.77 b (± 0.22)	- 3.27 bc (± 0.52)	- 4.02 a (±1.76)
Protonal SF 40	- 4.62 b (± 0.34)	- 3.41 bcd (± 0.71)	- 4.01 a (± 2.21)
Protonal SF 40 + Ca^{2+}	- 5.18 b (± 0.39)	- 2.38 ab (± 0.51)	- 3.78 a (± 1.80)
Protonal 686	- 6.23 b (± 0.38)	- 2.58 ab (± 0.88)	- 4.41 a (± 3.15)
Protonal 686 + Ca^{2+}	- 4.45 b (± 0.73)	- 3.36 bcd (± 0.18)	- 3.90 a (± 1.40)
Lecithin	- 4.81 b (± 0.31)	- 2.69 abc (± 0.53)	- 3.75 a (± 1.47)
Olive oil	- 4.96 b (± 1.04)	- 2.19 ab (± 0.07)	- 3.57 a (± 2.76)
Time effect [3]	- 4.57 a (± 1.87)	- 2.06 a (± 0.93)	

[1] Means with different letters in a column are significantly different ($p < 0.05$)

[2] Standard deviations are given in parenthesis

[3] Mean values in row with different letters are significantly different ($p < 0.05$)

[4] $a > 0$ indicates redness whereas $a < 0$ indicates greenness

Table 9: The effect of storage (at 5°C) on the b-values(yellowness/blueness) of minimally processed avocados treated with different coatings

Treatment	b value [4]		
	Day 1 [1]	Day 7 [1]	Treatment effect [1]
Control	36.24 ab (± 3.18) [2]	34.33 a (± 1.31)	35.28 a (± 2.73)
Dextrin 10 DE	39.65 b (± 1.43)	34.44 a (± 1.84)	37.04 a (± 4.06)
Dextrin 17 DE	36.74 ab (± 1.27)	36.39 bc (± 0.59)	36.57 a (± 3.06)
Dextrin 20 DE	39.78 b (± 1.67)	38.51 bcde (± 1.03)	39.14 b (± 5.55)
Emulac 50	37.55 ab (± 2.92)	34.14 a (± 0.53)	35.84 a (± 4.57)
Casinella QN	35.64 a (± 0.46)	37.94 bcd (± 2.47)	36.79 a (± 4.59)
Protonal SF 40	35.28 a (± 1.53)	35.70 ab (± 0.82)	35.49 a (± 3.20)
Protonal SF 40 + Ca^{2+}	37.94 ab (± 1.83)	38.56 bcde (± 0.32)	38.25 a (± 3.68)
Protonal 686	39.20 b (± 2.59)	36.87 bc (± 0.17)	38.01 a (± 3.91)
Protonal 686 + Ca^{2+}	35.41 a (± 1.17)	37.26 bcd (± 0.51)	36.33 a (± 2.19)
Lecithin	36.19 ab (± 0.64)	39.66 cde (± 0.39)	37.93 a (± 3.55)
Olive oil	35.94 ab (± 0.29)	37.32 bcd (± 1.33)	36.62 a (± 3.76)
Time effect [3]	37.08 a (± 3.82)	36.64 a (± 3.97)	

[1] Means with different letters in a column are significantly different ($p < 0.05$)

[2] Standard deviations are given in parenthesis

[3] Mean values in row with different letter are significantly different ($p < 0.05$)

[4] $b > 0$ indicates yellow colour, whereas $b < 0$ indicates blue colour

5.1.4 Microbiology tests

5.1.4.1 Total plate count

The effect of storage for a period of 7 days at 5°C on the growth of microbes on minimally processed avocados treated with different coatings is presented in Table 10.

Overall, time of storage had a significant increase in the growth of microbes. On day 1 of storage, samples treated with Lecithin had a significantly lower microbial growth than samples treated with Dextrin 10 DE, Protonal SF 40, Protonal 686 and Olive oil. However the following samples had a significantly higher microbial level than the control after 7 days of storage: Dextrin 10 DE, Dextrin 20 DE, Protonal SF 40, Protonal SF 40 + Ca^{2+}, Protonal 686, and Olive oil.

5.1.4.2 Anaerobic sporeformers

Table 11 shows the effect of storage for a period of 7 days at 5°C on the growth of anaerobic sporeformers of minimally processed avocados treated with different coatings.

Time of storage did not have any statistical significant effect on the growth of anaerobic sporeformers of the avocados. However, there seemed to be a general trend towards an increase in the number of sporeformers organisms during storage at 5°C. On day 1 of storage the samples treated with Protonal SF 40, Protonal SF 40 + Ca^{2+}, Protonal 686, and Lecithin had a significantly lower anaerobic sporeformes than samples treated with Dextrin 10 DE, Casinella QN, and Protonal 686 + Ca^{2+}. Overall, the following samples, had significantly higher anaerobic sporeformers counts than the control: Dextrin 10 DE, Casinella QN, Protonal 686 + Ca^{2+}.

Table 10: The effect of storage (at 5°C) on total plate counts of minimally processed avocados treated with different edible coatings

Treatment	Total plate count (log_{10} cfu/g)		
	Day 1 [1]	Day 7 [1]	Treatment effect [1]
Control	3.34 ab (± 0.66) [2]	5.27 a (± 0.26)	4.30 a (±1.12)
Dextrin 10 DE	5.48 bc (± 0.17)	7.23 bcd (± 0.68)	7.35 a (± 1.04)
Dextrin 17 DE	3.86 ab (± 0.29)	5.52 a (± 0.02)	4.69 a (± 1.04)
Dextrin 20 DE	4.97 abc (± 1.65)	6.46 bcd (± 0.17)	5.71 a (± 1.21)
Emulac 50	4.60 abc (± 1.07)	6.11 abc (± 0.36)	5.35 a (± 1.02)
Casinella QN	5.07 abc (± 1.78)	6.10 abc (± 0.69)	5.39 a (± 1.15)
Protonal SF 40	5.25 bc (± 1.86)	6.76 bcd (± 1.52)	6.00 a (± 1.53)
Protonal SF 40 + Ca^{2+}	4.28 ab (± 1.26)	6.64 bcd (± 0.46)	5.46 a (± 1.45)
Protonal 686	6.54 c (± 0.42)	7.26 bcd (± 0.29)	6.89 a (± 0.47)
Protonal 686 + Ca^{2+}	3.31 ab (± 0.11)	6.53 bcd (± 0.25)	4.92 a (± 1.73)
Lecithin	2.71 a (± 0.65)	5.83 a (± 0.76)	4.28 a (± 1.76)
Olive oil	5.34 bc (± 2.01)	7.64 cd (± 0.83)	6.49 a (± 1.69)
Time effect [3]	4.56 a (± 1.46)	6.44 b (± 0.96)	

[1] Means with different letters in a column are significantly different ($p < 0.05$)

[2] Standard deviations are given in parenthesis

[3] Mean values in row with different letter are significantly different ($p < 0.05$)

Table 11: The effect of storage (at 5°C) on anaerobic sporeformers of minimally processed avocados treated with different coatings

Treatment	Anaerobic sporeformers (MPN/g)[4]		
	Day 1 [1]	Day 7 [1]	Treatment effect [1]
Control	37.50 ab (± 3.54)	79.50 a (± 41.92)	58.50 a (± 29.69)
Dextrin 10 DE	567.00 c (± 753.00)	850.00 a (± 670.71)	708.50 c (± 200.11)
Dextrin 17 DE	27.50 ab (± 17.68)	35.00 a (± 14.14)	31.25 a (± 5.30)
Dextrin 20 DE	25.50 ab (± 20.61)	76.50 a (± 39.09)	51.00 a (± 36.06)
Emulac 50	37.50 ab (± 3.54)	56.50 a (± 23.33)	47.00 a (± 13.43)
Casinella QN	400.00 c (± 265.68)	565.00 a (± 256.60)	482.5 bc (± 116.68)
Protonal SF 40	13.50 a (± 3.54)	158.00 a (± 200.81)	85.75 ab (± 102.17)
Protonal SF 40 + Ca^{2+}	18.00 a (± 16.97)	25.50 a (± 4.67)	21.75 a (± 5.30)
Protonal 686	15.50 a (± 0.71)	153.00 a (± 207.88)	84.25 ab (± 97.22)
Protonal 686 + Ca^{2+}	555.50 c (± 770.03)	555.50 a (± 770.03)	555.50 bc (± 0.00)
Lecithin	11.50 a (± 6.36)	20.50 a (± 8.36)	16.00 a (± 6.36)
Olive oil	22.50 ab (± 10.61)	13.50 a (± 3.55)	18.00 a (± 6.36)
Time effect [3]	144.29 a (± 222.75)	215.67 a (± 279.25)	

[1] Means with different letters in a column are significantly different ($p < 0.05$)

[2] Standard deviations are given in parenthesis

[3] Mean values in row with a different letter are significantly different ($p < 0.05$)

[4] Means, most probable number

5.1.5 Organoleptic evaluation

The effect of storage for a period of 7 days at 5°C on the appearance, taste and colour of minimally processed avocados treated with different coatings is illustrated in Tables 12, 13 and 14 respectively.

Overall, time of storage had an effect on the appearance of the avocados stored at 5°C. Appearance of samples was less liked after storage. On day 7 of storage, samples treated with Dextrin 17 DE and Protonal 686 + Ca^{2+} were significantly less liked than control, Casinella QN, Lecithin and Olive oil samples.

Overall, time of storage had a significant effect on the colour of the avocados. During storage the colour of the samples seemed to be less liked. Overall the colour of the sample treated with Dextrin 10 DE was significantly more liked than the sample coated with Casinella QN.

Time of storage did not seem to affect the taste of the avocados significantly. However there seemed to be a trend towards avocados being less liked in terms of taste after 7 days of storage. Overall, avocados treated with Dextrin 20 DE was significantly more liked than the other treatments. On day 7 of storage avocados treated with Lecithin, had a less acceptable taste than the control and samples treated with Dextrin 10 DE, Dextrin 17 DE, Dextrin 20 DE, Casinella QN, Protonal SF 40, Protonal SF 40 + Ca^{2+}, and Protonal 686.

Table 12: The effect of storage (at 5°C) on the appearance of minimally processed avocados treated with different edible coatings

Treatment	Appearance [4] Rating Day 1 [1]	Day 7 [1]	Treatment effect [1]
Control	6.50 a (± 0.71) [2]	5.50 bc (± 0.71)	6.50 a (± 0.71)
Dextrin 10 DE	8.00 a (± 1.30)	5.00 abc (± 1.41)	6.50 a (± 1.91)
Dextrin 17 DE	6.50 a (± 0.71)	4.00 a (± 1.41)	5.25 a (± 1.70)
Dextrin 20 DE	7.00 a (± 1.25)	4.50 ab (± 0.71)	5.75 a (± 1.50)
Emulac 50	6.00 a (± 1.32)	5.00 abc (± 0.84)	5.50 a (± 0.57)
Casinella QN	6.00 a (± 1.41)	6.00 bcd (± 1.41)	6.00 a (± 1.15)
Protonal SF 40	6.50 a (± 0.71)	5.00 abc (± 1.86)	5.75 a (± 0.95)
Protonal SF 40 + Ca^{2+}	6.50 a (± 0.71)	5.00 abc (± 1.43)	5.75 a (± 0.95)
Protonal 686	6.50 a (± 0.71)	4.50 ab (± 0.71)	5.50 a (± 1.29)
Protonal 686 + Ca^{2+}	6.50 a (± 0.71)	4.00 a (± 0.67)	5.25 a (± 1.50)
Lecithin	6.00 a (± 1.25)	6.00 bcd (± 0.64)	6.00 a (± 1.45)
Olive oil	6.00 a (± 1.41)	7.00 d (± 1.98)	6.50 a (± 1.00)
Time effect [3]	6.50 a (± 2.06)	5.12 b (± 2.84)	

[1] Means with different letters in a column are significantly different ($p < 0.05$)

[2] Standard deviations are given in parenthesis

[3] Mean values in row with different letters are significantly different ($p < 0.05$)

[4] 1 = dislike extremely and 9 = like extremely

Table 13: The effect of storage (at 5°C) on the colour of minimally processed avocados treated with different edible coatings

Treatment	Colour [4] Rating Day 1 [1]	Day 7 [1]	Treatment effect [1]
Control	6.50 a (± 0.71) [2]	5.50 ab (± 0.71)	6.00 ab (± 0.81)
Dextrin 10 DE	8.00 a (± 1.67)	6.00 b (± 0.63)	7.00 b (± 1.15)
Dextrin 17 DE	6.50 a (± 0.71)	4.50 a (± 0.71)	5.50 ab (± 1.29)
Dextrin 20 DE	6.50 a (± 0.71)	5.00 ab (± 1.96)	5.75 ab (± 0.95)
Emulac 50	5.50 a (± 0.71)	5.00 ab (± 1.41)	5.25 ab (± 0.95)
Casinella QN	5.50 a (± 2.12)	4.00 a (± 1.41)	4.25 a (± 1.70)
Protonal SF 40	7.00 a (± 1.44)	5.00 ab (± 0.84)	6.00 ab (± 1.41)
Protonal SF 40 + Ca^{2+}	7.00 a (± 1.94)	6.50 b (± 0.71)	6.75 ab (± 0.50)
Protonal 686	6.50 a (± 0.71)	6.00 b (± 0.93)	6.25 ab (± 0.50)
Protonal 686 + Ca^{2+}	7.00 a (± 1.23)	5.00 ab (± 0.53)	6.00 ab (1.15)
Lecithin	6.50 a (± 0.71)	5.00 ab (± 1.09)	5.75 ab (± 0.95)
Olive oil	7.00 a (± 1.41)	6.00 b (± 1.97)	6.50 ab (± 1.00)
Time effect [3]	6.62 a (± 1.94)	5.29 b (± 2.84)	

[1] Means with different letters in a column are significantly different ($p < 0.05$)

[2] Standard deviations are given in parenthesis

[3] Mean values in row with a different letters are significantly different ($p < 0.05$)

[4] 1 = dislike extremely and 9 = like extremely

Table 14: The effect of storage (at 5°C) on the taste of minimally processed avocados treated with different edible coatings

Treatment	Taste Rating[4]		
	Day 1 [1]	**Day 7** [1]	**Treatment effect** [1]
Control	6.50 bcd (± 0.71) [2]	6.00 bc (± 1.41)	6.25 c (± 0.95)
Dextrin 10 DE	7.00 bcd (± 1.23)	6.50 c (± 0.71)	6.75 c (± 0.50)
Dextrin 17 DE	7.00 bcd (± 1.92)	5.00 bc (± 1.84)	6.00 abc (± 1.15)
Dextrin 20 DE	7.50 cd (± 0.71)	6.50 c (± 0.71)	7.00 d (± 0.57)
Emulac 50	5.00 ab (± 1.23)	4.00 ab (± 0.75)	4.50 ab (± 0.57)
Casinella QN	5.50 abc (± 0.71)	5.00 bc (± 1.41)	5.25 abc (± 0.95)
Protonal SF 40	6.50 bcd (± 0.71)	5.00 bc (± 1.93)	5.75 bc (± 0.95)
Protonal SF 40 + Ca^{2+}	6.50 bcd (± 0.71)	5.00 bc (± 1.41)	5.75 bc (± 1.25)
Protonal 686	6.00 bcd (± 1.41)	5.00 bc (± 0.74)	5.50 abc (± 1.00)
Protonal 686 + Ca^{2+}	6.00 bcd (± 1.41)	4.50 abc (± 0.71)	5.25 abc (± 1.25)
Lecithin	4.00 a (± 1.93)	3.00 a (± 1.98)	3.50 a (± 0.57)
Olive oil	7.50 cd (± 0.71)	3.50 ab (± 0.71)	5.50 abc (± 2.38)
Time effect [3]	6.25 a (± 2.82)	5.00 a (± 2.24)	

[1] Means with different letters in a column are significantly different ($p < 0.05$)

[2] Standard deviations are given in parenthesis

[3] Mean values in row with a different letter are significantly different ($p < 0.05$)

[4] 1 = dislike extremely and 9 = like extremely

5.2 Phase 2

In this phase, the general Hass cultivar (a browning cultivar) was used. This cultivar was provided by the suppliers in a ripened state.

