THE IMPROVEMENT OF THE SHELF LIFE OF VEGETABLES THROUGH PRE- AND POSTHARVEST TREATMENTS

By

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Dedication

"This work is dedicated to my father Seyoum Workneh and my mother Tafesu Dagne for their love, encouragement and sharing the small subsistence income they were getting from the small farm product, through the grace of God, to keep me in school without themselves had the opportunity to be educated."

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LIST of ABBREVIATIONS

Abbreviation	**Description**
AA	Ascorbic acid
ABS	Absorbancy
ANOVA	Analysis of variance
C.V.	Coefficient of variation
CFU	Colony forming unit
ComCat	Communication and Catalization
e.g.	For example
EC	Evaporative cooling
g	Gram
HDPE	High-density polyethylene
i.e.	That is
kg	Kilogram
LDPE	Low-density polyethylene
LL	DPE Linear low density polyethylene
LSD	Least significance difference
MAP	Modified atmosphere packaging
MDPE	Medium-density polyethylene
mg	Milligram
ml	Milliliter
MSE	Mean square error
NS	Not significant
P	Significance level
PG	Polygalacturonase activity
POX	Peroxidase activity
PP	Polypropylene
PS	Polystyrene
PVC	Polyvinylchloride

PVC	Polyvinylchloride, plasticized
PVDC	Polyvinylidine chloride
PWL	Physiological weight loss
RH	Relative humidity
S.E.	Standard error
SH	Sucrose to hexose ratio
TAC	Total available carbohydrate
TSS	Total soluble solid
TTA	Total titratable acidity
VA	Ethylene vinyl acetate copolymer

CHAPTER 1

GENERAL INTRODUCTION

1.1. Background

Research results suggested that selection and careful handling of food products determine the quality of fresh produce (Zagory and Kader, 1988). Harvesting products at optimum maturity, maintaining higher sanitation standards, decreasing injury incidence and maintaining optimum environmental conditions will guarantee excellent postharvest quality. Value that does not exist at harvesting time, however, cannot be added with the correct postharvest handling procedures.

The response of fruit and vegetables during storage to postharvest factors in part depends on preharvest practices (Salunkhe *et al.*, 1991). Therefore, it seems that preharvest operations or treatments that are applied to improve yield and quality of vegetables play an important role in postharvest quality changes. Preharvest treatments have had significant effects on the postharvest quality of vegetables (Bialezyk *et al.*, 1996; Gao *et al.*, 1996 and Carmer *et al.*, 2001). Recently, a preharvest treatment called Communication Catalysation (ComCat®) has been developed and it was shown in different countries to result in increased yields of vegetables (Hüster, 2001). Unlike the other preharvest chemical treatments at agricultural input level, the most important advantage of ComCat® is that it is both environmentally and ecologically friendly. ComCat® consists of biocatalysts of plant origin and induces resistance via activating plant defence mechanisms against pathogens, and biotical and abiotical stress factors. ComCat® treatment is an alternative to chemical treatments and can fit into future research trends to have a balance between yield and ecologisation. This treatment makes crops stronger and competent without destroying the other plant species in the ecology. However, at present there is no information on the postharvest quality aspects of the high harvest yield ComCat® treated vegetables, and the following questions arise:

- How do these complex plant growth regulators and natural metabolites affect the quality of vegetables at harvest?
- How do ComCat® treated vegetables perform when subjected to different postharvest treatments and during storage?

The effect of combined preharvest ComCat® and postharvest (prepackaging and packaging) treatments on quality parameters should therefore be investigated to understand postharvest performance of these ComCat® treated vegetables.

It would not be possible to investigate the effect of ComCat® treatment on postharvest performance on a wide variety of vegetables at once. Representatives of the vegetable part of a plant i.e. root, stem or leaf, and also the fruit part should be investigated first. The selection should also be from vegetables, which are of economic importance, in developed as well as developing countries, and should be ones that are commonly used for pre-packaged storage for a few weeks. From several options, carrots and tomatoes were selected as subjects of this study.

Regarding postharvest techniques, the current growing demand for "fresh-like" quality and shelf-stable intact vegetables has spurred the development of many innovations, especially in the packaging sector. Among these, controlled-atmosphere storage and modified atmosphere packaging are the dominant means of storage.

Packaging of fresh and lightly processed vegetables has several benefits: easy consumer packaging, protection from damage, reduction of water loss, shrinkage, and wilting, reduction of decay, reduction of physiological disorder (chilling damage), reduction of ripening and senescence processes, reduction of growth and sprouting and control of insects in some commodities (Gibbons, 1973; Kelsey, 1978; Crosby, 1981; Kumar and Balasubrahanyam, 1984; Myers, 1989).

Modified atmosphere packaging (MAP) involves depletion of O_2 and emanation of CO_2 within a sealed plastic packaging headspace, which controls the metabolism. Decay is also reduced due to the prevention of moisture condensation (Zagory and Kader, 1988). MAP is therefore a very convenient way to protect vegetables during storage.

The quality properties of packaged vegetables are, however, governed by cultivars, growing conditions and maturity stage at harvest (Cameron and Reid, 1982). Therefore, an optimum packaging system is required for new cultivars and products grown under different treatment conditions. It would therefore be important to investigate the effect of MAP storage on ComCat® treated vegetables.

The microbial load associated with vegetables during storage also plays an important role on quality deterioration and is one of the postharvest quality parameters (Brackett, 1992). The initial microbial load of vegetables from the field determines the microflora of vegetables during storage periods if one assumes good sanitation prevails during and after harvest. The shelf life and protection of microbial spoilage of vegetables stored with MAP can be improved by the application of any treatment that can reduce the initial microbial load (Bolin *et al.*, 1977; Brackett, 1992). The present research trend is towards developing environmentally and ecologically safe disinfectants for biocontrol. In most of the cases, vegetables are washed with cold chlorinated water prior to packaging or are disinfected with different types of chemicals. Several workers showed the effectiveness of chlorine solution in killing or reducing the microbiological flora and pathogenic organisms (Escudero *et al.*, 1999; Beuchat *et al.*, 2001; Li *et al.*, 2001; Ukuku and Sapers; 2001). Furthermore, emphasis was given to postharvest microbiological changes without due consideration to the possible effect of chlorine solutions on the postharvest physiology and biochemical changes of fruits and vegetables. Some chemicals are not easily available in the marketplace while others need technical knowledge for use. Simons and Sanguansri (1997) in Delaguis *et al.* (1999) reported that even though alternatives or modified treatments have been proposed, none have yet gained widespread acceptance. Recently a product, electrochemically activated water (anolyte), based on a Russian development, was developed for use in dental unit water lines (STEDS, Radical Waters, Midrand, South Africa). The original development was applied in fields varying from agriculture, cooling towers, swimming pools, dermatology, dressing and cleaning of wounds and disinfecting of instruments (Leonov, 1997; Bakhir, 1997; Prilutski and Bakhir, 1997). Anolyte contains free radicals and is considered totally harmless to human tissue, yet highly microbiocidal. It was further established that anolyte solutions return to a stable, inactive state of pure water, within a period of 48 hours after production, therefore being environmentally friendly. Since the effect of chlorine treatment on postharvest physiology and biochemical changes of fruits and vegetables has not yet been studied, the question arises what the effect of anolyte would be on these properties. Anolyte and chlorine as disinfecting treatments should therefore be investigated in parallel to add to the optimisation of postharvest handling of vegetables in general, and to investigate postharvest performance of ComCat® treated vegetables when subjected to these treatments in specific.

In underdeveloped countries there is a need for higher crop productivity, followed by appropriate means of reducing losses after harvest, aiming at sufficient food security and nutrition for their society. Fruits and vegetables are major food crops rich in vitamin C, magnesium and sometimes in carbohydrate, compared to the percentage of total food supply (Salunkhe *et al.*, 1991). Obviously, improving productivity, reduction of postharvest losses and maintaining quality after harvest should be the main strategy in underdeveloped regions of the world.

The second part of this study, therefore, deals with the appropriate postharvest technologies as a potential means to reduce the excessive losses of ComCat® treated and control vegetables after harvest in developing countries like Ethiopia.

Agriculture is the major branch of the Ethiopian economy. The climatic and soil conditions of Ethiopia allow cultivation of a wide range of fruit and vegetable crops. It has a vast potential for the internal market for fresh fruits and vegetables, primarily in densely populated urban areas, such as the Addis Ababa region, and is also located close to important foreign markets, such as Saudi Arabia, Djibouti, Somalia, etc.

However, growing and marketing of fresh produce in Ethiopia are complicated by high postharvest losses which are estimated as high as 25 - 35% of the produced volume for vegetables (Agonafir, 1991) and may reach even higher figures for fruits. These losses discourage farmers from producing and marketing fresh produce and limit the urban consumption of fresh vegetables to as low as 25-30% of that in the Western world (Wolde, 1991).

Lack of proper packaging is one of the major causes of postharvest losses of fresh fruits and vegetables in Ethiopia (Wolde, 1991). The lack of modern refrigeration and packaging house facilities in these countries results in severe food losses. A cooling chamber that works on the principle of evaporative cooling was developed to counter this problem (Seyoum and W/Tsadik, 2000). This chamber was shown to maintain temperature and also control humidity. Should ComCat® treatment also be able to increase yield of vegetables in Ethiopia, it would be important to know what the postharvest performance of these vegetables would be under evaporative cooling at storage temperatures other than the optimised refrigeration temperatures.

1.2. Objective

1. Investigations of the effect of preharvest ComCat® treatment on postharvest quality of stored carrots and tomatoes.

2. Investigation of the effect of pre-packaging and storage treatments (washing, dipping in anolyte and chlorine supplemented water) on the quality of preharvest ComCat® treated and untreated carrots and tomatoes.

3. Investigation of the storage quality of preharvest ComCat® treated carrots and tomatoes using modified atmosphere packaging.

4. Investigation of the storage performance of preharvest ComCat® treated carrots and tomatoes at different storage temperatures, being optimum refrigeration temperatures (1°C for carrots and 13°C for tomatoes), room temperature and evaporative cooling temperature.

CHAPTER 2

LITERATURE REVIEW

Abstract

The aim of this literature survey was threefold: First to explore the effect of different preharvest treatments on postharvest quality of fruits and vegetables. Second the principles of biological, chemical and biochemical changes in fruits and vegetables during development, maturation, ripening and storage were reviewed. Third postharvest handling and factors affecting quality of fruits and vegetables were examined. These include disinfecting, packaging and storage temperature. Pre- and postharvest treatments were found to have an effect on postharvest quality of fruit and vegetables, suggesting that postharvest quality of produce subjected to preharvest treatments should be assessed from a quality improvement, maintenance and consumer safety point of view. Cold storage is one of the most important postharvest unit operations. Low-cost alternative evaporative cooling systems are suggested as alternatives when cold storage conditions cannot be met.

2.1. Introduction

An understanding of the changes of fruits and vegetables during storage entails more than just knowledge of packaging methods. Preharvest as well as postharvest physiological properties have to be understood. Therefore, in this chapter the survey on the effect of preharvest treatments on postharvest quality of fresh commodities is presented first. This is followed by the survey of postharvest handling and storage including pre-packaging treatments and storage methods available for fresh commodities. Since tomatoes and carrots were selected for this study, the survey will mainly concentrate on these vegetables.

2.2. Development physiology of fruit and vegetables

2.2.1. Development physiology of tomatoes

Tomato physiology begins with fertilisation of the ovules of the blossom (Salunkhe *et al.*, 1991). Hormone production by the developing seeds and young ovary walls are highly responsible for growth. The period from the end of flowering to and including the ripening stage, during which chemical changes take place and new tissue is formed and brought to morphological completion, is known as the development period (Salunkhe *et al.*, 1991). It includes stages of permutation, physiological maturation, ripening, and senescence (Kader *et al.*, 1985). These stages are followed by continued changes in the chemical composition, which in turn is governed by a range of enzyme activities (Kader *et al.*, 1985).

Tomatoes are commercially mature at a fully developed fruit stage. Endogenous ethylene is present in measurable quantities during the entire development of the tomato fruit (Lyons and Pratt, 1964). It has been reported that soluble peroxidase activity increases dramatically during the early stages of tomato fruit development, reaching a maximum at the green mature stage (Thomas *et al.*, 1981). The soluble peroxidase activity remained higher during the breaker and light pink stages, but decreases in the later stages of development (Thomas *et al.*, 1981). Simultaneously IAA (indoleacetic acid) oxidase activity in the soluble fraction followed a parallel pattern to the peroxidase activity (Frenkel, 1972, Thomas *et al.*, 1981). Frenkel (1972) reported that the induction of the major isozyme component of peroxidase and IAA oxidase was enhanced as the tomato fruit ripens. The amount of auxin protectors also increased as the fruit developed (Thomas *et al.*, 1981).

A relationship between $NADP^+$-malic enzyme and organic acid levels exists in tomatoes from flowering through to ripening, and both increase during development, reaching maximum levels at the green mature stage (Knee and Finger, 1992). However, a decline in malate concentration is followed by a decrease in NADP-malic enzyme activity and citrate concentration. Their data reported demonstrated that it is possible that an enzyme is involved in cytoplasmic pH regulation. The sugar content of tomato fruits increases progressively throughout maturation and ripening, with a pronounced increase with the appearance of yellow pigmentation (Winsor *et al.*, 1962a, b, in Hobson and Davies, 1971). The starch content of tomatoes also increases with maturation, reaching a maximum at 8 weeks after fertilisation, but is not detected in young fruit up to 10 days. Results on the ascorbic acid content of tomatoes during the development and ripening seemed inconsistent. However, recent studies have indicated an increase in ascorbic acid contents of tomato fruit during maturation, with either a continuing increase or a slight decrease during the final stages of ripening (Dalal *et al.*, 1965, Mohammed *et al.*, 1999). The malic acid concentration decreases as tomatoes ripen, while citric acid increases up to the green-yellow stage and then generally decreases (Hobson and Davies, 1971).

2.2.2. Development physiology of carrots

Phan *et al.* (1973) gathered comprehensive information on the carrot roots during growth up to harvest. The main constituents of carrot roots are soluble carbohydrates comprising of non-reducing sugars, mostly sucrose, and reducing sugars, mostly glucose. Their data showed that there was active biosynthesis of carbohydrates, mainly sucrose, and carotenes, such as β-carotene (Phan *et al.*, 1973). The sugars and carotene contents of carrot roots consistently increase during the 3 months after seeding and reach their maxima at the end of 3 months. After 3 months of the development period, the sugar content of both groups of substances remains almost constant. The total soluble carbohydrate concentration increases rapidly a few days after the initiation of an entire ring of cambium in the carrot roots (Hole and McKee, 1988). Their data concerning the relationship between enzyme activity and carbohydrates during the development of carrot roots revealed the existence of little correlation.

The amount of organic acids and amino acids increases slowly with age during root development and these components are present in rather low concentrations (Phan *et al.*, 1973). However, pyruvic acid occurs in high amounts in growing carrot roots up to 3 months, which is followed by isocitric acid and malic acid (Phan *et al.*, 1973). These results indicated that a 3-month growth period for carrots could be sufficient before harvest, after which there is no more increase in chemical components responsible for good quality characteristics. However, this "biochemical maturity" is reached while carrots are still growing in diameter and can therefore not be taken as a criterion for determining the harvest date (Phan *et al.*, 1973).

2.3. Preharvest treatments of vegetables

2.3.1. Effect of preharvest factors on storage quality of vegetables

Preharvest treatments of fruits and vegetables are primarily aimed at increasing yields, while postharvest storage performance is normally neglected. Several research results were reported on methods to increase harvest yield and qualities of fruits and vegetables (Mitchell et al, 1997). Most research work has been targeted on cultural practices, such as rootstock/plant age, soil management, nutrition, training and pruning practices, crop loads, product size, and growth regulators (Rosenfeld, 1999). Bramlage (1993) in Watkins and Pritts (1999) highlighted the almost overwhelming number of preharvest variables that contribute to the variety of postharvest responses of the crops. Watkins and Pritts (2001) hypothesise that the diversified postharvest responses of fruits and vegetables during storage are in part due to preharvest cultural practice. A literature review has shown that the major factors affecting yield and quality of vegetables are cultivars, soil plant systems and fertiliser practices, and the environmental factors such as temperature, relative humidity, light intensity and rainfall during production (Rosenfield, 1999).

2.3.1.1. Soil plant system and fertiliser practice

From a literature review on the effect of cultural practice on quality of vegetables with emphasis on tomatoes, carrots and lettuce (Rosenfeld, 1999), it was deduced that in general, the objectives of optimal fertilisation strategy were maximization of yield, the maximization of fruit and vegetable quality, minimization of environmental pollution caused by fertilisers and the minimisation of fertiliser expenses. The yield of potatoes, soybeans, cabbages, carrots, onions, cucumbers, strawberries, and eggplants oscillated because of the different soil and climatic conditions (Data given by Polus, in Schnabl *et al.*, 2001). These could also suggest that there could be changes in postharvest quality based on different soil and climatic conditions.

Postharvest quality parameters of tomatoes and carrots vary with the fertilisation practice during production. Even under unfavourable climatic conditions, application of phosphorous - potassium (PK) fertiliser could be used to increase carotene content in carrots (Evers, 1989a). Photosynthetic products like sugar are slightly affected with fertilisation (Evers, 1989a). However, Evers (1989a, b and c) showed that seasonal variations and genetic variations are often larger than variations caused by soil and fertiliser practices.

Qualities of carrots are reduced as a result of increased use of mineral fertiliser, but are not affected by measure fertilisers (Lieblein, 1993) whereas increased use of composted manure had no effect on quality of carrots. The postharvest response of carrots is often dependent on the level of fertiliser applied during the growth period (Petrichenko *et al.*, 1996).

Vitamin B concentrations are higher in plants grown with organic fertilisers as compared with plants grown with inorganic fertilisers (Mozafar, 1994). Nitrogen fertilisers, especially at high rate, seem to decrease the concentration of vitamin C in different fruits and vegetables, among them potatoes, tomatoes and citrus fruits (Mozafar, 1994).

Positive and negative effects of preharvest treatments on tomatoes, either to increase yield or improve nutritional quality, have been reported (Bialezyk *et al.*, 1996; Gao *et al.*, 1996 and Carmer *et al.*, 2001). Carmer *et al.* (2001) supplied tomato plants with either low electric conductivity (EC = 0.25 Sm^{-1}) nutrient solutions or with nutrient solutions supplied with 55 mM NaCl to generate at high electric conductivity (0.75m^{-1}). Their results showed that high electric conductivity increased the total soluble solids

(TSS) by ca. 18% and titratable acids by ca. 32% relative to low electric conductivity treatments. The report also indicted that after storage of 2 weeks at 15°C, fruits of high electric conductivity treated plants were 12% less firm than those of low electric conductivity plants, and no difference in TSS or acidity were found.

Salunkhe et al (1971) has shown the effect of Telone (1,3-dichloropropene and other chlorinated hydropropane) and Nemagon (1,2-dibromo-3-chloropropane) on essential nutritive components and the respiratory rates of carrots and sweet corn seeds. The respiration of carrots treated with Telone and Nemagon was below 94 $\mu l\ O_2\ h^{-1}\ g^{-1}$ and 82.8 $\mu l\ O_2\ h^{-1}\ g^{-1}$ respectively, while that of untreated carrots was 108.8 $\mu l\ O_2\ h^{-1}\ g^{-1}$. This result clearly showed that preharvest treatments could affect the physiology of carrots at harvest. A significant increase in the content of total carotene, β-carotene and total sugars, was observed, with a simultaneous decrease in respiratory rates in carrots (Salunkhe *et al.*, 1971). The low respiration rates indicated that the metabolic activities of the treated carrots were low, which lead to increased shelf life without quality deterioration. Pre-planting soil fumigation with Telone and Nemagon also resulted in increased carotene, β-carotene and total sugars, and decreased the respiratory rates in carrot roots (Singh *et al.*, 1970).

Most researchers reported that there are either positive or negative effects of any type of preharvest practices on quality of carrots and tomatoes, especially at harvest (Watkins and Pritts, 2001; Tittonell *et al.*, 2001; Ozeker *et al.*, 2001; Sen *et al.* 2001). Salt concentrations of nutrient solutions were shown to affect quality of celery more than yield (Pardossi *et al.*, 1999), and preharvest Ethephon (2-chloroethylphosphonic acid) spray directly onto pepino fruits advanced colour changes (Maroto *et al.*, 1995; Lopez *et al.*, 2000). Nutritional treatments had a positive effect in reducing the peel disorder of fruits under commercial conditions (Zilkah *et al.*, 2001). More research is needed on the storage of fresh produce subjected to various preharvest practices. It is also recommended that after each preharvest practice a study on the quality of vegetables and their storability needs to be conducted in order to secure favourable storage conditions for these products and maintain freshness and nutritional quality.

2.3.1.2. Effect of environmental factors on vegetable quality

The effect of climate on quality of vegetables is normally mostly higher than the effect of fertiliser on photosynthetic products such as sugar (Rosenfeld, 1999). Temperature is the major environmental factor affecting quality of vegetables. Vegetables like carrots and tomatoes respond to various levels of temperature. Carrots grown at high temperature were shown to have a higher total sugar content, whereas those grown at low temperature were sweeter, specifically due to a higher sucrose content (Rosenfeld, 1999). Apple watercore, ethylene evolution, flesh firmness, membrane permeability and sorbitol levels were shown to be affected by preharvest fruit temperature (Yamada and Kobayashi, 1999).

Hao and Papadopoulos (1999) have shown that supplemental lighting during growth of cucumber increases biomass allocation to fruits, fruit dry matter content and skin chlorophyll content. Leonardi *et al.* (2000) grew tomato plants in two glasshouse compartments under two vapour pressure deficit (vpd) levels, showing that fruit growth and transpiration rates greatly varied during daylight hours, which has enhanced under high vpd conditions. As a result a significant reduction in fruit weight and in fruit water content, and an increase in soluble solids was found. Environmental vapour pressure increases can therefore affect not only growth but also quality of tomato fruits. It was shown that air humidity has effects on growth, flowering, and finally on keeping quality of some greenhouse species (Mortensen, 2000).

2.3.1.3. Water management

Sørensen *et al.* (1997) showed the effect of drought stress on carrot quality. Glucose, fructose and sucrose concentrations in carrots exposed to drought stress at different growth stages were not affected in any consistent manner. The results also indicated that the concentration of dry matter was low when drought was induced at an early growth stage in coarse and sandy soil. Averaging the effect of drought periods and cultivars, drought stress was observed to increase the concentration of sucrose in taproots from the coarse, sandy soil. It was also shown that drought stress increased storage losses due to the development of disease (Sørensen *et al.*, 1997).

Research on deficit-irrigating micro-irrigated tomatoes showed that occurrence of early blight disease (caused by the fungus *Alternaria solani*) was increased by 50%, while blossom end rot incidence was five times more severe compared with full irrigation (Obreza *et al.*, 1996). This result indicated that deficit irrigation could cause substantial economic loss of tomatoes through decreased crop marketable quality.

Dodds *et al.* (1996) studied the influence of water table depth and found that 0.6 m depth gave the best yield and largest fruit size, however, a higher incidence of catfacing, cracking, sunscald and loss of firmness of tomatoes were found. A balance between yield and quality at a water table depth between 0.6 and 0.8 m was recommended for tomato production on sandy loam soils.

On the other hand, irrigation deficit in the first growth period of tomato reduced the number of flowers leading to a decrease in the number of fruits and in the marketable yield (Colla *et al.*, 1999). The soil moisture deficit resulted in increased soluble solids and acidity of the fruit. However, reducing irrigation by 25% before fruit onset and by as much as 50% in the fruit development and ripening stages resulted in no significant decrease of soluble solid yield.

2.3.1.4. Hormone treatment

Hormones are essential for plant growth and development. The quality of hormone present and tissue sensitivity to hormones has an effect on plant physiology. Plant hormones are responsible for cell elongation, cell division, inhibition of senescence, abscission of leaves and fruits, dormancy induction of buds and seeds, promotion of senescence, epinasty and fruit ripening. The preharvest as well as postharvest physiological processes in fruits and vegetables are responsible for changes in composition and quality of these horticultural crops and depend on the plant species. The major classes of plant hormones include auxins, gibberelins, cytokinins, abscisic acid, brassinosteroids and ethylene (Mauseth, 1991; Raven *et al.*, 1992; Salisburg and Ross, 1992; Davies, 1995).

Gibberellins are known to stimulate the physiological processes such as stem elongation, bolting, breaking seed dormancy, enzyme production, induce maleness in dioecious flowers, cause parthenocarpic (seedless) fruit development and can delay senescence in leaves and citrus fruits (Mauseth, 1991; Raven *et al.*, 1992; Salisburg and Ross, 1992; Davies, 1995). Cytokinin stimulates cell division, morphogenesis in tissue, the growth of lateral buds, release of apical dominance, leaf expansion resulting from cell enlargement, enhances stomatal opening in some species and promotes the conversion of etioplasts into chloroplasts via stimulation of chlorophyll synthesis (Mauseth, 1991; Raven *et al.*, 1992; Salisburg and Ross, 1992; Davies, 1995). The functions of abscisic acid are to stimulate the closure of stomata, inhibition of shoot growth, induction of seeds to synthesise storage proteins, induction of gene transcription, especially for proteinase inhibitors in response to wounding, which may explain an apparent role in pathogen defence (Mauseth, 1991; Raven *et al.*, 1992; Salisburg and Ross, 1992; Davies, 1995). Ethylene has been the most studied plant hormone in relation to fruit ripening and postharvest storage. Some of the functions of ethylene are to stimulate the release of dormancy, shoot and root growth, and differentiation of adventitious root formation, leaf and fruit abscission, bromiliad flower induction, induction of femaleness in dioecious flowers, flower opening, flower and leaf senescence and fruit ripening (Mauseth, 1991; Raven *et al.*, 1992; Salisburg and Ross, 1992; Davies, 1995).

The other growth regulating compounds are brassinosteroids, salicylates, jasmonates and polyamines. An abundance of research has been devoted on the chemistry and physiology of natural growth regulators resulting in the recognition of brassinosteroids as a new class of phytohormones (Sasse, 1997; Schnabl *et al.*, 2001). Some of the effects of brassinosteroids include stimulation of stem elongation, inhibition of root growth and development and promotion of ethylene biosynthesis and epinasty. Recently, the brassinosteroids has gained a broad spectrum of application and extremely low toxicity and mutagenicity have received increasing attention (Schnabl *et al.*, 2001). Previous work showed that brassinosteroids are plant growth promoting regulators, which are effective in cell elongation and division, source/sink metabolism, chlorophyll synthesis and reproductive and vascular development (Clouse, 1996; Mandalla, 1988). Sasse *et al.* (1995) and Takatsuto *et al.* (1996) reported that they enhance nutrient contents, improve shape and taste of fruits, have beneficial effects on germination, growth and seed quality. Much research has been conducted on the potential performance and investigations of the potential applications of brassinosteroids in agriculture (Schnabl *et al.*, 2001). This will be elaborated upon section 2.3.1.5 below.

2.3.1.5. Communication Catalization (ComCat®) treatment

Recently, a hormone containing treatment has been introduced as an alternative agricultural input to the use of chemicals to increase production of vegetables and other crops. ComCat® is a natural biocatalyst, which is extracted from seeds of plants and mainly consists of amino acids, gibberellin, kitenins, auxin (indole-3-acitic acid), brassinosteroids, natural metabolites, pathogen-related PR-proteins with defence reactions, terpenoids, flavonoids, vitamins, inhibitors, other signal molecules, biocatalysts and cofactors. The yields of ComCat® treated vegetables were shown to be increased for cabbage (8%), tomato (16-19%), potato (9-19%), soybeans (26-30%), eggplants (37%), cucumbers (25-32%), carrots (32%), onions (49%) and strawberries (50%), compared to control vegetables and fruits (Schnabl *et al.*, 2001). These vegetables were also shown to have better root development, improved resistance induction, less chance of deficiencies with fertiliser, higher resistance to pathogens prior to harvest and they seem to have a slightly better resistance to environmental stress, and increased protein content (Hüster, 2001; Schnabl *et al.*, 2001).

A reduction of plant disease symptoms of up to 45% in comparison to untreated control plants has been found. This is the result of induction of the PR-proteins (pathogenesis-related proteins) namely peroxidase, chitinase and 1-3 gluconase. These enzymes protect cell walls and prevent infection by fungi (Hüster, 2001). Advantages of ComCat® treatment are that only low doses are necessary to show measurable effects of these brassinosteroids containing plant extract in crop plants, their environmental safety and the possibility of reducing the amount of pesticides needed (Schnabl *et al.*, 2001). Apart from studies on yield increase, no data is yet available on the effect of preharvest ComCat® treatment on quality of fruits and vegetables at harvest, as well as during storage. ComCat® was approved by, and registered with the Federal German Biological Centre of Agriculture and Forestry (BBA), Institute for Integrated Plant protection, as a harmless plant strengthening substance of plant origin. It is also licensed for use in Ecological Farming, according to the EU-regulation 2092/91.

As discussed, the effects of ComCat® include (a) serving the plant as a general strengthening agent for the organic development, (b) inducing resistance through activating plant own defence mechanisms against pathogens, and biotic and abiotical stress factors, (c) improve root development, (d) increase yield in agricultural cash crops as well as horticultural crops and (e) increase protein content.