The quality of the raw materials provided was not consistent. Two batches of avocados were supplied (supply 1 and supply 2), at different times from which two replicates were made respectively. The first batch (supply 1) was perceived to be of poorer quality than the second batch (supply 2) due to the presence of black spots. Supply was introduced as a block factor in the statistical model, to eliminate the variability in raw materials caused by the different supplies.

5.2.1 Moisture content

Figure 19 and Table 15 show the effect of storage for 9 days on the moisture content of minimally processed avocados treated with Dextrin 20 DE alone and in combination with other coatings.

Statistical analysis indicated that overall there was no significant time and treatment effect, but there seemed to be an overall trend towards a loss in moisture during storage. There was no significant difference between treatments on different days of storage. Supply effect indicated that the moisture content for supply 1 was significantly higher than that of supply 2.

5.2.2 Level of O_2 and CO_2 surrounding the avocados in the package

Figures 20 and 21 and Tables 16 and 17 show the effect of storage for 9 days on the level of O_2 and CO_2 respectively in the package of minimally processed avocados treated with Dextrin 20 DE alone and in combination with other coatings.

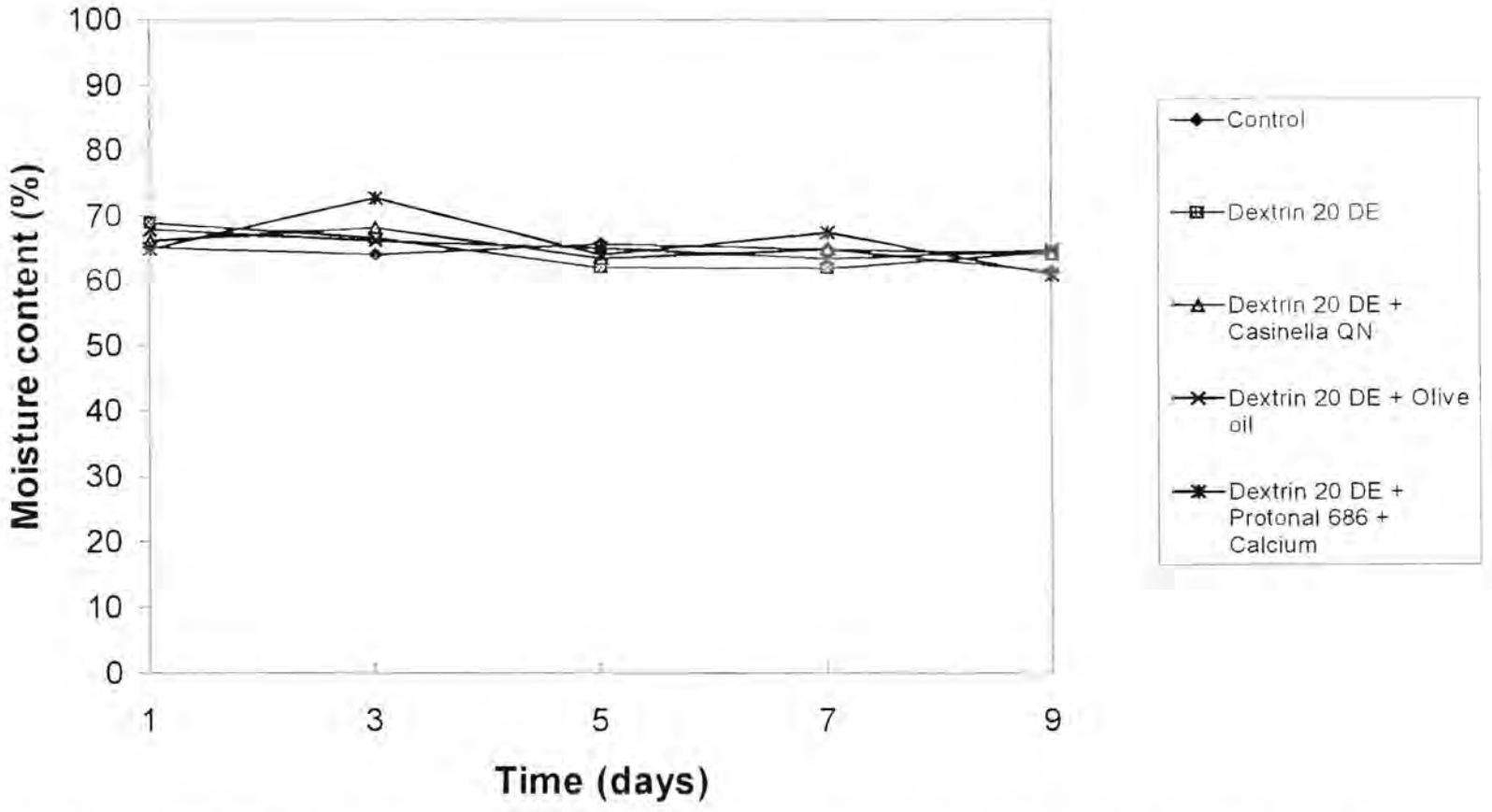

Fig. 19: Effect of storage for 9 days at 5°C on the moisture content of minimally processed avocados treated with Dextrin 20 DE alone and in combination with other coatings

Table 15: The effect of storage (at 5°C) on the moisture content of minimally processed avocados treated with Dextrin 20 DE alone and in combination with other coatings, with supply as blocking factor

Days	Treatments					Time effect [1]
	Control	Dextrin 20 DE	Dextrin 20 DE + Casinella QN	Dextrin 20 DE + Olive oil	Dextrin 20 DE+ Protonal 686 + Ca^{2+}	
1	65.12 a [2] (± 2.69) [3]	68.89 a (± 1.99)	66.09 a (± 7.56)	67.82 a (± 8.04)	64.82 a (± 0.53)	66.55 a (± 4.86)
3	64.03 a (± 5.26)	66.54 a (± 5.56)	68.13 a (± 5.69)	66.15 a (± 4.92)	72.65 a (± 2.72)	67.50 a (± 5.30)
5	65.59 a (± 2.37)	61.94 a (± 4.46)	63.43 a (± 5.39)	65.06 a (± 3.75)	64.00 a (± 3.32)	64.01 a (± 3.78)
7	64.81 a (± 7.20)	61.89 a (± 5.07)	64.89 a (± 8.84)	63.30 a (± 7.25)	67.37 a (± 6.44)	64.45 a (± 6.55)
9	61.35 a (± 7.19)	64.35 a (± 4.85)	63.98 a (± 7.54)	64.71 a (± 2.06)	60.85 a (± 5.99)	63.04 a (± 5.47)
Treatment effect [2]	64.18 a (± 5.01)	64.72 a (± 4.91)	65.31 a (± 6.55)	65.41 a (± 5.26)	65.94 a (± 5.62)	
Supply effect [2]	66.85 a (± 3.29)			63.38 b (± 6.51)		

[1]Means with different letters in a column are significantly different ($p < 0.05$)

[2]Means values in row with different letters are significantly different ($p < 0.05$)

[3]Standard deviation are given in parenthesis

Overall, time of storage had a significant effect on the O_2 level in the air surrounding the avocados in the pack. During storage, the O_2 levels decreased. On days 1 and 3 of storage the O_2 levels were significantly higher than on days 5, 7 and 9. On day 5 of storage the O_2 level was significantly higher than on days 7 and 9. Overall, there was no treatment effect. Supply effect showed that the O_2 levels for supply 1 were significantly higher than that for supply 2.

Overall, there was a significant time and treatment effect on the CO_2 level in the air surrounding the avocados in the packs. During storage, the CO_2 levels increased except for control. On days 1 and 3 of storage the CO_2 level was significantly lower than on days 7 and 9. On day 9 of storage, the CO_2 level in the control pack was significantly lower than all the other treatments. CO_2 levels of supply 1 were significantly lower than that of supply 2.

5.2.3 Colour

The effect of storage for 9 days on the L (lightness), a (redness to greenness) and b (yellowness to blueness) values of minimally processed avocados treated with Dextrin 20 DE alone and in combination with other coatings are illustrated in Figures 22, 23 and 24 and Tables 18, 19 and 20 respectively.

Overall, time of storage had a significant effect on the L-value. During storage the L-values decreased. L-values measured on Day 1 of storage were significantly higher than those measured on days 5, 7 and 9. Also the L- values measured on day 3 of storage was significantly higher than those measured on day 9, clearly showing a decrease in the L-values. Overall, no difference in the L-values were observed between the treatments. However, the L-values obtained for avocados from supply 1 were significantly higher than those of supply 2.

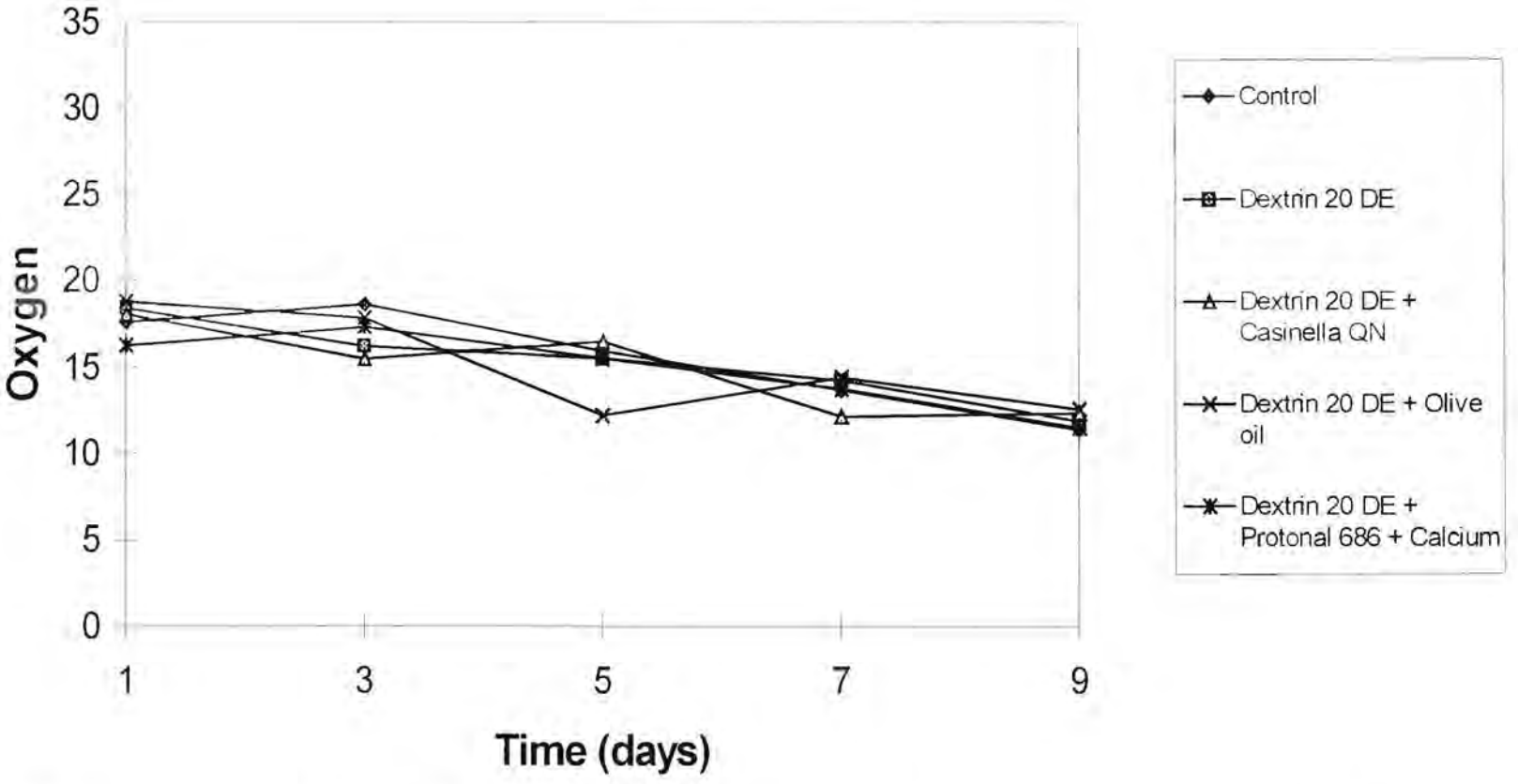

Fig. 20: Effect of storage for 9 days at 5°C on the oxygen level for minimally processed avocados treated with Dextrin 20 DE alone and in combination with other coatings

Table 16: The effect of storage (at 5°C) on the O_2 levels of minimally processed avocados treated with Dextrin 20 DE alone and in combination with other coatings, with supply as blocking factor

Days	Treatments					Time effect [1]
	Control	Dextrin 20 DE	Dextrin 20 DE + Casinella QN	Dextrin 20 DE + Olive oil	Dextrin 20 DE+ Protonal 686 + Ca^{2+}	
1	17.59 a [2] (± 1.71) [3]	18.43 a (± 0.24)	18.04 a (± 1.09)	18.80 a (± 1.17)	16.26 a (± 1.96)	17.82 c (± 1.51)
3	18.64 a (± 1.11)	16.23 a (± 2.53)	15.48 a (± 2.92)	17.85 a (± 1.25)	17.33 a (± 1.71)	17.10 c (± 2.15)
5	15.92 a (± 2.65)	15.51 a (± 2.61)	16.48 a (± 0.93)	12.20 a (± 3.86)	15.51 a (± 2.42)	15.13 b (± 2.82)
7	13.63 a (± 4.36)	14.20 a (± 2.68)	12.11 a (± 4.58)	14.37 a (± 2.15)	13,71 a (± 1.63)	13.61 a (± 3.04)
9	11.36 a (5.68)	11.85 a (± 5.41)	12.34 a (± 4.17)	12.55 a (± 2.18)	11.47 a (± 11.47)	11.91 a (± 3.86)
Treatment effect [2]	15.43 a (± 4.15)	15.24 a (± 3.58)	14.87 a (± 3.66)	15.16 a (± 3.46)	14.86 a (± 2.89)	
Supply effect [2]	16.67 a (± 1.71)			13.55 b (± 4.11)		

[1]Means with different letters in a column are significantly different ($p < 0.05$)

[2]Means values in row with different letters are significantly different ($p < 0.05$)

[3]Standard deviation are given in parenthesis

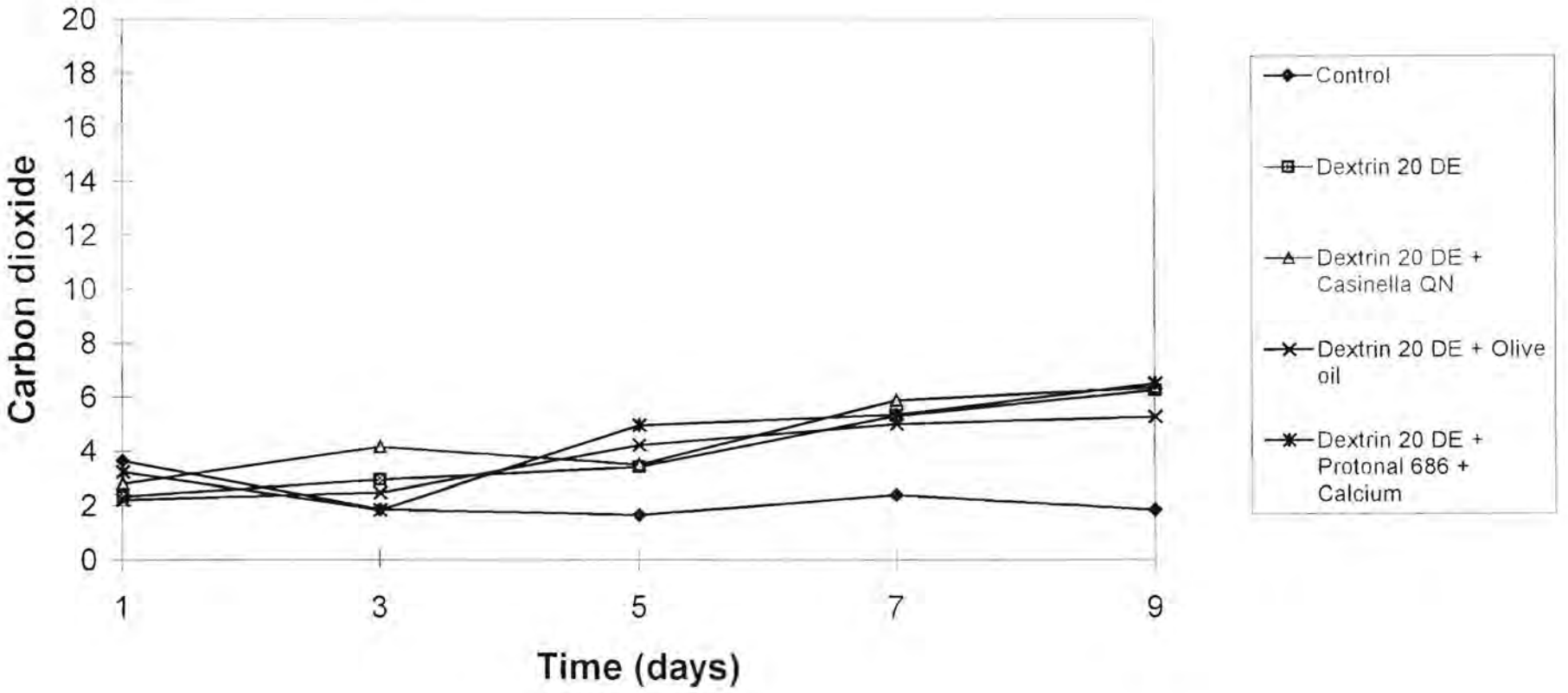

Fig. 21: Effect of storage for 9 days at 5°C on the carbon dioxide level for minimally processed avocados treated with Dextrin 20 DE alone and in combination with other coatings

Table 17: The effect of storage (at 5°C) on the CO_2 levels of minimally processed avocados treated with Dextrin 20 DE alone and in combination with other coatings, with supply as blocking factor

Days	Treatments					Time effect [1]
	Control	Dextrin 20 DE	Dextrin 20 DE + Casinella QN	Dextrin 20 DE + Olive oil	Dextrin 20 DE+ Protonal 686 + Ca^{2+}	
1	3.65 a [2] (± 1.64) [3]	2.33 a (± 1.47)	2.80 a (± 1.21)	2.21 a (± 1.21)	3.24 a (± 1.65)	2.84 ab (± 2.33)
3	1.86 a (± 1.22)	2.95 a (± 1.00)	4.16 a (± 1.79)	2.48 a (± 1.04)	1.85 a (± 1.31)	2.66 a (± 1.46)
5	1.65 a (± 0.67)	3.42 a (± 1.46)	3.51 a (± 0.49)	4.23 a (± 2.25)	4.96 a (± 1.64)	3.56 b (± 1.73)
7	2.40 a (± 1.93)	5.30 a (± 1.74)	5.89 a (± 1.29)	5.00 a (± 1.23)	5.35 a (± 1.36)	4.78 c (± 1.85)
9	1.85 a (± 1.31)	6.25 b (± 1.75)	6.36 b (± 1.06)	5.26 b (± 1.09)	6.49 b (± 2.31)	5.24 c (± 2.28)
Treatment Effect [2]	2.28 a (± 1.48)	4.05 b (± 2.02)	4.54 b (± 1.79)	3.84 b (± 1.82)	4.38 b (± 2.26)	
Supply Effect [2]	3.39 a (± 1.91)			4.24 b (± 2.06)		

[1]Means with different letters in a column are significantly different ($p < 0.05$)

[2]Means values in row with different letters are significantly different ($p < 0.05$)

[3]Standard deviation are given in parenthesis

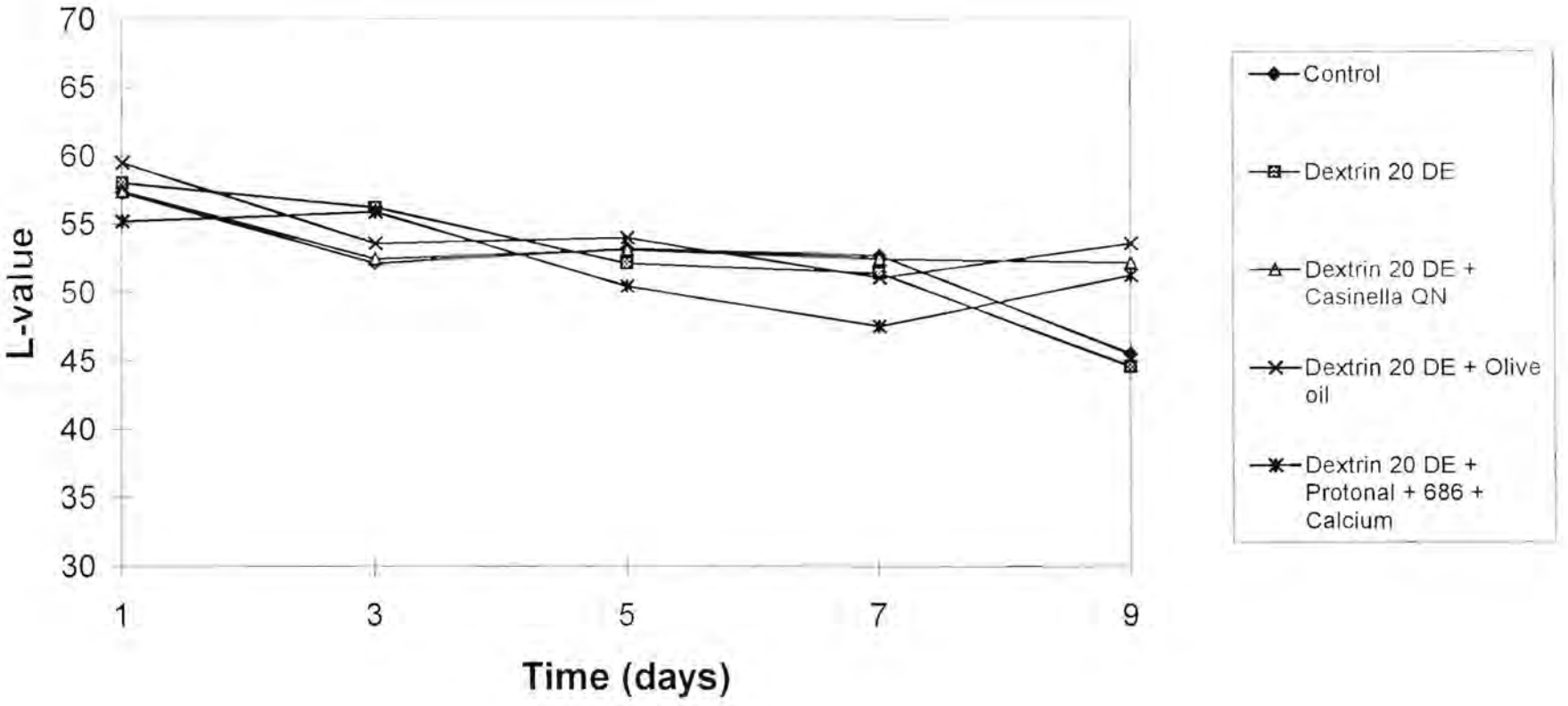

Fig. 22: Effect of storage for 9 days at 5°C on the L-value (lightness) for minimally processed avocados treated with Dextrin 20 DE alone and in combination with other coatings

Table 18: The effect of storage (at 5°C) on the L-values of minimally processed avocados treated with Dextrin 20 DE alone and in combination with other coatings, with supply as blocking factor

Days	Treatments					Time effect[1]
	Control	Dextrin 20 DE	Dextrin 20 DE + Casinella QN	Dextrin 20 DE + Olive oil	Dextrin 20 DE+ Protonal 686 + Ca^{2+}	
1	57.32 a [2] (± 7.38) [3]	58.01 a (± 6.32)	57.38 a (± 9.61)	59.49 a (± 8.62)	55.21 a (± 4.46)	57.48 c (± 6.83)
3	52.11 a (± 12.02)	56.21 a (± 6.07)	52.47 a (± 10.51)	53.57 a (± 6.46)	55.90 a (± 8.91)	54.05 bc (± 8.26)
5	53.23 a (± 12.83)	52.14 a (± 7.22)	53.13 a (± 12.77)	53.99 a (± 5.94)	50.48 a (± 3.78)	52.59 ab (± 8.26)
7	52.72 a (± 5.91)	51.44 a (± 6.29)	52.46 a (± 6.61)	51.07 a (± 3.97)	47.52 a (± 9.10)	51.04 ab (± 6.16)
9	45.50 a (± 6.94)	44.58 a (± 8.00)	52.16 a (± 8.36)	53.59 a (± 2.52)	51.27 a (± 3.36)	49.42 a (± 6.76)
Treatment effect [2]	52.18 a (± 9.26)	52.47 a (± 7.71)	53.52 a (± 8.93)	54.34 a (± 5.96)	52.07 a (± 6.76)	
Supply effect [2]	57.75 a (± 6.52)			48.09 b (± 5.40)		

[1]Means with different letters in a column are significantly different ($p < 0.05$)

[2]Means values in row with different letters are significantly different ($p < 0.05$)

[3]Standard deviation are given in parenthesis

L = 0 indicates blackness; L = 100 indicates whiteness

Statistical analysis indicated that overall, time of storage had a significant effect on the a-values. During storage the a-values significantly decreased. On day 1 the a-values were significantly more negative (more green) than on days 5, 7 and 9. Also on days 5 and 7 of storage a values were significantly negative (more green) than on the last day of storage. Overall, there was no treatment effect. There was also no significant difference between treatments on different days of storage. No supply effect was observed.

Overall, there was a significant time and treatment effect on the b-values. During storage, the b-values decreased significantly. Values measured on day 1 of storage were significantly higher (more yellow) than those measured on days 5, 7 and 9. Moreover, b-values measured on day 5 were significantly higher (more yellow) than those on days 7 and 9. Overall, the samples treated with Dextrin 20 DE + Casinella QN had significantly lower b-values when compared to the other treatments. However, b values measured for avocados from supply 1 were significantly higher than that of supply 2.

5.2.4 Texture

The effect of storage for 9 days on the texture of minimally processed avocados treated with Dextrin 20 DE alone and in combination with other coatings is illustrated in Figure 25 and Table 21.

Overall, time of storage had a significant effect on the texture of the avocados. During storage, the texture values decreased. On day 1 of storage the texture value was significantly higher than on all the other days of storage. Moreover on days 3 and 5 the texture values were significantly higher than on days 7 and 9 of storage. Overall, no difference in texture was observed between the treatments. However, texture measurements obtained for supply 1 were significantly lower (i.e. softer) than that of supply 2.