2.4. Postharvest physiology of fruit and vegetables

2.4.1. Respiration

Respiration is defined as a process by which stored organic materials (carbohydrates, proteins, and fats) are broken down into simple end products with a release of energy. In the process O_2 is used and CO_2 is liberated. Vegetables continue to respire after harvest and during storage. Each type of vegetable and cultivars requires a specific range of CO_2 and O_2 concentration levels for safe storage without the occurrence of physiological disorder. The level of physiological activity and potential storage life can be indexed by the rate of respiration. Respiration is one of the basic physiological factors, which speeds up ripening of fresh commodities and is directly related to maturation, handling, and ultimately to the shelf life (Ryall and Lipton, 1979; Ryall and Pentzer, 1982). Generally, the loss of freshness of perishable commodities depends on the rate of respiration. An increase in respiration rate hastens senescence, reduces food value for consumers, and increases the loss of flavour and sellable dry weight.

Stored intact fruits and vegetables face desiccation and chilling injury after harvest and during storage. Due to wounding stress, as a result of chilling or mechanical injuries, respiration rate and overall metabolic activities usually increase. The main physiological manifestation of metabolic activities include increased respiration rate and, in some cases, ethylene production (Rosen and Kader, 1989).

The index of physiological activity and potential shelf life have a direct relationship with respiration of fruits and vegetables. Since sugars in fruits and vegetables play a role in the respiration process, the quantity of sugars in fruits and vegetables available for respiration is the dominant factor for the shelf life of these commodities at a given temperature (Paez and Hultin, 1972). For normal respiration, removal of respiratory CO_2 needs more emphasis than supply of O_2, because some fruits and vegetables are highly sensitive and could be suffocated with a high level of CO_2 (Duckworth, 1966; Kader *et al.*, 1985). Removal of respiratory heat requires attention because it increases the product temperature and surrounding air temperature, which in turn is responsible for increasing respiration and causes acceleration of substrate utilization, predominantly sugars (Ryall and Lipton, 1979; Ryall and Pentzer, 1982). The rate of respiration depends on the quantity of available O_2 as well as the storage temperature. A decrease in rate of respiration increases the shelf life of fruits and vegetables. In order to achieve long storage life of fruits and vegetables, the rate of respiration should be reduced through decreasing the O_2 level, slightly increasing the CO_2 level, and decreasing the storage temperature, which includes removal of respiratory heat (Duckworth, 1966; Kader *et al.*, 1985). The optimum gas composition is the range of O_2 and CO_2 levels that would minimise physiological disorder, reduce respiration rate and reduce ethylene production during storage. The limit of tolerance to low O_2 and high CO_2 levels depends on several parameters including temperature, physiological conditions, maturity, and previous treatment.

2.4.2. Temperature quotient of respiration

Temperature highly affects the metabolic activities of fruits and vegetables. Relatively higher temperatures increase the rate of respiration and ethylene production during storage. The rate of chemical reactions in fruits and vegetables is also controlled by temperature. It was reported that theoretically, the rate of respiration doubles for each 10°C increase in temperature. Depending on the maturity and anatomical structure of the fruits or vegetables, the temperature quotient of respiration may be more than double (Ryall an Lipton, 1979; Ryall and Pentzer, 1982; Sargent *et al.*, 1991). These researchers showed that the respiration rate of topped carrots increased by 79% at 25 - 27°C, when compared to topped carrots at 0°C. Similarly, the rate of respiration (rate of CO_2) of bunched carrots increased by about 71% at 25 - 27°C, compared to those at 0°C (Hardenburg *et al.*, 1986), and the respiration rate of mature green tomatoes increased by 100% when stored at 25 - 27°C compared to storage at 0°C (Hardenburg *et al.*, 1986).

Preharvest treatments on a farm, or in an orchard, affects postharvest physiology of fruits and vegetables, such as the respiration rates. Physiology of fruits and vegetables begins at the time of blossoming or bud formation and is affected by preharvest factors such as fertilisation, variety, and irrigation, and by environmental factors such as sunlight duration and quality, temperature, humidity etc., as well as preharvest spray of hormones and growth regulators (Ryall and Lipton, 1979; Ryall and Pentzer, 1982; Schnabl *et al.*, 2001). These were reviewed in section 2.3 above. These treatments could possibly have positive or negative effects on the postharvest quality and shelf life of fruits and vegetables, indicating the importance of further integrated research on pre-and postharvest physiology when implementing new preharvest treatment agents or methods.

2.4.3. Ethylene production and effects

Ethylene advances the onset of an irreversible rise in respiration rate in climacteric fruit and increases the ripening process. The effect of low O_2 and high CO_2 levels on the production of ethylene adds to the nature of the ethylene production or inhibition process. Fruits and vegetables are classified into five groups according to ethylene production rates within the ranges of 0.1 ml ethylene $kg^{-1}h^{-1}$ at 20°C to 100.0 ml ethylene kg^{-1} h^{-1} at the same temperature (Kader *et al.*, 1985). In general, the ethylene production rates of tomatoes range from 1.0 - 10 ml $kg^{-1}h^{-1}$ at 20°C, which classifies them in the moderate class according to ethylene production rate (Ryall and Lipton, 1979; Kader *et al.*, 1985). Tomato is one of the few vegetables to which a known phytohormone, ethylene, is applied commercially to influence the rate of ripening. Ethylene plays a significant role in the physiological and biochemical changes that occur with the climacteric onset. Lyons and Pratt (1964) reported that endogenous ethylene was present in measurable quantities during the entire growth phase of the tomato fruit. The concentration of ethylene increased 10-fold when fruit growth reached 70-93% of its total fruit growth (Lyons and Pratt, 1964). It was also shown that the concentration of ethylene increased 400 times that of the average measured during growth, at the climacteric onset of ethylene production and onset of ripening. External introduction of ethylene to tomatoes at all stages of development induces ripening and climacteric onset along with phenotypic changes common to normal ripening, such as red color development, fruit softening, and characteristic flavour (McGlasson, 1978 and 1985). However, the acceptable edible quality of tomatoes can only be attained with those that were 93% mature (McGlasson, 1978 and 1985).

In shelf life improvement, maintaining low ethylene concentration and reducing ethylene biosynthesis plays a significant role. Adams and Yang (1979) have shown that aminoethoxyvinylglycine (AVG) block the conversion of s-adenosyl-methionine to 1-aminocyclopropane-1-carboxylic acid. Aminoethoxyvinylglycine was also effective in inhibiting ethylene synthesis in slices of green tomatoes, but was only relatively effective in pink and red tomato fruits. Low temperature also reduces induction of ethylene in tomato fruits during storage, so that the shelf life of tomatoes is increased, when stored at low temperature. Higher temperatures increase ethylene production and result in advanced physiological and biochemical changes in fruit (Wiley, 1994).

Ethylene-induced formation of isocoumarin was also characterised in relation to ethylene-enhanced respiration in whole or cut carrots (Lafuente *et al.*, 1996). Sarker and Phan (1979) reported that ethylene induces the formation of isocoumarin (8-hydroxy-3-methyl-6-methoxy-3,4-dihydro-isocomarin) in carrots, a compound associated with bitterness in carrots (Carlton *et al.*, 1961; Simon, 1985). Concentrations of ethylene ranging from 0.1 to 5 ppm, and temperatures from 1 to 15°C, increased respiration, resulting in a more rapid formation of isocoumarin (Lafuente *et al.*, 1996). It was also shown that exposure to low levels of ethylene (0.5 ppm) for 14 days at 1 or 5°C resulted in isocoumarin contents of 20 and 40 mg/100g peel, respectively. These levels were sufficient to bring a detectable bitter flavour in intact carrot roots. These results clearly indicated that carrot quality is highly sensitive to ethylene during storage and transportation, suggesting that ethylene concentration as well as its biosynthesis should be controlled during commercial storage of carrots.

The presence of the commonly identified phytohormones and various growth regulators are believed to have an inductive effect on ethylene production of fruits and vegetables during ripening (Abdel-Rahman *et al.*, 1975; Davey and Van Staden, 1978; Ryall and Lipton, 1979; Ryall and Pentzer, 1982). Some chemicals applied to bring about abscission of fruits and vegetables that are important in fruit thinning and mechanical harvesting have been shown to induce ethylene production. These can cause premature ripening in fruits and bitterness in carrots.

2.4.4. Transpirational loss

Storage temperature and relative humidity play an important role in the physiological changes of fresh produce including physiological weight loss. Water loss is rapid at low relative humidity, since the vapour pressure difference between the commodity and surrounding air is a driving force for moisture transfer from the wet product to the air. In most of the cases moisture content of fresh fruits and vegetables are very high (usually greater than 70%). Therefore, the air inside the flesh is nearly saturated i.e. close to 100% relative humidity. Berg and Lentz (1966) noted that the lower humidity ratio causes desiccation and marked softening of carrots, together with some increase in decay. High relative humidity is therefore desirable for reducing physiological weight loss during storage of fruits and vegetables.

Temperature is the other major environmental factor that considerably affects the postharvest physiological weight loss of stored vegetables (Salunkhe *et al.*, 1991). The commodity temperature is highly dependent on the surrounding air temperature. Usually weight loss from perishable commodities is high if surrounding air temperature, flesh moisture content and temperature are high. Vapour pressure increases as air, flesh temperature and moisture content increases. Depending on the magnitude of temperature gradients and relative humidity of the surrounding air the physiological moisture loss varies. In summary, the most important ways to reduce physiological weight loss are by increasing relative humidity and decreasing storage temperature.

2.4.5. Postharvest physiological disorder

Postharvest physiological disorders affect mainly fruits. Susceptibility to disorders was shown to be dependent on a number of factors, such as maturity at harvest, cultural practices, climate during the growth season, produce size, harvesting, and handling practices. Adverse environmental conditions or a nutritional deficiency during growth and development of fruits and vegetables cause postharvest physiological disorders (Brown, 1973; Eckert *et al.*, 1975; Eckert, 1978a, b and c). They may be classified as low temperature disorders, postharvest physiological disorders and mineral deficiency disorders. Low temperature storage is beneficial because it reduces respiration and metabolic activities. Tropical and subtropical fruit and vegetables require specific ranges of storage temperature. On the other hand, low temperature does not reduce all aspects of metabolism to the same extent as it reduces respiration. This could lead to a metabolic imbalance due to an accumulation of reaction by-products and possibly a shortage of substrates. Chilling injury is a disorder long observed in plant tissues, especially those of tropical or subtropical origin. This injury is due to the exposure of plant tissue to temperatures below their critical temperature, which is usually below 15°C (Ryall and Lipton, 1979; Bramlage, 1982; Couey, 1982; Wills *et al.*, 1989). The physical symptom of chilling injury and the lowest safe storage temperature for some fruits and vegetables varies. Pitting of the skin due to the collapse of cell beneath the surface and browning of flesh tissues are some of the common symptoms of chilling injury. Therefore, selection of a proper storage temperature range for fruits and vegetables is a critically important factor in order to maintain the best quality and increase shelf life. The approximate lowest safe storage temperature for tomato fruit varies between 7.2-12.8°C (Hardenburg *et al.*, 1986), while carrot roots are not susceptible to chilling injury when stored at temperatures as low as 0°C.

2.4.6. Chemical and biochemical changes during ripening and storage

During ripening of fruits, several biodegradation processes take place, such as depolymerization, substrate utilization, loss of chloroplasts, and pigment distraction, mainly due to the action of hydrolytic enzymes (esterases, dehydrogenases, oxidases, phosphatases and ribonucleases) (Baker, 1975; Mattoo *et al.*, 1975). There are also some biosyntheses associated with these processes such as syntheses of proteins and nucleic acids, maintenance of mitochondria, oxidative phosphorylation, phosphate ester formation and syntheses linked to the metabolic path way (Baker, 1975; Mattoo *et al.*, 1975).

2.4.6.1. Carbohydrates

Among the changes that may occur during ripening of flesh fruits, such as tomatoes, is a change in the carbohydrates composition mainly due to substrate utilization and action of hydrolytic enzymes (Pratt, 1975). The presence of free or combined sugars with other constituents plays an important role in flavours of vegetables through a sugar to acid ratio balance. During ripening of fruit, carbohydrates undergo metabolic transformations, both qualitatively and quantitatively. Starch is completely hydrolysed into glucose, and fructose and sucrose formed as ripening progresses (Mattoo *et al.*, 1975). However, structural carbohydrates are decreased slightly. Mattoo *et al.* (1975) reported that pentosans and cellulose are stored carbohydrates, which may also serve as potential sources of acids, sugars, and other respiratory substances during ripening.

In tomatoes natural sugars such as arabinose, rhamnose, and galactose are steadily decreased in cell walls as the color changes (Campbell *et al.*, 1990). Several studies have also shown that changes in cell wall carbohydrates similar to those reported for intact fruits occurred in cell walls from pericarp discs (model for intact tomato fruits) (Gross and Wallner, 1979; Gross, 1984; Campbell *et al.*, 1990). Other studies showed that the total sugar content of tomatoes increased during ripening, and may be followed by no further changes or a slight decrease during ripening (Baldwin *et al.*, 1991). Similar findings were reported by other researchers, who showed that sucrose content in tomatoes also decreased with the progress of ripening (Goodenough *et al.*, 1982; Baldwin *et al.,* 1991).

During storage of fruit and vegetables, free sugars show a general initial increase followed by a decrease. The increase in free sugars in some fruit and vegetables are due to the breakdown of polysaccharides. In some fruits approximately equal quantities of glucose and fructose are formed due to hydrolysis of starch. However, as storage time advances, especially in fruit, the content of all three free sugars (sucrose, glucose and fructose) declines. Several factors contribute towards the excessive decline of sugars during storage, such as fruit maturity, storage temperature, concentration of O_2, N_2, ethylene and CO_2. Higher temperature favours faster utilisation of sugars as substrate in the respiration process (Wiley, 1994).

In carrots, the ratio of nonreducing to reducing sugars exhibits a sharp decrease after 14 to 18 weeks of storage at 1.1°C. Simultaneously an active synthesis of reducing oligosaccharides as raffinose together with the formation of new rootlets is observed (Phan *et al.*, 1973). Temperature also regulates the rate at which biochemical changes occur during storage. Higher temperature activates enzymatic catalysis and leads to chemical and biochemical breakdown of chemicals in fruits and vegetables during storage. As a result, fruits and vegetables loose firmness faster at higher temperature due to high enzymatic activities (Yoshida *et al.,* 1984).

2.4.6.2. Organic acids

Common acids found in fruit includes citric, malic and ascorbic acid. During ripening, organic acids are among the major cellular constituents undergoing changes (Salunkhe *et al.*, 1991). Studies have shown that there is a considerable decrease in organic acid during ripening of fruits. Modi and Reddy (1966), in Salunkhe *et al.* (1991), showed that concentrations of citric, malic and ascorbic acids declined 10, 40 and 2.5 fold respectively, in fruits such as tomatoes. The titratable acidity decreases with storage time, especially at higher temperatures (Mohammed *et al.*, 1999). Higher storage temperatures are known to have an increasing effect on the rate of decrease in ascorbic acid content in tomatoes during storage (Salunkhe et al, 1991). However, Mohammed *et al.* (1999) showed that the ascorbic acid content in tomatoes slightly increased during ripening during storage at 20°C for 14 and 21 days. In general, the ascorbic acid content decreases rapidly after full ripening of tomatoes stored at higher storage temperatures.

In carrots, titratable organic acidity decreases slowly during storage (Phan *et al.*, 1973). However, Phan *et al.* (1973) reported that stored carrots had high contents of iso-citric and malic acids, which they could not explain. Pyruvic and oxaloacetic acids were not detected in these stored carrots.

2.4.6.3. Enzyme activity

As was mentioned above, the biochemical changes are responsible for development of off-flavours, discoloration and loss of firmness of fruit and vegetables and are affected by enzymes. The most important enzymes related to food quality include lipolytic acyl hydrolase, lipoxygenase, peroxidase, catalase, protease, polyphenol oxidase, amylase, pectin methylesterase, polygalacturonase, ascorbic acid oxidase and thiaminase (Svensson, 1977). Lipolytic acyl hydrolase, lipoxygenase, peroxidase, catalase and protease are responsible for the changes in flavour of minimally processed as well as intact fruits and vegetables, while polyphenol oxidase is responsible for the changes in colour, especially during ripening of fruits and vegetables (Wiley, 1994). The changes in softness of fruits and vegetables are due to the activities of amylase, pectin methylesterase and polygalacturonase, which result in loss of texture. The reaction catalysed by ascorbic acid oxidase and thiaminase leads to the loss of nutritional quality of food in terms of vitamins C and B content (Wiley, 1994). Catalase and peroxidase are also known for their oxidative activity during ripening and their activity levels increases considerably during ripening of fruit and vegetables (Mattoo *et al.*, 1975). Glycolytic enzymes are groups of enzymes responsible for the glycolytic breakdown of sugars, and they increase considerably during ripening of fruits. Other classes of enzymes active during storage of fruit and vegetables include hydrolytic enzymes, invertase, transminases, citrate cleavage enzymes, enzymes of the tricarboxylic acid cycle and chlorophyllase (Mattoo *et al.*, 1975), which are responsible for the hydrolysis of starch, flesh softening in most fruits, the degradation of sucrose, liberation of monosaccharides, turnover of amino acids, and ensuing chlorophyll degradation during ripening.

El-Zoghbi (1994) reported effects of enzyme activity on alcohol-insoluble solid dietary fibres, and texture and firmness changes during ripening of tropical fruit such as mango, guava, date and strawberry. The results showed that alcohol-insoluble solid, dietary fibres, texture and firmness declined in these fruit during ripening. However, both polygalacturonase as well as cellulase activities of the fruits increased markedly, during ripening.

The pectin-degrading polygalacturonase (PG) isoenzymes are highly responsible for softening of fruit (Hobson, 1964; Tigchelaar *et al.*, 1978; Marangoni *et al.*, 1995). Bruinsma *et al.* (1990) demonstrated that these enzymes are highly responsible for most chemical and biochemical changes associated with deterioration of fruit and vegetable quality during ripening and storage. Postharvest treatment had also an effect on biochemical changes related to enzymatic activities during storage (Marangoni *et al.*, 1995; Yoshida *et al.*, 1984; Dodds *et al.*, 1996; Assi *et al.*, 1997). PG normally accumulates to high levels in ripe tomatoes (Kramer *et al.*, 1992). Similarly, Brummell and Harpster (2001) reported that polygalacturonase activity is largely responsible for pectin depolymerization and solublization of polysaccharides. The loss of firmness of tomatoes during ripening is due to the increased activities of PG and pectin esterase (El-Zoghbi, 1994). In tomato it was shown that ethylene production was induced prior to polygalacturonase production and that it is responsible for triggering PG synthesis indirectly (Grierson and Tucker, 1983). Extracted PG activity in non-chilled tomato fruit significantly correlated with softening of the fruit. However, chilling-associated softening correlated with higher initial extracted pectinmethylesterase (PME) activity. It was suggested that loss of turgor from translocation of water to the PME-modified cell walls was responsible for loss in firmness as a consequence of chilling. Heat treatment, on the other hand, strongly reduces PG activity of tomatoes during ripening and storage (Yoshida *et al.*, 1984). In general, relatively higher temperatures (about 22°C) favour development of PG activity in tomatoes and high temperature treatment suppresses this enzyme activity (Yoshida *et al.*, 1984).

Biochemical processes are also responsible for colouration and loss of firmness of fruits and vegetables (Kertesz, 1951; Doesburg, 1965; Hildebrand, 1989). As a result of increased enzymatic activity occurring due to storage temperature, postharvest quality of tomatoes such as red colour formation, firmness, titratable acidity and decay during ripening are affected (Batu, 1998). Changes in colour from green to red are delayed in the case of tomatoes stored at lower temperature as compared with relatively higher temperature, since the lower storage temperature reduces enzymatic activity. This could lead to increased acidity during storage.

2.4.7. Postharvest microbiology of fruit and vegetables

The freshness quality and consumer safety of fruits and vegetables depend on the microbial population at harvest, as well as during storage (Brackett, 1992). Fruit and vegetables are highly susceptible to microbial contamination during growth, harvest and postharvest operations (Madden, 1992). Most fruits have pH values below 4.5. Consequently, under such acidic conditions most of the microorganisms cannot grow easily. Conversely, most vegetables have pH's above 4.5, which creates favourable conditions for various types of microorganisms. Carrots have pH values varying from 4.9-6.3 while tomatoes have lower pH values ranging between 3.9 and 4.7 (Banwart, 1989). Microorganisms account for up to 15% of postharvest decay of fruits and vegetables. Bacteria, yeast and moulds are the three important groups of microorganisms that affect the quality of fruits and vegetables during storage. The sources of these microorganisms could be from environmental air, soil, and poor sanitation during postharvest unit operations.

Several distinct mechanisms for preharvest infection are known. Lesions on stems, leaves and flower parts of infected plants as well as dead plant materials are sites for sporulation of pathogenic fungi (Nelsen, 1965; Salunkhe *et al.*, 1991). Rain and wind transport spores of these fungi to flowers and fruits at every stage of their development. When free water is present and the climatic conditions favourable, these spores germinate and rapidly increase the microbial population. The population and type of microorganisms associated with fruit vegetables are different from those of stem and root vegetables, the reason being the differences in chemical composition such as moisture content, titratable organic acid, and free sugar content. Usually the microbial population is higher on stem and root vegetables than in most fruits mainly due to acidity and may be due to the contact with soil. The most important postharvest diseases of tomatoes include alternaria, rhizopus, buckeye, grey mold, soft rot, acid rot, bacterial soft and ripe rot, which are caused by *Alternaria alternata, Phytophthora* species, *Botrytis cinerea, Rhizopus stolonifer, Alternaria tenuis, Geotrichum candidum, Erwinia cartovora or Pseudomonas* species and *Colletotrichum* species, respectively (Senter *et al.*, 1987; Splittstoesser, 1987; Buick and Damoglou, 1987; Brackett 1988a; Bulgarelli and Brackett, 1991; Salunkhe et al 1991). The major postharvest diseases of fresh carrots are gray moulds rot, centrospora rot, watery soft rot and bacterial soft rot which is due to *Botrytis cinerea, Centtrospora acerina, Sclerotinia sclerotiorium* and *Erwinia cartovora,* respectively. Similarly, losses of other vegetables in general are due to watery soft rot, cottony leak, fusarium rot, and bacterial soft rot that are caused by *Sclerotinia, Pythium butleri, Fusarium and Erwinia or Pseudomonas* species, respectively.

As ripening of fruits and vegetables progresses, the firmness decreases and the intrinsic factors, which confer resistance during development, can no longer protect against microbial decay (Eckert *et al.*, 1975 and 1978). The high moisture and nutrient content of fruits and vegetables adds to susceptibility to invasion by specific pathogenic microorganisms (Eckert, 1978 a, b and c).

The microbial deterioration of fruits and vegetables are highly influenced by environmental factors, such as temperature, moisture content of crops, relative humidity of air, and storage air gas composition. In general, control of postharvest decay of fruits and vegetables are based upon the prevention of infection before as well as after harvest, eradication of incipient infections and retarding the progress of the pathogen in the host by postharvest treatments such as disinfection (Eckert *et al.*, 1975).

2.5. Post-harvest handling and storage

2.5.1. Pre-packaging treatments

2.5.1.1. Postharvest disease control

As mentioned above, the postharvest quality and safety of fresh fruits and vegetables mainly depends on their microbial population (Brackett, 1992). Different approaches have been used to reduce postharvest loss, classified in chemical and non-chemical methods. Various synthetic fungicides and bactericides have been used alone or in combination with chlorination to limit the growth and transmission of diseases caused by microorganisms during storage of fresh commodities. However, chemicals may have a negative impact on agriculture in developing countries (Eckert, 1990; Conway *et al.*, 1999; Qadir and Hashinaga, 2001). Biological products such as Aspire, BioSave, and Yield Plus are now available in the marketplace of developed countries to control growth of bacteria or fungi. *Pseudomonas syringae van Hall* (strain ESC-11) is sold commercially under the names BioSave-11 and BioSave-110 (EcoScience Corp., Orlando, Florida), and is currently being used to control decay on apples and pears caused by *Botrytis cinerea*, *Mucorpiriformis fischer*, and *Pseudomonas expansum* (Janisiewicz and Marchi, 1992; Janisiewicz and Jeffers, 1997).

Other methods used to control postharvest diseases are modification of pH, chemical treatments or a combination (Huxsoll and Bolin 1989; King and Bolin, 1989). The success of these products, however, remains limited (Wisniewski *et al.*, 2001). The reason may include the following: (a) there is an increasing consumer concern over the use of pesticide as a postharvest decay control of food (Wisniewski and Wilson, 1992), (b) the use of fungicides may result in predominance of fungicide resistant strains (Elmer and Gaunt, 1994), (c) the variability experienced in the efficiency of these products, and lack of understanding of how to adapt "biological approaches" to a commercial setting (Wisniewski *et al.*, 2001). Researchers and industries have come up with some noble ideas such as using combinations of different antagonists. However, these approaches seem to meet with consumer resistance (Wisniewski *et al.*, 2001).

Much work as been done on non-chemical approaches and efficient postharvest disease control methods including various physical treatments such as biocontrol agents, heat, ultra-violet light and ionising radiation. Some novel heat treatment approaches to postharvest decay control of fruits and vegetables were developed and applied in Israel (Rodov *et al.*, 1995; Afeke *et al.*, 1998; Fallik *et al.* 1999, Fallik *et al.*, 2000). The postharvest decay of bell pepper was significantly decreased by hot water treatment dipping at temperatures of 45 and 53°C for 15 and 4 minutes, respectively (Gonzalez *et al.*, 1999). Heat treatment, when combined with fungicide treatments, was also reported to be effective and can be a useful alternative method to control postharvest decay (Conway *et al.*, 1999). In this case the effect of fungicide residues on human health and costs associated with combinations of treatments has to be considered. However, the application of some of these methods, such as heat treatment, requires equipment that is complex and costly for use in under-developed countries (Rodov *et al.*, 1996).

Intensive research was conducted on the efficiency of chlorine solutions to control the growth of pathogenic microorganisms on fresh produce and minimally processed "ready-to-eat" fruits and vegetables (Wei *et al.*, 1995; Zhuang *et al.*, 1995; Park *et al.*, 1995; Velazques *et al.*, 1998, Beuchat *et al.*, 1998; Delaguis *et al.*, 1999; Escudere *et al.*, 1999; Li *et al.*, 2001; Beuchat *et al.*, 2001; Ukuku and Sapers, 2001; Cantwell *et al.*, 2001; Prusky et al, 2001). These studies were carried out on the survival of inoculated spoilage fungi such as *Alternaria hydrophila* (Velazquez *et al.,* 1998; Beuchat *et al.*, 2001), *Alternaria alternata* (Prusky *et al.* 2001) and human pathogenic bacteria such as *Salmonella montevideo* (Zhuang *et al.*, 1995), *Salmonella stanley* (Ukuku and Sapers, 2001) and *Yersinia enteroocolitica* (Escudero *et al.*, 1999). Similarly, studies on the normal microbial populations of harvested vegetables were also reported (Beuchat *et al.*, 1998; Prusky *et al.* 2001). The concentration of free chlorine in these studies varied from 50-2000 and ppm and dipping times from 1-10 minutes.

Chlorination, when used in combination with non-chemical methods, is effective in reducing postharvest disease losses. Warm chlorinated water was shown to reduce microbial loads by 3 log cfu. g^{-1} in lettuce washed in chlorinated water at 47°C, and 1 log cfu. g^{-1} at 4°C (Delaquis *et al.*, 1999). Similarly, the combination of different chlorine concentrations (50 to 400 mg/ml NaOCl, pH 7.0) with 52.5°C water were shown to be more effective than use of water or chlorine solutions at 20°C for reducing initial microbiological flora in minimally processed green onions (Cantwell *et al.*, 2001). Park *et al.* (1995) reported that the microbial load of the minimally processed watercress and onion was effectively reduced with chlorine (100 ppm). However, high concentrations of chlorine resulted in greater microbial proliferation after 7 days of storage, loss of ascorbic acid and significant colour changes in stored cut vegetables.

Preharvest dipping treatment in the organic chlorine compound Troclosene Sodium extended the storage life of fruit by delaying development of black-spot disease (Prusky *et al.*, 2001). All the research work cited here, has been devoted to the control of pathogens and postharvest decay by microorganisms without any emphasis on the effect of free chlorine on the quality, or the physiological and biochemical quality changes.

In general, chlorination is an effective method especially in reducing microbial load in intact fruits and vegetables. For several reasons, such as availability of chlorinated solutions and cost, chlorine disinfection seems to be a good method for application in developing countries. The efficacy of chlorine solution on postharvest physiological as well as chemical and biochemical changes still needs to be explored.

2.5.1.2. Electrochemically activated water (anolyte water)

Anolyte water is prepared from an aqueous solution of NaCl and this electrochemically-activated water is known to be a powerful, non-toxic, non-hazardous disinfectant. Two kinds of electrochemically activated water are produced, anolyte and catholyte. The anolyte water is described as having an oxidation-reduction potential (ORP) in the region of +1000 mV and catholyte an ORP of –800 mV, and the pH value of catholyte is in the alkaline region while the pH value of anolyte is in the acidic region. Current thinking around the concept is that the ORP of both solutions fluctuates between these values at a rate too rapid to measure. Some of the biocidal agents (free radicals) in the solutions are ClO_2, HClO, Cl_2, ClO^-, H_2O_2, HO_2^-, NaHO, O_2, O_3, $H^\bullet$ and $^\bullet OH$. Prilutski and Bakhir (1997) investigated properties of anolyte and catholyte produced by diaphragm electrolysis of aqueous solutions. Their results showed that catholyte and anolyte have different physical-chemical properties. Anolyte is thought to have the antimicrobial effect and catholyte a detergent or cleaning effect (Popova *et al.*, 1999). The presence of the free radicals with their oxidising effects in the solutions is considered of great importance. It is the free radicals (working substances), which gives anolyte bactericidal and sporicidal activity (Aquastel, 2000), since higher organisms possess antioxidant defence systems, whereas microorganisms generally do not.