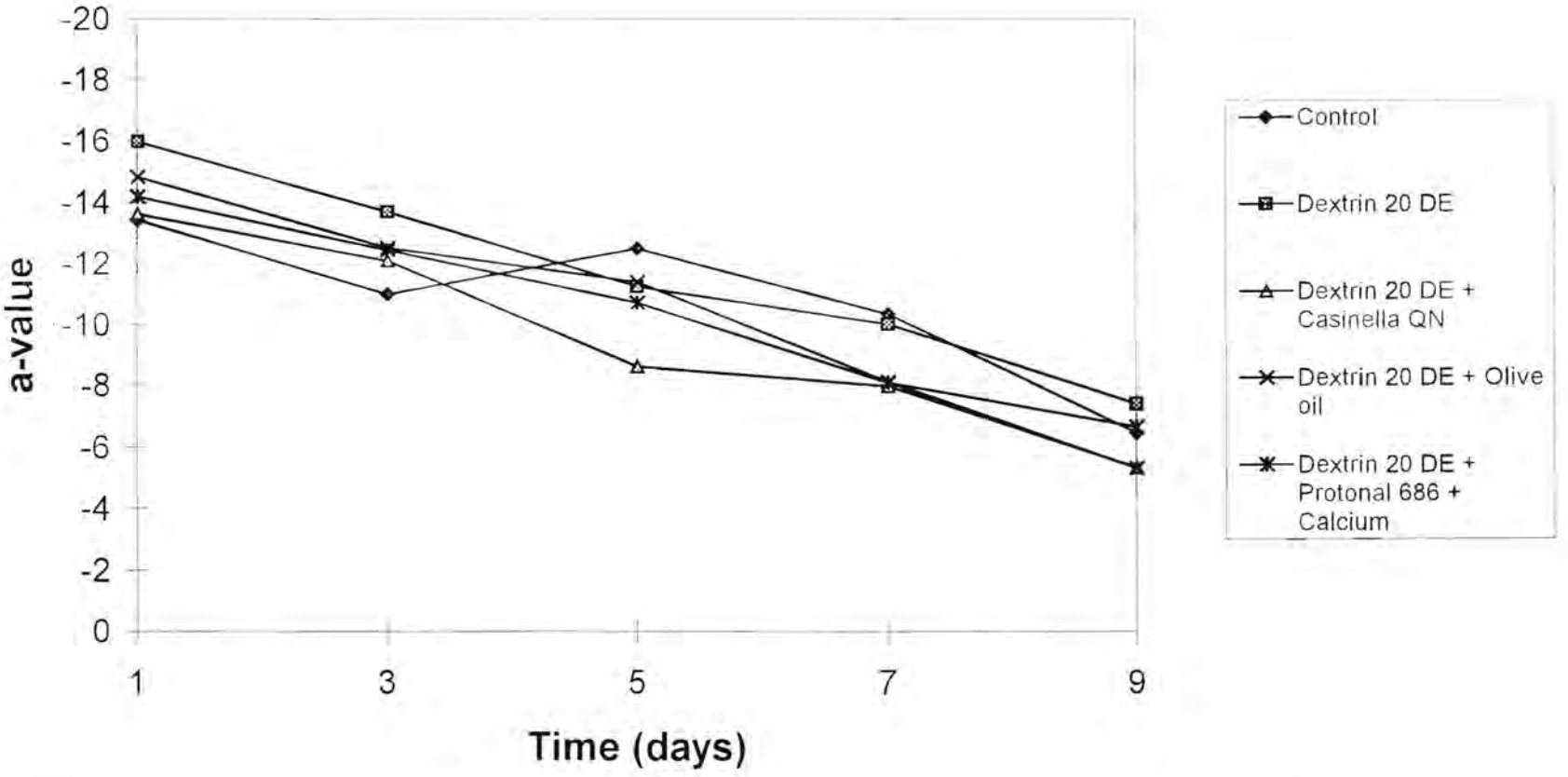

Fig. 23: Effect of storage for 9 days at 5°C on the a-value (red/green) for minimally processed avocados treated with Dextrin 20 DE alone and in combination with other coatings

Table 19: The effect of storage (at 5°C) on the a-values of minimally processed avocados treated with Dextrin 20 DE alone and in combination with other coatings, with supply as blocking factor

Days	Treatments					Time effect [1]
	Control	Dextrin 20 DE	Dextrin 20 DE + Casinella QN	Dextrin 20 DE + Olive oil	Dextrin 20 DE + Protonal 686 + Ca^{2+}	
1	-13.42 a [2] (± 5.92) [3]	-15.98 a (± 2.97)	-13.61 a (± 4.44)	-14.83 a (± 2.30)	-14.18 a (± 4.95)	-14.41 d (± 3.96)
3	-10.99 a (± 3.14)	-13.68 a (± 2.79)	-12.07 a (± 2.27)	-12.51 a (± 1.84)	-12.43 a (± 2.83)	-12.34 cd (± 2.49)
5	-12.49 a (± 4.38)	-11.23 a (± 1.74)	-8.62 a (± 3.42)	-11.40 a (± 0.33)	-10.72 a (± 1.84)	-10.89 c (± 2.76)
7	-10.34 a (± 3.83)	-10.01a (± 3.94)	-7.99 a (± 1.60)	-8.11 a (± 4.13)	-8.09 a (± 0.87)	-8.91 b (± 3.02)
9	-6.45 a (± 3.03)	-7.40 a (± 1.81)	-5.32 a (± 1.30)	-5.31 a (± 3.17)	-6.66 a (± 2.84)	-6.23 a (± 2.40)
Treatment effect [2]	-10.74 a (± 4.47)	-11.66 a (± 3.91)	-9.53 a (± 3.97)	-10.43 a (± 4.19)	-10.42 a (± 3.88)	
Supply effect [2]	-11.08 a (± 4.69)			-10.03 a (± 3.27)		

[1]Means with different letters in a column are significantly different ($p < 0.05$)

[2]Means values in row with different letters are significantly different ($p < 0.05$)

[3]Standard deviation are given in parenthesis

a > 0 indicates redness whereas a < 0 indicates greenness

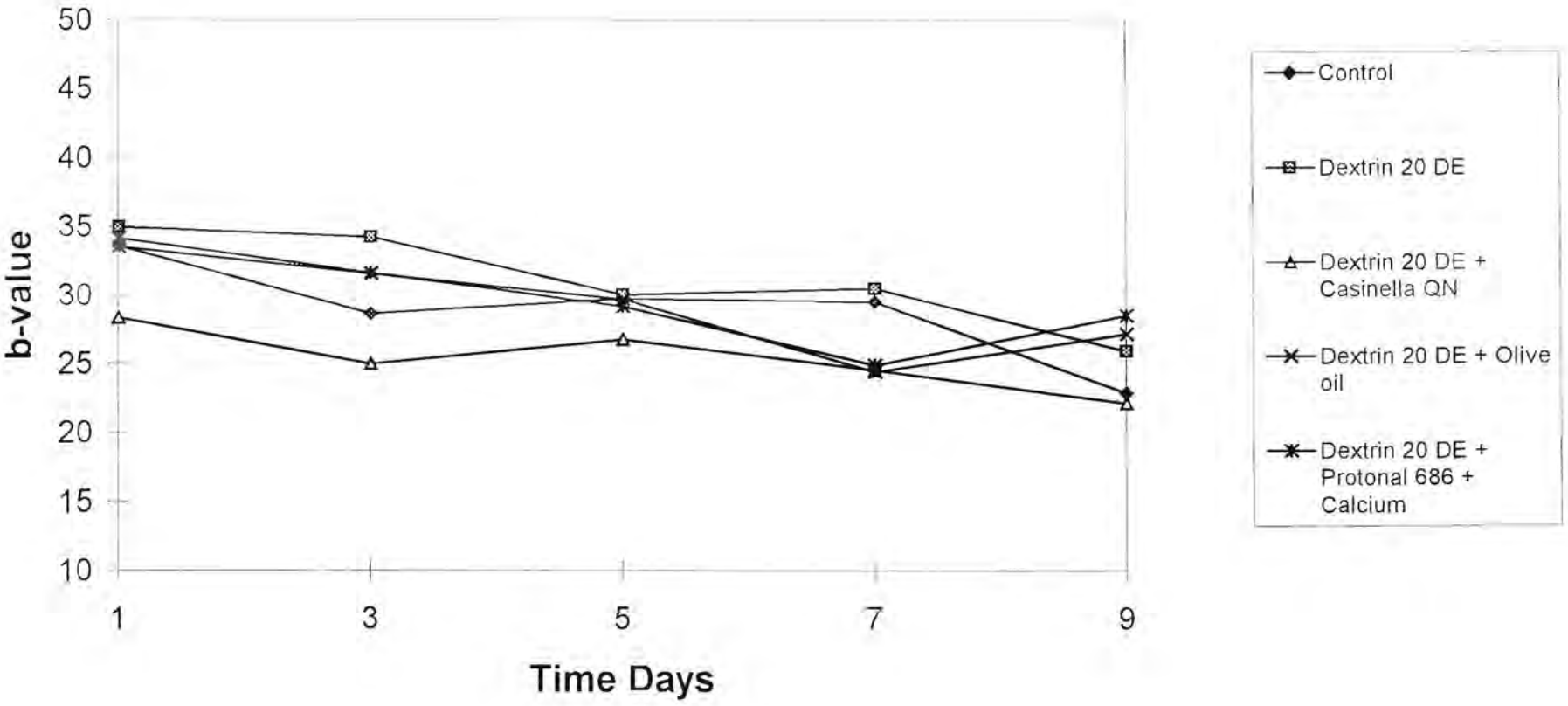

Fig. 24: Effect of storage for 9 days at 5°C on the b-value (yellow/blue) for minimally processed avocados treated with Dextrin 20 DE alone and in combination with other coatings

Table 20: The effect of storage (at 5°C) on the b-values of minimally processed avocados treated with Dextrin 20 DE alone and in combination with other coatings, with supply as blocking factor

Days	Treatments					Time effect [1]
	Control	Dextrin 20 DE	Dextrin 20 DE + Casinella QN	Dextrin 20 DE + Olive oil	Dextrin 20 DE+ Protonal 686 + Ca^{2+}	
1	33.58 a [2] (± 4.81) [3]	34.93 a (± 3.17)	28.36 a (± 4.84)	33.54 a (± 4.49)	34.15 a (± 3.04)	32.91 c (± 4.39)
3	28.62 b (± 9.93)	34.21 c (± 3.09)	24.99 a (± 2.69)	31.52 bc (± 3.06)	31.62 bc (± 5.93)	30.19 bc (± 5.97)
5	29.75 a (± 10.71)	30.02 a (± 5.76)	26.76 a (± 8.75)	29.71 a (± 3.15)	29.17 a (± 3.62)	29.08 b (± 6.36)
7	29.50 a (± 4.17)	30.45 a (± 2.89)	24.51 a (± 3.54)	24.42 a (± 2.52)	24.89 a (± 6.87)	26.76 a (± 4.68)
9	22.86 a (±7.15)	25.92 a (± 5.44)	22.14 a (± 1.90)	27.18 a (± 1.67)	28.51 a (± 4.35)	25.32 a (± 4.80)
Treatment effect [2]	28.86 b (± 7.79)	31.12 b (± 5.04)	25.35 a (± 4.91)	29.27 b (± 4.30)	29.67 b (± 5.45)	
Supply effect [2]	31.31b (± 5.18)			26.40 a (± 5.45)		

[1]Means with different letters in a column are significantly different ($p < 0.05$)

[2]Standard deviation are given in parenthesis

[3]Means values in row with different letters are significantly different ($p < 0.05$)

$b > 0$ indicates yellow colour, whereas $b < 0$ indicates blue colour

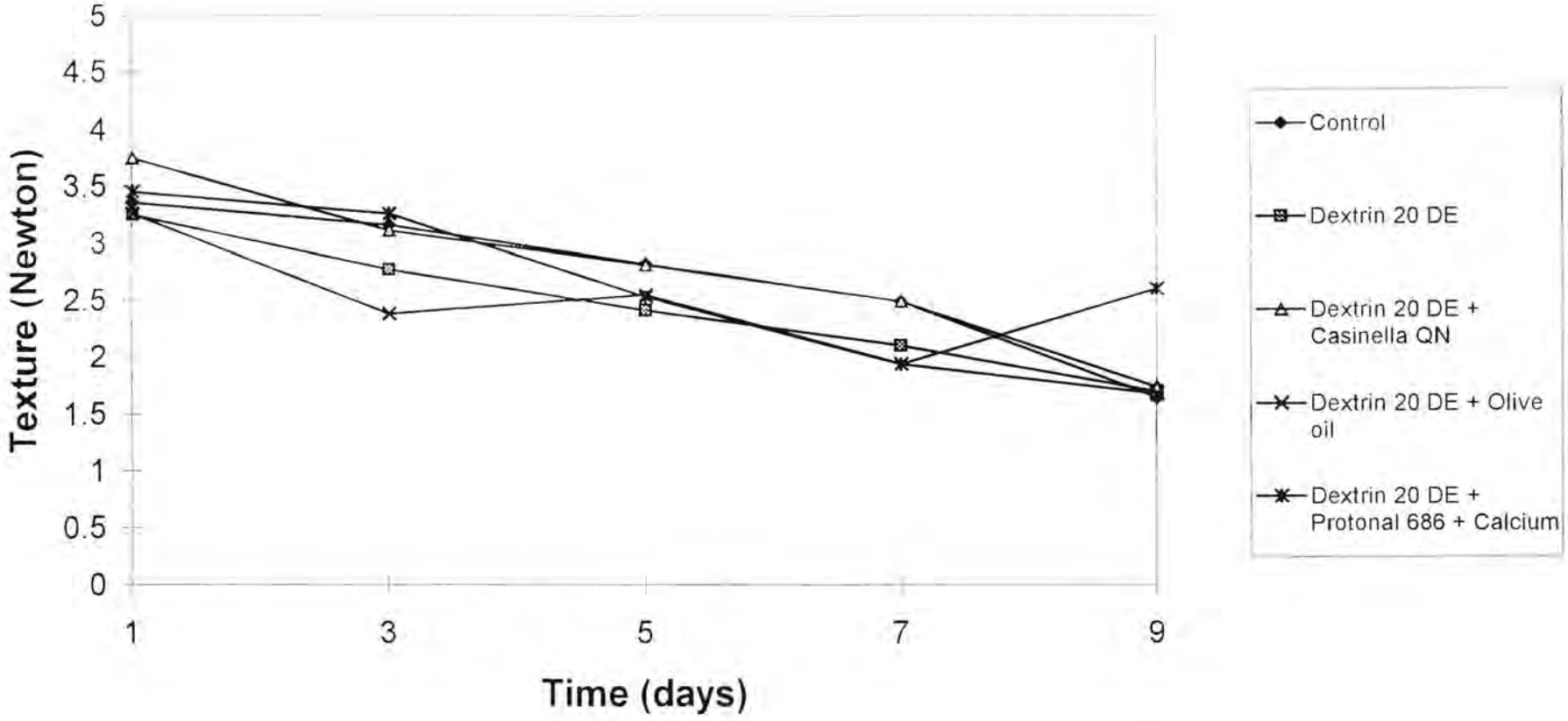

Fig. 25: Effect of storage for 9 days at 5°C on the texture of minimally processed avocados treated with Dextrin 20 DE alone and in combination with other coatings

Table 21: The effect of storage (at 5°C) on the texture values of minimally processed avocados treated with Dextrin 20 DE alone and in combination with other coatings, with supply as blocking factor

Days	Treatments					Time effect [1]
	Control	Dextrin 20 DE	Dextrin 20 DE + Casinella QN	Dextrin 20 DE + Olive oil	Dextrin 20 DE+ Protonal 686 + Ca^{2+}	
1	3.36 a [2] (± 0.36) [3]	3.25 a (± 0.82)	3.75 a (± 1.31)	3.26 a (± 0.32)	3.45 a (± 0.92)	3.41 c (± 0.72)
3	3.16 a (± 0.50)	2.77 a (± 0.17)	3.11 a (± 1.33)	2.38 a (± 0.59)	3.26 a (± 0.85)	2.93 b (± 0.78)
5	2.82 a (± 0.64)	2.41 a (± 0.77)	2.81 a (± 0.60)	2.55 a (± 0.64)	2.53 a (± 0.58)	2.62 b (± 0.60)
7	2.49 a (± 0.86)	2.10 a (± 0.55)	2.49 a (± 0.52)	1.94 a (± 0.64)	1.94 a (± 0.59)	2.19 a (± 0.63)
9	1.64 a (± 0.69)	1.69 a (± 0.52)	1.73 a (± 0.80)	1.67 a (± 0.69)	2.60 a (± 1.99)	1.87 a (± 1.03)
Treatment effect [2]	2.69 a (± 0.83)	2.45 a (± 0.77)	2.78 a (± 1.07)	2.36 a (± 0.77)	2.76 a (± 1.13)	
Supply effect [2]	2.35 a (± 0.93)			2.87 b (± 0.86)		

[1]Means with different letters in a column are significantly different ($p < 0.05$)

[2]Means values in row with different letters are significantly different ($p < 0.05$)

[3]Standard deviation are given in parenthesis

5.2.5 Microbiological analysis

Figures 26, 27, 28, 29 and 30 and Tables 22, 23, 24, 25, and 26 show the effect of storage for 9 days on the total plate count, coliforms, yeasts, moulds and anaerobic sporeformers, respectively, of minimally processed avocados treated with Dextrin 20 DE alone and in combination with other coatings.