The electrochemically produced anolyte water was effectively used in drinking water disinfecting units (Aquastel, 2000). The determination of organic chlorine compounds and trihalogenmethanes showed significantly less formation of these by-products. Moreover, the formation of halogens was significantly less than defined standard requirements and the pH-value only slightly decreased (Aquastel, 2000). Anolyte attracts interest since it is an environmentally and ecologically friendly substance for use as a postharvest fruit and vegetables disinfectant. Based on these and the antimicrobial action of anolyte, its potential to be used as a postharvest disinfectant of fruit and vegetables should be explored.

2.5.2. Packaging

2.5.2.1. Controlled atmosphere packaging

Controlled atmosphere storage refers to storage of food in a gas atmosphere that is different from the normal composition of air. In controlled atmosphere the headspace gas is precisely adjusted to the atmosphere composition required by a specific fruit or vegetable (Perry, 1993). Controlled atmosphere storage is mostly used for the long-term storage of whole fruits and vegetables in warehouses, bulk storage and transportation.

Over 4000 research papers have been published giving information on the optimal modified atmosphere conditions for cultivars of fruits and vegetables (Zagory and Kader, 1988; Kader *et al.*, 1989). These literatures have built good basic principles of controlled atmosphere storage and significantly contributed towards the success of this technology in developing countries (Lioutas, 1988).

The major influence on the choice of controlled atmosphere conditions is the susceptibility of particular cultivars to superficial scald (Johnson, 1999). Using controlled atmosphere storage methods, the gas compositions have to be adjusted to a certain level specific to each fruit and vegetable type, and it is unlikely that there will be benefits from further adjustment of the compositions of oxygen and carbon dioxide without risking the damage to certain fruits and vegetables (Johnson, 1999).

The controlled atmosphere gas compositions required by a given commodity is obtained by mixing appropriate volumes of gases. Certain machinery is needed to mix and control the gas composition of the air during the storage period. In controlled-atmosphere storage packaging, atmospheric conditions (level of gases) are continuously controlled and adjusted to maintain the optimal concentrations required by specific fresh produce. As a result of this, it is capital intensive and expensive to operate for short-term storage (Kader *et al.*, 1989). The factors that could probably determine the success of controlled atmosphere technology in developing countries are the cost and complexity (multidisciplinary nature) of the technology. However, controlled atmosphere storage is not appropriate for some commodities. For example, Lipton (1977) has shown that controlled atmosphere storage was not advantageous for carrot storage, and recommended the use of modified atmosphere packaging.

Low O_2 or high CO_2 levels may be of benefit for very short-term preservation of vegetables, as for example, with asparagus (Torres-Penarada and Saltveit, 1994), banana (Wills *et al.*, 1990) and some vegetables (Wills *et al.*, 1979). Low O_2 or high CO_2 conditions result in partial or full anaerobic respiration and produce volatile compounds (acetaldehyde and ethanol) responsible for aroma of fresh fruits (Morris *et al.*, 1979; Paz *et al.*, 1981). However, excessive accumulation of volatile compounds may lead to off-flavours and some physiological disorders (Ke and Kader, 1989). A study on the effects of low O_2 and high CO_2 levels on tomato physiology and composition showed that fruits treated with low O_2 or high CO_2 had skin injury and blotchy ripening (Klieber *et al.*, 1996). The incidence of fruits showing disease when ripe, increase with increasing treatment time with O_2 and CO_2 (Klieber *et al.*, 1996). Sozzi *et al.* (1999), on the other hand, reported that keeping tomatoes in controlled atmospheres, even in the presence of ethylene, had marked residual effects. These results suggested an antagonism between elevated CO_2 or low O_2, and exogenous ethylene levels, which could determine most of the physiological behaviour under controlled atmosphere storage, though a direct regulatory mechanism by O_2 and/or CO_2 should not be discarded. The literature cited indicated that controlled atmosphere storage of tomatoes in conditions of low O_2 and CO_2 atmosphere might have economic benefits for very short-term storage of a few days.

2.5.2.2. Modified atmosphere packaging (MAP)

Historically, modified atmosphere packaging (MAP) has been erroneously described as synonymous with controlled-atmosphere storage (CAS) or controlled-atmosphere packaging (CAP). Modified atmosphere packaging (MAP) is defined as the "packaging of perishable products in an atmosphere which has been modified so that its composition is other than that of air" (Hintlian and Hotchkiss, 1986). MAP storage implies a lower degree of control of gas concentrations as compared with controlled atmosphere and vacuum packaging. Typically, initial atmospheric conditions are established for a transient period, and the interplay of the commodity physiology and the physical environment maintain those conditions within broad limits (Zagory and Kader, 1988). Modified atmosphere is depletion of oxygen (O_2) and emanation CO_2, occurring within a sealed plastic packaging headspace. This happens when fruit and vegetables are respiring. Modified atmosphere packaging can be created either as a result of commodity respiration (passive) or intentionally through active packaging (Zagory and Kader, 1988). Passive modified atmosphere can be achieved in hermetically sealed packages due to commodity respiration. Levels of O_2 and CO_2 concentrations then adjusted themselves to the commodity-desired range as a result of O_2 consumption and CO_2 evolution during storage. If a commodity's respiration characteristics are matched to the film permeability value, the optimum favourable modified atmosphere can be created for a specific product (Smith *et al.*, 1987b).

The atmosphere created by MAP depends on product, environmental and packaging film factors. Product factors include respiration rate, respiration quotient, quantity of the product, and O_2 and CO_2 concentrations necessary to approximately achieve optimum reduction of product aerobic respiration rate. Packaging film factors consists of permeability of polymeric packaging materials to O_2 and CO_2, and water vapour at a selected storage temperature. Temperature, relative humidity, light and sanitation represent the environmental factors. This indicates that as many factors as possible should be included during MAP of fresh vegetable trials (Zagory and Kader, 1988). A proper understanding of the basic principles of the physiology, chemical, biochemical and microbiological properties of vegetables during MAP storage is required for the use of this technology.

2.5.2.2.1. Physiological changes

The beneficial effect of MAP on increasing shelf life of perishable products is partially due to the decrease in O_2 and the increase in CO_2 levels, and partially to the decrease in physiological loss in weight (Biale, 1946 and 1960; Fidler *et al.*, 1973; Isenberg, 1979; Smock, 1979; Ben-Yehoshua *et al.*, 1983). The role of MAP is to primarily reduce respiration rate of fruit and vegetables by retarding metabolic activities. Reduced respiration also retards softening, and slows down various compositional changes associated with ripening (Kader, 1986).

It is obvious that plant tissue responds quickly to low O_2 or high CO_2 levels during MAP storage. Low O_2 levels were shown to reduce the rate of respiration and resulted in a delay in climacteric onset of the rise in ethylene levels and a decrease in the rate of ripening (Fidler *et al.*, 1973; Kader, 1986; Kannelis, *et al.*, 1990; Yang and Chinnan, 1988a, b; Kannelis *et al.*, 1991). Hypoxia conditions (low O_2 levels) have shown to inhibit the accumulation of RNA, protein, and DNA synthesis associated with the wounding of vegetables (Butler *et al.*, 1990). The lowest limit of O_2 level, which can be tolerated by vegetables, is a critical factor that must be taken into consideration in selecting proper MAP for fruits and vegetables such as carrots and tomatoes (Butler *et al.*, 1990).

Burg and Burg (1967) have shown that the CO_2 level is a competitive inhibitor of ethylene production during storage, and metabolic activities are reduced through an increase in CO_2 levels in the headspace. Low O_2, high CO_2 or both decreases the production of ethylene during MAP storage, which means lower rates of respiration, and prolongs the shelf life of produce (Blackman, 1954; Aligue, 1995; Agar and Streif, 1996; Petracek *et al.*, 2002). This is for the benefit of both climacteric and non-climacteric fruits and vegetables during storage. The levels of CO_2 and O_2 in the headspace of packages balance each other and have a coupled effect on the metabolic activities of fresh produce during storage. The effect of lower O_2 and higher CO_2 levels on respiration is additive and can reduce respiration more than effected separately (Kader *et al.*, 1989). These means high levels of CO_2, when coupled with low O_2 levels have a cumulative effect on the respiration rate.

Oxygen and CO_2 levels below or above the optimum levels specific to each fruit or vegetable can cause physiological damage. Exposure of fresh commodities to extreme levels of O_2 and CO_2 below and above that required for normal respiration, may initiate anaerobic respiration and lead to the development of off-flavours due to the accumulation of ethanol and acetaldehyde (Zagory and Kader, 1988). Increased CO_2 is also associated with an increase of acidity in plant tissues with a subsequent reduction in pH. This acidification could lead to inactivation of enzymes, or phytotoxicity by CO_2.

2.5.2.2.2. Chemical and biochemical changes

The loss in firmness, development of off-flavours, and discoloration of fresh and minimally processed commodities after harvest or during ripening or storage, is the result of various biochemical changes and was reviewed in section 2.4.6. A review on the biochemical basis for effects of MAP on fruits and vegetables by Kader (1986) indicated that biochemical and compositional changes such as color, texture (firmness), flavour, and nutritive value can be reduced due to the modified atmosphere created by MAP during storage. MAP may inhibit enzymatic activities responsible for the deterioration in the mentioned quality parameters of fresh commodities during storage. The loss of chlorophyll and lycopene synthesis is reduce when tomatoes are stored in a modified atmosphere of less than 4% O_2 and 4% CO_2 for 2 months (Goodenough and Thomas, 1980). The appearance of PG is delayed for up to 8 weeks in green mature tomatoes stored in 5% O_2 and 5% CO_2 at 12.5°C (Goodenough *et al.*, 1982), suggesting delayed fruit softening. Burton (1974) reported that the changes in starch to sugar conversion is slower in packaged fruit and vegetables with a headspace containing 5-20% CO_2 or less than 3% O_2 at 2°C.

Under MAP the effect of polyphenoloxidase and tyrosinase, the enzymes responsible for browning of plant tissue, decreases as a result of limited availability of O_2 as a substrate (Anese *et al.*, 1997). A reduction in O_2 levels or increase in CO_2 levels in the microenvironment of a crop by MAP could reduce enzymatic activities responsible for quality deterioration (Duckworth, 1966; Ruall and Lipton, 1979; Ryall and pentzer, 1982; Kader *et al.* 1985). However, the effect of low O_2 created during MAP on biochemical changes cannot be separated from the effect of increased CO_2 (Murr and Morris, 1974; Bown, 1985; Siriphanich and Kader, 1986; Ballantyne *et al.*, 1988b). Barth *et al.* (1993) reported that MAP of broccoli stored at 20°C for 96 hr resulted in a significant decrease of POX after 24 hrs compared to POX activities in unpackaged samples. The chemical compositions such as ascorbic acid and total chlorophyll also remained higher in packaged broccoli, but O_2 levels below 3% increases the concentrations of ethanol and acetaldehyde, and activities of pyruvate decarboxylase and alcohol dehydrogenase, which results in ethanolic fermentation in fresh-cut carrots (Kato-Noguchi, 1997).

This review shows that MAP has an effect on the biochemical as well as chemical changes in fruits and vegetables during ripening and storage through reducing the levels of O_2 and increasing the levels of CO_2 in the headspace. Generally, the lower the O_2 concentrations achieved by the MAP the lower the rate of chemical as well as biochemical changes, resulting in reduced respiratory metabolism and other biochemical processes, and leading to retention of chemical quality characteristics. However, maintenance of optimum minimum O_2 concentration inside the packages is very important to avoid accumulation of excessive volatile compounds responsible for off-flavour and physiological disorder. This entails the need for selection of appropriate packaging material for the specific fruits and vegetables.

2.5.2.2.3. Microbiological changes

The extended shelf life of many MAP may allow extra time for microorganisms to reach dangerously high levels in a food. Even in the case of slow growing photogenic microorganisms, extended shelf life using MAP gives them sufficient time to multiply, if not properly managed during storage. Additional research in the microbial control on MAP foods is still needed.

Hao *et al.* (1999) studied microbiological quality and production of botulinum toxin in MAP of broccoli, carrots, and green beans. Types of packaging material affected the quality of vegetables without noticeably influencing the growth of microorganisms. These results created awareness that the use of MAP could have both positive as well as negative effects on the microbial flora associated with fruits and vegetables during storage based on the pre-packaging treatment and storage conditions. However, their result showed that the toxin was not detected in the vegetables under study.

Microorganisms require certain conditions to grow and reproduce. These conditions are either properties of food such as nutrients, pH and water activity or extrinsic properties of food associated with storage environment such as oxygen, light, temperature and time. The most important environmental factors are gaseous composition and temperature. Modified atmosphere packaging in principle controls these factors to reduce or suppress spoilage and extend shelf life of fresh produce. Anese *et al.* (1997), studying the effect of modified atmosphere packaging on microbial growth, have shown that CO_2 and N_2 levels were the most effective in inhibiting microbial growth. Concentrations of CO_2 in excess of 5% inhibit the growth of microorganisms of most foods. However, the pH of MAP foods increases with increase in carbon dioxide concentration in packages. Direct suppression of microorganisms by MAP has only been possible with the horticultural commodities that can withstand carbon dioxide levels at 20% or above without development of off-flavours, odours, and colours (Harvey, 1982; Aaron 1989). The ability of modified atmosphere alone is not sufficient to limit the incidence of spoilage of fruits and vegetables. The necessity of pre-packaging treatments to reduce initial microbial load, as well as limiting their growth during storage, is critical.

Conversely, unsuitable MAP can prevent wound healing, speed up senescence, or incidence of physiological breakdown, making fruits and vegetables more susceptible to postharvest pathogens. EL-Goorani and Sommer (1981) suggested that O_2 levels below 1% of CO_2 levels above 10% could be utilised to limit the growth of yeasts and moulds.

In summary, the growth of microorganisms is likely to continue inside MAP vegetables and fruits, especially for those that need higher O_2 and lower CO_2 levels. Additional pre-packaging treatments are therefore still required.

2.5.2.2.4. Postharvest factors affecting MAP

The microenvironment created and maintained in the headspace of a package is the net result of the interaction of commodity factors, environmental factors and packaging material characteristics. Zagory and Kader (1988) described the effects and development of modified atmospheres in the packaging of fresh produce. In order to maintain quality of fruits and vegetables, the product, environmental and packaging material factor must be considered in creating favourable conditions for MAP of fresh produce in order to keep the quality. Therefore, in this section a brief description of commodity factors and environmental and packaging material factors for MAP will be presented.

2.5.2.2.4.1. Commodity factors

In order to develop an appropriate modified atmosphere environment in packages several product factors needs to be understood. These includes the rate of O_2 uptake and CO_2 evolution, permeability of packaging films to O_2 and CO_2, tolerance of plant materials to levels of these gases and diffusivity of fruits and vegetable skin and flesh to O_2 and CO_2 (Fidler *et al.* 1973; Isenberg, 1979; Smock, 1979; Kader, 1985; Salveit, 1989). Zagory and Kader (1988) classified the commodity factors affecting the effectiveness of MAP according to resistance to gas diffusion, respiration, ethylene production and sensitivity, optimum temperature, relative humidity, and gas concentrations

Resistance to gas and water diffusion across the plant material varies with different vegetables or fruits, cultivars, plant organs and stage of maturity (Cameron and Reid, 1982). The resistance to gas diffusion is affected more by anatomical difference than biochemical differences among various vegetables and fruits (Burton, 1974). However, the rate at which O_2 passes through skin or epidermal tissue depends on the O_2 gradient (Kader *et al.,* 1989; Burg, 1990). In some commodities the skin and epidermal tissue may posses pores, called stomata, and lenticles, which are the passages for the O_2. Cranberry, tomato and green pepper skins contain no pores, and all gases diffuse through holes in the pedicel scar. The resistance of gas diffusion through the boundary gas layer and skin of most fruits and vegetables varies with the type of gases, i.e. O_2, CO_2 and ethylene (Burg, 1990). The rate of gas diffusion from the surface of the product to the interior also depends on the proportion of gas space. In the case of minimally processed fruits and vegetables, the released cellular fluid flows into the gas space and blocks gas transfer. Diffusion of gas would be much slower in cell sap than in the gas-filled intercellular spaces (Burg, 1990). In general, the structures such as the skin, the cell wall, the intercellular space and the cytosol are the barriers of O_2 movement to the mitochondria in the cell.

The respiration of fruits and vegetables was discussed in section 2.4.1., but without taking into account packaging films and MAP. The effectiveness of packaging films can be evaluated by their ability in keeping the normal respiration rate of produce without decomposition. Based on the rate of respiration, fruits and vegetables are classified as climacteric or non-climacteric. Tomato and carrot are classified as climacteric fruit and root vegetables, respectively, with moderate respiration rates compared to the other fruits and vegetables. The respiration rate increases consistently with an increase in storage temperature. The respiration rate of topped carrots is from 10-20 mg kg^{-1}. h^{-1} and 46-95 mg kg^{-1}. h^{-1} at 0°C and 20-21°C respectively. For mature green tomatoes the respiration rate varies from 5-8 and 28-41 mg kg^{-1}. h^{-1} at 4-5°C and 20-21°C storage (Salunkhe *et al.*, 1991). Thus, it is apparent that a reduction in storage temperature is an effective means of extending the commercial life of tomatoes and carrots using MAP.

The rate of ethylene production of fresh produce, and the sensitivity to this hormone, determines the type of packaging film appropriate for secured quality maintenance and shelf life extension (Zagory and Kader, 1988). Tomato produces 1-10 mg CO_2. kg^{-1}. h^{-1} ethylene, which is moderate compared to some other fruits (Kader *et al.*, 1985). Very low concentrations of ethylene are required to advance biochemical and physiological changes during storage and accelerate the ripening processes of carrots and tomatoes. The effect of ethylene on fruits and vegetables that can be stored at a temperature range of 0 to 4.4°C is not possible to detect. The recommended storage temperatures for carrots range from 0 to 1°C and hence the effect of ethylene on carrot during storage at this temperature is negligible. The recommended storage temperature for tomatoes is varying from 8 to 13°C, which is sufficiently high for production of ethylene, and should be considered as a postharvest factor affecting the shelf life of tomatoes.

A reduction in ethylene production and sensitivity associated with MAP can delay the onset of climacteric and prolong the storage life of these fruits (Zagory and Kader, 1988). Even non-climactreric fruits and vegetables can benefit from reduced ethylene sensitivity and lower respiration rate attributed to MAP. Ethylene production is reduced by ether low O_2, high CO_2, or both, and the effects are additive (Zagory and Kader, 1988).

2.5.2.2.4.2. Environmental factors

Air temperature, relative humidity and natural microbial flora of perishable produce are the main environmental factors affecting the effectiveness of modified atmosphere packaging (Zagory and Kader, 1988). Packaged fruits and vegetables have specific ranges of temperature optima for their normal respiration. Below this limit, fruits and vegetables can easily by affected by chilling injury, which advances to high respiration, hastened senescence and short shelf life. The optimum storage temperature for a specific fruit or vegetable is the one that delays senescence, and maintains quality the longest without causing chilling, freezing, or other injury (Zagory and Kader, 1988). The optimum temperature for tropical fruits is above 13°C, because these fruits are sensitive to chilling injury. Non-chilling-sensitive commodities can be stored near 0°C without negative effect. Previous studies indicated that the effect of reduced O_2 and elevated CO_2 had an effect on chilling injury (Lyons and Breidenbach, 1987). Chilling injury of fruits and vegetables reduced with low O_2 and high CO_2 concentrations in the storage atmosphere (Lyons and Breidenbach, 1987). The optimum temperature range for carrot and tomatoes are 0-5 and 8-13°C, respectively (Salunkhe *et al.*, 1991). In general, optimum temperature is a key factor in reducing respiration, production of ethylene, chilling injury, enzymatic activities, proliferation of microorganisms during storage of fruits and vegetables, although modified atmosphere packaging expands the limit of optimum temperature.

The danger associated with high relative humidity is the condensation in packages, which creates favourable conditions for microbial growth. Condensation on the package film affects the permeability property and creates unfavourable interior conditions during storage. Proper temperature maintenance throughout the postharvest handling lines is central to prevent the incidence of condensation in films. On the other hand, low relative humidity increases the loss of moisture from fruits and vegetables (Kader, 1987). The optimum range of relative humidity for carrot storage varies from 90-100%, whereas for tomatoes the range can vary from 85-90% (Salunkhe *et al.*, 1991)

2.5.2.3. Packaging films

Selection of appropriate films for MAP storage of fresh vegetables on their effectiveness in protecting produce during shelf life is critical (Arthey and Dennis, 1991; Perry, 1993; Wiley, 1994; Farber and Dodds, 1995). The principal plastic materials for MAP that can be used with intact vegetables and fruits include polybutylene, LDPE, HDPE, PP, PVC, PS, VA, ionomer, Pliofilm, and PVDC (Schlimme and Rooney, 1994). Permeability of packaging film to O_2 and CO_2 varies with type of packaging film material or composition. The characteristics of the main types of plastic films with their potential uses in MAP are summarized in Table 2.1 (Schlimme and Rooney, 1994). The table shows that the transmission rate for O_2 varies from 8 up to as high as 13,000 cm^3 m^{-2} day^{-1} at 1 atm for PVC and LDPE packaging films, respectively. The transmission rate of packaging materials for CO_2 also varies from 59 up to 77,000 (cm^3 m^{-2} day^{-1} at 1 atm), which is quite a large variation, the lowest permeability being for PVDC and highest for LDPE. The water vapour transmission rate also varies from 1.5 to 155 g m^{-2} day^{-1}. The variation in permeability of packaging films to O_2, CO_2 and H_2O could give a wide range of choices for films appropriate for specific commodities.

Table 2.1. Permeability characteristics of several plastic films with potential for use as MAP of fresh and lightly processed produce (Schlimme and Rooney, 1994).

	Transmission Rate		
Film Type	O_2*	CO_2*	H_2O vapour**
Low-density polyethylene (LDPE)	3900-13000	7700-77000	6-23.2
Linear low density polyethylene (LL DPE)	7000-9300	-	16-31
Medium-density polyethylene (MDPE)	2600-8293	7700-38750	8-15
High-density polyethylene (HDPE)	52-4000	3900-10000	4-10
Polypropylene (PP)	1300-6400	7700-21000	4-10.8
Polyvinylchloride (PVC)	620-2248	4263-8.138	>8
Polyvinylchloride (PVC), plasticized	77-7750	770-55000	>8
Polystyrene (PS)	2000-7700	10000-26000	108.5-155
Ethylene vinyl acetate copolymer (12% VA)	8000-13000	35000-53000	60
Ionomer	3500-7500	9700-17800	22-30
Rubber hydrochloride (Pliofilm)	130-1300	520-5200	>8
Polyvinylidine chloride (PVDC)	8-26	59	1.5-5

*Expressed in terms of cm^3 m^{-2} day^{-1} at 1 atm.
**Expressed in terms of g m^{-2} day^{-1} at 37.8°C and 90% RH.

Temperatures have effects on the permeability of packaging films. As temperature increases, the film permeability increases, which may affect the passive modified atmosphere that the film is designed for. Permeability of CO_2 is affected more than O_2

permeability, implying that a film that is a suitable packaging material at one temperature may not be so at another (Zagory and Kader, 1988). Kader *et al.* (1989) reported that most common films are relatively good barriers to moisture vapour, because they maintain high internal humidity even in dry, ambient conditions. This means that relative humidity affects the permeability of packaging films less than storage temperature. However, condensation inside films decreases the permeability of CO_2 and O_2 (Rodov, 1995).

It is obvious that the quality of fresh commodities can be dependent on the types of packaging film used for MAP. Under poor modified atmosphere storage management, occurrence of anaerobic metabolism is also possible in commodities packaged in films with low permeability to O_2, CO_2 and water vapour. Losses in physiological weight are higher from fresh commodities packaged in low-density packaging films than in high-density films. However, because near ambient O_2 level is maintained in the headspace of packages, the occurrence of anaerobic metabolism of fresh commodities could be avoided with the use of packaging films with higher permeability to O_2, CO_2 and water vapour during storage. Lower weight loss and firmness of tomatoes were maintained better in polyolefin bags (0.015 mm thick) than in PVC (0.0177 mm thick) during 5 weeks of storage at 10°C and 80% relative humidity (Frezza *et al.*, 1997). The shelf life of tomatoes was extended better when packaged in polyethylene and PP films as compared with PVC bags (Batu and Thompson, 1998). The lowest weight losses and the highest soluble solids contents after 60 days of storage were found in tomatoes packaged in polyethylene and PP films. Shelf life studies of pink ripe tomatoes packaged in polyethylene films with 100, 200 or 300 gauge showed that freshness was best maintained in perforated films compared with the other (Singh *et al.*, 1996). The reason for this may be due to higher permeability to gases of the two films as compared with polyvinyl chloride.

MAP using LDPE delayed changes in acidity, soluble solids, texture, colour and PG activity, and resulted in substantial reductions of weight loss of tomatoes, while higher density films showed the lowest weight losses, and the highest soluble solids content after 60 days of storage (Nakhasi *et al.*, 1991). However, it could be possible that the higher microbial populations found in these packages were due to relatively a higher storage temperature (13°C) and moisture accumulation in the headspace of packages.

Ben-Yehoshua *et al.* (1998) has shown that perforating the film affected the concentrations of both O_2 and CO_2 and water condensation without affecting the relative humidity of the air in the package headspace. The perforated film prevented the decay and maintained the postharvest quality of vegetables and fruits compared to storage in open boxes. Polyethylene bags with 2-4 diffusion holes per 4 kg tomatoes gave the best results, as the CO_2 permeability was improved (Esquerra and Bautista, 1990). Similarly LDPE bags were found to be better in maintaining the quality of carrots during storage compared with high-density polypropylene bags (Seyoum *et al.*, 2001). This indicated that the microperforated packaging films maintain the best quality of tomatoes during MAP. The strategies for combining films or film and micropore combinations could lead to realistic solutions to meet the required gas transfer properties (Exama *et al.*, 1993).

The common films, such as PVC and LDPE, can satisfy the gas flux requirements of low respiring tissues of carrots (Exama *et al.*, 1993). Emond *et al.* (1995), on the other hand, showed that perforated films significantly reduced water loss and condensation in packages of carrots during storage at 2°C and 95% relative humidity, compared to unperforated films. Nazar *et al.* (1996) showed that perforations in polyethylene bags equivalent to 0.5% of the total effective surface area of the package had no effect on the shelf life of carrots. The packaging film permeability also greatly influences disease incidence and sprouting of carrots during storage (Lingaiah and Reddy, 1996), as did non-ventilated polyethylene bags (Lingaiah and Reddy, 1996).

2.5.3. Progress on evaporative cooling of vegetables

The effect of storage temperature as an environmental factor during packaging was reviewed in 2.5.2.2.2. with emphasis on optimum temperatures for specific fruits and vegetables, e.g. 1°C for carrots and 13°C for tomatoes. To maintain these temperatures, refrigeration is normally required. Due to the mechanical complexity and expense, such practices are not available in most developing countries. Concurrently, postharvest losses are high, e.g. estimated at 25-35% in Ethiopia (Agonifer, 1991) or even at 40% in developing countries in general (FAO, 1981). Alternative storage methods with controlled temperature and humidity could be sought that are mechanically less complex and also less expensive. Evaporative cooling methods could be an answer.

Little work has been devoted towards the application of evaporative cooling principles in the field of fruit and vegetable preservation, with most of the work having been carried out in India and USA (Rama *et al.*, 1990; Rama and Narasimham, 1991; Roy and Pal, 1994; Christenbury *et al.*, 1995; Thompson and Kasmire, 1981; Thompson *et al.*, 1998.).

Rama *et al.* (1990), in India, showed that a metallic EC chamber reduced weight loss of potatoes compared to those stored at ambient conditions (20-30°C, RH 40-80%). Similar findings were obtained on physiological weight loss during EC storage of fresh commodities by Dzivama et al (1999). Roy and Pal (1993) have developed a zero energy cool chamber. The cooler increased the shelf life of tropical fruits by 2 to 14 days (15 - 27% increase) compared to storage at ambient temperature. Lower PWL and loss of vitamin A and C content were observed in fruits stored in a zero-energy cool chamber compared with fruits stored at room temperature (Kumar and Nath, 1993). Other findings also showed that weight loss was 3 times greater under ambient conditions than in a zero energy evaporative cooling chamber until tomatoes reached a red-ripe stage (Gopalakrishna *et al.*, 1991). It was indicated that the ripening time was extended with extended shelf life from 6.25 (ambient) to 10 days by cool storage. Acedo-AL (1997) showed that tomato fruits harvested at mature-green and stored in evaporative cooling storage had much less weight loss and shrivelling and a longer storage life than fruits kept at ambient conditions with significantly delayed ripening. Dzivama *et al.* (1999) evaluated application of evaporative cooling for storing mangoes, bananas and tomatoes. Results indicated that the produce remained in good condition for 18 days in the cooler, compared to 9 days in ambient conditions.

Under Ethiopian conditions a naturally ventilated evaporative cooler was designed and constructed using locally available and low cost materials (Seyoum and W/Tsadik, 2000). The cooler was able to significantly reduce the storage temperature and increase the relative humidity of air within, compared to that of the surrounding climatic conditions. The average reduction in air temperature inside the cooler was found to be 5°C. below environmental air temperature, with an average rise of 26% relative humidity. As a result it was possible to store mangoes for more than two weeks in the cooler without deterioration in appearance quality. Twenty-two percent of the mangoes stored at ambient conditions perished after 10 days of storage compared to 0.87% of the cooled samples.

The studies cited here were more devoted to natural ventilation cooling of fruits and vegetables during which only few postharvest quality parameters such as weight loss were monitored. However, a wider range of quality parameters should be included in order to understand the extent of postharvest quality (physiological, chemical and microbiological) changes occurring during EC storage. For instance, fresh produce may appear good, while microbial populations might thrive as a result of higher than optimum storage temperatures achieved in cold storage facilities and other than optimum humidities for specific fruits or vegetables.

2.6. Summary

The literature survey shows that any preharvest treatment, either to improve yield or quality, has an effect on fresh commodity quality at harvest and during storage. It is clear that not all the reviewed conditions could be optimised simultaneously. The reviewed conditions were also not described for specific cultivars of a vegetable type. This means that many of these conditions will have to be accepted as constant in a study, e.g., agricultural practices and environmental conditions, with focus on a few selected variables, e.g. ComCat® treatment, postharvest disinfection and storage temperature.

The postharvest performance of high yield ComCat® treated vegetables, e.g. carrots and tomatoes have not yet been investigated. The reviewed literature on postharvest performance of carrots and tomatoes stretches over several years and the research was carried out in many countries, while using different experimental approaches. These could therefore not be used as references against which to measure the ComCat® treated vegetables. Experiments will have to be designed carefully and proper controls will have to be incorporated.

Postharvest treatments of vegetables include disinfecting, and use of MAP and cold storage. According to the literature survey, disinfecting methods can generally be classified as chemical, biological, and physical methods. Although several chemicals and biological products are available on the market their success remains limited due to variability experienced in their effectiveness and lack of experience on how to adapt them to the commercial settings. The literature shows that chlorine treatment is currently the most commonly used, easiest to use and the cheapest method, but not necessarily the most effective. It is very effective for control of microorganisms, but almost no knowledge is available on its effect on the physiological aspects of harvested vegetables. It would therefore be necessary to include a second disinfectant in the study in order to avoid misleading results of disinfectant on ComCat® treated vegetables. Anolyte, an electrochemically activated water seems to be a good candidate for such a control. It was shown to kill and limit the growth of microorganisms. Chemical methods have residues that have negative impacts on health and environment or ecology, while physical methods need high initial investment. This encourages further research not only for microbiological safety, but also to develop environmentally and ecologically safe sustainable disinfectants as a partial or complete substitution for chemicals.

Regarding extended shelf life, the literature pointed to MAP and specific storage temperature for best results. New packaging material is constantly reaching the market, so that those aspects should be the final methods to test the postharvest performance of ComCat® treated vegetables. MAP, combined with cold storage are the most popular method as a result of its efficiency in increasing the shelf life of fresh commodities without much quality deterioration, less operational and material cost and easy consumer packaging. The optimum temperatures to store carrots and green mature tomatoes were reported to be 1°C and 13°C, respectively.

The observation that ComCat® can increase harvest yield of vegetables may be effectively used for food security purposes in underdeveloped countries when production is immediately followed with sustainable preservation technology. Despite high market demand after production, 20-50% of commodities is lost before reaching consumers in these countries due to a shortage of cooling facilities. The optimum storage temperatures, such as those for carrots and tomatoes mentioned above, can thus not be met. An attempt will be made to explore a low-cost vegetable preservation technology and look at the storability of the high harvest yield ComCat® treated carrots and tomatoes under higher storage temperature using EC. According to reviewed literature, evaporative cooling is cheap, easy to install and maintain, and was therefore proposed to be explored as an alternative cool storage tool in developing countries.

CHAPTER 3

EFFECT of PRE- and POSTHARVEST TREATMENTS on PHYSIOLOGICAL, CHEMICAL and MICROBIOLOGICAL QUALITIES of CARROTS

Abstract

Quality changes in preharvest ComCat® treated and untreated control carrots stored at 1 ± 0.5°C and ambient temperatures (16.7-29.5°C) and relative humidity (31-68%) were studied for more than 4 weeks. The carrots were analysed for headspace gases (O_2, CO_2, and N_2), physiological weight loss (PWL), total soluble solids (TSS), total available carbohydrates (TAC), sucrose, glucose, fructose, ascorbic acid (AA) content, peroxidase (POX) activity, aerobic bacteria, yeasts and fungi, and coliforms. The effect of chlorinated and anolyte water disinfecting treatments coupled with MAP was investigated. ComCat® treated carrots had less aerobic and coliform bacteria at harvest as well as higher levels of TSS, TAC sucrose and sucrose-hexose ratio, and lower glucose and fructose concentrations. Preharvest ComCat® treatment reduced PWL in carrots during storage, TSS content was better maintained, and a general trend of lower sucrose, glucose, fructose and total soluble sugar concentrations were found during storage. The concentrations of TAC seemed to accumulate better in ComCat® treated than in control carrots stored at room temperature. The preharvest ComCat® treatment had no significant effect on AA content and POX activity of carrots during storage, although it had a slightly higher AA at harvest. Two disinfecting treatments, chlorinated and anolyte water dipping, were compared. During storage, these treatments when coupled with MAP also had significant effects on PWL, TSS, TAC, sucrose and fructose content of carrots. Chlorine disinfecting resulted in an etched surface, which was not observed for anolyte treatment. Anolyte water showed a better positive effect on PWL compared with chlorine solution. Disinfecting carrots in anolyte water

significantly maintained the AA content and decreased the level of POX activity. A short time of 5 min dipping of carrots in anolyte water was found to be as effective as a 20 min in chlorinated water treatment, significantly reducing growth of aerobic bacteria, fungi and coliforms in carrots during storage. Storage temperature significantly affected all postharvest quality parameters tested in carrots during storage. Higher temperatures rapidly deteriorated microbiological, physiological and chemical quality characteristics of carrots. The combined effect of pre-and postharvest treatments such as ComCat®, disinfecting, packaging and low temperature storage treatments had a significant positive effect on maintaining postharvest quality and improvement of the shelf life of carrots.

3.1. Introduction

Horticultural commodities are different from other foods with their high levels of respiration and other metabolic processes associated with maturation, ripening, and senescence after harvest. During development and storage, carrots undergo a complex series of physiological, biochemical and microbiological events involving changes in postharvest quality (Phan *et al.*, 1973, Nilsson, 1987, Hole and McKee, 1988, Rosefeld *et al.*, 1998, Suojala, 1999, Suojala, 2000). During storage, levels of O_2 and CO_2 are critical for carrot quality. With the use of modified atmosphere packaging (MAP) metabolic activities may be reduced by controlling the levels of O_2 and CO_2 in packages of fresh commodities (Zagory and Kader, 1988). Temperature and relative humidity are other important factors that affect the quality and shelf life. Low temperature reduces the rate of respiration and biochemical activities, which are responsible for quality changes of carrots (Zagory and Kader, 1988). It was also shown that the respiration rate of carrots can be affected by preharvest treatments, so that the respiration rate is lower, and therefore leading to longer shelf lives (Salunkhe *et al.*, 1971). The preharvest histories of vegetables are therefore very important, as fresh produces are responding differently to postharvest factors. A preharvest treatment, ComCat®, has been developed recently from plant extracts, which was shown to improve vegetable yield, general strength and development of plants, and activate inherent plant defence mechanisms via induced resistance (Hüster, 2001). In South Africa, the preharvest ComCat® treatment increased carrot yield by 32% (Schnabl *et al.*, 2001). Investigation on quality of ComCat® treated carrots both at harvest and during storage is yet to be explored.

During storage microorganisms are the most important factors in postharvest vegetable quality management and postharvest vegetable and fruit decay (Harvey, 1978). Vegetables are usually treated with chlorinated water after washing to reduce microbial load prior to packaging (Bolin *et al.*, 1977). Much work has been done on the development of alternative methods to control postharvest decay (Rodov *et al.*, 1995 and 1996; Fallik *et al.*, 1999; Fallik *et al.*, 2000; Afek *et al.*, 1999).

One alternative could be the use of electrochemically activated saline water (anolyte water), which is safe from both an environmental as well as an ecological point of view. The objectives of this chapter were: (1) to investigate postharvest properties of ComCat® treated carrots; (2) to investigate anolyte water as an alternative disinfectant to a chemical treatment such as chlorine to reduce microbial load and suppress their growth thereafter during storage; and (3) investigate the storage quality of preharvest ComCat® treated carrots using MAP at 1°C and ambient temperature.

3.2. Materials and Methods

3.2.1. Carrot production

Carrots were planted in the Bloemfontein area, South Africa. During the growing period, carrots were treated twice with ComCat®. Experimental plants were treated with 10 g ha^{-1} ComCat® in 350 l of water, and control plants with 0 g ha^{-1}. Carrots were sprayed once at the three leaves stage, and a second time at a vegetative stage. All other agricultural practices were kept the same between the treatments during carrot production. At a maturity stage of 5 months, carrots were harvested and topped in the field, and were immediately transported the vegetable laboratory of the University of Free State. The topping, harvesting and transportation of carrots were made early in the morning before the temperature was too high. For protection against mechanical injury during transportation, carrots were packed in plastic crates. ComCat® treated and untreated carrots were harvested manually from four randomised blocks each and delivered to the laboratory immediately after harvest. After delivery to the laboratory, carrots were topped by carefully retaining the crown of the root. Carrots free of blemishes or defects were selected. The working surfaces and all tools used for topping carrots were washed and disinfected prior to use by 1% Chlorobac (Syndachem, Pty, LTD). Immediately after delivery to the laboratory, the carrots were hand washed with water at a temperature of 4°C, to remove field heat, soil particles and to reduce microbial populations on the surface. Prepackaging disinfecting treatments and packaging of carrots were performed on the same day.

3.2.2. Postharvest treatment

After washing, a total amount of 144 kg ComCat® treated carrots were sub divided into three groups of 48 kg each, in preparation for dipping treatments in chlorinated water, anolyte water or tap water, at 4°C. Plastic containers were washed, disinfected and rinsed with distilled water prior to use for the dipping treatments. Plastic containers were used to avoid losses of charged ions from anolyte water to metal containers. Tap water in these plastic containers was adjusted to 100µg. ml^{-1} free chlorine with sodium hypochlorite (5% NaOCl). A 20 minutes dipping time in 100µg. ml^{-1} chlorine supplemented water solution was selected, as this was reported to be the optimum effective dipping time without significant effect on the overall quality of vegetables (Nunes and Emond, 1999). The free chlorine was determined using a test kit from Hach (Model CN-66; USA). The temperature was maintained at 4°C during the measurements of free chlorine.

Anolyte water was prepared electrochemically from saline water containing 5% NaCl with an ionyzer (Radical Waters Pty Ltd, South Africa) operating at a pressure of 50 kpa. The pH of anolyte water was adjusted to 6.1 and it contained 3.55% total dissolved solids. Immediately after preparation, anolyte water was cooled to 4°C, and carrots were dipped without delay to avoid loss of inhibitory characteristics. The optimum dipping time of carrots in anolyte water was determined as 5 minutes in a trial experiment where carrots were dipped in anolyte water for 5, 10, and 20 minutes and compared with the efficacy of 20 minutes dipping in chlorinated water (100 μg. ml^{-1}) on microorganisms (Seyoum *et al.*, 2002). Carrots that were dipped in tap water were dipped for 20 minutes.

3.2.3. Modified atmosphere packaging

Low-density polyethylene (LDPE) bags were found to be the preferred packaging film above polypropylene (PP) for storage of carrots at 1°C in a trial study (Seyoum, *et al.*, 2001). Other researchers recommend, microperforated LDPE for MAP of carrots and tomatoes (Lipton 1977; Seyoum *et al.*, 2001). In this study the microperforated bags specifically designed and manufactured for carrot and tomato storage were therefore used (Xtend® Film, Patent No. 6190710, StePac L.A., Ltd., Israel). Carrots were subdivided and packaged as 1 kg-samples, and stored at 1°C or room temperatures. The unpackaged 1 kg-sample carrots, for each treatment combination, were placed on perforated plastic bags and left open during storage at 1°C or room temperatures.

On each sampling date, packages of carrots (1kg each) were randomly taken in triplicate from each treatment for quality analyses. A new package was taken each time in order to maintain the microenvironment and avoid contamination through repeated opening and sealing.

3.2.4. Gas sampling and analysis

Micro atmosphere gas analysis was performed at days 0, 3, 5, 9, 17, 25 and 32 of the storage period. Gas samples were withdrawn from the package by piercing the test film with a pressure lock syringe (Precision Sampling Crop, Baton Rouge, Lousiana) with a fine needle and withdrawing a 5 ml gas sample (Ballantyne *et al.*, 1988 a and b; Gunes *et al.,* 1997; Jeon and Lee, 1999). At each sampling date, three packages from each storage condition were tested separately. CO_2, O_2 and N_2 concentrations were analysed by gas chromatography (GC) on a Varian 3300 equipped with a Porapak Q 1.2 m X 2.3 mm stainless steel column, with thermal conductivity detection at 200 mA and H_2 as carrier at 19.5 ml. min^{-1} flow rate for CO_2 determination. For analysis of N_2 and O_2, a 0.8 m Molsieve column (PHASE SEP.) was used, with thermal conductivity detection operated at 200 mA and H_2 as carrier at 12.5 ml. min^{-1}. The GC oven temperature was set at 45°C for both analyses. The opening made by the needle during gas sampling was immediately sealed with drawing tape, to avoid leaking of gas from the packages.

3.2.5. Physiological weight loss

The physiological weight loss (PWL) was determined using the methods as described by Pirovani *et al.* (1997) and Waskar *et al.* (1999). The PWL was determined by periodical weighing carrots on days 0, 7, 14, 21, 28, 35, 42 and 50 after packaging. The differential weight loss was calculated for each interval and converted into percentage by dividing the change with the initial weight recorded on each sampling interval. The cumulative PWL was expressed in per cent with respect to different treatments.

3.2.6. Chemical analysis

3.2.6.1. Total soluble solids (TSS)

The TSS was determined using the procedures as described by Waskar *et al.* (1999). An aliquot of juice was extracted using a Kenwood juice extractor, and 50 ml of the slurry was centrifuged for 15 minutes at 5000 x g at 4°C. The TSS was determined by an Atago N1 hand refractometer with a range of 0 to 32 °Brix, and resolutions of 0.2 °Brix by placing 1 to 2 drops of clear juice on the prism. Between samples the prism of the referactrometer was cleaned with tissue paper soaked in methanol, washed with distilled water and dried before use. The refractometer was standardised against distilled water (0% TSS).

3.2.6.2. Free sugar analysis

Free sugars, sucrose, glucose and fructose, were determined by the method of Riaz and Bushway (1996). A 50 g carrot sample was homogenised for 2 minutes. Sugars were extracted by placing a 10 g aliquot in a 100 ml beaker and stirring for 1 minute with 35 ml 95% ethanol. The samples were shaken 20 times and kept at room temperature overnight. Samples were transferred to a 50-ml volumetric flask and made to volume with 80% ethanol. After filtration, aliquots of 5 ml were placed in vials and centrifuged for 5 minutes at 3000 x g (Beckman, Microfuge E) before analysis by high performance liquid chromatography (HPLC). HPLC was carried out on a Waters system (501 pump) and Biorad Aminex column (7.8 mm X 300 mm) with a differential refractive index detector (R401) operated at 42°C and a mobile phase of de-ionized water at a flow speed of 0.6 ml. min^{-1} and temperature of 85°C.

3.2.6.3. Total available carbohydrate (TAC)

Total available carbohydrate was estimated by the Automated Clegg Anthrone method (Clegg, 1956, and Osborne & Voogt, 1978). A carrot puree of 2 g was homogenised for approximately 2 minutes with 15 ml distilled water and 20 ml 99% H_2SO_4 and placed in an oven at 50°C for 15 minutes. A series of trail studies were conducted on the effect of heating time and type of acid (perchloric and sulphuric acids) to be used for hydrolysis to determine any variation in the values, however, no difference was observed. The warm solution was thoroughly mixed for 5 minutes with an electric stirrer, transferred to a 250-ml volumetric flask and made up to the mark with distilled water. The extracted samples were refrigerated until analysed the next day. Aliquots were placed in vials and analysed by a Technicon AutoAnalyzer.

3.2.6.4. Ascorbic acid analysis (AA)

The ascorbic acid content (AA) of carrots was determined by the 2,6–dichlorophenolindophenol method (AOAC, 1970). An aliquot of 10 ml carrot juice extract was diluted to 50 ml with 3% metaphosphoric acid in a 50 ml volumetric flask. An aliquot was centrifuged at 10 000 x g for 15 minutes and titrated with the standard dye to a pink end-point (persisting for 15 sec). The ascorbic acid content (%) was calculated from the titration value, dye factor, dilution and volume of the sample.

3.2.6.5. Peroxidase activity (POX)

Carrot tissue (12.5 g) was homogenised in 25 ml of citrate-phosphate buffer (pH 6.5) (made with 0.05 M citric acid and 0.1 M Na_2HPO_4) for 2 minutes (Howard *et al.*, 1994). The homogenate was held at 4°C for 2 hr and centrifuged at 10 000 X g for 15 minutes at 4°C. Prior to POX activity determination a reaction mixture was prepared by adding 0.05 ml of 1% o-dianisidine dye in methanol to 6 ml of 30% H_2O_2 substrate. Then 2.9 ml of this dye-substrate mixture was transferred to a 3 ml test cuvette. Enzyme supernatant (0.1 ml) was introduced into the test cuvette from a 0.1 ml pipette with the tip below the surface. The control consisted of the same reaction mixture, but with 0.1 ml extraction buffer instead of enzyme extract. The cuvettes were covered with parafilm, and the solution mixed by inversion. The rate of peroxidase activity was followed spectrophotometrically at 460 nm (Howard *et al.*, 1994). The increase in absorbancy was recorded at 15 seconds intervals for 2 minutes. POX activity was expressed as increase in absorbancy at 460 nm g^{-1} tissue min^{-1} at 25°C.

3.2.7. Microbiological analysis

Microbial populations were estimated by the procedure followed by Brackett (1988a and 1990). Three carrots were cut aseptically into pieces with sterile knives. Samples of 25 g were blended with 225 ml 0.1% peptone water (pH 7.0) in a stomacher for 3 minutes. The slurries were serially diluted in 9 ml 0.1% peptone water. To determine populations of total aerobic microorganisms, duplicate samples were plated on plate count agar (Oxoid CM463, and pH 7.0±0.2) and incubated at 30°C for 2 days. For the estimation of *E. coli* and coliform population, duplicate samples were plated on violet red bile agar (VBRA with MUG, Oxoid CM978) and incubated at 37°C for 1 day. To determine moulds and yeasts, duplicate samples were plated on Rose-Bengal Chloramphenicol Agar Base (Oxoid CM549) and incubated at room temperature for 3 to 5 days. In all the cases pour plate methods were used. The mean log10 of viable counts from two duplicate plates were noted. .Microbial populations were not analysed immediately after disinfection, as they were assumed to be around 0 log10 CUF.g^{-1} as was shown by Seyoum et al. (2003).

3.2.8. Subjective quality attributes

The descriptive quality attributes of carrots were carried out after 28 days of storage at 1°C or room temperature according to the methods used by Ballantyne *et al.* (1988a and b) and Brackett (1990). This descriptive quality attributes were determined subjectively by observing the level of visible moulds growth, decay, shrivelling or dehydration overall, as well as interior firmness and the surface appearance characteristics such as smoothness and shininess of carrots. A rating with poor (unmarketable), fair (some marketable), good (marketable), very good and excellent was used (Mohammed et al., 1999).

3.2.9. Experimental design and data analysis

A factorial experiment with 2 preharvest treatments, 3 prepackaging disinfecting treatments, 2 storage temperatures and 3 replications were used in the study. The experimental design was arranged in a factorial type of randomised complete block design (RCBD), with three samples from each treatment combination. A pack of carrots were taken randomly from each treatment group on each sampling day and used for the different quality analyses. Each replicate sample for analysis of microbiological quality and free sugar content (sucrose, glucose and fructose) was analysed in duplicate. Statistical significant differences between the treatments were determined by analysis of variance (ANOVA) with an MSTAT-C software package (MSTAT, Michigan State Univ., East Lansing) and multiple comparison of the treatment means by Duncan's multiple range test (Duncan, 1955). The effect of two different types of packaging films with different levels of permeability to O_2, CO_2 and H_2O vapour on microbiological, physiological and chemical quality of stored carrots were investigated earlier (Seyoum, *et al.*, 2001). It was shown that an appropriate MAP improved the shelf life of carrots through controlling physiological activities such as allowing lower respiration rates, prevention of condensation, but preventing moisture loss, maintaining optimum gas composition and normal respiration of carrots without the occurrence of anaerobic respiration during storage. Therefore, during the current investigation the statistical analysis of the disinfecting treatment was coupled with MAP in order to see the overall effect of these treatments on the quality parameters. The individual effect of MAP and disinfecting treatments was analysed using multiple comparison of each treatment means by mean separation of Duncan's multiple range test.

3.3. Results

3.3.1. Headspace gas concentration

Storage temperature had the greatest effect on changes in headspace gas concentration (Fig. 3.1, 3.2 and 3.3). There was a significant difference ($P \leq 0.001$) in O_2 consumption between carrots stored at 1°C and ambient temperature during the 32 days of storage. During the first 3 days, the O_2 level decreased faster in packages of carrots stored under room temperature than in the packages of carrots stored at 1°C. After 3 days of storage at both temperatures, the O_2 concentrations remained near equilibrium i.e. 19%-20% at 1°C and 17.5%-18.5% at room temperature. The effect of storage temperature on the headspace CO_2 concentration was also significant ($P \leq 0.001$). The CO_2 level increased rapidly in packages of carrots stored at room temperature during the first 9 days. In the packages of carrots stored at 1°C, the CO_2 increased the first 3 to 5 days, and then decreased slightly to stabilise after 9 days. The CO_2 level equilibrated after 9 days, i.e. below 1% at 1° and 2%-4% at room temperature. Storage temperature was also an important factor affecting N_2 concentration. The headspace N_2 concentration was significantly ($P \leq 0.001$) affected with the storage temperature. The N_2 levels increased during the first 3 days with a rapid rise in packages of carrots stored at room temperature and equilibrated after 3 days, i.e. at 80%-81% at 1°C and 81.5%-82.5% at room temperature.

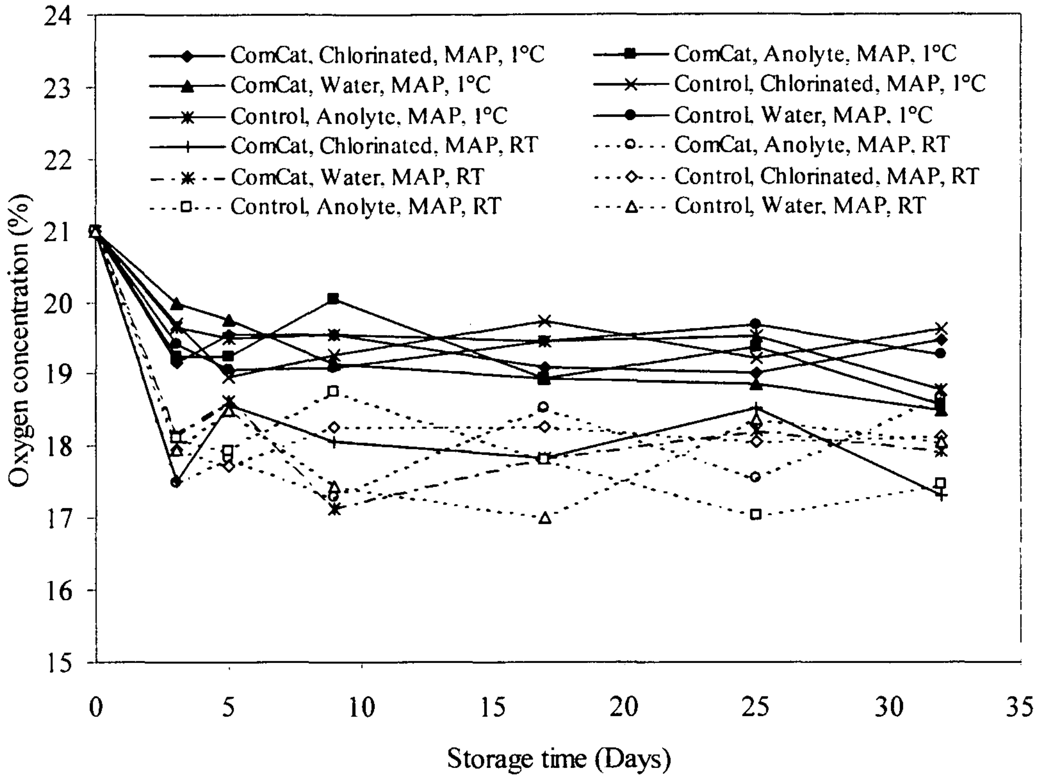


Figure 3.1. Changes in O_2 content (%) in packages of carrots in Xtend® film stored at 1°C and room temperature (RT) for 32 days (n = 3 over six storage times).

Significance level showing the effect of pre- and postharvest treatment on O_2 concentrations

Significance	
Preharvest treatment (A)	NS
Disinfecting (B)	NS
Storage temperature (C)	***
A X B	NS
A X C	NS
B X C	NS
A X B X C	NS

NS, *** Nonsignificant or significant at P ≤ 0.01, 0.05 or 0.001 respectively. $LSD_{0.05}$ Value = 0.378, S.E. = 0.024 and C.V. = 0.035.

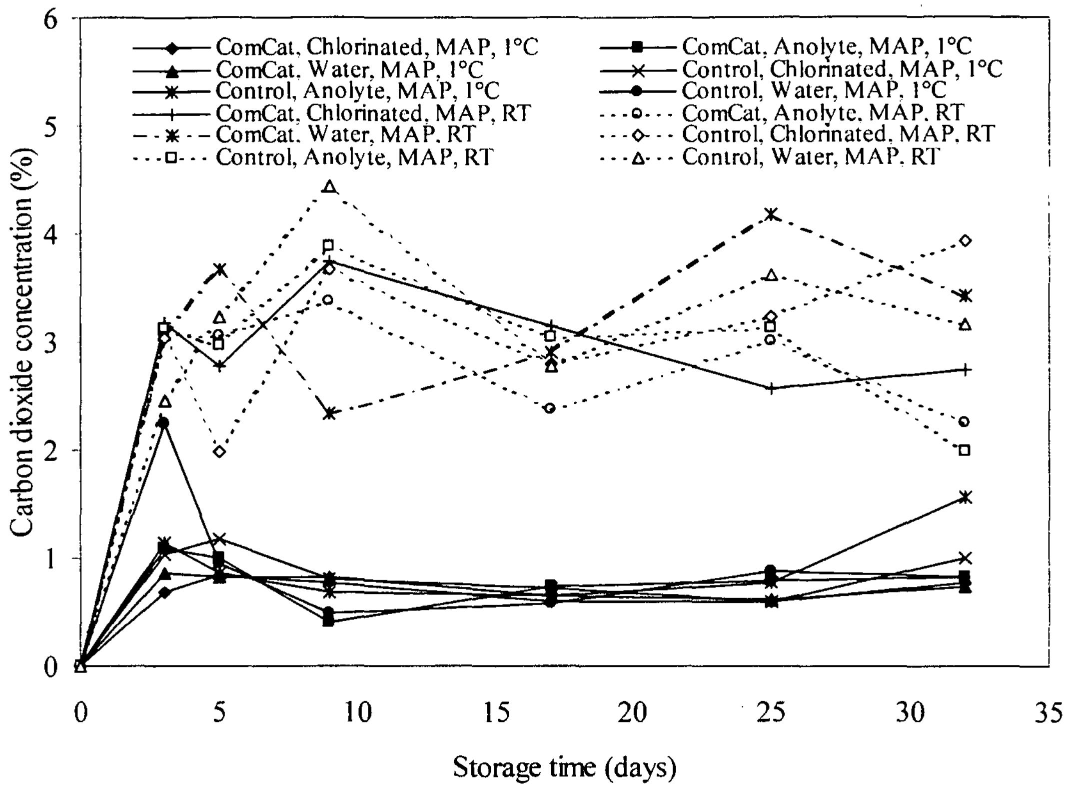


Figure 3.2. Changes in CO_2 content (%) in packages of carrots in Xtend® film stored at 1°C and room temperature (RT) for 32 days (n = 3 over six storage time).

Significance level showing the effect of pre- and postharvest treatment on CO_2 concentrations

Significance	
Preharvest treatment (A)	NS
Disinfecting (B)	NS
Storage temperature (C)	***
A X B	NS
A X C	NS
B X C	*
A X B X C	NS

NS, *, **, *** Nonsignificant or significant at $P \leq 0.01$, 0.05 or 0.001 respectively. $LSD_{0.05}$ Value = 0.826, S.E. = 0.074 and C.V. = 0.277.