Overall, there was a significant time and treatment effect on the microbial growth. During storage, the microbial growth increased. Overall, all 9 days of storage were significantly different from each other. Initially, the microbial counts of control and the sample treated with Dextrin 20 DE + Olive oil were significantly lower than the samples treated with Dextrin 20 DE, Dextrin 20 DE + Casinella QN and Dextrin 20 DE + Protonal 686 + Ca^{2+}. Overall, the sample treated with Dextrin 20 DE + Olive oil had significantly lower total plate counts than the samples treated with Dextrin 20 DE and Dextrin 20 DE + Protonal 686 + Ca^{2+}. However, there was no supply effect.

Statistical analysis indicated that overall, time of storage and treatment had a significant effect on the coliform count. During storage the coliform count increased significantly. Overall, the coliform count of the control was significantly lower than all the other treatments. Also the coliforms of the sample treated with Dextrin 20 DE + Protonal 686 + Ca^{2+} were significantly higher than the samples treated with Dextrin 20 DE and Dextrin 20 DE + Olive oil. No supply effect was observed.

Overall, there was a significant time and treatment effect on the yeast growth. During storage, the yeast counts increased significantly. Overall, the sample treated with Dextrin 20 DE + Olive oil was significantly lower than the control, and samples treated with Dextrin 20 DE + Casinella QN and Dextrin 20 DE + Protonal 686 + Ca^{2+}. On day 7 of storage, the yeast counts of the sample treated with Dextrin 20 DE + Olive oil was significantly lower than samples treated with Dextrin 20 DE + Casinella QN and Dextrin 20 DE + Protonal 686 + Ca^{2+}. However, there was no supply effect.

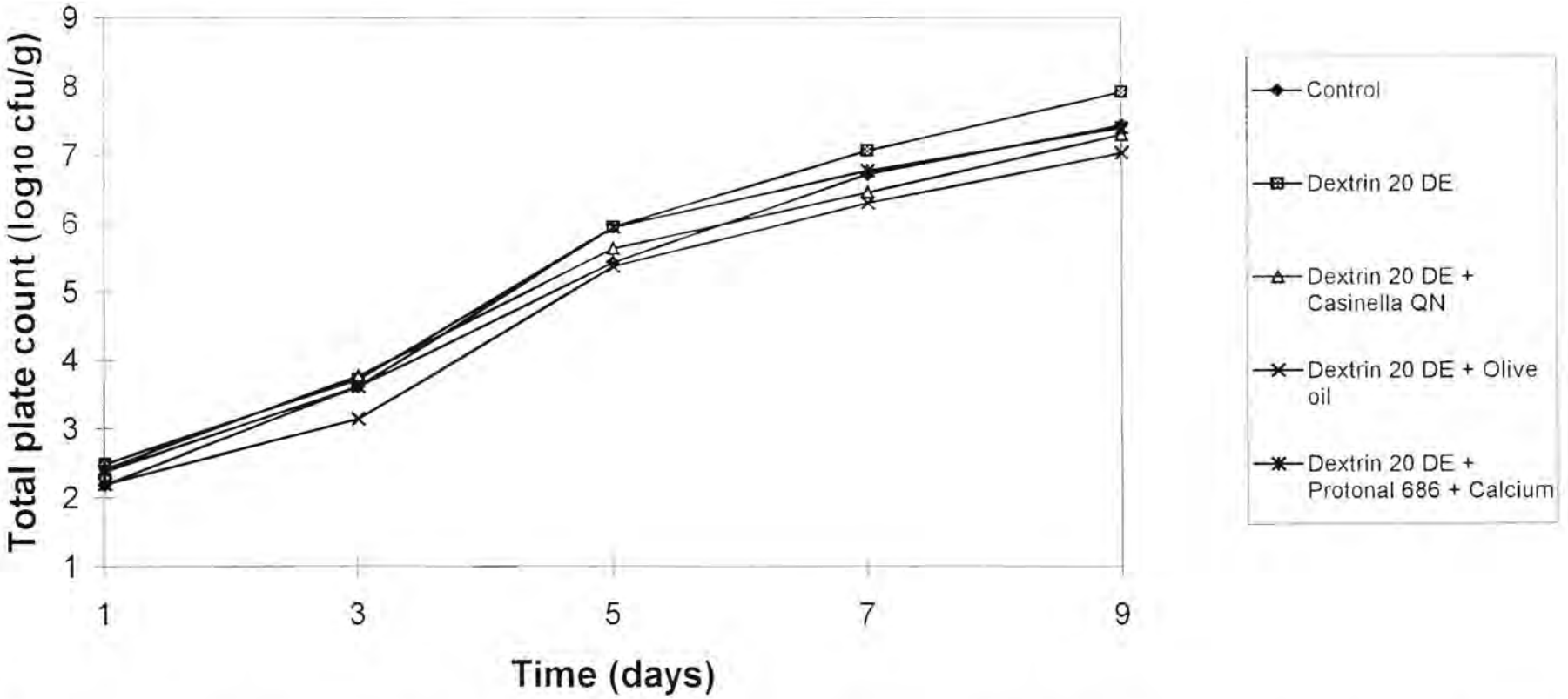

Fig. 26: Effect of storage for 9 days at 5°C on the total plate count of minimally processed avocados treated with Dextrin 20 DE alone and in combination with other coatings

Table 22: The effect of storage (at 5°C) on the total plate count (log_{10} cfu/g) of minimally processed avocados treated with Dextrin 20 DE alone and in combination with other coatings, with supply as blocking factor

Days	Treatments					Time effect [1]
	Control	Dextrin 20 DE	Dextrin 20 DE + Casinella QN	Dextrin 20 DE + Olive oil	Dextrin 20 DE+ Protonal 686 + Ca^{2+}	
1	2.19 a [2] (± 0.08) [3]	2.49 b (± 0.07)	2.42 b (± 0.16)	2.20 a (± 0.15)	2.38 b (± 0.16)	2.34 a (± 0.17)
3	3.61 a (± 0.86)	3.72 a (± 0.81)	3.77 a (± 0.75)	3.14 a (± 0.46)	3.61 a (± 0.64)	3.57 b (± 0.68)
5	5.43 a (± 0.52)	5.95 a (± 0.75)	5.63 a (± 0.89)	5.37 a (± 0.61)	5.97 a (± 0.57)	5.66 c (± 0.66)
7	6.72 a (± 0.43)	7.06 a (± 0.83)	6.45 a (± 0.66)	6.30 a (± 1.12)	6.77 a (± 0.43)	6.66 d (± 0.71)
9	7.43 a (± 0.56)	7.91 a (± 0.03)	7.29 a (± 0.44)	7.02 a (± 0.65)	7.39 a (± 0.41)	7.41 e (± 0.51)
Treatment effect [2]	5.08 ab (± 2.05)	5.43 c (± 2.16)	5.11 ab (± 1.91)	4.81 a (± 1.98)	5.22 bc (± 2.00)	
Supply effect [2]	5.11 a (± 2.07)			5.15 a (± 1.93)		

[1]Means with different letters in a column are significantly different ($p < 0.05$)

[2]Means values in row with different letters are significantly different ($p < 0.05$)

[3]Standard deviation are given in parenthesis

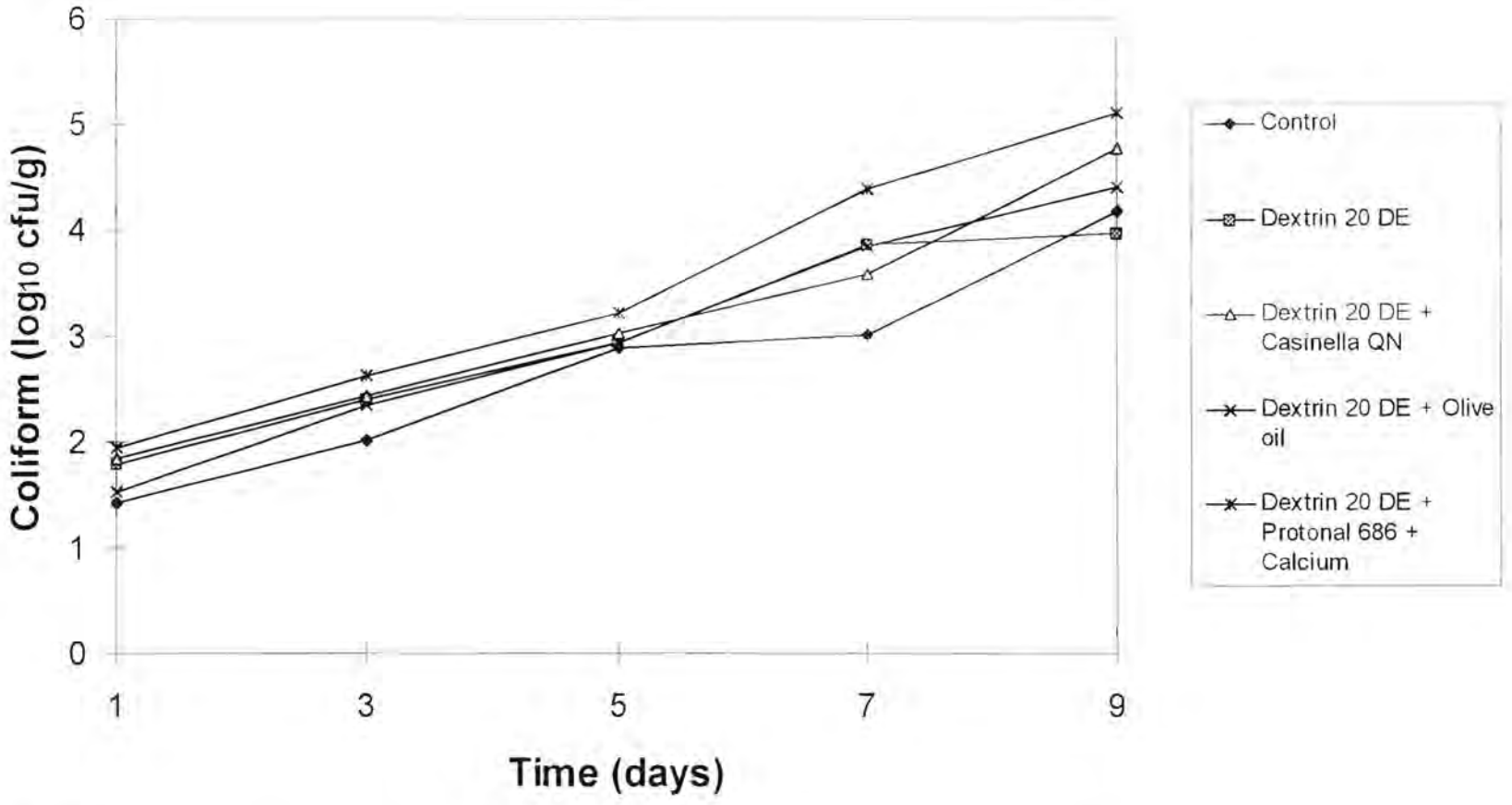

Fig. 27: Effect of storage for 9 days at 5°C on the coliform count for minimally processed avocados treated with Dextrin 20 DE alone and in combination with other coatings

Table 23: The effect of storage (at 5°C) on the coliform counts (log_{10} cfu/g) of minimally processed avocados treated with Dextrin 20 DE alone and in combination with other coatings, with supply as blocking factor

Days	Treatments					Time effect [1]
	Control	Dextrin 20 DE	Dextrin 20 DE + Casinella QN	Dextrin 20 DE + Olive oil	Dextrin 20 DE+ Protonal 686 + Ca^{2+}	
1	1.41 a [2] (± 0.38) [3]	1.78 a (± 0.56)	1.84 a (± 0.57)	1.52 a (± 0.51)	1.94 a (± 0.41)	1.69 a (± 0.49)
3	2.02 a (± 0.22)	2.39 a (± 0.31)	2.43 a (± 0.26)	2.35 a (± 0.32)	2.63 a (± 0.06)	2.36 b (± 0.30)
5	2.89 a (± 0.03)	2.94 a (± 0.07)	3.02 a (± 0.10)	2.94 a (± 0.09)	3.21 a (± 0.27)	2.99 c (± 0.17)
7	3.01 a (± 0.01)	3.87 a (0.77)	3.59 a (± 0.69)	3.85 a (± 1.06)	4.39 a (± 1.01)	3.74 d (± 0.85)
9	4.18 a (± 0.44)	3.97 a (± 0.53)	4.77 a (± 0.81)	4.41 a (± 0.96)	5.11 a (± 1.08)	4.49 e (± 0.83)
Treatment effect [2]	2.69 a (± 1.00)	2.99 b (± 0.97)	3.13 bc (± 1.14)	3.02 b (± 1.23)	3.46 c (± 1.34)	
Supply effect [2]	2.99 a (± 1.22)			3.12 a (± 1.07)		

[1]Means with different letters in a column are significantly different ($p < 0.05$)

[2]Means values in row with different letters are significantly different ($p < 0.05$)

[3]Standard deviation are given in parenthesis

Table 24: The effect of storage (at 5°C) on the yeast count (log_{10} cfu/g) of minimally processed avocados treated with Dextrin 20 DE alone and in combination with other coatings, with supply as blocking factor

Days	Treatments					Time effect [1]
	Control	Dextrin 20 DE	Dextrin 20 DE + Casinella QN	Dextrin 20 DE + Olive oil	Dextrin 20 DE+ Protonal 686 + Ca^{2+}	
1	2.01 a [2] (± 0.21) [3]	1.87 a (± 0.29)	2.12 a (± 0.52)	1.82 a (± 0.29)	1.90 a (± 0.20)	1.94 a (± 0.31)
3	2.35 a (± 0.23)	2.24 a (± 0.34)	2.50 a (± 0.47)	2.19 a (± 0.16)	2.27 a (± 0.08)	2.31 b (± 0.28)
5	3.21 a (± 0.67)	2.88 a (± 0.37)	3.22 a (± 0.46)	2.61 a (± 0.15)	3.16 a (± 0.44)	2.99 c (± 0.46)
7	3.93 ab (± 0.18)	3.82 ab (± 0.74)	4.22 b (± 0.26)	3.35 a (± 0.48)	4.41 b (± 0.51)	3.94 d (± 0.57)
9	4.45 a (± 0.31)	4.43 a (± 0.37)	5.11 a (± 0.22)	4.19 a (± 0.51)	4.85 a (± 0.56)	4.61 e (± 0.49)
Treatment effect [2]	3.17 b (± 0.99)	3.05 ab (± 1.06)	3.44 c (± 1.19)	2.83 a (± 0.92)	3.32 bc (± 1.24)	
Supply effect [2]	3.12 a (± 1.11)			3.12 a (± 1.07)		

[1]Means with different letters in a column are significantly different ($p < 0.05$)

[2]Means values in row with different letters are significantly different ($p < 0.05$)

[3]Standard deviation are given in parenthesis

Statistical analysis indicated that overall, time of storage had a significant effect on the mould growth. During storage, the mould growth increased, significantly. Initially, no mould growth was found. Mould growth was detected after the fifth day of storage for samples treated with Dextrin 20 DE + Casinella QN, Dextrin 20 DE + Olive oil and Dextrin 20 DE + Protonal 686 + Ca^{2+}. However, the mould growth for supply 1 was significantly lower than that of supply 2.