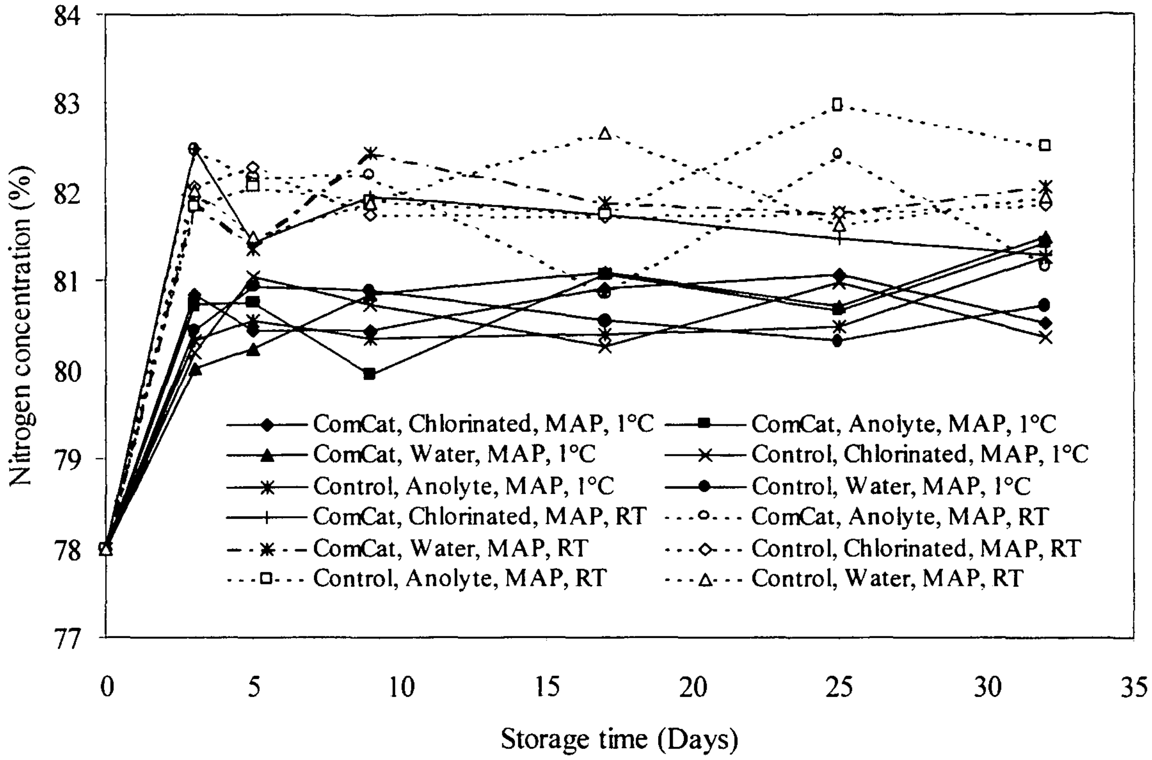


Figure 3.3. Changes in N_2 content (%) in packages of carrots in Xtend® film stored at 1°C and room temperature (RT) for 32 days (n = 3 over six storage time).

Significance level showing the effect of pre- and postharvest treatment on N_2 concentrations	
Significance	
Preharvest treatment (A)	NS
Disinfecting (B)	NS
Storage temperature (C)	***
A X B	NS
A X C	NS
B X C	NS
A X B X C	NS

NS, *, **, *** Nonsignificant or significant at $P \leq 0.01$, 0.05 or 0.001 respectively. LSD0.05 Value = 1.068, S.E. = 0.059 and C.V. = 0.081.

The effect of preharvest ComCat® treatment on changes in O_2, CO_2 and N_2 concentrations in the packages of carrots was not significant ($P \geq 0.05$). Since these results are indicative of the levels of respiration rate in carrots, they show that ComCat® treated carrots had the same respiration rate as the controls. No significant difference ($P > 0.05$) in changes of nitrogen concentration were observed in packages of carrots with respect to preharvest treatments. For all three gases, a greater fluctuation after equilibration was observed at room temperature, compared to storage at 1°C. There were no significant differences in gas concentrations inside packages of carrots treated with chlorinated and anolyte water. The effect of chlorinated and anolyte water dipping and water wash treatments was not significant ($P > 0.05$) on gas concentrations during the storage period. The interactive effect of disinfecting with storage temperature had a significant ($P \leq 0.05$) influence on the changes in the level of CO_2 during storage.

3.3.2. Physiological weight loss (PWL)

Table 3.1 shows PWL of carrots during the storage period of 50 days. Compared to packaged carrots, the PWL was 22.3% higher in unpackaged ComCat® treated carrots and 30.1% in control carrots stored at ambient temperature during the first two weeks (Table 3.1). Signs of dehydration, such as a dull, shrivelled appearance, were the first observable defects in unpackaged carrots. During 50 days storage, the PWL was in general slightly higher in control carrots than in ComCat® treated carrots stored at 1°C. This difference was not significant at $P > 0.05$, but at $P \leq 0.07$, and could show an advantage of ComCat® over the control carrots.

Compared to the water washed carrots, the pooled mean PWL was found to be more by 2.1% and 1.3% when dipped in chlorinated and anolyte water, respectively. In several samples, the PWL was higher ($P \leq 0.09$) in chlorine washed carrots, compared to anolyte disinfected ones.

Table 3.1. Changes in physiological weight loss of carrots subjected to pre- and postharvest treatments and stored at 1 °C and room temperature (RT) for 50 days.

	Physiological loss in weight (%)						
Treatment	day 7	day 14	day 21	day 28	day 35	day 42	day 50
ComCat®, Cl_2, MAP, 1°C	3.132^{cd}	5.036^{de}	6.385^{c}	8.889^{cd}	13.520^{c}	17.520^{de}	22.832^{c}
ComCat®, Anolyte, MAP, 1°C	2.717^{d}	5.624^{de}	7.112^{c}	8.998^{cd}	14.504^{c}	16.592^{fg}	21.958^{cd}
ComCat®, Cl_2, MAP, RT	10.226^{b}	12.772^{c}	22.256^{b}	32.079^{b}	48.412^{a}	53.087^{ab}	57.559^{ab}
ComCat®, Anolyte, MAP, RT	10.342^{b}	14.585^{c}	20.130^{b}	32.919^{b}	44.098^{b}	51.016^{bc}	54.906^{bcd}
ComCat®, H_2O, MAP, 1°C	2.486^{d}	3.446^{de}	6.095^{cd}	8.548^{cd}	14.265^{c}	15.987^{gh}	21.327^{cde}
ComCat®, H_2O, MAP, RT	9.107^{bc}	12.610^{c}	20.534^{b}	29.193^{b}	44.444^{b}	49.675^{c}	55.498^{abc}
ComCat®,H_2O,1°C	10.676^{b}	27.667^{d}	38.503^{a}	46.760^{a}	-	-	-
ComCat®, H_2O, RT	21.363^{a}	34.881^{b}	-	-	-	-	-
Control, Cl_2, MAP, 1°C	2.178^{d}	6.182^{d}	7.312^{c}	10.167^{c}	15.001^{c}	17.893^{d}	23.672^{c}
Control, Anolyte, MAP, 1°C	4.818^{bcd}	5.518^{d}	7.322^{c}	9.282^{c}	12.118^{c}	16.211^{gh}	20.777^{cdef}
Control, Cl_2, MAP, RT	9.899^{b}	12.173^{c}	21.525^{b}	30.524^{b}	45.696^{ab}	54.282^{a}	57.965^{ab}
Control, Anolyte, MAP, RT	11.239^{b}	12.229^{c}	22.683^{b}	28.022^{b}	49.144^{a}	51.196^{bc}	55.863^{bc}
Control, H_2O, MAP, 1°C	1.716^{d}	5.282^{d}	7.114^{c}	9.098^{cd}	13.378^{c}	17.211^{ef}	21.566^{cde}
Control, H_2O, MAP, RT	7.939^{bcd}	12.944^{c}	20.889^{b}	27.941^{b}	45.727^{ab}	51.094^{bc}	58.431^{a}
Control, H_2O, 1°C	10.657^{b}	34.058^{b}	37.259^{a}	43.837^{a}	-	-	-
Control, H_2O, RT	25.766^{a}	43.037^{a}	-	-	-	-	-

Significance	
Preharvest treatment (A)	NS
Disinfecting + MAP (B)	***
Storage temperature (C)	***
A X B	NS
A X C	**
B X C	***
A X B X C	***

NS, **, *** Nonsignificant or significant at $P \leq 0.01$ or 0.001 respectively. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The LSD Value = 5.536, S.E. = 0.613, MSE = 11.829 and C.V. = 0.121.

The storage temperature affected the PWL of carrots significantly ($P < 0.001$). After 50 days, the losses were found to be 36.9% and 34.2% more from packages of water washed control and ComCat® treated carrots stored at room temperature than from packages of water washed control and ComCat® treated carrots stored at 1°C, respectively. Based on the group of postharvest treatments, the rate of PWL was divided into three groups (Table 3.1). The highest moisture loss was from unpackaged carrots stored at room temperature with the lowest rate of moisture transfer from packaged carrots stored at 1°C. The three-way interaction between preharvest, disinfecting + MAP and storage temperature treatments was highly significant ($P \leq 0.001$) on the PWL of carrots.

3.3.3. Chemical changes

3.3.3.1. Total soluble solid (TSS)

Table 3.2 displays the changes in TSS of carrots subjected to different pre- and postharvest treatments. The TSS significantly increased ($P \leq 0.001$) with storage time in all samples (Appendix A.1). The TSS content of carrots raised with increased storage temperature as well as with storage time. Individually, MAP highly influenced the changes in the TSS contents ($P \leq 0.001$) of carrots stored at 1°C as well as room temperature. The TSS of carrots increased at a lower rate in packaged carrots when compared to the rates of increase of TSS in unpackaged carrots. Storage temperature also had a highly significant ($P \leq 0.001$) effect on the changes in TSS content of carrots during storage. As shown in Table 3.2, the TSS content increased faster and to higher levels in carrots stored at room temperature than at 1°C.

At harvest the ComCat® treated carrots had a higher TSS than control carrots, however, the difference was not significant ($P \leq 0.05$). A general trend of higher TSS was also observed in ComCat® treated carrots compared to control carrots during 28 days of storage. It might therefore not be excluded that ComCat® treated carrots are of better chemical quality than untreated ones.

Table 3.2. Changes in total soluble solid (TSS) of carrots subjected to both pre- and postharvest treatment and stored at 1°C and room temperature for 28 days.

	Total soluble solid TSS (°Brix)			
Treatment	Day 0	day 14	day 21	day 28
ComCat®, Cl_2, MAP, 1°C	9.267^{a}	9.200^{ef}	9.917^{cd}	9.667^{f}
ComCat®, Anolyte, MAP, 1°C	9.267^{a}	9.533^{ef}	9.200^{de}	9.333^{fg}
ComCat®, Cl_2, MAP, RT	9.267^{a}	10.400^{cd}	10.667^{bc}	12.133^{abc}
ComCat®, Anolyte, MAP, RT	9.267^{a}	10.200^{cd}	10.800^{b}	12.500^{ab}
ComCat®, H_2O, MAP, 1°C	9.267^{a}	9.467^{ef}	8.933^{de}	9.533^{f}
ComCat®, H_2O, MAP, RT	9.267^{a}	9.933^{cde}	10.067^{bcd}	11.267^{cd}
ComCat®, H_2O, 1°C	9.267^{a}	10.433^{cd}	11.033^{ab}	11.633^{bc}
ComCat®, H_2O, RT	9.267^{a}	12.867^{a}	-	-
Control, Cl_2, MAP, 1°C	8.800^{ab}	9.200^{ef}	9.500^{cd}	9.657^{f}
Control, Anolyte, MAP, 1°C	8.800^{ab}	9.268^{ef}	8.867^{de}	9.800^{f}
Control, Cl_2, MAP, RT	8.800^{ab}	10.267^{cd}	10.133^{bcd}	11.333^{bc}
Control, Anolyte, MAP, RT	8.800^{ab}	9.567^{ef}	10.600^{bc}	11.500^{bc}
Control, H_2O, MAP, 1°C	8.800^{ab}	9.133^{ef}	9.400^{cd}	9.333^{fg}
Control, H_2O, MAP, RT	8.800^{ab}	9.934^{cde}	10.233^{bcd}	10.767^{cde}
Control, H_2O, 1°C	8.800^{ab}	12.267^{ab}	12.000^{a}	13.133^{a}
Control, H_2O, RT	8.800^{ab}	12.467^{ab}	--	-

Significance

Preharvest Treatment (A)	NS
Disinfecting + MAP (B)	***
Storage Temperature (C)	***
A X B	NS
A X C	**
B X C	NS
A X B X C	NS

NS, **, *** Nonsignificant or significant at P≤ 0.01 or 0.001 respectively. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The LSD Value = 1.104, S.E. = 0.133, MSE = 0.466 and C.V. = 0.067.

During 21 days at 1°C, the ComCat® treated carrots dipped in anolyte water had a higher TSS content, when compared to the TSS content of control carrots dipped in anolyte water, although not significant at $P \leq 0.05$ level. The ComCat® treated carrots dipped in chlorinated water had a slightly higher TSS content compared to those in control carrots treated with chlorine, although not significant at $P < 0.05$ significance level. Similarly, the control carrots dipped in tap water had a slightly lower TSS content, except during the third week of storage at 1°C when compared to the TSS content of carrots dipped in the disinfectant. Surprisingly, the packaged ComCat® treated carrots dipped both in anolyte as well as chlorinated water had consistently higher TSS contents during the storage period of 28 days at room temperature, when compared to the TSS content of packaged control carrots subjected to the same postharvest treatment. Even if there was no significant difference ($P > 0.05$) between TSS content of the ComCat® treated and untreated carrots, the data could possibly indicate a difference in metabolic activities. The group mean differences in TSS of ComCat® treated and untreated carrots were found to be 0.467, 0.228, 0.142, and 0.339 °Brix for 0, 14, 21 and 28 days of storage, respectively.

The multiple comparison test showed that the TSS did not differ significantly for the individual effects of disinfecting with chlorine, anolyte water dipping and control ($P > 0.05$ for all treatments). The TSS content of carrots dipped in anolyte water seemed to remain approximately constant and slightly lower than the TSS content of carrots dipped in chlorinated water, especially during the first 10 days at both storage temperatures. At room temperature, the TSS seemed to build up and become slightly higher in carrots dipped in anolyte water, when compared with those dipped in chlorinated water. However, a higher group mean TSS was observed in carrots dipped in chlorinated water than in the other two treatments (water washed and anolyte treatments). Relatively, the lowest group means TSS was found in water washed carrots. The two-way interaction between preharvest ComCat® treatment and storage temperature was significant ($P \leq 0.01$) on the changes in TSS content of carrots during storage. The three-way interaction between preharvest ComCat® treatment, disinfecting together with packaging and storage temperature was only significant at $p \leq 0.096$.

3.3.3.2. Total available carbohydrate (TAC)

The TAC significantly decreased ($P \leq 0.001$) during the storage time of all samples from around 13 g. $100g^{-1}$ to as low as 8 g. $100g^{-1}$. A rapid decrease in TAC was observed within the first two weeks of storage, followed by a slow decrease thereafter for most treatments (Table 3.3). The TAC content was better maintained in packaged carrots during storage at room temperature, as well as at 1°C, during 28 days. However, the difference in TAC between packaged and unpackaged carrots were not significant at $P \leq 0.05$ during the entire storage interval. Even if there was no significant difference ($P > 0.05$) in the TAC change during this short storage period, there were generally higher concentrations of TAC in packaged carrots than in unpackaged carrots stored at 1°C as well as room temperature.

Storage temperature significantly ($P \leq 0.001$) affected the TAC in carrots during storage. For example, the ComCat® treated carrots dipped in chlorinated, anolyte and tap water and stored at 1°C had 14.1%, 4.6% and 14.2% higher TAC contents than the ComCat® treated carrots subjected to the same disinfecting treatment but stored at room temperature for 28 days. Similarly, after 28 days at 1°C, the TAC contents in control carrots dipped in chlorinated, anolyte and tap water were respectively 15.1%, 14.3% and 21.3% higher compared to the control carrots subjected to the same disinfecting treatment and stored at room temperature. As it was mentioned earlier in this section, a rapid decrease of carbohydrate was observed right after harvest and during the first week of storage at both temperatures. During the later weeks of storage the carbohydrate concentrations decreased faster in carrot stored at room temperature due to the effect of higher temperature on the metabolic processes.

Table 3.3. Changes in total available carbohydrate contents of carrots subjected to both pre- and postharvest treatments and stored at 1°C and room temperature (RT) for 28 days.

	Total available carbohydrate (g. 100g^{-1} FW)				
Treatment	day 0	day 7	day 14	day 21	day 28
ComCat®, Cl_2, MAP, 1°C	13.880^{a}	10.373^{bc}	9.371^{abc}	9.663^{ab}	9.589^{a}
ComCat®, Anolyte, MAP, 1°C	13.880^{a}	9.632^{bcd}	9.334^{abc}	9.352^{abcd}	9.288^{ab}
ComCat®, Cl_2, MAP, RT	13.880^{a}	10.714^{bc}	9.810^{a}	8.294^{bcd}	8.236^{abc}
ComCat®, Anolyte, MAP, RT	13.880^{a}	12.430^{a}	9.983^{a}	9.477^{abc}	8.858^{abc}
ComCat®, H_2O, MAP, 1°C	13.880^{a}	11.413^{ab}	9.925^{a}	9.295^{abc}	9.277^{ab}
ComCat®, H_2O, MAP, RT	13.880^{a}	10.105^{bcd}	9.597^{ab}	8.280^{bcd}	7.957^{c}
ComCat®, H_2O, 1°C	13.880^{a}	9.594^{bc}	9.127^{bcd}	8.193^{def}	8.994^{abc}
ComCat®, H_2O, RT	13.880^{a}	9.597^{bc}	9.568^{ab}	-	-
Control, Cl_2, MAP, 1°C	12.590^{ab}	9.810^{bcd}	9.758^{a}	9.490^{abc}	9.192^{ab}
Control, Anolyte, MAP, 1°C	12.590^{ab}	10.432^{bc}	9.608^{ab}	9.479^{abc}	9.341^{ab}
Control, Cl_2, MAP, RT	12.590^{ab}	8.940^{ef}	8.900^{bcd}	8.018^{def}	7.803^{c}
Control, Anolyte, MAP, RT	12.590^{ab}	9.052^{def}	9.461^{ab}	7.929^{ef}	8.006^{bc}
Control, H_2O, MAP, 1°C	12.590^{ab}	10.762^{b}	9.282^{bcd}	9.837^{a}	9.518^{a}
Control, H_2O, MAP, RT	12.590^{ab}	9.553^{cd}	9.412^{ab}	8.256^{bcd}	7.493^{c}
Control, H_2O, 1°C	12.590^{ab}	10.479^{bc}	9.934^{a}	9.428^{abc}	9.313^{ab}
Control, H_2O, RT	12.590^{ab}	9.032^{ef}	8.927^{bcd}	-	-

Significance

Preharvest Treatment (A)	NS
Disinfecting + MAP (B)	NS
Storage Temperature (C)	***
A X B	*
A X C	***
B X C	*
A X B X C	*

NS, *, ***Significant at $P \leq 0.05$ or 0.001 respectively. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The LSD Value = 1.116, S.E. = 0.127, MSE = 0.478 and C.V. = 0.069.

The TAC content was slightly higher in ComCat® treated carrots at harvest and was found to be 13.88 g. 100 g^{-1} and 12.59 g 100 g^{-1} for preharvest ComCat® treated and control carrots, respectively. The effect of preharvest treatment on the TAC during storage of carrots was not significant at $P \leq 0.05$, but at $P \leq 0.09$ level. During the 28 days of storage at 1°C, there was a consistent trend in the changes of TAC content between those concentrations in ComCat® treated and control carrots. The reason for this may be maintenance of a kind of steady state in TAC in both preharvest ComCat® treated and untreated carrots, which could be attributed to the effect of low temperature. The TAC content remained to be slightly higher in ComCat® treated carrots subjected to different postharvest treatments during 28 days at room temperature, although not significant at $P > 0.05$. The comparison of group means over the storage period of 28 days indicated that the mean TAC remained higher (by 0.062 g. g-1 FW) in ComCat® treated carrots than in control carrots. This may indicate that slightly less conversion of carbohydrates to free sugars took place in ComCat® treated carrots. During the 28 days of storage at room temperature, the TAC was found to be higher in ComCat® treated carrots, when compared to those concentrations in control carrots.

A general trend ($P < 0.05$) of higher TAC content was found in water washed control carrots stored at 1°C compared to carrots dipped in chlorinated and anolyte water, except for the 14th day storage interval. Although there were some discrepancies, similar trends were observed in the changes in TAC content of control carrots subjected to these disinfecting treatments and stored at room temperature. The two-way interaction between the ComCat® treatment and storage temperature had a highly significant ($P < 0.001$) influence on the changes in TAC during storage. These interactions indicate that the ComCat® treatment had a positive effect on the changes in TAC content of carrots stored at low temperatures. The three-way interaction between pre- and postharvest treatments was also significant ($P < 0.05$) on the changes of TAC in carrots during storage. Indicating a general synergistic effect of pre- and postharvest treatments on TAC content.

3.3.3.3. Free sugar

The contents of individual sugars (sucrose, glucose and fructose) in carrots stored at 1 °C and ambient temperatures are presented in Table 3.4 (a), (b) and (c). The sucrose content varied between 5.37 g.100^{-1}g and 3.09 g.100^{-1}g, and decreased during the first 1 to 2 weeks of storage, after which it increased up to 21 days of storage, and finally decreased at 28 days.

At harvest, the glucose content was significantly lower in ComCat® treated than in control carrots, while the fructose and sucrose contents were higher, however, not significantly ($P > 0.05$). MAP seemed to have a greater effect on the sucrose content of carrots during storage. After 14 days at room temperature, the unpackaged ComCat® treated and control carrots had 18.2% and 12.9% less sucrose, respectively, when compared with the sucrose contents in packaged ComCat® treated and control carrots. In general, the sucrose contents remained higher in packaged carrots, compared to those in unpackaged ones stored at room temperature. The sucrose content also decreased faster in unpackaged carrots stored at room temperature than in carrots stored at 1 °C.

During the fourth week of storage the concentration of sucrose decreased more in control carrots stored at room temperature compared to those stored at 1°C. During storage, the preharvest ComCat® treatment had no significant ($P > 0.05$) effect on sucrose concentration in carrots. However, a general trend of slightly higher sucrose contents in the ComCat® treated carrots was observed.

The trends in sucrose concentration in carrots dipped in chlorinated, anolyte and tap water were complex, when these carrots were stored at optimum temperature of 1°C, although the concentrations of sucrose was significantly lower ($P \leq 0.05$) in water washed carrots on the 7th and 28th days of storage interval (Table 3.4 (a)). Nevertheless, these trends seemed more clear when carrots dipped in different disinfectants and stored at room temperature were compared.

Table 3.4. (a). Changes in sucrose content of carrots subjected to both pre-and postharvest treatments and stored at 1°C and room temperature (RT) for 28 days.

Treatment	Sucrose ($g.100^{-1}g$)				
	day 0	day 7	day 14	day 21	Day 28
ComCat®, Cl_2, MAP, 1°C	5.370^{a}	4.254^{bcd}	4.005^{bc}	5.615^{ab}	5.004^{abc}
ComCat®, Anolyte, MAP, 1°C	5.370^{a}	4.510^{bcd}	4.446^{abc}	5.020^{abc}	4.630^{bcd}
ComCat®, Cl_2, MAP, RT	5.370^{a}	4.122^{bcd}	4.376^{abc}	4.313^{cd}	4.068^{cde}
ComCat®, Anolyte, MAP, RT	5.370^{a}	5.373^{a}	4.123^{bc}	4.887^{bc}	4.890^{ab}
ComCat®, H_2O, MAP, 1°C	5.370^{a}	3.707^{de}	4.409^{abc}	5.334^{abc}	3.533^{e}
ComCat®, H_2O, MAP, RT	5.370^{a}	3.726^{cde}	4.012^{bc}	4.579^{cd}	3.974^{de}
ComCat®, H_2O, 1°C	5.370^{a}	3.987^{cde}	3.737^{cde}	4.287^{cde}	3.330^{e}
ComCat®, H_2O, RT	5.370^{a}	3.088^{e}	3.494^{de}	-	-
Control, Cl_2, MAP, 1°C	5.122^{ab}	4.955^{ab}	4.744^{abc}	5.141^{abc}	5.111^{ab}
Control, Anolyte, MAP, 1°C	5.122^{ab}	4.327^{bcd}	3.985^{bc}	4.489^{cd}	5.774^{a}
Control, Cl_2, MAP, RT	5.122^{ab}	4.764^{abc}	5.297^{a}	4.156^{cde}	4.202^{cde}
Control, Anolyte, MAP, RT	5.122^{ab}	4.054^{cde}	4.777^{ab}	3.913^{cde}	4.139^{cde}
Control, H_2O, MAP, 1°C	5.122^{ab}	3.791^{de}	4.063^{bc}	5.968^{a}	4.885^{ab}
Control, H_2O, MAP, RT	5.122^{ab}	4.530^{bcd}	4.462^{abc}	3.999^{de}	3.535^{e}
Control, H_2O, 1°C	5.122^{ab}	4.468^{bcd}	4.285^{bc}	4.683^{cd}	4.648^{bcd}
Control, H_2O, RT	5.122^{ab}	3.278^{e}	3.652^{de}	-	-

Significance

Preharvest treatment (A)	NS
Disinfecting + MAP (B)	***
Storage temperature (C)	***
A X B	**
A X C	NS
B X C	**
A X B X C	NS

NS, **, *** Nonsignificant or significant at $P \leq 0.01$ or 0.001 respectively. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The LSD Value = 0.813, S.E. = 0.062, MSE = 0.254 and C.V. = 0.111.

After 28 days at room temperature, the sucrose content was significantly higher ($P \leq 0.05$) in ComCat® treated carrots dipped in anolyte water than when dipped in chlorinated and tap water. After approximately 18 days of storage at 1°C the sucrose contents in ComCat® treated carrots, dipped in chlorine supplemented water, dropped below those in control carrots which were also dipped in chlorinated water. The sucrose concentration remained significantly ($P \leq 0.05$) higher (by 15.4% after 28 days) in ComCat® treated carrots dipped in anolyte water and stored at room temperature, compared to control carrots that received the same postharvest treatments up to 28 days. During the first 2 weeks of storage at room temperature, sucrose concentrations decreased faster in ComCat® treated carrots dipped in chlorine supplemented water or water washed compared to those in control carrots subjected to the same postharvest treatment. In general, the ComCat® treatment had a positive effect on the sucrose content when coupled with anolyte water treatment after 2 weeks of storage at room temperature, although the differences in sucrose concentration in ComCat® and control carrots dipped in anolyte water was not significant ($P > 0.05$) on most of the storage intervals (Table 3.4 (a)).

Although the glucose content was significantly lower ($P \leq 0.001$), almost half, in ComCat® treated carrots at harvest, it did not differ much from the controls after 28 days of storage (Table 3.4 (b)). A general pattern of an increase in glucose contents of carrots was noticed during the first two to the third week of storage stating the obvious. The fructose content followed a similar trend (Table 3.4 (c)).

The preharvest ComCat® treatment had a significant ($P \leq 0.01$) effect on the changes in concentrations of glucose in carrots at both storage temperatures (Table 3.4. (b) and Appendix A.1). Closer inspection shows that the differences are mainly at harvest and not so much during storage. During the first 21 days of storage, glucose concentrations were higher in controls than in ComCat® treated carrots treated with chlorine and stored at 1°C (Table 3.4 (b)).

Table 3.4. (b). Changes in glucose content of carrots subjected to both pre-and postharvest treatments and stored at 1°C and room temperature (RT) for 28 days.

Treatment	Glucose (g.100^{-1}g)				
	day 0	day 7	day 14	day 21	day 28
ComCat®, Cl_2, MAP, 1°C	0.635^{b}	0.960^{bcd}	1.326^{abc}	2.758^{a}	1.084^{ab}
ComCat®, Anolyte, MAP, 1°C	0.635^{b}	1.198^{bcd}	1.084^{bcd}	1.778^{bc}	1.118^{ab}
ComCat®, Cl_2, MAP, RT	0.635^{b}	1.157^{bcd}	1.303^{abc}	1.514^{cde}	0.921^{ab}
ComCat®, Anolyte, MAP, RT	0.635^{b}	1.019^{bcd}	1.048^{bc}	1.467^{cde}	0.870^{bc}
ComCat®, H_2O, MAP, 1°C	0.635^{b}	0.834^{cd}	0.983^{bc}	2.177^{b}	1.315^{a}
ComCat®, H_2O, MAP, RT	0.635^{b}	1.835^{a}	0.903^{bc}	1.561^{cd}	0.959^{ab}
ComCat®, H_2O, 1°C	0.635^{b}	1.111^{bcd}	1.082^{bcd}	1.284^{cde}	1.434^{a}
ComCat®, H_2O, RT	0.635^{b}	1.283^{abc}	1.147^{bcd}	-	-
Control, Cl_2, MAP, 1°C	1.134^{a}	1.227^{abcd}	1.516^{a}	1.766^{bcd}	1.026^{ab}
Control, Anolyte, MAP, 1°C	1.134^{a}	1.148^{bcd}	1.270^{abc}	1.717^{bcd}	1.241^{a}
Control, Cl_2, MAP, RT	1.134^{a}	1.388^{abc}	1.324^{abc}	1.015^{de}	0.816^{bc}
Control, Anolyte, MAP, RT	1.134^{a}	1.186^{bcd}	1.385^{abc}	1.015^{de}	1.119^{ab}
Control, H_2O, MAP, 1°C	1.134^{a}	0.997^{bcd}	1.260^{abc}	2.300^{ab}	0.966^{ab}
Control, H_2O, MAP, RT	1.134^{a}	1.448^{ab}	1.487^{a}	1.347^{cde}	1.027^{ab}
Control, H_2O, 1°C	1.134^{a}	1.067^{bcd}	1.135^{bcd}	1.809^{bc}	1.186^{a}
Control, H_2O, RT	1.134^{a}	1.238^{abcd}	1.681^{a}	-	-

Significance

Preharvest treatment (A)	**
Disinfecting + MAP (B)	NS
Storage temperature (C)	*
A X B	NS
A X C	NS
B X C	*
A X B X C	NS

NS, *, ** Nonsignificant or significant at P≤ 0.05 or 0.01 respectively. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The LSD Value = 0.497, S.E. = 0.010, MSE = 0.095 and C.V. = 0.253.

The glucose content remained lower (except at 21 days interval) in ComCat®treated carrots dipped in chlorine solution compared to untreated carrots subjected to the same postharvest treatment and stored at room temperature. On the contrary, during the first 21 days at 1°C, the glucose consistently remained lower in water washed ComCat® treated carrots when compared to those concentrations in untreated carrots. Although some deviations were observed over some storage intervals, changes in glucose followed similar patterns in carrots dipped in anolyte water and stored at 1°C. However, after 28 days of storage the concentrations of glucose seemed to drop faster in the control carrots dipped in chlorinated and tap water than in ComCat® treated carrots stored at both temperatures. In general, the glucose content seemed to remain slightly lower during the first two weeks, and slightly higher during the third and fourth weeks of storage in control carrots than in ComCat® treated carrots.

The glucose content was maintained better in disinfected carrots during storage at 1°C compared to those washed in distilled water. Changes in glucose content in unpackaged carrots were not significantly different from packaged ones, and disinfecting + MAP also had no significant ($P > 0.05$) effect on the glucose content of carrots (Table 3.4 (b)). The results also show that the glucose content remained higher in ComCat® treated carrots, which were dipped in chlorinated water compared to those dipped in anolyte water during storage at both temperatures, except during the second and forth weeks of storage at 1°C. The two-way interaction between disinfecting + MAP and storage temperature had a significant influence on the changes in glucose content of carrots during storage.

The fructose content also showed a general increase up to week three of storage, followed by a decrease. The effect of ComCat® treatment on the changes in fructose content of carrots was significant at $P \leq 0.01$. Initially, ComCat® treated carrots had a lower fructose content compared to untreated carrots ($P > 0.05$) (Table 3.4 (c)). During storage of 7 days at 1°C, the fructose content remained lower in ComCat® treated carrots, but increased thereafter.

Table 3.4. (c). Changes in fructose content of carrots subjected to both pre-and postharvest treatments and stored at 1 °C and room temperature (RT) for 28 days.

Treatment	Fructose ($g.100^{-1}g$)				
	day 0	day 7	day 14	day 21	day 28
ComCat®, Cl_2, MAP, 1 °C	0.573^{ab}	0.858^{cd}	1.117^{a}	2.000^{a}	0.975^{abc}
ComCat®, Anolyte, MAP, 1 °C	0.573^{ab}	0.901^{bcd}	0.925^{abc}	1.296^{bcd}	0.951^{abc}
ComCat®, Cl_2, MAP, RT	0.573^{ab}	0.786^{cd}	0.919^{abc}	1.045^{def}	0.692^{bc}
ComCat®, Anolyte, MAP, RT	0.573^{ab}	0.960^{bcd}	0.805^{bc}	1.028^{def}	0.618^{c}
ComCat®, H_2O, MAP, 1 °C	0.573^{ab}	0.678^{d}	0.835^{bc}	1.470^{bc}	1.007^{ab}
ComCat®, H_2O, MAP, RT	0.573^{ab}	1.348^{a}	0.730^{bc}	1.120^{cde}	0.717^{bc}
ComCat®, H_2O, 1 °C	0.573^{ab}	0.836^{cd}	0.766^{bc}	0.952^{efg}	1.325^{a}
ComCat®, H_2O, RT	0.573^{ab}	1.094^{abc}	0.871^{abc}	-	-
Control, Cl_2, MAP, 1 °C	0.895^{a}	0.946^{bcd}	1.106^{a}	1.202^{cde}	0.863^{bc}
Control, Anolyte, MAP, 1 °C	0.895^{a}	0.921^{bcd}	0.967^{abc}	1.302^{bcd}	0.975^{abc}
Control, Cl_2, MAP, RT	0.895^{a}	1.009^{bc}	0.877^{abc}	0.795^{ef}	0.566^{c}
Control, Anolyte, MAP, RT	0.895^{a}	0.896^{bcd}	1.035^{ab}	0.831^{ef}	0.906^{bc}
Control, H_2O, MAP, 1 °C	0.895^{a}	0.877^{cd}	0.931^{abc}	1.622^{b}	0.852^{bc}
Control, H_2O, MAP, RT	0.895^{a}	1.113^{abc}	1.065^{ab}	0.995^{def}	0.818^{bc}
Control, H_2O, 1 °C	0.895^{a}	1.285^{ab}	0.901^{abc}	1.355^{bc}	1.017^{ab}
Control, H_2O, RT	0.895^{a}	1.038^{bc}	0.744^{bc}	-	-

Significance	
Preharvest treatment (A)	**
Disinfecting + MAP (B)	**
Storage temperature (C)	***
A X B	*
A X C	*
B X C	**
A X B X C	NS

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01 or 0.001 respectively. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The LSD Value = 0.327, S.E. = 0.010, MSE = 0.041 and C.V. = 0.215.

Mostly packaging slightly reduced the rate of fructose reduction during the storage at room temperature, compared to unpackaged carrots (Table 3.4 (c)). Disinfecting + MAP had a significant ($P \leq 0.01$) effect on the changes in fructose content of ComCat® treated and untreated carrots stored at 1°C and room temperature. Storage temperature was one of the dominant factors affecting the fructose concentration in carrots during storage ($P \leq 0.001$). The higher storage temperature increased the rate at which the sugars were depleted in carrots during storage. A rapid decrease in fructose content, especially after three weeks of storage, was evident from the data presented in Table 3.4 (c). During 21 days of storage at room temperature, the water washed control carrots had a higher fructose content compared to disinfected ones. With storage at room temperature, the water washed ComCat® treated carrots had a higher fructose content compared to the disinfected ones except during the second week of storage. Although no great difference was observed, fructose content remained higher in ComCat® treated disinfected carrots during the first 14 days at 1°C, and seemed to remain approximately the same thereafter.

3.3.3.4. Total soluble sugar

The total soluble sugar was calculated from the experimental sucrose, glucose and fructose content of carrots (Table 3.5). At harvest the total sugar was found to be 6.58 and 7.15 $g.100^{-1}g$ fresh weight for ComCat® treated and control carrots, respectively.

The preharvest ComCat® treatment had no significant ($P \geq 0.05$) effect on the total soluble sugar during storage. The ComCat® treated carrots dipped in chlorine water had a higher total soluble sugar from 2 weeks storage onwards at 1°C, when compared to control carrots dipped in chlorine. The total soluble sugar remained to be lower in packaged as well as unpackaged ComCat® treated, water washed carrots during 28 days at 1°C, although not significant ($p>0.05$). During the first 7 days at room temperature, the total soluble sugar was found in higher amounts in control carrots dipped in chlorinated or distilled water compared to the total soluble sugar in ComCat® treated carrots subjected to the same treatments. Thereafter, the concentration became higher in ComCat® treated carrots subjected to the same treatments.

Table 3.5. Changes in total sugar content of carrots subjected to both pre-and postharvest treatments and stored at 1 °C and room temperature (RT) for 28 days.

Treatment	Total soluble sugar (g.100^{-1}g FW)				
	Day 0	Day 7	Day 14	Day 21	Day 28
ComCat®, Cl_2, MAP, 1 °C	6.578^{ab}	6.072^{bcd}	6.448^{bcde}	8.780^{ab}	7.064^{abc}
ComCat®, Anolyte, MAP, 1 °C	6.578^{ab}	6.609^{abcd}	6.598^{bcde}	8.095^{abc}	6.699^{bcd}
ComCat®, Cl_2, MAP, RT	6.578^{ab}	6.065^{bcd}	7.366^{abc}	6.872^{cde}	5.681^{cd}
ComCat®, Anolyte, MAP, RT	6.578^{ab}	7.353^{a}	7.498^{a}	7.383^{bcd}	6.378^{bcd}
ComCat®, H_2O, MAP, 1 °C	6.578^{ab}	5.219^{d}	5.975^{bcde}	6.524^{cde}	5.855^{cd}
ComCat®, H_2O, MAP, RT	6.578^{ab}	6.909^{abc}	7.197^{abc}	7.260^{bcde}	5.650^{cd}
ComCat®, H_2O, 1 °C	6.578^{ab}	5.935^{bcd}	6.454^{bcde}	8.981^{a}	7.484^{ab}
ComCat®, H_2O, RT	6.578^{ab}	5.465^{cd}	6.221^{bcde}		
Control, Cl_2, MAP, 1 °C	7.151^{a}	7.128^{ab}	6.227^{bcde}	8.109^{abc}	7.000^{bcd}
Control, Anolyte, MAP, 1 °C	7.151^{a}	6.396^{bcd}	5.645^{bcde}	7.509^{bc}	7.989^{a}
Control, Cl_2, MAP, RT	7.151^{a}	7.162^{ab}	6.254^{bcde}	5.966^{de}	5.584^{cd}
Control, Anolyte, MAP, RT	7.151^{a}	6.136^{bcd}	7.014^{bcd}	5.760^{e}	6.164^{bcd}
Control, H_2O, MAP, 1 °C	7.151^{a}	5.665^{bcd}	5.585^{cde}	8.224^{ab}	6.703^{abcd}
Control, H_2O, MAP, RT	7.151^{a}	7.091^{ab}	6.320^{bcde}	6.341^{de}	5.380^{d}
Control, H_2O, 1 °C	7.151^{a}	6.820^{abcd}	5.512^{de}	7.847^{bc}	6.851^{abcd}
Control, H_2O, RT	7.151^{a}	5.554^{cd}	6.543^{bcde}		

Significance	
Preharvest treatment (A)	NS
Disinfecting + MAP (B)	**
Storage temperature (C)	***
A X B	NS
A X C	NS
B X C	NS
A X B X C	NS

NS, **, *** Nonsignificant or significant at $P \leq 0.01$ or 0.001 respectively. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The LSD Value = 1.251, S.E. = 0.066, MSE = 0.741 and C.V. = 0.129.

Disinfecting + MAP had a significant ($P < 0.001$) effect on the total soluble sugar. The ComCat® treated carrots dipped in chlorinated or anolyte water had a higher total soluble sugar content during 21 days at 1°C, when compared to those that were water washed. Similarly, the total soluble sugar was better maintained in control carrots dipped in chlorinated or anolyte water compared to water washed ones, both stored at 1°C for 28 days, except for the 21 days storage interval. The total soluble sugar in ComCat® treated carrots dipped in anolyte water remained higher than those in untreated carrots dipped in anolyte water and stored at 1°C up to the 21 days and room temperature up to 28 days, being significant for the latter ($P < 0.001$). The effect of MAP was positive on the changes in total soluble sugar of ComCat® treated carrots stored at room temperature, but no clear trends were obtained.

The storage temperature was highly significant ($P \leq 0.001$) on the changes in total soluble sugar content of carrots during storage (Table 3.5). In general, the total soluble sugar increased during storage at 1°C until 28 days, peaking at 21 days, whereas it decreased during storage at room temperature. After 28 days of storage, the percentage difference in total soluble sugar content in carrots stored at 1°C, as well as room temperature, was greater in control carrots than in ComCat® treated carrots. After 28 days of storage at 1°C, the total soluble sugar was 19.6%, 4.8% and 3.5% more in packaged ComCat® treated carrots dipped in chlorinated, anolyte and tap water, respectively, when compared to the ComCat® treated carrots subjected to the same postharvest treatments, but stored at room temperature.

The unpackaged ComCat® treated carrots dipped in tap water had 3.6% more total soluble sugar after 14 days of storage at 1°C compared to the concentrations of this sugar in the unpackaged ComCat® treated carrots stored at room temperature. The greatest difference in total soluble sugar (19.6%), due to the effect of storage temperature, was found for ComCat® treated carrots dipped in chlorinated water.

The packaged control carrots dipped in chlorinated, anolyte and tap water had 20.2%, 22.8% and 19.7% more total soluble sugar at the end of the 28 days of storage at

1 °C compared to the concentrations of this sugar in control carrots subjected to the same postharvest treatments and stored at room temperature. After the first 14 days of storage at 1 °C, the total soluble sugar was 19.7% more in unpackaged control carrots dipped in tap water compared to the concentrations in unpackaged control carrots stored at room temperature. Although these results are not significant, they indicated a tendency that the greatest effect of storage temperature on total soluble sugar content during storage, was on control carrots, rather than ComCat® treated ones.

The total sugar content was not significantly ($P > 0.05$) influenced due to the interaction between the preharvest ComCat® treatment and storage temperature. None of the interactions between pre- and postharvest treatment had a significant influence on the changes in total soluble sugar content of carrots during this short period of storage.

3.3.3.5. Sucrose-hexose ratio

At harvest, ComCat® treated carrots had a significantly ($P \leq 0.05$) higher, almost double, sucrose-hexose (SH) ratio (Table 3.6). During storage the SH was also significantly ($P \leq 0.05$) affected by the preharvest ComCat® treatment. The interaction between preharvest and postharvest disinfecting and MAP treatments had a significant ($P \leq 0.05$) effect on the changes in SH during storage. The two-way interaction between the postharvest treatments was highly significant ($P < 0.001$) on the changes of SH during storage.

3.3.3.6. Ascorbic acid content (AA)

The changes in ascorbic acid (AA) content of carrots in the course of storage were highly dependent ($P \leq 0.001$) on the storage temperatures (Table 3.7). As a general trend, the AA content of carrots decreased during storage at both temperatures. Although a general trend of decrease in AA of carrots was observed during storage at 1 °C, the rate of reduction with the progression of storage time was not significant ($P > 0.05$) (Appendix A1).

Table 3.6. Changes in sucrose-hexose ratio of carrots subjected to both pre-and postharvest treatments and stored at 1°C and room temperature (RT) for 28 days.

Treatment	Sucrose-hexose ratio				
	Day 0	Day 7	Day 14	Day 21	Day 28
ComCat®, Cl_2, MAP, 1°C	4.445^{a}	2.351^{abc}	1.712^{cde}	1.266^{c}	2.496^{abcd}
ComCat®, Anolyte, MAP, 1°C	4.445^{a}	2.158^{bcd}	2.364^{abc}	1.847^{abc}	2.237^{cde}
ComCat®, Cl_2, MAP, RT	4.445^{a}	2.124^{bcd}	1.978^{bcde}	1.684^{bc}	2.612^{abcd}
ComCat®, Anolyte, MAP, RT	4.445^{a}	2.714^{a}	2.378^{bcd}	1.954^{abc}	3.357^{a}
ComCat®, H_2O, MAP, 1°C	4.445^{a}	2.478^{ab}	2.567^{ab}	1.510^{bc}	1.856^{cde}
ComCat®, H_2O, MAP, RT	4.445^{a}	1.171^{e}	2.599^{a}	1.756^{abc}	2.399^{cd}
ComCat®, H_2O, 1°C	4.445^{a}	2.061^{bcde}	2.137^{bcd}	2.100^{abc}	1.720^{de}
ComCat®, H_2O, RT	4.445^{a}	1.298^{de}	1.746^{cde}		
Control, Cl_2, MAP, 1°C	2.525^{b}	2.276^{abc}	1.816^{bcde}	1.742^{bc}	2.709^{abc}
Control, Anolyte, MAP, 1°C	2.525^{b}	2.080^{bcd}	1.852^{bcde}	1.588^{bc}	2.681^{abc}
Control, Cl_2, MAP, RT	2.525^{b}	1.989^{cde}	2.415^{abc}	2.591^{a}	3.324^{ab}
Control, Anolyte, MAP, RT	2.525^{b}	1.944^{bcde}	1.989^{bcde}	2.225^{ab}	2.069^{cde}
Control, H_2O, MAP, 1°C	2.525^{b}	2.132^{bcd}	2.015^{bcde}	1.578^{bc}	2.712^{abc}
Control, H_2O, MAP, RT	2.525^{b}	1.769^{bcde}	1.795^{bcde}	1.881^{abc}	2.356^{cde}
Control, H_2O, 1°C	2.525^{b}	1.899^{abcde}	2.146^{bcde}	1.482^{bc}	2.144^{cde}
Control, H_2O, RT	2.525^{b}	1.455^{cde}	1.282^{e}		

Significance

Preharvest treatment (A)	*
Disinfecting + MAP (B)	**
Storage temperature (C)	NS
A X B	*
A X C	NS
B X C	***
A X B X C	NS

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01 or 0.001 respectively. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The LSD Value = 0.705, S.E. = 0.046, MSE = 0.191 and C.V. = 0.188.

The other important factor that significantly affected ($P \leq 0.05$) the AA content of carrots during storage, was disinfecting treatment (Table 3.7). The AA content of carrots dipped in chlorinated water decreased and remained lower during the storage period at both temperatures than in carrots dipped in anolyte water. The decrease in AA content of carrots dipped in either chlorinated or anolyte water was higher during storage at room temperature than at 1 °C.

The AA of carrots was not significantly ($P > 0.05$) affected by the preharvest ComCat® treatment during the storage period. The ComCat® treated carrots had approximately 0.5 $mg.100^{-1}g$ more ascorbic acid at harvest than the control carrots, suggesting that ComCat® treatment had a positive effect on the nutritional quality characteristics in terms of AA content. Although not significant ($P > 0.05$), a general trend of higher AA content was found in ComCat® treated carrots during the storage period of 28 days at both storage temperatures. However, some deviations in carrots dipped in chlorinated water and stored at room temperature were observed. The interactive effect of pre- and postharvest treatment had no significant ($P > 0.05$) influence on the changes in AA content of carrots (Table 3.7). Therefore, combining pre- and postharvest treatment showed to have strong benefits to consumers in terms of better nutritional value of carrots after storage.

3.3.3.7. Peroxidase activity (POX)

The activity of POX of carrots rapidly increased during the first one or two weeks of storage (Figure 3.4). The POX was found to be lower in carrots stored at room temperature during the first week of storage, when compared to the activities of POX in carrots stored at 1°C at a significance level of $P \leq 0.07$. Hence, it seemed that carrots stored at 1°C are suddenly exposed to different conditions than those in the soil, resulting in more respiratory enzyme activity during the early adjustment period. After one week of storage, the level of activities of POX seemed to equilibrate in carrots stored at 1°C, while it continued to increase in the carrots stored at room temperature

Table 3.7. Changes in ascorbic acid content (AA) of carrots subjected to both pre and postharvest treatment and stored at 1°C and room temperature (RT) for 28 days.

	Ascorbic acid content (mg.100^{-1}g FW)				
Treatment	Day 0	day 7	Day 14	day 21	day 28
ComCat®, Cl_2, MAP, 1°C	10.512^{a}	10.137^{a}	9.761^{a}	8.447^{abc}	9.011^{ab}
ComCat®, Anolyte, MAP, 1°C	10.512^{a}	9.855^{a}	9.949^{a}	9.386^{a}	9.574^{a}
ComCat®, Cl_2, MAP, RT	10.512^{a}	7.959^{cde}	7.957^{de}	5.913^{de}	3.942^{de}
ComCat®, Anolyte, MAP, RT	10.512^{a}	9.496^{ab}	9.386^{ab}	7.040^{bc}	4.224^{d}
Control, Cl_2, MAP, 1°C	9.574^{ab}	9.198^{ab}	9.157^{ab}	8.072^{bcd}	7.884^{bc}
Control, Anolyte, MAP, 1°C	9.574^{ab}	9.715^{a}	8.729^{ab}	9.011^{ab}	8.823^{ab}
Control, Cl_2, MAP, RT	9.574^{ab}	8.447^{bcd}	8.443^{cd}	6.180^{cde}	2.816^{e}
Control, Anolyte, MAP, RT	9.574^{ab}	8.898^{ab}	8.635^{ab}	6.814^{cd}	4.029^{d}

Significance

Preharvest Treatment (A)	NS
Disinfecting (B)	*
Storage Temperature (C)	***
A X B	NS
A X C	NS
B X C	NS
A X B X C	NS

NS, *, *** Nonsignificant or significant at $P \leq 0.05$ or 0.001 respectively. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The LSD Value = 2.065, S.E. = 0.252, MSE = 1.610 and C.V. = 0.150.

until two weeks of storage. Generally, the overall effect of storage temperature was highly significant ($P < 0.001$) on the changes in levels of POX of carrots during the third and fourth weeks of storage. The higher levels of POX in carrots stored at room temperature could indicate the increased oxidation status of the carrots compared to those stored at 1°C. In fact, the level of POX seemed to equilibrate in carrots stored at 1°C after one week of storage, and could be responsible for the protection of cell walls and normal respiration and metabolism during the long-term storage of carrots at this temperature. The level of activities of POX in carrots was not significantly ($P > 0.05$) affected due to the interactive effect between pre- and postharvest treatments with storage temperature.

The data presented in Figure 3.4 clearly demonstrated the effect of pre-packaging treatment (chlorinated or anolyte water) on the biochemical changes of carrots during storage. The overall effect of disinfecting treatment on the level of POX in carrots was significant ($P \leq 0.01$). The level of POX was generally higher in carrots dipped in chlorinated water, when compared to the POX in carrots dipped in anolyte water during storage at both temperatures, at a significance level of $P \leq 0.09$. At the end of day 7 at room temperature, the POX in carrots was significantly ($P \leq 0.09$) lower in carrots dipped in anolyte water. After 28 days at 1°C the levels of POX were again significantly lower in carrots dipped in anolyte water. The levels of POX in carrots were not significantly ($P > 0.05$) affected by the preharvest ComCat® treatment both at harvest as well as during storage.

The interactive effect of postharvest treatment with preharvest treatments was not significant ($P > 0.05$) on the changes of the level of POX in carrots during storage. This similarity in their biochemical changes could suggest that the shelf life of carrots treated with ComCat® was as good as normal carrots at both temperatures. Polygalacturonase activity was also tested for, but was not detected in carrots during storage, supporting the previous findings reported by Stratilova *et al.* (1998).

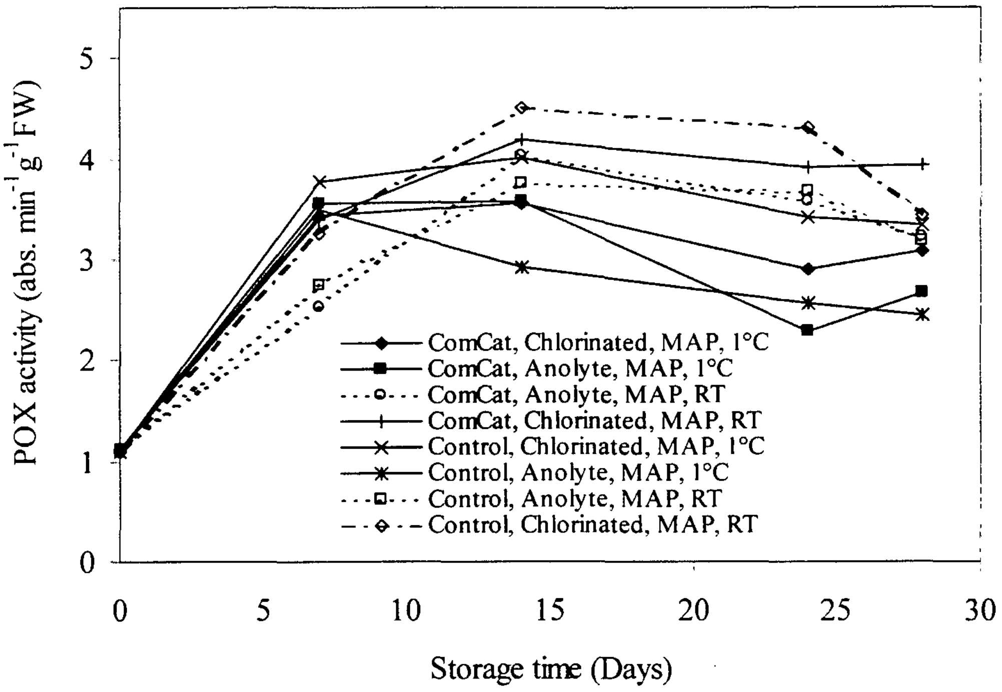


Figure 3.4. Changes in levels of activities of peroxidase in carrots subjected to different pre- and postharvest treatments and stored at 1°C and room temperature (RT) for 28 days.

Significance level showing the effect of pre- and postharvest treatment on POX activity	
Significance	
Preharvest Treatment (A)	NS
Disinfecting (B)	**
Storage Temperature (C)	***
A X B	NS
A X C	NS
B X C	NS
A X B X C	NS

NS, **, *** Nonsignificant or significant at $P \leq 0.01$ or 0.001 respectively. The $LSD_{0.05}$ Value = 0.517, S.E. = 0.063 and C.V. = 0.171.

3.3.4. Microbiological changes

Storage temperature was one of the most important factors that significantly ($P \leq 0.001$) controlled the growth of microorganisms (Table 3.8). Total aerobic bacteria grew and exceeded initial populations in packaged as well as unpackaged carrots stored at room temperature. The overall treatment means for room temperature and 1°C storage proved that cold storage was highly significant ($P \leq 0.001$) in controlling growth of microorganisms. The populations of total aerobic bacteria in carrots stored at room temperature was more than 40.5% higher after 28 days, than in carrots stored at 1°C.

The unpackaged carrots allowed greater growth of total aerobic bacteria during storage at 1°C, except for the 14th day storage interval for control carrots, although this was not significant ($P > 0.05$). However, packaging allowed significantly ($P \leq 0.05$). greater growth of total aerobic bacteria in control carrots when stored at room temperature. In general, this showed that MAP helped to control the aerobic bacteria during storage of carrots at 1°C. The population of total aerobic bacteria remained higher (by 7.0% after 14 days) in packaged control carrots stored at room temperature, than in storage without packaging.

The effect of disinfecting and MAP treatment was highly significant ($P \leq 0.001$) on reduction of aerobic bacteria on carrots during storage. The chlorinated and anolyte water significantly ($P \leq 0.001$) reduced the populations of aerobic bacteria in carrots stored at both temperatures in comparison with the populations of these microorganisms associated with water washed carrots. The populations of aerobic bacteria decreased in carrots dipped in chlorinated and anolyte water during storage at 1°C and did not exceed initial populations at this temperature. The efficacy of chlorinated water on the killing and suppression of the growth of total aerobic bacteria was not significantly ($P \geq 0.05$) different from that of anolyte water as a disinfectant (Table 3.8).

Although not significant ($P > 0.05$), the populations of aerobic bacteria were slightly, 3.5%, higher in control samples than in ComCat® treated carrots at harvest. However, the ComCat® treatment had a significant ($P \leq 0.05$) effect on the total aerobic bacteria

Table 3.8. Total aerobic bacteria populations on carrots dipped in chlorinated and anolyte water and storage at 1 °C and room temperature (RT) for 28 days.

	Total aerobic bacteria (log CFU.g^{-1} FW)				
Treatment	Day 0	Day 7	day 14	day 21	Day 28
ComCat®, Cl_2, MAP, 1°C	4.408^{ab}	2.076^{jk}	1.544^{m}	2.458^{mno}	2.513^{jkl}
ComCat®, Anolyte, MAP, 1°C	4.408^{ab}	1.541^{k}	1.724^{lm}	2.427^{no}	2.134^{klm}
ComCat®, Cl_2, MAP, RT	4.408^{ab}	3.604^{h}	4.832^{def}	5.659^{cd}	4.843^{ef}
ComCat®, Anolyte, MAP, RT	4.408^{ab}	4.131^{gh}	4.765^{ef}	5.372^{e}	4.796^{f}
ComCat®, H_2O, MAP, 1°C	4.408^{ab}	4.627^{f}	3.261^{ghi}	3.401^{hijk}	3.272^{gh}
ComCat®, H_2O, MAP, RT	4.408^{ab}	6.330^{a}	6.073^{ab}	6.112^{abc}	5.942^{abc}
ComCat®, H_2O, 1°C	4.408^{ab}	4.942^{ef}	3.371^{ghi}	3.601^{hi}	3.348^{gh}
ComCat®, H_2O, RT	4.408^{ab}	6.328^{a}	6.251^{ab}	-	-
Control, Cl_2. MAP, 1°C	4.568^{a}	2.183^{ij}	1.993^{ij}	2.463^{no}	2.534^{jkl}
Control, Anolyte, MAP, 1°C	4.568^{a}	1.954^{jk}	2.065^{ij}	2.540^{no}	2.865^{ijk}
Control, Cl_2, MAP, RT	4.568^{a}	4.896^{f}	4.601^{lf}	5.196^{f}	5.412^{cde}
Control, Anolyte, MAP, RT	4.568^{a}	4.363^{gh}	4.566^{f}	4.798^{g}	4.814^{ef}
Control, H_2O, MAP, 1°C	4.568^{a}	4.905^{f}	3.765^{g}	3.642^{h}	3.027^{hij}
Control, H_2O, MAP, RT	4.568^{a}	6.016^{a}	6.340^{a}	6.618^{a}	6.268^{a}
Control, H_2O, 1°C	4.568^{a}	$5.468b^{cd}$	3.444^{gh}	3.738^{h}	3.397^{gh}
Control, H_2O, RT	4.568^{a}	5.291^{ef}	5.886^{abc}	-	-
Significance					
Preharvest treatment (A)		*			
Disinfecting + MAP (B)		***			
Storage temperature (C)		***			
A X B		**			
A X C		***			
B X C		***			
A X B X C		***			

*, **, *** Significant at $P \leq 0.05$, 0.01 or 0.001 respectively. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The LSD Value = 0.513, SE= 0.051, MSE = 0.101 and C.V. = 0.074.

on carrots during storage. The numbers of viable aerobic bacteria was consistently higher in the control carrots dipped in chlorine supplemented and anolyte water during storage at 1 °C, when compared to the populations of aerobic bacteria in ComCat® treated carrots dipped in the same disinfectants. The populations of aerobic bacteria remained to be lower in ComCat® treated carrots dipped in tap water during the first 16 days of storage at 1 °C, after which the populations became approximately equal in both ComCat® treated and control carrots, although not significantly different ($P > 0.05$). The unpackaged ComCat® treated carrots had significantly ($P \leq 0.05$) higher populations of aerobic bacteria during 16 days compared to those in control carrots stored at room temperature.