Overall, there was a significant time and treatment effect on the anaerobic sporeformers growth. During storage the anaerobic sporeformers increased significantly. Overall, the days of storage were significantly different from each other. Overall and on day 3 of storage, the anaerobic counts of the control were significantly lower than the other treatments. However, there was no supply effect.

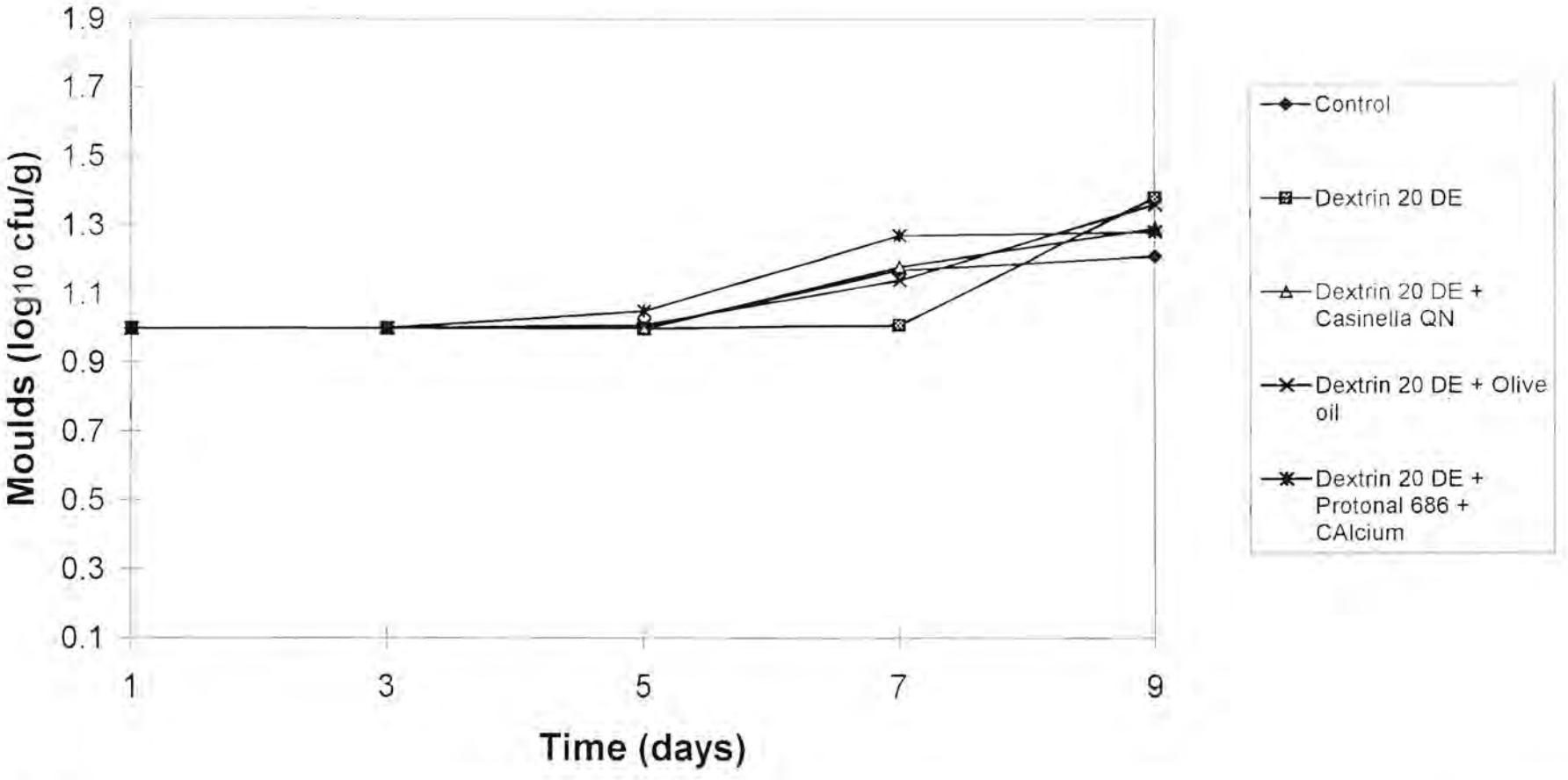

Fig. 29: Effect of storage for 9 days at 5°C on the moulds count of minimally processed avocados treated with Dextrin 20 DE alone and in combination with other coatings

Table 25: The effect of storage (at 5°C) on the mould growth (log_{10} cfu/g) of minimally processed avocados treated with Dextrin 20 DE alone and in combination with other coatings, with supply as blocking factor

Statistical analysis on moulds was done only on the three last days of storage, due to no mould growth on the first two days of storage

Days	Treatments					Time effect [1]
	Control	Dextrin 20 DE	Dextrin 20 DE + Casinella QN	Dextrin 20 DE + Olive oil	Dextrin 20 DE+ Protonal 686 + Ca^{2+}	
1	< 1 a	< 1 a	< 1 a	< 1 a	< 1 a	< 1
3	< 1 a	< 1 a	< 1 a	< 1 a	< 1 a	< 1
5	< 1 a	< 1 a	< 1 a	1.20 a [2] (± 0.01) [3]	1.05 a (± 0.10)	1.01a (± 0.05)
7	1.17 a (± 0.34)	1.20 a (± 0.01)	1.10 a (± 0.27)	1.14 a (± 0.19)	1.27 a (± 0.34)	1.15 b (± 0.25)
9	1.21 a (± 0.39)	1.38 a (± 0.34)	1.29 a (± 0.36)	1.36 a (± 0.26)	1.28 a (± 0.31)	1.31 c (± 0.31)
Treatment effect [2]	1.12 a (± 0.28)	1.13 a (± 0.26)	1.15 a (± 0.27)	1.17 a (± 0.23)	1.20 a (± 0.28)	
Supply effect [2]	1.03 a (± 0.09)			1.16 b (± 0.27)		

[1]Means with different letters in a column are significantly different ($p < 0.05$)

[2]Means values in row with different letters are significantly different ($p < 0.05$)

[3]Standard deviation are given in parenthesis

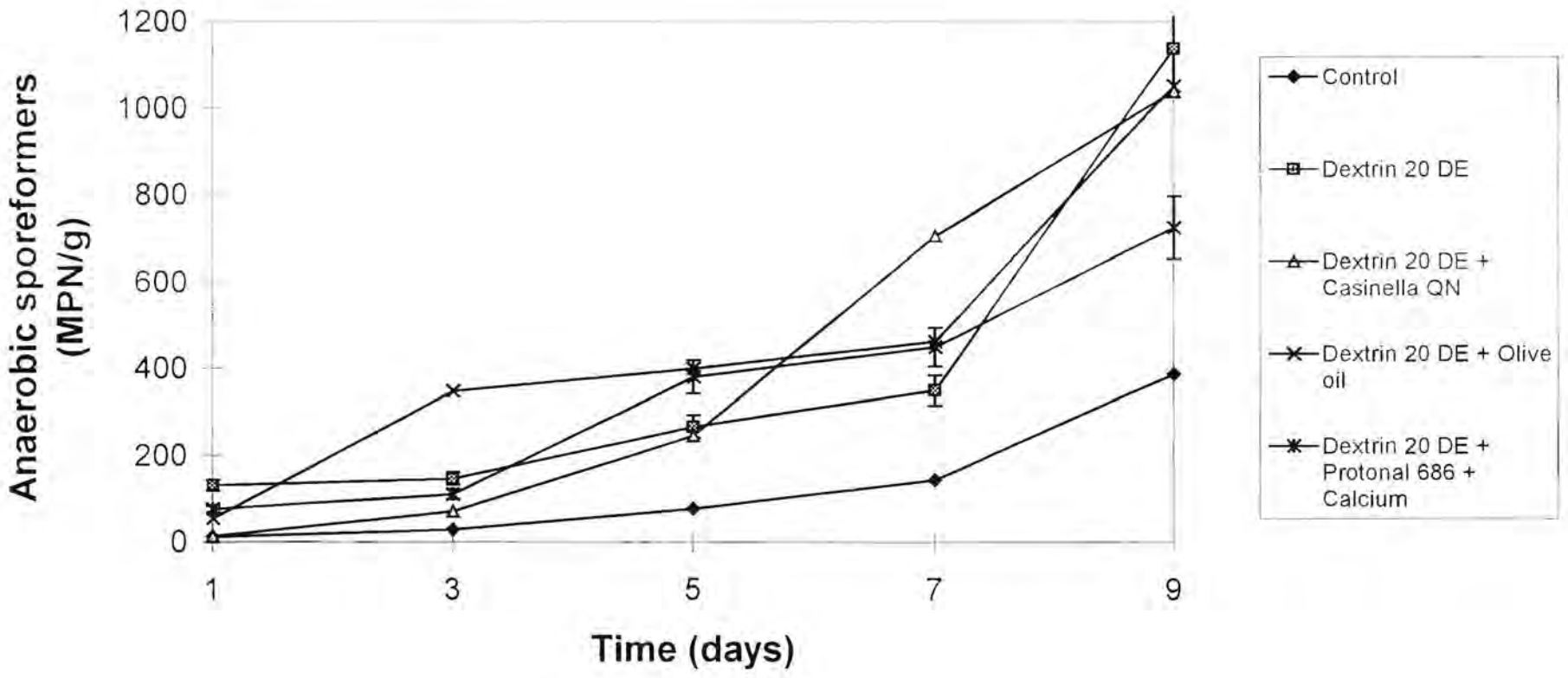

Fig. 30: Effect of storage for 9 days at 5°C on the anaerobic sporeformers of minimally processed avocados treated with Dextrin 20 DE alone and in combination with other coatings

Table 26: The effect of storage (at 5°C) on the anaerobic sporeformers (MPN/g) of minimally processed avocados treated with Dextrin 20 DE alone and in combination with other coatings, with supply as blocking factor

Days	Treatments					Time effect [1]
	Control	Dextrin 20 DE	Dextrin 20 DE + Casinella QN	Dextrin 20 DE + Olive oil	Dextrin 20 DE+ Protonal 686 + Ca^{2+}	
1	11.63 a [2] (± 6.75) [3]	130.63 a (± 128.00)	12.87 a (± 7.94)	53.25 a (± 66.25)	75.00 a (± 51.96)	56.68 a (± 76.02)
3	28.50 a (± 13.50)	146.25 b (± 128.54)	70.00 ab (± 56.57)	347.50 ab (± 503.08)	110.00 b (± 60.96)	140.45 b (± 237.91)
5	76.25 a (± 50.89)	266.25 a (± 155.10)	245.00 a (± 340.63)	400.00 a (± 467.26)	381.25 a (± 513.72)	273.75 c (± 335.94)
7	142.75 a (± 53.82)	350.00 a (± 135.40)	705.00 a (± 706.94)	462.50 a (± 438.51)	450.00 a (± 449.07)	422.05 d (± 423.49)
9	387.50 a (± 256.17)	1137.56 a (± 495.60)	1037.50 a (± 434.69)	1050.00 a (± 533.85)	725.00 a (± 473.46)	867.50 e (± 490.51)
Treatment effect [2]	129.33 a (± 176.02)	406.12 b (± 445.27)	414.07 b (± 540.41)	462.65 c (± 511.88)	348.25 bc (± 412.16)	
Supply Effect [2]	333.10 a (± 424.34)			370.98 a (± 465.64)		

[1]Means with different letters in a column are significantly different ($p < 0.05$)

[2]Means values in row with different letters are significantly different ($p < 0.05$)

[3]Standard deviation are given in parenthesis

CHAPTER 6

DISCUSSION

The primary objective of the project was to investigate the use of edible coatings on the shelf life of minimally processed (MPR) avocados stored at 5°C. Minimally processed avocados deteriorate fast due to two main mechanisms: physiological deterioration (respiration, transpiration, enzymic reactions) and microbiological deterioration (Ohlsson, 1994; Wiley, 1994). Figure 31 illustrates the various factors affecting the shelf life of MPR avocados. Edible coatings are reported to function as gas barriers (slowing down respiration rate), moisture barriers (slowing down transpiration) and may also retain the colour by preventing oxidative degradation processes. They may also delay firmness loss by means of reduction in respiration rates therefore delaying ripening which results in reduction of firmness loss during storage (Park, Chinnan & Shewfelt, 1994; Wiley, 1994; Anonymous, 1997). Moisture barrier properties of coatings may also indirectly affect microbial growth. According to Avena-Bustillos, Krochta & Saltveit (1997), edible coating may be responsible for rapid spoilage of produce especially in moist environment because of their high nutrient value for microbial growth. Therefore they shorten the shelf life of produce. However, the effectiveness of any edible coating is depended on the nature and thickness of the coating. Therefore if coatings are not properly applied, their functionality may be affected adversely.

Although edible coatings can potentially slow down physiological processes of MPR avocados, other factors also play a role, i.e. variety, stage of ripening, permeability of packaging material, surface-to-volume ratio (wounding through cutting), temperature (Smith, Geeson & Stow, 1987; Avena-Bustillos *et al.*, 1994; Park, *et al.*, 1994). It is expected that different varieties of Hass cultivar avocados will have different physiological and biochemical behaviour. The stage of ripening of avocados directly impacts on the rate of respiration.

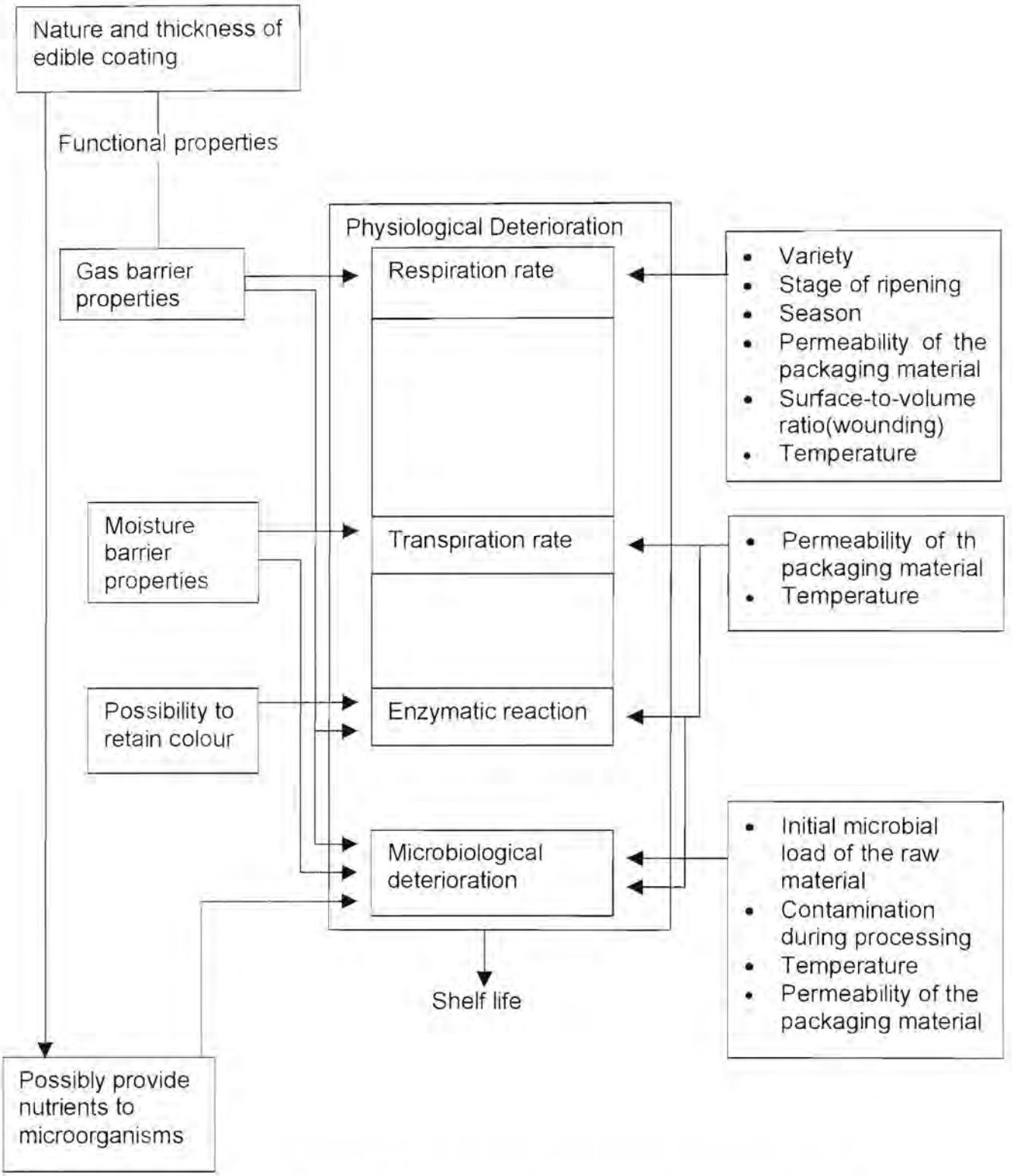

Fig 31. Factors affecting the shelf life of minimally processed avocados coated with an edible coating

The respiration pattern is divided into three stages: the pre-climacteric minimum (a period of low respiration); the climacteric maximum (a period when respiration is at its maximum), and the post-climacteric period (with a decline in respiration). Cutting and Wolstenholwe (1992b) indicated that there is a seasonal trend in quality for avocados. Less acceptable avocados are produced early and towards the end of the marketing season. A rainy season may also produce poor quality avocados, since the trees do not require a lot of water.

The permeability of packaging materials can affect respiration and transpiration rate of MPR avocados. If the packaging material does not allow vapour to escape, the moisture present may allow microbes to proliferate. Also if packaging materials allow a built up of CO_2, anaerobic microorganisms may grow. Avocado size also play a role; where smaller pieces of avocados have a higher respiration rate, because larger surface areas are exposed to oxygen (Pantastico, 1975). Wounding avocados through minimal processing also induces elevated ethylene production rates, which may accelerate deterioration (Baldwin *et al.*, 1995; Brecht, 1995; Anena-Bustillos *et al.*, 1997). Low temperature storage, in general, extends the shelf life of MPR produce by slowing down physiological and microbiological deterioration. Microbiological deterioration is affected by the initial microbial load of the raw material, contamination during processing and permeability of packaging materials as indicated before.

6.1 Difference between the two phases

Avocados used in Phase 1 were ripened at the University of Pretoria at ambient temperatures (± 27°C), whereas avocados used in Phase 2 were supplied already ripened (using ethylene). Due to the different methods of ripening used, Phase 1 and 2 avocados were probably at different stages of ripening thereby impacting on the results obtained. Different varieties of the Hass cultivar were used during the two phases; Westalia Hass (a non browning variety) for Phase 1 and general Hass cultivar (a browning variety) for Phase 2. This could also have affected the results obtained. Avocados for Phase 1 were harvested towards the

mid summer season, resulting in good quality produce, whereas Phase 2 avocados were harvested towards the end of spring season which is a less optimal time for producing quality Hass avocados. During Phase 2, two supplies of avocados were used. The avocados obtained from supply 1, had black spots inside the fruit, probably due to rainy conditions during the harvesting period. Supply 2 avocados were of a better quality.

6.2 Phase 1

The primary objective of Phase 1 was to study the effect of individual edible coatings (carbohydrates, protein and lipid based), on the microbiological, physico-chemical and sensory quality of MPR avocados. Based on these results, the best coatings would be selected to be investigated further in Phase 2.

According to Salunkhe and Kadam (1995), Hass cultivar has moisture contents ranging between 74.4 and 76.0%. Therefore the moisture values obtained during the research are in agreement with those reported in literature. These relatively high values were expected since avocados used were harvested early in the season.

Although it was expected that the lipid based coatings (i.e. lecithin and olive oil) would provide better water barrier properties than polysaccharides and proteins based coatings (Avena-Bustillos *et al.*, 1994; Cuq *et al.*, 1995; Krochta & Mulder-Johnston, 1997), this was not the case. This might have been due to non-uniform application of lipid coating (olive oil). Olive oil could not be sprayed evenly on MPR avocados due to its high viscosity, thereby necessitating the additional use of brushes. Furthermore, it was difficult to transfer the avocados from the sifted metallic tray, where the coatings were being applied, to the pack itself . This could have resulted in damage to the applied coatings thus allowing moisture to evaporate more freely. Avocados treated with protein based coatings had significantly lower moisture contents than those treated with

polysaccharides. This was expected, since protein based coatings are reported to have poor water barrier properties (Fennema, 1986; Anonymous, 1997).

During storage there was a loss in moisture content of MPR avocados probably due to transpiration. Transpiration cannot be completely prevented, but can potentially be slowed down by low temperature and the use of edible coatings (Cutting and Wolstenholwe, 1992a; Park *et al.*, 1994). Overall, samples treated with polysaccharides with added calcium (Protonal 686 + Ca^{2+} and Protonal SF 40 + Ca^{2+}) had significantly higher moisture contents than other treated samples. This was probably due to the formation of a gel when calcium is combined with alginate. This entraps water resulting in less water evaporation losses (Moe *et al.*, 1995). According to Fennema (1986), to form a gel (through reactions with calcium), alginate has to contain a certain proportion of guluronic acid, and the guluronic acid monomers must occur in series. The calcium divalent cation, Ca^{2+}, fits into the guluronic acid structure like eggs into an egg box. This may be visualized as a "molecular cross-linking glue" binding the alginate polymers together by forming junction zones, leading to a gelling of the alginate solution. After gelation, the water molecules are physically trapped by the alginate matrix or network, but still free to migrate. Therefore the water-holding capacity of the gel is due to capillary force.

Protein, polysaccharide and lipid based coatings are reported to retard gas transport by slowing down the respiration rate (Smith *et al.*, 1987; Park *et al.*, 1994; Anonymous, 1997). The presence of an artificial barrier to gas diffusion around fruit may result in reduced O_2 and increased CO_2 concentrations as well as altered water and ethylene concentrations. The extent to which these factors are changed depends on variety, cultivar, extent of wounding through minimal processing and handling as well as respiration rate (Smith *et al.*, 1997). These factors probably had a direct effect on the results obtained for the control sample.

During the experiment the exact stage of ripening was not known. It was

assumed that avocados used in this phase might have been at the post-climacteric stage. A slow respiration rate is usually associated with the post-climacteric stage of ripening (Salunkhe & Kadam, 1995). This appeared to be the case during Phase 1 since high oxygen levels were observed in the packs throughout storage. However, the influence of the packaging materials must also be considered. According to the supplier of the packaging material used (Modern Packaging Company), the tub itself was able to trap oxygen and CO_2 by allowing it to come into the pack but not allowing it to escape. On the other hand, the packaging film (used to seal the tubs) allowed CO_2 and ethylene to escape whereas some oxygen was allowed to enter the pack, but not escape. One would therefore expect a build up of oxygen levels and a decrease in carbon dioxide levels in the pack over time. This phenomenon was observed during the experiment.

Initially, the O_2 levels in all the packs were the same. On day 7 of storage the avocados treated with Protonal 686 + Ca^{2+} and Lecithin had higher O_2 levels surrounding the MPR avocados in the packs than most other samples. This probably indicates that these edible coatings acted as an O_2 barrier, not allowing diffusion of O_2 into the avocados. Initially, lower CO_2 levels were observed in the samples coated with Casinella QN compared with the control. Initially all the Dextrin coated samples together with the sample coated with olive oil had higher CO_2 levels than Casinella QN. Casinella QN performed differently than the other coatings by having an increase in CO_2. The reason for this is unclear. Overall, the following samples had lower levels of CO_2 in the packs than the control: Emulac 50, Casinela QN, Protonal 686, Protonal 686 + Ca^{2+} and Lecithin. It is possible that some of the CO_2 produced by avocados diffused into the coating itself (Avena-Bustilos *et al.*, 1994). These results seem to indicate that the use of the above mentioned coatings had some gas barrier properties.

According to Shapton & Shapton (1991), pigments of whole avocados are stable compounds and remain intact in the tissue even when extensive senescence has

occurred. However, these pigments are disturbed by minimal processing, exposing them to oxygen, which lead to oxidative breakdown. Cutting and shredding also generally result in changes in food colour because many of the plant pigments are organised in tissue cells and pigments bodies, such as the chloroplasts, which contain green chlorophyll. When these cells are broken, the pigments leach out and are partially destroyed on contact with air (Jen, 1989; Salunkhe *et al.*, 1991; Brody, 1993). Enzymatic browning, if not prevented, can also cause browning of MPR avocados.

It has been reported that some polysaccharide coatings are able to maintain the colour of MPR products by preventing oxygen from coming into contact with the produce, which may lead to oxidative degradation processes (Park *et al.*, 1994; Anonymous, 1997). However, results from this study showed that coatings other than those based on polysaccharides, had the potential to retain colour. Initially, higher L-values were observed for avocados treated with Protonal 40 + Ca^{2+}, Protonal 686 and Lecithin. This advantage was however not maintained during storage. In general there was a decrease in the L (lightness) value after 7 days of storage. This showed that avocados became darker with time. This could have been the result of oxidation of pigments (chlorophyll) as explained above, loss in water and ageing. Normally, a loss in moisture can result in shriveling and accelerate ageing, resulting in a change in colour. However, this did not seem to be the case in this experiment.

Initially, most of the samples were perceived to be greener than the control sample. After 7 days of storage it was observed that the control and samples treated with Dextrin 10 DE had less negative a (greenness) values, than samples treated with Dextrin 20 DE, Casinella QN, Protonal 40 and Protonal 686 + Ca^{2+}. This was expected for the control, due to its unprotected flesh, which makes it easier to react with oxygen in the pack. Most of the polysaccharide coatings (Dextrin 20 DE, Protonal 40 and Protonal 686 + Ca^{2+}) seemed to have a greener colour or higher negative a-values after 7 days of storage as expected. Overall,

only avocados treated with Dextrin 20 DE had significantly higher b-values (yellowness) than samples treated with other coatings. The reason for this could probably be due to its viscous nature, perhaps affecting the reflection of light from the surface of the product.

Edible coatings do not in general retard the growth of microorganisms. In fact, they can only perform such a function if antimicrobial agents are added to them (Brandenburg *et al.*, 1993). They can however potentially slow down the growth of microbes, because aerobic microbes require oxygen for survival. In general, fruits and vegetables are free of any signs of microbial spoilage when aerobic plate counts of less than 10^6 cfu/g are obtained, while counts of 10^7 to 10^8 cfu/g generally denote off odours and/or off-flavours. Most of the South African MPR fruits companies, exporting products to Europe, use10^7 cfu/g as their cut off point for total plate counts (TPC) (Binder, Quality Control manager, Spring Valley Foods, 2000 - personal communication). However, from a sensory view-point, all the samples remained acceptable for at least 7 days of storage. Therefore, it is suggested that for MPR avocados a cut-off point of 10^8 is used, to denote the end of the shelf life (from TPC point of view).

Initially, samples treated with Lecithin had a significantly lower microbial load than samples treated with Dextrin 20 DE, Protonal SF 40, Protonal 686 and Olive oil. After 7 days of storage, the control, and samples treated with Dextrin 20 DE and Lecithin seemed to perform better in terms of microbiological quality. The proliferation of microbes could be due to nutrients that coatings are providing to the microbes or due to cross-contamination during processing.

Since the initial microbial counts for Protonal 686 were quite high, it was thought that the coatings used might have contaminated the samples. After conducting microbiological analyses of all the edible coatings used, it was confirmed that the coatings could not have been the source of contamination and that all samples were well within manufacturers' specifications. The growth and increase in

microbes could therefore be because of the following: cross-contamination during and after processing; coatings providing nutrients to the microbes; as well as favourable environmental conditions (high O_2 levels). Miller & Krochta (1997) as well as Avena-Bustillos *et al.*, (1994), reported that edible coatings based especially on proteins might enhance the nutritional quality of the food, which is one of the requirements of microorganisms. It is useful to note that microorganisms normally gain access to fruits and vegetables before harvesting. During and after processing, high microbial counts are considered to be a disadvantage, which could affect the shelf life of the final products adversely (Hsu and Beuchat, 1986; Amanatidou, Smid and Gorris, 1999).