The two-way interaction between ComCat® treatment and disinfecting + MAP was highly significant ($P \leq 0.001$) on the changes of the populations of total aerobic bacteria. Populations of total aerobic bacteria were also significantly ($P \leq 0.001$) influenced with the interaction between ComCat® treatment and storage temperature. Similarly, the interactive effect of postharvest treatments (disinfecting, MAP and storage temperature) highly ($P \leq 0.001$) reduced the growth of aerobic bacteria during storage. The three-way interactions of preharvest and postharvest treatment had a significant ($P \leq 0.01$) effect on the changes in the populations of total aerobic bacteria in packages of carrots during 28 days of storage.

The population of moulds and yeast was also significantly ($P \leq 0.001$) affected with storage temperature (Table 3.9). Higher storage temperature favoured growth of moulds and yeasts on carrots. After 28 days at 1 °C, there were 25% to 46.9% more populations of moulds and yeasts in ComCat® treated and untreated carrots dipped in tap water and stored at room temperature compared to those stored at 1 °C, respectively. The number of yeasts and moulds decreased much and remained lower after disinfecting when stored at 1 °C.

Table 3.9. Changes in the population of yeasts and moulds in carrots subjected to different disinfecting treatments and stored at 1°C and room temperature (RT) for 28 days.

	Total Fungi Count (log CFU.g^{-1} FW)				
Treatment	day 0	day 7	day 14	day 21	day 28
ComCat®, Cl_2, MAP, 1°C	3.934^{a}	1.439^{ghi}	1.541^{fg}	1.673^{fgh}	1.831^{efg}
ComCat®, Anolyte, MAP, 1°C	3.934^{a}	1.511^{ghi}	1.424^{g}	1.634^{gh}	1.501^{fg}
ComCat®, Cl_2, MAP, RT	3.934^{a}	3.093^{de}	3.269^{c}	3.431^{abc}	4.083^{a}
ComCat®, Anolyte, MAP, RT	3.934^{a}	3.476^{de}	3.002^{cd}	3.244^{bc}	3.473^{bc}
ComCat®, H_2O, MAP, 1°C	3.934^{a}	2.322^{f}	3.052^{cd}	2.997^{bcd}	2.855^{cd}
ComCat®, H_2O, MAP, RT	3.934^{a}	3.988^{bcd}	4.287^{ab}	4.120^{a}	3.823^{ab}
ComCat®, H_2O, 1°C	3. 934^{a}	2.818^{ef}	2.467^{de}	2.793^{cde}	2.475^{de}
ComCat®, H_2O, RT	3. 934^{a}	5.589^{a}	4.488^{a}	-	-
Control, Cl_2, MAP, 1°C	3.866^{a}	0.952^{i}	1.842^{fg}	1.695^{fgh}	2.244^{def}
Control, Anolyte, MAP, 1°C	3.866^{a}	1.073^{ghi}	1.415^{g}	1.598^{gh}	2.179^{def}
Control, Cl_2, MAP, RT	3.866^{a}	2.580^{ef}	2.688^{cde}	3.886^{ab}	3.823^{ab}
Control, Anolyte, MAP, RT	3.866^{a}	2.282^{fg}	2.678^{cde}	3.797^{ab}	3.637^{ab}
Control, H_2O, MAP, 1°C	3.866^{a}	2.077^{fg}	2.126^{ef}	2.167^{efg}	2.270^{def}
Control, H_2O, MAP, RT	3.866^{a}	4.228^{bc}	4.118^{ab}	3.992^{a}	4.272^{a}
Control, H_2O, 1°C	3. 866^{a}	2.769^{ef}	2.414^{de}	2.270^{ef}	3.078^{bcd}
Control, H_2O, RT	3. 866^{a}	5.211^{ab}	4.148^{ab}	-	-

Significance	
Preharvest treatment (A)	NS
Disinfecting + MAP (B)	***
Storage temperature (C)	***
A X B	NS
A X C	*
B X C	NS
A X B X C	***

NS, *, *** Nonsignificant or significant at $P \leq 0.05$ or 0.001 respectively. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The LSD Value = 0.633, SE= 0.053, MSE = 0.154 and C.V. = 0.126.

The numbers of fungi were significantly ($P \leq 0.001$) affected with chlorinated and anolyte water dipping treatments as compared to those in water washed controls. Significantly ($P \leq 0.001$) higher numbers of yeast and mould populations were found in water washed than in disinfected carrots when stored at 1°C temperature. The efficacy of chlorinated and anolyte water in reducing the number of yeasts and moulds was not significantly ($P > 0.05$) different during the storage period of 28 days. Yeast and mould growth was not significantly ($P > 0.05$) affected by the preharvest ComCat® treatment.

The interactive effect of pre- and postharvest treatments significantly improved the microbiological quality of carrots in terms of fewer populations of fungi. The three-way interaction between all the pre- and postharvest treatments was highly significant ($P \leq 0.001$) on the growth of fungi on carrots during storage.

The storage temperature greatly affected ($P \leq 0.001$) the populations of coliforms as shown in Table 3.10. Similar trends were obtained for control carrots during storage at 1°C as well as at room temperature. The results show that higher temperature storage combined with MAP favoured more growth of coliforms in control carrots. MAP coupled with cold storage temperature suppressed the growth of coliforms as compared to storage at room temperature.

Although in most of the instances the populations of total coliforms in carrots seemed to be lower in packaged carrots than in unpackaged carrots stored at 1°C and room temperature, these trends were not consistent for all the treatments. Disinfecting + MAP treatments resulted in a significant ($P \leq 0.001$) reduction of the populations of total coliform bacteria. Treatment with chlorinated or anolyte water reduced ($P \leq 0.001$) the numbers of coliforms compared to water washed carrots. After 28 days at 1°C, the populations of coliform were significantly ($P \leq 0.05$) lower in carrots dipped in chlorinated or anolyte water, when compared to water washed ones, but not in carrots stored at room temperature.

Table 3.10. Total coliform populations on carrots dipped in chlorinated and anolyte water and storage at 1°C and room temperature (RT) for 28 days.

	Total coliform bacteria (log CFU.g^{-1} FW)				
Treatment	day 0	day 7	Day 14	day 21	day 28
ComCat®, Cl_2, MAP, 1°C	3.400^{ab}	0.492^{i}	0.515^{k}	2.458^{efg}	1.700^{ijk}
ComCat®, Anolyte, MAP, 1°C	3.400^{ab}	1.798^{gh}	1.800^{ij}	2.427^{efg}	1.399^{jk}
ComCat®, Cl_2, MAP, RT	3.400^{ab}	2.802^{de}	3.552^{cd}	2.573^{defg}	3.508^{abcd}
ComCat®, Anolyte, MAP, RT	3.400^{ab}	2.725^{de}	2.728^{efg}	2.971^{bcde}	3.024^{bcde}
ComCat®, H_2O, MAP, 1°C	3.400^{ab}	3.255^{cd}	3.060^{def}	3.734^{ab}	2.722^{defg}
ComCat®, H_2O, MAP, RT	3.400^{ab}	3.517^{bc}	3.491^{de}	3.870^{a}	4.150^{a}
ComCat, H_2O, 1°C	3.400^{ab}	3.321^{cd}	2.350^{fgh}	3.268^{abcd}	3.348^{bcde}
ComCat®, H_2O, RT	3.400^{ab}	3.996^{ab}	5.065^{a}	-	-
Control, Cl_2, MAP, 1°C	3.449^{a}	1.837^{fgh}	1.989^{ghi}	2.463^{efg}	1.088^{kl}
Control, Anolyte, MAP, 1°C	3.449^{a}	1.994^{fg}	2.035^{fgh}	2.133^{fgh}	1.725^{ijk}
Control, Cl_2, MAP, RT	3.449^{a}	2.198^{efg}	2.254^{fgh}	3.290^{abcd}	3.492^{abcd}
Control, Anolyte, MAP, RT	3.449^{a}	2.707^{de}	2.731^{efg}	3.941^{a}	3.256^{bcde}
Control, H_2O, MAP, 1°C	3.449^{a}	3.167^{cde}	3.213^{de}	3.093^{bcde}	2.785^{cdef}
Control, H_2O, MAP, RT	3.449^{a}	4.305^{a}	4.520^{ab}	4.217^{a}	3.691^{abc}
Control, H_2O, 1°C	3.449^{a}	3.964^{ab}	3.444^{de}	3.072^{bcde}	3.304^{bcde}
Control, H_2O, RT	3.449^{a}	4.128^{a}	4.225^{ab}	-	-

Significance	
Preharvest treatment (A)	*
Disinfecting + MAP (B)	***
Storage temperature (C)	***
A X B	NS
A X C	NS
B X C	***
A X B X C	**

NS, *, **, *** Nonsignificant or significant at $P \leq 0.01$, 0.05 or 0.001 respectively. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The LSD Value = 0.740 S.E. = 0.049, MSE = 0.210 and C.V. = 0.147.

As a general trend, populations of total aerobic and coliform bacteria remained lower in disinfected ComCat® treated carrots in comparison with control carrots stored at 1°C ($P \leq 0.05$). The interaction between postharvest treatments had a highly significant ($P \leq 0.001$) influence on the reduction of growth of total coliforms.

The three-way interaction between pre- and postharvest treatments was highly significant ($P \leq 0.001$) on the total coliform populations during storage. No viable *E. coli* was found in any one of the samples tested during the storage period.

3.3.5. Subjective quality attributes

The visual appearance observations on carrots are presented in Table 3.11. There were distinctively different types of visual qualities, especially based upon the different postharvest treatments and storage temperature. The appearance of carrots remained excellent during the 4 weeks of storage at 1°C. The visual appearance of carrots was highly affected by storage temperature. Visible mould growth and sprouting were serious problems encountered with carrots stored at room temperature. These carrots became dull with a firm surface with soft inner tissues. Carrots stored at 1°C temperature remained firm, shiny and juicy during the 28 days of storage.

MAP improved the visual appearance of carrots during storage at 1°C, when compared to the quality of unpackaged carrots (Table 3.11). The packaged carrots were firm and shiny during storage at 1°C, while unpackaged carrots became soft and bendable after a few weeks of storage at 1°C. It was noticed that the softening process was faster in smaller carrots than in larger ones. Similarly, the visual appearance of packaged carrots also remained better during storage at room temperature.

In open air there was excessive moisture loss, which resulted in dehydration and shrivelling within a few days. Also, there was visible mould growth during the first few days, which was soon slowed down due to dehydration of the carrots. MAP, when combined with disinfecting treatment, improved the descriptive quality characteristics of carrots during storage especially at 1°C (Table 3.11).

Table 3.11. Changes in descriptive quality attributes of pre- and postharvest treated carrots after 28 days of storage at 1°C and room temperature (RT).

Treatment	Final state of carrots	Rating
ComCat®, Cl_2, MAP, 1°C	very shiny, firm and large size, slightly etched	very good
ComCat®, Anolyte, MAP, 1°C	very shiny, firm and large size	excellent
ComCat®, Cl_2, MAP, RT	most sprouting, rooting, slight mold, etched, some dull, firm on surface, some soft interior tissue	fair
ComCat®, Anolyte, MAP, RT	most sprouting, rooting, some dull, firm on surface, some soft interior tissue	good
ComCat®, H_2O, MAP, 1°C	shiny, firm	very good
ComCat®, H_2O, MAP, RT	most sprouting, rooting, enhanced mould growth, most dull, some decay, firm on surface, some soft interior tissue	fair
ComCat®, H_2O, 1°C	some soft, marketable	fair
ComCat®, H_2O, RT	moulds growth first, shrivelling, some dried out, all unmarketable	poor
Control, Cl_2, MAP, 1°C	very shiny, some, firm and small size	very good
Control, Anolyte, MAP, 1°C	very shiny, firm and small size,	excellent
Control, Cl_2, MAP, RT	most sprouting, rooting, slight mold, etched, some dull, firm on surface, some soft interior tissue	fair
Control, Anolyte, MAP, RT	most sprouting, rooting, some dull, firm on surface, some soft interior tissue	good
Control, H_2O, MAP, 1°C	shiny, firm,	very good
Control, H_2O, MAP, RT	most sprouting, rooting, enhanced mould growth, most dull, some decay, firm on surface, some soft interior tissue	fair
Control, H_2O, 1°C	most soft, light and some marketable	fair
Control, H_2O, RT	moulds growth first, excessively shrivelling, most dried out, all unmarketable	poor

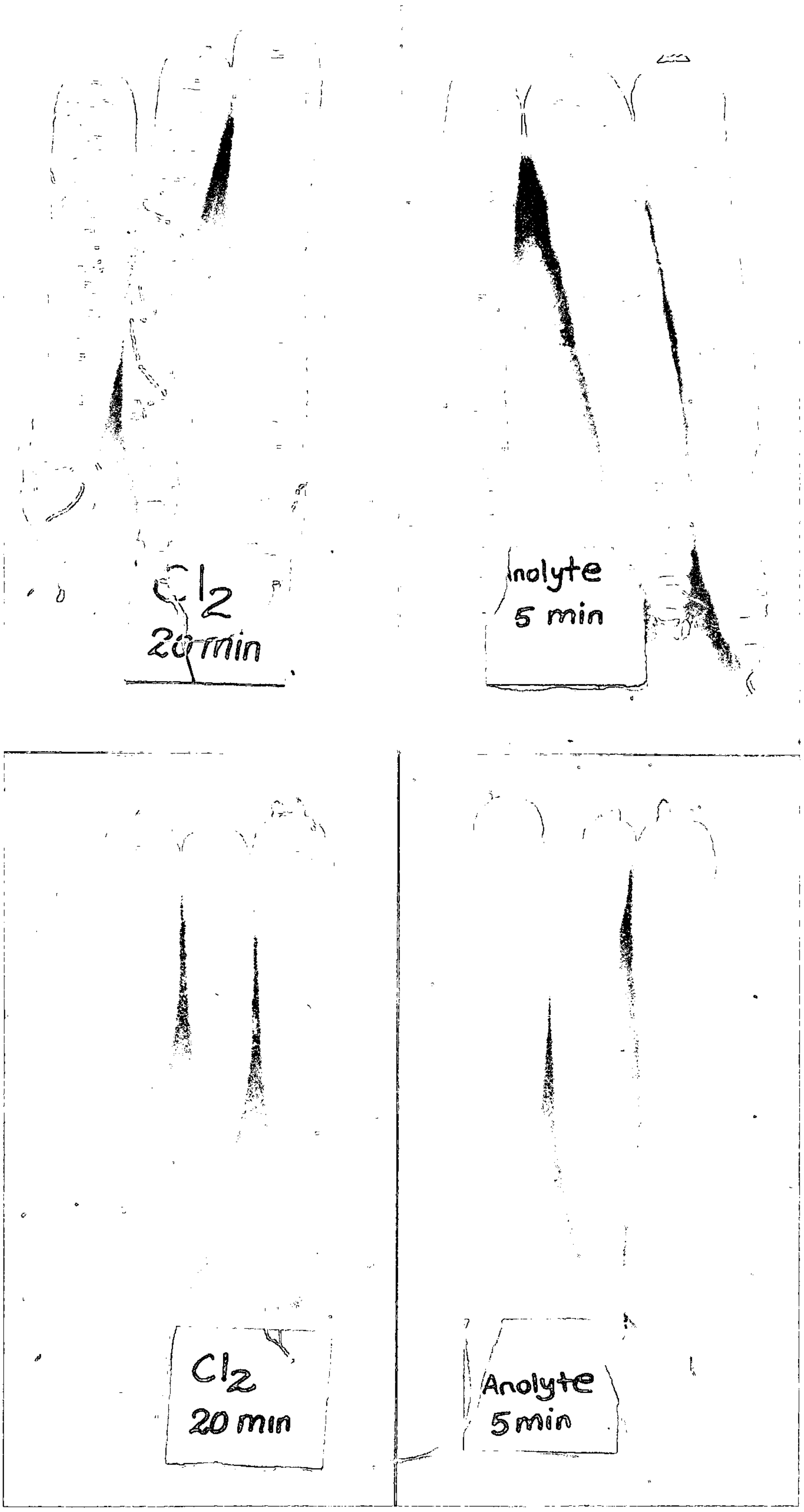


Figure 3.5. The effect of chlorinated and anolyte water dipping treatment on the visual appearance of carrots stored at 1°C for 8 days (above) and 16 days (below), showing the etched surface of the chlorine treated carrots.

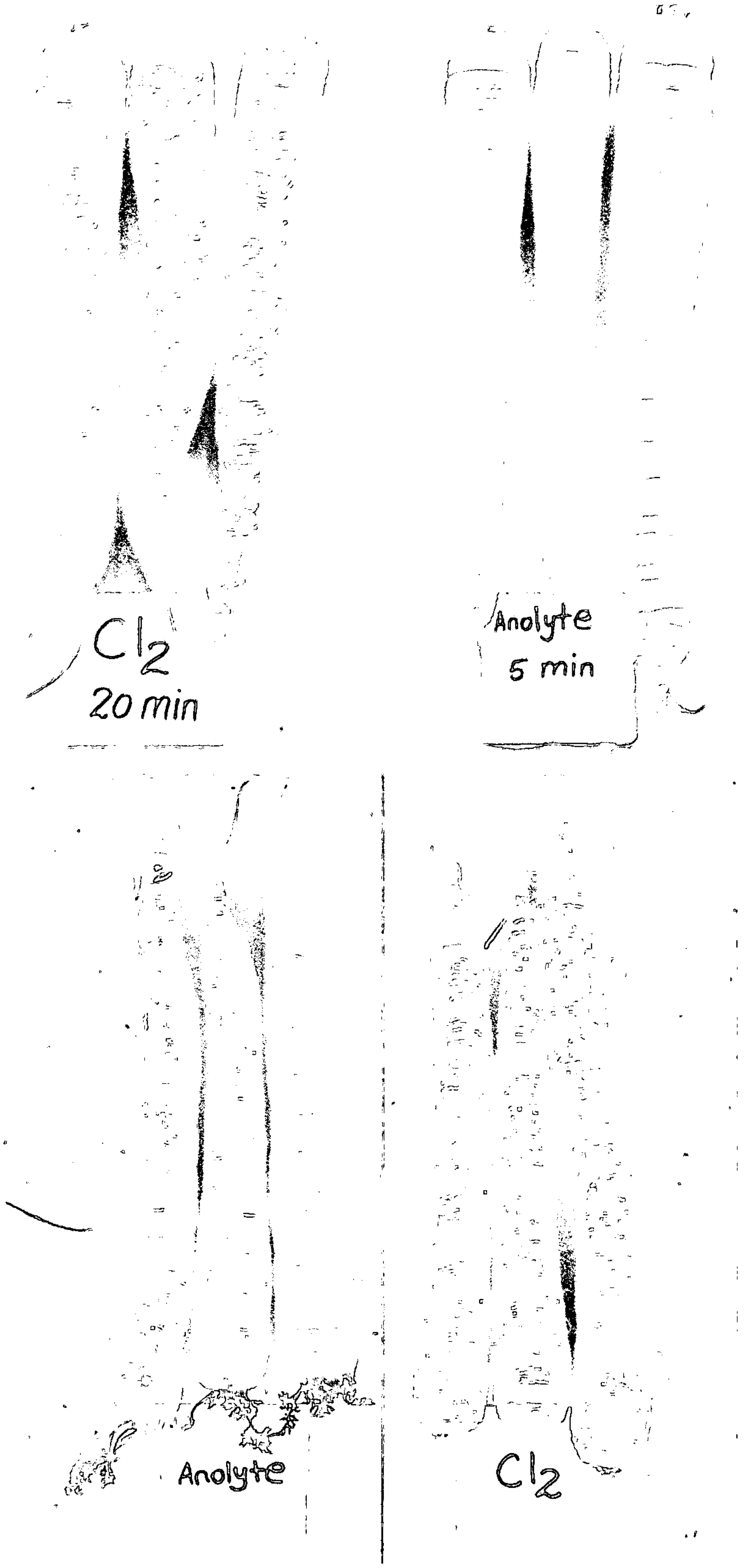


Figure 3.6. The effect of chlorinated and anolyte water dipping treatment on the visual appearance of carrots stored at room temperature for 8 days (above) and 16 days (below), showing the etched surface of the chlorine treated carrots.

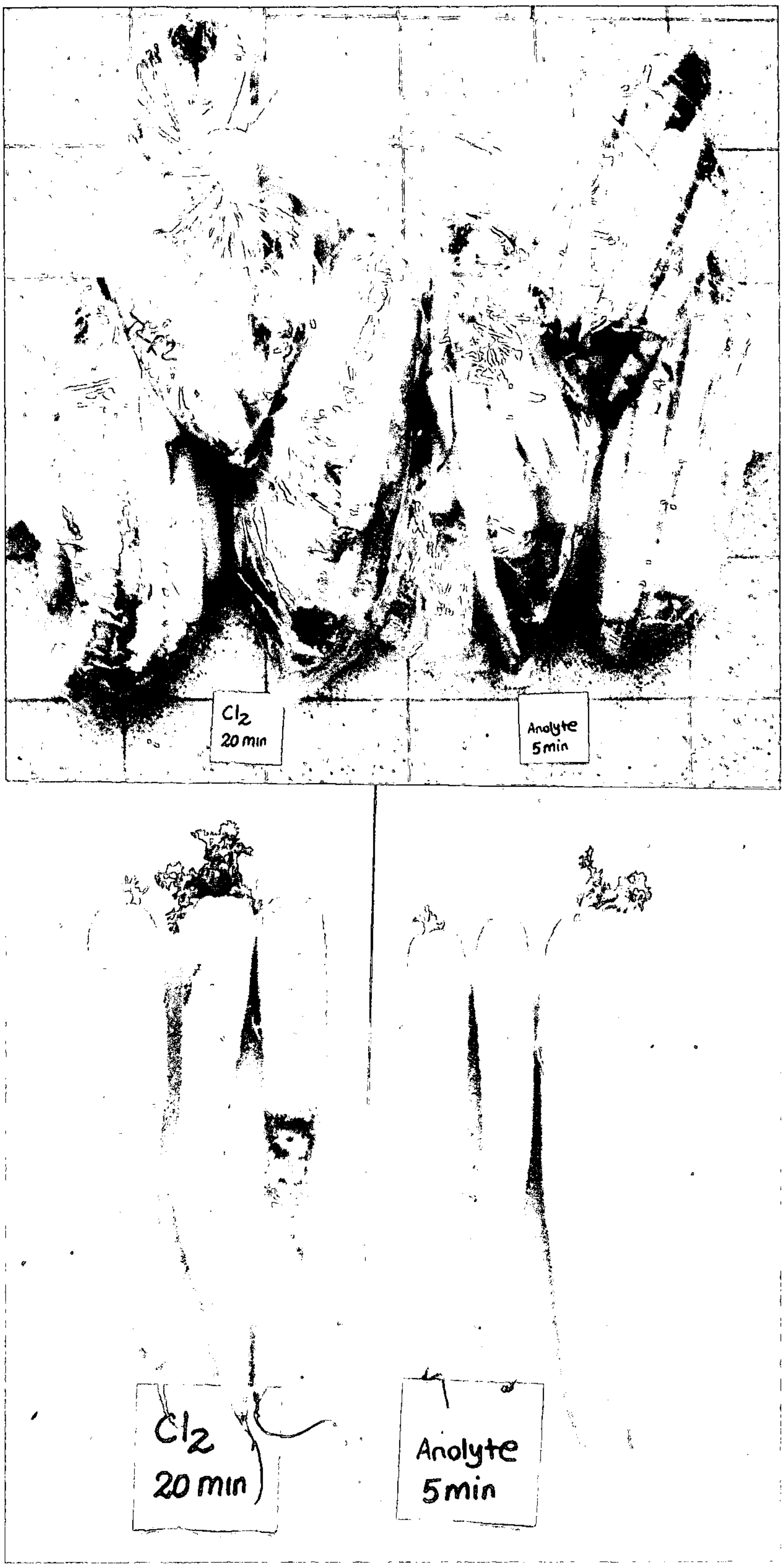


Figure 3.7. The effect of chlorinated and anolyte water dipping treatment on the visual appearance of carrots stored at room temperature for 30 days, showing the levels of decay and moulds growth on the chlorine treated carrots.

The anolyte water treatment coupled with MAP and low temperature storage treatment showed the best visual appearance quality of carrots and was the optimum operation for shelf life improvement of carrots. During storage at 1°C, carrots dipped in anolyte water had a better visual appearance when compared to carrots dipped in chlorinated water (Figure 3.5). The carrots dipped in chlorinated water for 20 minutes had etched marks while the carrots dipped in anolyte water remained shiny and smooth. The etching effect of chlorine on the surface tissue of carrots was more explicit when stored at room temperature (Figure 3.6) compared to storage at 1°C (Figure 3.5). The carrots dipped in anolyte water had no visual sign of decay after 30 days at room temperature while carrots dipped in chlorinated water had signs of wet rot decay (Figure 3.7). Carrots dipped in anolyte water began to sprout one week earlier than the carrots dipped in chlorinated water (Figure 3.6).

3.4. Discussions

The permeability and microperforations of the Xtend® packaging film used, allowed sufficient O_2 and CO_2 concentrations in the headspace of packages of carrots, which was enough to balance the normal respiration of carrots (Figure 3.1-3.3). The O_2 and CO_2 concentrations remained above 15% and below 5% during the storage period of 32 days, respectively, in packages of carrots (Figure 3.1 and 3.2). This is an indication for normal respiration of carrots in microperforated film during storage without the incidence of anaerobic metabolism (Exama *et al.*, 1993), and was in the same order as recommended by other researchers (Berg and Lentz, 1966; Phan, 1994). The ability of this microperforated film to control the respiration gases (CO_2 and O_2) established within the sealed bags (Figure 3.1 and 3.2), maintained the freshness quality of carrots (Table 3.11) without the incidence of anaerobic metabolism and decomposition, and is in agreement with the views of Geeson (1988) who suggested suitable permeable plastic film for the keeping quality of vegetables. At room temperature the Xtend® film did not cause excessive depletion of O_2 and accumulation of CO_2, although the rate of respiration of the carrots were higher (Wiley, 1994).

The Xtend® film not only maintained optimum gas composition for normal respiration but also reduced PWL from carrots during storage at both storage temperatures (Figure 3.1), and no vapour condensation was observed. The unpackaged carrots stored at room temperature lost the highest moisture content, while the lowest rate of moisture transfer was observed for packaged carrots stored at 1°C. Similar trends in changes in PWL from green chile stored at 8°C and 24°C were previously reported by Wall and Berghage (1996). These data are in agreement with that of Kader et al. (1989) and Jeon and Lee (1999). This has its own great advantage because the loss of moisture of unpackaged carrots not only resulted in fruits and vegetable shrinkage, dehydration and softening (Table 3.2 and 3.11), but triggers the other physiological and biochemical processes (Table 3.4 and Figure 3.4), which was also shown by Ben-Yehoshua (1967).

The TSS content of carrots was significantly ($P \leq 0.001$) increased during storage especially at room temperature (Table 3.2), which is in agreement with other research (Lingaiah and Huddar, 1991; Jitender-Kumar *et al.*; 1999) that the TSS content of carrots increases with the progression of storage duration, as well as storage temperature. MAP significantly ($P \leq 0.05$) affected the changes in TSS of carrots during storage at 1°C as well as room temperature (Table 3.2). The increase in TSS of unpackaged carrots was rapid, while the TSS content of packaged carrots was maintained better during storage. The excessive increase in TSS of carrots during storage is an indication of quality deterioration (Pal and Roy, 1988; Wasker et al., 1999). The changes in TSS content of carrots and the PWL had a direct relationship during storage (Table 3.1 and 3.2). Such a correlation was reported earlier by Jitender-Kumar *et al.* (1999), that the TSS increases with increasing PWL. The evidences obtained that the TSS increased with the increase of PWL of carrots during storage suggests the importance of reducing PWL through increasing storage air relative humidity and by the use of MAP. The increase in TSS of carrots may partially result from desiccation of carrots, which in turn leads to a concentrating effect on the TSS content. Since packaging prevented excessive moisture loss, the TSS content of carrots was maintained better in samples stored at 1°C (Table 3.2).