Anaerobic sporesporeformers exceeding 10^4 MPN in fruits is regarded to be unacceptable (Bester, Associate Professor, University of Pretoria, 2000 – personal communication). All samples were deemed to be acceptable in this regard, probably because aerobic conditions prevailed in the packs throughout the storage period. Overall, the control sample, and samples treated with Dextrin 17 DE, Dextrin 20 DE, Emulac 50 Protonal 40 + Ca^{2+}, Lecithin and Olive oil had significantly lower anaerobes sporeformers than the other samples. Although no literature was available on the naturally occurring counts of anaerobic sporeformers on avocados, it is likely that the level of contamination would depend on the environment in which the avocados was grown and harvested.

Appearance, taste, and colour (as perceived organoleptically) deteriorated upon storage, probably due to ageing, loss in moisture and breakdown or oxidation of pigments. The appearance of the samples on day 7 of storage became less acceptable. Avocados treated with Olive oil based coating gave a glossy appearance, which was better than all the polysaccharides based coatings after 7 days of storage. The avocados lost some of their green colour with time.

Overall the taste of sample treated with Dextrin 20 DE was significantly better than the other treatments. Dextrin 20 DE gave a sweeter taste, which

complimented that of the avocados, while Lecithin gave an unacceptable metallic taste.

From a microbiological view-point, the shelf life of MPR avocados stored at 5 °C, was approximately 7 days (including the control sample). Low temperature of storage and the use of permeable packaging material therefore seem to play a more important role than the edible coatings in extending the shelf life of MPR avocados. Since the thickness of the applied coatings was not measured, it is uncertain whether the coatings were applied uniformly. Moreover, Baldwin *et al.* (1996) found that edible coatings only improved the storage life of cut apples when preservatives were also used. From a sensory view-point all the samples remained acceptable for at least 7 days of storage.

In an attempt to select the best coatings for Phase 2, the following functional properties of the various coatings were compared: O_2 , CO_2 and moisture barrier properties as well as the retention of colour. Microbiological and sensory aspects were also deemed important. It was expected that the different categories of edible coatings would compliment each other so that a logical choice could be made in combining them. However, in most instances, the edible coatings did not perform as expected. The probable reason for this was due to other factors (e.g. ripening stage, packaging permeability, season) having a more dramatic effect on the physiological behaviour of MPR avocados than edible coatings.

It was decided to choose Dextrin 20 DE to be used in combination with other coatings in Phase 2 due to the following reasons: colour retention ability as well as a high acceptability for taste. It was also decided to select one coating from each category (i.e. lipid, protein or carbohydrate based) to be combined with Dextrin 20 DE. Although Lecithin performed better than Olive oil in all functional properties, it was rejected due to an unacceptable metallic taste. Olive oil performed well in terms of colour retention (best appearance on day 7 of storage) and was therefore the selected lipid based coating for Phase 2. Polysaccharide

(alginate) coatings with calcium provided a good moisture barrier. However alginate Protonal 686 + Ca^{2+} was chosen since it seemed to provide an oxygen barrier. Casinella QN a protein based coating was selected due to better colour (b-values) than Emulac 50.

6.3 Phase 2

The initial intention of Phase 2 was to combine the most effective coatings. Due to the poor performance of the individual coatings, it was decided to combine Dextrin 20 DE with other better performed coatings from other categories on the quality and shelf life of MPR avocados stored for nine days at 5°C. The individual coatings that performed the best in Phase 1 were combined in this phase to investigate their possible synergistic effect. However the quality of the raw material supply was not consistent. Two batches of avocados were supplied (supply 1 and supply 2), at different times, from which two replicates were made respectively. The first batch (supply 1) was perceived to be of poorer quality than the second batch (supply 2) due to the presence of black spots.

Moisture values obtained in this phase were below the cited literature values (Salunkhe and Kadam, 1995) probably due to the late season of harvesting. During the late season, avocados have lower moisture contents as they have difficulty in growing due to unfavourable environmental conditions (Salunkhe and Kadam, 1995). During storage the moisture content of avocados was not significantly reduced, probably because of their low initial values. However, the moisture content of avocados from supply 1 was significantly higher than that of supply 2. This is most likely because supply 1 avocados were harvested 3 weeks before supply 2 avocados. Both of these can be regarded as late season avocados. As stated above, avocados harvested towards the end of the avocado season are less able to retain water while still attached to trees than those harvested earlier on in the season. Samples treated with Dextrin 20 DE + Protonal 686 + Ca^{2+} were expected to prevent moisture losses as alginate

combined with calcium were found to have good moisture barrier properties in Phase 1. However, the edible coatings did not seem to provide any moisture barrier properties during this phase. This unexpected finding could possibly be due to the late season of harvesting.

Although the edible coatings investigated during the first phase did not perform as expected in terms of their gas barrier properties, it was anticipated that using them in combination during Phase 2 would provide better functionality. However, the gas barrier properties of the combined coatings still seemed to be negligible. The reason for this is most likely due to factors such as stage of ripening and season of harvesting. As was the case with avocados used during Phase 1, the exact stage of ripening of the avocados was not known for Phase 2. Based on the O_2 and CO_2 levels found in the packs during storage, it was assumed that the avocados used in this phase might have been at the initial stage of the climacteric phase, which is associated with a fast respiration rate (Salunkhe & Kadam, 1995). Moreover, wounding through minimal processing can further accelerate the respiration rate. This increase in respiration in wounded plant tissue is thought to be a consequence of elevated ethylene due to cutting, which stimulates ripening (Brecht, 1995). According to Avena-Bustillos *et al.* (1997), the wounding reaction in fresh produce is suppressed by edible coatings. However, MPR fruits undergo wounding before the coating can be applied. Therefore, coatings may suppress wounding only to a limited extent if at all.

It is doubtful whether the edible coatings could reduce the increased respiration rate significantly under these conditions. This is reflected by the results obtained for the O_2 levels in the packs. During storage it was observed that avocados from all treatments (including the control) significantly lost O_2 (but not to the extent that it would slow down the respiration rate significantly). Overall, control performed differently by having a significantly decrease in CO_2 whereas the treated samples showed a gradual built up of CO_2 in the packs. The exact

reason for this is unclear, but variations found might be due to non-uniform application of coatings.

Usually transpiration rate is also accelerated during the climacteric stage. This also explains the lower moisture values obtained for all samples. The time interval (3 weeks) between the two suppliers might also have played a role in the results obtained.

During storage, L, a and b values were significantly decreased. Therefore, avocados became darker and lost some of their greenness and yellowness during storage. Supply had a significant effect on the colour measurements. Supply 1 performed better than supply 2 in term of the L-value (light colour) and of b-values (yellowness). Season might have played a role. Both supplies of avocados were late season avocados. It was expected that combination of different coatings would have a better colour retention than coatings acting alone.

Similar L and b values were obtained for all samples including the control due to the late season avocado usage. Overall, samples coated with Dextrin 20 DE + Casinella QN were less yellow in colour than all the other sample probably to non-uniform coating which allowed pigment oxidation. Based on the respective a-values, avocados from Phase 2 seemed to have a greener colour than that from Phase 1. This might be due to the different varieties used (i.e. the non-browning and browning Hass varieties). In addition, a more effective anti-browning dip (including sodium metabisulphite) was used in Phase 2.

In general, the texture of avocados, like colour did not remain constant. There was a significant softening in texture for all samples during storage. This softening could be due to the failure of coatings to act as gas barriers (slowing down respiration) therefore accelerating the softening process. Also the stage of maturity might have played a role, as there are enzymes produced during the senescence period responsible for fruit softening. On days 7 and 9 of storage the

avocados were significantly softer probably due to ageing and an increase in cellulase activity, which lead to softening. Supply also played a role in the results, where avocados from supply 1 were significantly softer than those of supply 2; but the actual difference seems to be relatively small.

Although fruits are regarded as perishable produce, they possess potent defense mechanisms e.g. their peels that keep them from decaying fast. This first line of defense is a physical barrier against invasion by microbes (Wiley, 1994). However cutting can affect the microorganisms by circumventing the normal protection provided by outer skins or peeling. Sometimes, microbes that are not normally considered to be spoilage organisms can serve as such when normal protection mechanisms are eliminated (Brackett, 1993; Ohlsson, 1994).

In this research a number of microbiological tests (TPC, coliforms, yeasts and moulds and anerobic sporeformers) were done, which were all used to determine the shelf life of the MPR avocados. Various microbial cut-off points were carefully considered when determining the shelf life.

Total plate counts in this phase were below their cut off point (10^8 cfu/g). According to Jay (1985), foods are free of any signs of microbial spoilage when coliforms counts are less than 10^3 cfu/g. Growth of coliforms was expected, as it is impossible to eliminate them. Coliforms were under control until day 5 of storage where most of the samples were close to the cut-off point. The growth of coliforms could be due to poor hygiene or cross contamination during processing which is aggravated by no heat treatment which could have killed some of the microbes. Coliforms are good indicators of poor hygiene and contamination. Their presence reflects the microbiological quality of foods relative to product shelf life or their safety from foodborne pathogens. While the presence of large number of coliforms in foods is highly undesirable, it would be virtually impossible to eliminate all from fresh foods (Güntensperger, 1994). Coliforms are not depended on the product but depend on the sanitation of the process from

harvesting till consumption. Method such as hazard analysis critical control point (HACCP) are recommended for lowering of coliforms or maintenance of sanitation conditions (Banwart, 1989).

According to Jay (1992), the wider pH growth range of moulds and yeasts are good spoilage agents of fruits. Bester (2000 - personal communication), pointed out that fruits are mostly spoiled by yeast. Yeast levels higher than 10^5 cfu/g and moulds higher than 10^3 cfu/g is deemed to be unacceptable (Bester, 2000 - personal communication). Yeast counts significantly increased during storage, but counts started to become close to the cut-off point on the last day of storage. Overall, samples treated with Dextrin 20 DE + Olive oil had lower yeast counts than all the other samples except for the sample coated with Dextrin 20 DE. The possible growth of fungi might have been due to the pH of the avocados (± 6.3) which favours the growth of yeasts (Cutting & Wolstenholwe, 1992a). On the other hand there was no growth of moulds on day 1 to 5 of storage except for avocados treated with Dextrin 20 DE + Olive oil and Dextrin 20 DE + Protonal 686 + Ca^{2+}. The actual growth started on day 7 of storage. Mould growth was expected because normally fruits are spoiled by mould due to their pH range and presence of moisture content higher than 50 %. Despite the presence of mould growth the count was below the cut off point on day 9 of storage. Supply also played a role showing that supply 2 avocados had higher moulds counts than supply 1 possibly due to late harvest. In addition yeasts grow faster than moulds, and often precede the moulds in the spoilage process of fruits in certain circumstances and this was the case in this research Banwart (1989).

It seemed that anaerobic sporeformers were not a problem when considering their cut off point (greater than 10^4 MPN). The numbers of organisms were still below the cut-off point. This could be because of the low initial anaerobic sporeformers counts as well the aerobic conditions in the packs. It was noted that overall sample treated with Dextrin 20 DE + Olive oil had the highest surviving anaerobic counts and control the lowest.

Most researchers use TPC to predict the shelf life of MPR products. During this phase TPC, yeast and moulds counts as well as anaerobic spore counts for MPR avocados were below their cut off points on the last day of storage. Coliforms, which are indicator organisms, however, were above their cut off point (10^3 cfu/g) for all samples after day 5 of storage. Therefore it is of importance to consider coliforms when investigating the shelf life of MPR avocados. Thus, if all the above results of microbes tested are considered then it could be said that the shelf life of MPR avocados in this phase was approximately 5 days. This is less than the proposed shelf life of 9 days at 5°C to facilitate exporting. By day 7 of storage the perceived colour, as indicated by the L-values of avocados, was unacceptable. It was noted that with time microbes proliferated. The possible growth of microbes could be due to cross-contamination and nutrients provided by the fruits and coatings.

From a microbiological view-point, all the samples (including the control), seemed to have a shelf-life of approximately 5 days at 5°C. However, the sensory quality of the MPR avocados (although not tested formally), was not deemed to be of an acceptable standard by day 7. Avocados used in the second phase were perceived to be of poor quality, compared with the avocados used in the first phase.

It is evident that the use of edible coatings did not extend the shelf life of MPR avocados significantly during this phase. Therefore, it is apparent that there were factors other than the use of coating, impacting on the shelf life of MPR avocados. These include factors such as stage of ripening, packaging permeability, season, and behaviour of the variety. All these factors must therefore be considered when researching minimally processed fruits.

CHAPTER 7

CONCLUSION AND RECOMMENDATION

The primary objective of Phase 1 was to study the effect of individual edible coatings (carbohydrates, protein and lipid based), on the microbiological, physico-chemical and sensory quality of MPR avocados. Based on these results, the best coatings would be selected and combined for use in Phase 2.

From a microbiological (TPC) and sensory viewpoint the use of permeable packaging alone (control sample) and in combination with selected edible coatings extended the shelf life of minimally processed (MPR) avocados to at least 7 days at 5°C. This could be due to the use of high quality avocados (harvested during the mid-season) at the post-climacteric respiration stage (with low physiological activity). Low temperature storage and permeability of packaging material probably played a more significant role in extending the shelf life of the avocados than the use of edible coatings.

It was expected that the different categories of edible coatings would compliment each other so that a logical choice could be made in combining them. However, in most instances the edible coatings did not perform as expected. This could probably be attributed to non-uniform application of the coatings. In addition, the edible coatings appeared to provide additional nutrients to spoilage microorganisms.

The initial intended objective was to combine the most effective coatings in Phase 1. However due to the poor performance of the coatings it was decided to study the effect of selected edible coatings, in combination, on the quality and shelf life of MPR avocados stored for nine days at 5 °C.

The Dextrin 20 DE coating was used in combination with other coatings in Phase 2 due to the following reasons: colour retention ability as well as a high

acceptability for taste. The best performing coating from each category (i.e. lipid, protein or carbohydrate based) was combined with Dextrin 20 DE. These included: Olive oil (good colour retention); Protonal 686 + Ca^{2+} (moisture and gas barrier) and Casinella QN (colour retention).

The use of combined coatings packed in permeable material in Phase 2 did not extend the shelf life of MPR avocados significantly (approximately 5 days). The use of edible coatings was even less effective here than in Phase 1. This can be attributed to the following factors: poor and inconsistent quality of the raw material, inappropriate season of harvest (end of the spring season), rain during harvesting, and probably climacteric stage of ripening with high respiration and transpiration rates and possibly non uniform application of coatings

It is recommended that in order to extend the shelf life of MPR avocados for export purposes (with a shelf life of more than 7 days at 5°C) using edible coatings the following factors must be taken into consideration: high and consistent quality of raw material, harvesting season (mid season) stage of ripening (post-climacteric), uniform application of edible coating and the use of anti-microbial agents (e.g. potassium sorbate). It is essential to establish the exact stage of ripening before commencing processing. Studying respiration behaviour of the fruit can assist with regard to this. Uniformity of application of coatings could be measured by using a tracer component, 0.5% zinc silicate phosphor that emit fluorescent light under ultraviolet illumination.