The TAC content rapidly decreased within the first two weeks of storage (Table 3.3). This decrease was reported to be due to the conversion of carbohydrates into free sugars, such as sucrose, fructose and glucose (Nilsson, 1987; Suojala, 2000). Phan *et al.* (1973) have shown that the changes in TAC content was due to the transfer of the carrots from underground conditions to atmospheric environment, whereupon active respiration takes place, which causes a demand for low molecule weight sugars, as is discussed below for individual sugars. The TAC content of carrots was slightly lower ($P \geq 0.05$) in unpackaged than in packaged ones, especially towards the end of the storage period (Table 3.3). In general, this result showed that MAP reduced the rate of conversion of TAC into free sugars during storage at both temperatures, when compared to unpackaged ones.

While the TAC decreased in packaged carrots, the sucrose content seemed to be consistently higher in packaged carrots, while the glucose and fructose content showed higher concentrations in unpackaged carrots during most of the storage intervals (Table 3.4 (a-c). These changes in carbohydrates can be explained by a higher metabolic activity in unpackaged carrots with a subsequent hydrolysis of carbohydrates.

The excessive moisture loss in unpackaged carrots may also contribute to the concentration of free sugars in unpackaged carrots (Table 3.1, 3.4 b and c). Similarly, the total soluble sugar was found to be slightly higher in unpackaged carrots (Table 3.5) while these carrots had a lower TAC content (Table 3.3). This data thus provides evidence that there was more hydrolysis of carbohydrates, e.g. starch, into glucose and fructose at higher rate than the amount of free sugar required for the glycolysis process of the respiration pathway. Due to higher hexose sugar content and lower concentrations of sucrose in unpackaged carrots, the SH generally remained lower in unpackaged carrots (Table 3.6), which is in agreement with the results of Nilsson (1987) and Suojala (2000), who reported that the SH sharply decreased during the early stages of storage.

Conversely, Carlin *et al.* (1990) and Chervin and Boisseau (1994) reported that sucrose, glucose and fructose rapidly decreased in minimally processed carrots, which was mainly as a result of a high respiration rate due to wounds and losses of nutrients (leakage) associated with this product. In the current results, as well as the data reported by Nilsson (1987) and Suojala (2000), the SH seemed to equilibrate towards the later storage period. This was because the fructose and glucose contents of carrots rapidly increased during this period, while the sucrose content of carrots was decreased, mainly during the early storage period. The SH of carrots stored both at 1°C as well as room temperature, rapidly decreased during the first week of storage, and showed a general decrease during the following two weeks. These results were in agreement with the previous findings by Nilsson (1987) and Suojala (2000), who reported that the SH ratio sharply decreased during the early stages of storage. In these results, as well as the data reported by Nilsson (1987) and Suojala (2000), the SH seemed to equilibrate towards the later storage period. This is because the fructose and glucose contents of carrots rapidly increased during this period, while sucrose contents of carrots decreased, especially during the first week of storage. The SH increased or equilibrated after three weeks of storage, which was due to either an increase in sucrose, or a decrease in fructose and glucose contents of carrots. The increasing effect of hexose on the total sugar accumulation during the storage period was noticed. In general, the stabilisation of SH after a few days of storage, could indicate that carrots need a self-adjustment time after being dug from the soil, separated from leaves and exposed to the open air, before finally having normal respiration and metabolism rates.

As a general trend, the total aerobic bacteria, and mould and yeast populations were higher in unpackaged carrots during storage at 1°C (Table 3.8 and 3.9). The higher glucose and fructose concentrations in unpackaged carrots (Table 3.4. b and c) could partially contribute to higher populations of natural microbiological flora of control carrots (Table 3.8 – 3.10). In packaged carrots stored at 1°C, the total aerobic bacteria remained lower, which could partially be due to lower concentrations of glucose and fructose (Table 3.4 b and c) in these carrots, and the modified atmosphere created in the headspace. For example, the microbial populations seemed to increase more during the third week of storage in most of the samples, coinciding with the sudden increase in free sugars in carrots during this storage interval (Table 3.4 and 3.8 - 3.10).

The visual appearance of unpackaged carrots highly deteriorated at room temperature, when compared to packaged carrots (Table 3.11). During the early stages of storage, due to availability of moisture and the favourable temperature, visible mould growth occurred on unpackaged carrots, so that they were unmarketable after one week. As the storage time advanced, the dehydration process seemed to stunt growth of microorganisms and sprouting of unpackaged carrots. Loss of moisture was not a problem in the packaged carrots during MAP storage at room temperature, however, this resulted in sprouting of carrots. In general the results showed the benefits of MAP to reduce physiological as well as biochemical changes and maintain the freshness quality of carrots, and improving the effectiveness of other postharvest treatments such as washing, disinfecting and low temperature storage, to improve the shelf life of carrots.

Storage temperature was the most important factor that significantly ($P \leq 0.001$) affected the postharvest quality of carrots (Table 3.1 - 3.11). The gas concentrations, PWL, TSS, TAC, sucrose, fructose and glucose content, total aerobic bacteria, yeasts and moulds and total coliform bacteria were significantly influenced by the storage temperature. Varoguaux and Wiley (1994) showed that storage temperature highly affects the physiology and biochemistry of vegetables, which was also demonstrated in the current study, and resulted in significant physiological and biochemical changes in carrots stored at room temperature (Table 3.1 - 3.11). Room temperature storage increased the utilization of O_2 and production of CO_2 by carrots compared to those stored at 1°C, which, according to Wiley (1994), was due to the higher temperature effect. The main physical change that occurred with carrots was loss of moisture, which is one of the most important aspects related to fresh perishable vegetables that reduces the saleable weight and can induce senescence of the products (Grierson and Wardwski, 1978). The PWL was much higher in carrots stored at room temperature than in carrots stored at 1°C. At room temperature the relative humidity of air usually is lower than the vapour pressure exerted by water in the carrots, which creates vapour pressure differences that are the deriving force for moisture movement outward (Salunkhe et al., 1991; Ryall and Lipton, 1979). The surface tissue and skin of carrots seemed to be poor barriers to moisture transfer from the flesh to the surrounding air. This results in a higher rate of moisture transfer when stored at room temperature.

The AA content of carrots stored at 1°C as well as room temperature decreased during storage (Table 3.7.). After two weeks of storage at room temperature, the decrease of AA was much more in carrots stored at room temperature, which was due to the interactive effect of storage temperature and storage time ($P \leq 0.001$) on the physiology and biochemical activities of the carrots (Appendix A.1). Odebode and Unachukwu (1997) reported that the AA in infected carrots decreased as the storage period increased. With the microbiological flora as indicator of levels of infection of carrots (Table 3.8 – 3.10), it may also be an explanation for the decrease in AA content.

Storage temperature was an important factor that had a highly significant ($P \leq 0.001$) effect on the microbiological flora in carrots during storage (Table 3.8-3.10). The microbiological qualities of carrots were better in carrots stored at 1°C, with significantly ($P \leq 0.001$) lower populations of aerobic bacteria, moulds and yeasts, and coliform bacteria. The reason for this could be attributed to several factors associated to storage temperature. Apart from the physiological activity of microorganisms, the physiological and biochemical changes in carrots are also reduced by the lower temperature, which lead to slow respiration and metabolism of carrots. This in turn resulted in the firm tissue that is resistant to microbial infection (Bracket, 1992). Higher temperatures increased the hydrolysis of carbohydrates into free sugar (Table 3.3 and 3.4), and could lead to availability of nutrients for the microorganisms to grow (Table 3.8-3.10).

Low temperature maintained the TSS content better in carrots during storage (Table 3.2). The rate of increase in TSS was rapid and significant ($P \leq 0.001$) at room temperature compared to refrigerated storage. The TSS content increased faster and to higher levels in carrots stored at room temperature than at 1°C (Pal and Roy, 1988; Waskar et al., 1999; Lingaiah and Huddar, 1991; Jitender-Kumar *et al.*; 1999). This was partly attributed to higher moisture loss due to high temperature and low relative humidity. Pal and Roy (1988) and Waskar et al (1999) reported similar trends on the changes in the TSS during storage at different storage temperatures. Increased reduction of TAC at room temperatures storage could be attributed to hydrolysis of carbohydrates at a faster rate compared to storage at 1°C.

Since POX is a respiratory enzyme, the results show that the rates of oxidative processes in the respiration increase during the first few days of storage. Berg and Lentz (1966) and Phan *et al.* (1973) suggested that the rates of metabolic processes increase in order to adjust the carrot roots to the new conditions immediately after they are detached from the plant and exposed to environmental conditions that were different from the underground conditions. The effect of storage temperature on POX activity of carrots was significant ($P \leq 0.05$) during storage. The higher levels of POX in carrots stored at room temperature during the later weeks of storage, could indicate the increased oxidation status of the carrots, compared to those stored at 1°C. Similar to MAP, storage temperature influenced the effectiveness of pre- and postharvest treatments on keeping the qualities of carrots during storage, which was demonstrated by the interactive effect of storage temperature with the other pre- and postharvest treatments (Table 3.1 - 3.10). Low temperature storage supported the postharvest washing, disinfecting and MAP towards the achievement of the improvement of shelf life of ComCat® treated and untreated carrots. The coupled effect of MAP and storage temperature seemed to have a synergistic effect on storage quality maintenance of carrots. As mentioned above, MAP reduced O_2 concentration in the headspace of packages of carrots, and reduced the rate of respiration (Wiley, 1994), while low temperature reduces both biological and biochemical activities in the produce. In this result, the combined effect of MAP and storage temperature significantly improved the shelf life, as well as maintained better chemical, microbiological, and visual appearance quality of carrots during storage (Table 3.1 - 3.11).

The prepackaging disinfecting of carrots in chlorine supplemented or anolyte water significantly ($P \leq 0.001$) reduced the populations of total aerobic bacteria, yeasts, moulds and total coliform bacteria during storage (Table 3.8, 3.9 and 3.10). The results of this study were in agreement with the previous work on efficacy of chlorine as a postharvest decay control for vegetables (Zhuang *et al.*, 1995; Velazquez *et al.*, 1998; Beuchat *et al.*, 1998; Escudero *et al.*, 1999; Beuchat *et al.*, 2001; Ukuku and Sapers, 2001; Prusky *et al.* 2001;). The effectiveness of chlorine solution was highly dependent on storage temperature with the most effective reduction of microbial populations during storage being at low storage temperature (Table 3.8 - 3.10). The reason for this is, that at low storage temperature, the deteriorative factors on carrots such as the etching effect of chlorine on the absorbing surface tissue (Figure 3.5 and 3.6), the PWL (Table 3.1) and chemical and biochemical changes (Figure 3.4 and Table 3.1-3.6), was at a minimum and resulted in firm tissue, which is resistant to microbiological infection. Once the natural microbiological flora of carrots was reduced by chlorine immediately after disinfection, it remained lower at low temperature (Table 3.8 - 3.10). The results of this chapter also showed that anolyte water significantly ($P \leq 0.001$) reduced the natural microbiological flora of carrots during storage. In general, anolyte water suppressed the growth of microbiological flora throughout the storage period as good as chlorine, if not better (Figure 3.7 and Table 3.8 and 3.9). A short dipping time of 5 minutes in anolyte water was found to be as effective as 20 minutes in chlorinated water on reducing and retarding development of microbial flora on carrots during storage (Seyoum et al., 2003).

In comparison to carrots dipped in chlorinated water, the carrots dipped in anolyte water displayed better fresh quality properties, such as a smooth and shiny surface, for one month of storage (Figure 3.5 - 3.7 and Table 3.11). Etching of the surface of carrots by chlorinated water was observed, while carrots dipped in anolyte water remained shiny and smooth (Figure 3.5). These results thus provide strong evidence that chlorinated water may not be the optimum disinfecting treatment for carrots. An alternative treatment is recommended, and anolyte water would be a good alternative.

The PWL was higher in carrots dipped in chlorinated water, when compared to those dipped in anolyte and tap water. In a separate investigation, the difference was shown to be significant (Seyoum et al., 2003). The higher loss of moisture from carrots dipped in chlorinated water was attributed to its etching effect of this disinfectant on the absorbing surface tissue of carrots (Figure 3.5 and 3.6).

The disinfecting treatment had no significant ($P > 0.05$) effect on the TAC, total soluble sugar and glucose content of carrots during storage, however, the total soluble sugars were better maintained in disinfected carrots. Disinfecting treatment had a significant ($P \leq 0.05$) effect on the reduction of AA (Table 3.7). The decrease of AA of carrots dipped in chlorinated water was higher than that of anolyte washed samples during storage at room temperature. The chlorinated water treatment also increased the level of POX in carrots during storage. Again this could be due to the wounding effect of chlorine on the surface tissue of carrots, resulting in increased metabolic activities (Figure 3.4, 3.5 and 3.6) during storage. While both disinfectants showed to be similar in effectiveness in killing and controlling postharvest decay during storage, these results thus showed that disinfecting with anolyte water proved to be superior to chlorinated water in maintaining the optimum physiological condition, nutritional value, as well as some biochemical processes.

The interactive effect of disinfecting treatment + MAP and storage temperature was significant on the changes in PWL ($P \leq 0.001$), TAC ($P \leq 0.05$), sucrose content ($P \leq 0.01$), SH ($P \leq 0.001$), total aerobic bacteria ($P \leq 0.001$) and population of total coliform bacteria ($P \leq 0.001$). The best quality properties of carrots were maintained due to the interactive effect between anolyte water disinfecting, use of MAP, and low temperature storage. Therefore, suitable combinations of preharvest practice, prepackaging treatments, packaging and control of environmental factors should be used to improve the quality and shelf life of packaged carrots.

The effect of preharvest ComCat® treatment on changes in oxygen, carbon dioxide and nitrogen concentrations in the packages of carrots were not significant ($P > 0.05$). These results are indicative of the levels of respiration rate in ComCat® treated and control carrots, suggesting that ComCat® treated carrots have the same respiration rate as the control. This similarity in their biochemical changes could then suggest that the shelf life of carrots treated with ComCat® was as good as normal carrots at both temperatures. Some of the results of this research showed that an improvement in some quality properties of carrots could be achieved by preharvest ComCat® treatment. As a general trend, the ComCat® treatment resulted in a reduction of the PWL of carrots during storage. One of the reasons for the higher losses of moisture from control carrots was their smaller size as compared with the size of ComCat® treated carrots, therefore having a relatively larger surface area for evaporation. The other reason could be attributed to the effect of plant growth hormones in ComCat®, such as gibberellin and auxin, which are known to reduce PWL in fruits and vegetables (Bartholomew *et al.*, 1983; Rodrigues and Subramanyam, 1966; Salunkhe, *et al.*, 1975; Stallknecht *et al.*, 1983).

The TSS was slightly higher in ComCat® treated carrots compared to the controls during storage, although only significant at $P \leq 0.09$. The interesting point was, that in most of the sampling intervals, the TSS values remained higher in ComCat® treated carrots (Table 3.2). This pattern of TSS was consistent in the case of carrots stored in MAP at 1°C as well as room temperature for 28 days, but not in unpackaged carrots. The interactive effect of preharvest ComCat® treatment and storage temperature was significant ($P \leq 0.01$) on the changes in TSS content of carrots during storage. This shows the necessity of proper storage temperature in order to maintain the TSS of fresh harvest ComCat® treated carrots. The plant growth regulators in ComCat®, such as gibberellin, indole-3-acetic acid and brassinosteroids, with their individual functions during plant growth and development, seemed to have promote the accumulation of TSS.

An increase in low molecular weight sugars during the early stages of storage of carrots was previously reported by Phan *et al.*, 1973, Nilsson, 1987 and Suojala, 2000. These researches also showed that the hexose sugars increase in carrots during storage, while the sucrose content decreases, and the total soluble sugar content seems to remain constant with the progress of storage time. In this study, the data also showed an increase in hexoses, with a simultaneous decrease of sucrose and TAC content during storage (Table 3.3 and 3.4 a - c). However, in Table 3.4 (b) and (c), it can be seen that the glucose and fructose contents are in the same order of magnitude as the respective contents of control carrots at harvest. These results therefore do not agree with those of Nilsson (1987) and Suojala (2000). However, the sugar contents do not decrease as drastically as was reported by Carlin *et al.* (1990) and Chervin and Boisseau (1994) for minimally processed carrots, where high respiration rates were observed due to wounding and leakage losses of nutrients. The change of sugar contents in intact fresh carrots during storage is lower, as compared to minimally processed carrots due to a relatively lower metabolism rate during storage.

General trends of the sugar data showed that the plant growth hormones reduced ($P \leq 0.1$) the hexose sugar content in ComCat® treated carrots (Table 3.4 and 3.5). The previous investigation on the fruit composition as effected by preharvest gibberellin treatment showed that this plant growth hormone resulted in lowering of both hesperidine and reducing sugars (Salunkhe *et al.*, 1991). In this study the free sugars such as glucose and fructose (Table 3.4 b and c) were found to be lower in ComCat® treated carrots at harvest, which may be attributed to the effect of the gibberellin in ComCat®. A general trend of lower glucose and fructose content was observed in ComCat® treated carrots during storage. The reason for this could be a lower rate of conversion from polysaccharides to free sugars (sucrose, glucose and fructose), which in turn would indicate higher rates of metabolic activities before harvest. The preharvest ComCat® treatment increased the TAC ($P \leq 0.05$), sucrose ($P > 0.05$) and TSS ($P > 0.05$) of carrots at harvest (Table 3.1, 3.3 and 3.4 (a)). This could indicate that ComCat® increased the biosynthesis of polysaccharide carbohydrates while efficiently utilizing free sugars for physiological processes during the growth and development period. A previous investigation on the fruit composition as effected by preharvest gibberellin treatment showed that this plant growth hormone resulted in lowering reducing sugar content (Salunkhe *et al.*, 1991), which is confirmed by the present findings. This may have its own advantage in keeping microbial population in carrots low in preharvest ComCat® treated carrots during storage (Table 3.8 - 3.10), which was also observed in lower populations of total aerobic bacteria, total coliform bacteria at harvest and during storage, which seemed to have had a direct relationship with the contents of total soluble sugar, glucose, fructose and sucrose contents of these carrots (Table 3.4 - 3.5 and Table 3.8 - 3.10). However, the relationship between the changes in sugar and the population of microorganisms in ComCat® treated carrots was not consistent and more complex, due to the other postharvest treatments applied.

When the carrots were stored at low temperature (1°C), TAC decreased to a steady state after 14 days of storage. At room temperature the TAC remained consistently higher in ComCat® treated carrots subjected to different postharvest treatments and storage (Table 3.3). These data show that ComCat® treatment had a positive effect on the maintenance of TAC in carrots stored at higher temperature, suggesting better postharvest quality.

The interactive effect of ComCat® treatment and postharvest treatments significantly ($P \leq 0.05$) influenced the PWL, TAC, and populations of microorganisms during storage (Table 3.1, 3.3 and 3.8 - 3.10). The results suggested that the ComCat® treated carrots, when combined with anolyte water dipping treatments and stored at the optimum low temperature, would help to increase the shelf life. Three-way interactions between the pre-and postharvest treatments were observed for the PWL ($P \leq 0.001$), TAC ($P \leq 0.05$) populations of aerobic bacteria ($P \leq 0.001$), fungi ($P \leq 0.001$) and coliform bacteria ($P \leq 0.01$). In general, the chemical quality characteristics were also maintained better in carrots subjected to proper combinations of postharvest treatments.

In summary, the results in this chapter showed that anolyte water dipping treatment may be a better disinfectant treatment for carrots than chlorine, as it has shown that it did not etch the surface tissues, significantly reduced the natural microbiological flora and resulted in higher contents of TSS and AA, perhaps due to induced lower metabolic rates, without affecting the other quality properties. Preharvest ComCat® treatment improved some of the quality properties of carrots at harvest and during storage. The advantages gained by the preharvest ComCat® treatment are:

1) higher TAC, TSS, AA, sucrose content and SH and lower populations of total aerobic bacteria and total coliform bacteria at harvest, although only significant at $P \leq 0.09$.
2) better chemical quality characteristics during storage in terms of higher TSS ($P > 0.05$) and lower PWL ($P > 0.05$) in ComCat® treated carrots during storage at 1°C, although only significant ($P \leq 0.09$)
3) better maintenance of TSS, AA and TAC in carrots during storage at room temperature
4) improved keeping quality in relation to better visual appearance during storage at 1°C

The findings that ComCat® treatment showed better maintenance of TSS, total soluble sugar, TAC and AA in carrots at room temperature, would encourage research on the performance of ComCat® treated carrots in evaporative cooling conditions at a storage temperature higher than the optimum 1°C. The results of such research are presented in chapter 5.

Carrots are a subsurface root part of a plant. The data obtained for carrots may perhaps not be appropriate to predict the effect of preharvest ComCat® treatment on biological and biochemical changes in other parts of plants, such as the fruit, during storage. For comparison, an investigation on the effect of preharvest ComCat® treatment on biological and biochemical changes in a fruit was consequently carried out. In this respect, the performance of ComCat® treated tomatoes during storage is presented in chapter 4.

CHAPTER 4

EFFECT of PRE- and POSTHARVEST TREATMENTS on PHYSIOLOGICAL, CHEMICAL and MICROBIOLOGICAL QUALITIES of TOMATOES

Abstract

Preharvest ComCat® treated tomatoes and untreated controls were evaluated for changes in physiological, chemical and microbiological quality characteristics during storage at 13°C and room temperature (16.9 - 25.2°C) and a relative humidity of 34 - 76% after 8, 16, 24 and 30 days. The effectiveness of MAP in Xtend® film, disinfection treatments with chlorine or anolyte water and different storage temperatures were also evaluated. The O_2 and CO_2 concentrations remained above 15% and below 6% during storage, respectively. MAP reduced utilisation of free sugars, reduced moisture loss, retained ascorbic acid (AA), and slightly suppressed growth of microorganism. Storage temperature was the most dominant factor that affected all the quality parameters tested. Faster ripening of tomatoes at room temperature was accompanied by rapid changes in O_2 and CO_2 and increased the physiological weight loss (PWL), pH, utilization of free sugar, peroxidase (POX) and polygalacturonase (PG) activities, and populations of microorganism.

Disinfecting treatments significantly reduced microorganisms. Chlorine disinfection resulted in higher O_2 consumption and CO_2 production than anolyte water, implying higher respiration and metabolism rates in these tomatoes. PWL was lower and the total soluble solids (TSS) content, total soluble sugar, POX activity, and marketability of tomatoes were generally maintained better in tomatoes dipped in anolyte water. The fructose content increased continuously in tomatoes dipped in anolyte water for up to 24 days while fructose increased only up to 16 days in tomatoes dipped in chlorinated

water during storage at 13°C. Disinfecting in anolyte water delayed senescence slightly.

At harvest, ComCat® treated tomatoes had a lower pH, lower levels of ascorbic acid, fructose and glucose, higher levels of TSS, total titratable acids (TTA) and sucrose, higher POX activity, and lower microbial populations, than controls. ComCat® treatment had a significant effect on O_2 and N_2 concentrations, PWL, pH, AA content, sucrose content, POX activity, aerobic bacteria, moulds and yeasts, coliform bacteria and marketability of tomatoes during storage. During storage at room temperature, the headspace of packages of ComCat® treated tomatoes had higher O_2, lower CO_2 and lower N_2 contents, which indicated a lower respiration rate, compared to controls.

During storage the AA and TSS contents were better retained in ComCat® treated tomatoes than in control tomatoes, while the sucrose and glucose contents stayed lower in ComCat® treated tomatoes. However, after full ripeness, the fructose content was higher in ComCat® treated tomatoes, compared to controls. The POX activity was generally lower in ripening ComCat® treated tomatoes than in control tomatoes. The difference in PG activity was only visible after 30 days at 13°C between the ComCat® treated and control tomatoes. The climacteric peak of ripening was delayed almost by one week in ComCat® treated tomatoes. Due to low concentrations of free sugar, high TTA, low pH and high POX activity in ComCat® treated tomatoes, the microbial populations were generally lower during the early stages of ripening in storage. Finally, the marketability of ComCat® treated tomatoes were significantly better than that of the control tomatoes, especially under room temperature storage conditions.

4.1. Introduction

Postharvest physiological, microbiological, chemical and biochemical qualities of tomatoes partly depend upon preharvest factors such as genetic, climatic, biotic, edaphic, chemical and hormonal factors, as well as combinations of these (Hobson, 1988; Jauregui *et al.*, 1999; Leonardi *et al.*, 2000; Prieto *et al.*, 1999;Salunkhe *et al.*, 1991; Shi *et al.*, 1999).

Five major classes of plant hormones are generally recognised: auxin, gibberellin, cytokinin, abscisic acid and ethylene (Davies, 1995; Mauseth, 1991; Raven *et al.*, 1992; Salisburg and Ross, 1992), which may change the rate of biological and biochemical changes in fruits. To achieve a given objective postharvest research is necessary whenever new plant growth regulators or other chemicals are externally applied during growth and development, because the treatment could have positive or negative effects on the quality of fruits at harvest and or during storage. ComCat® is a substance extracted (see Chapter 1 and 2) from plants, and consists of combinations of plant hormones (auxin, gibberellin, brassinosteroids, kinetins), aminoacids, natural metabolites and other ingredients that were shown to increase the yield of vegetables (Schnabl *et al.*, 2001). However, there are no data available on the postharvest physiology, microbiology, chemical and biochemical quality aspects of preharvest ComCat® treated tomatoes.

The aims of postharvest treatment of fruit are to reduce enzymatic activities and postharvest decay problems. Chemical treatments were found to be effective in reducing the occurrence of postharvest decay by pathogens (Eckert, 1990; Barth *et al.*, 1995; Beuchat *et al.*, 1998; Afek *et al.*, 1999, Prusky *et al.*, 2001) and hot water washing was also found to be very efficient to control postharvest decay in fruits and vegetables (Rodov *et al.*, 1995; Fallik *et al.*, 1999 and 2000). Most chemical treatments may leave residues and consequently encounter consumer acceptance problems (Johnson and Sangchote, 1994; Wisniewski and Wilson, 1992). This necessitates research in search of alternative disinfecting treatments. Anolyte is a good candidate, and has been shown to be effective in retarding growth and proliferation of microorganisms on carrots during storage (Chapter 3).

The objectives of this chapter were: (1) to investigate the postharvest properties of ComCat® treated tomatoes; (2) to investigate anolyte water as an alternative disinfectant to a chemical treatment such as chlorine to reduce microbial load and suppress their growth thereafter during storage; and (3) to investigate the storage quality of preharvest ComCat® treated tomatoes using MAP at 13°C and ambient temperature.

4.2. Materials and Methods

4.2.1. Tomato production

Tomatoes (*Leucopersicon esculentum,* var. Marglobe) were grown during the autumn season in the area of Bloemfontein South Africa. ComCat® was applied at 10g ha^{-1} in 350 litres, and 0g ha^{-1} as control, and was sprayed twice during the growth and development of tomatoes. The first spraying was performed prior to transplanting of the seedlings while the second spraying was at the start of flowering. The other preharvest practices were kept constant between the treatments during production of tomatoes in this experiment. Fruit were harvested manually at the green-mature stage and delivered to the laboratory immediately after harvest. In order to protect the tomatoes from mechanical injury during transportation, plastic crates were used. For each preharvest treatment, the tomato fruit were harvested from four random blocks in order to obtain representative samples. Fruit without observable mechanical injury, blemishes or defects were selected for the study. The working surfaces and all tools used for processing of tomatoes during disinfecting, packaging and storage were washed and disinfected with1% Chlorobac (Syndachem, Pty, Ltd.) prior to use. Immediately after delivery to the laboratory, the fruit were hand washed with water, to remove field heat, surface dust and reduce microbial populations on the surface. Prepackaging disinfecting treatments and packaging of tomatoes were performed on the same day.

4.2.2. Postharvest treatment

The postharvest disinfecting, packaging and storage treatments were conducted according to the methods described in chapter 3, section 3.2.2. After washing a total amount of 144 kg ComCat® treated tomatoes were subdivided into three groups of 48 kg each in preparation for dipping treatments in chlorinated water, anolyte water and tap water. Plastic containers were washed, disinfected and rinsed by distilled water prior to use for the dipping treatments. Plastic containers were used to avoid losses of charged ions from anolyte, which would be the case with metal containers. Tap water in plastic containers was adjusted to 100μg. ml^{-1} total chlorine with standard grade sodium hypochlorite (5% NaOCl), in which tomatoes were dipped for 20 minutes.

The pH of anolyte water was adjusted to pH of 6.7 and contained 0.87% total dissolved solids. Immediately after preparation, anolyte water was used for disinfecting tomatoes for 5 minutes.

4.2.3. Modified Atmosphere Packaging

The tomatoes were subdivided into 1 kg-samples, which were packed in commercial micro-perforated bags (Xtend® Film, Patent No. 6190710, StePac L.A., Ltd., Israel), which are specifically designed for tomato packaging and for storage at 13°C. The unpackaged 1 kg-sample tomato, for each treatment combination, were placed on perforated plastic bags and left open during storage at 13°C or room temperatures. Tomatoes were stored at 13°C and RH of 34-76% and at room temperature (16.9-25.2°C).

On each sampling date, packages of tomato (1kg each) were randomly taken in triplicate from each treatment for quality analyses. A new package was taken each time in order to maintain the microenvironment and avoid contamination through repeated opening and sealing.

4.2.4. Gas sampling and analysis

The analysis of headspace gas concentrations (O_2, CO_2 and N_2) during storage was conducted as described in Chapter 3, section 3.2.4.

4.2.5. Physiological weight Loss (PWL)

PWL was conducted as described in chapter 3, section 3.2.5.

4.2.6. Chemical and biochemical analysis

4.2.6.1. pH and titratable acidity (TTA)

An extract of an aliquot of juice was prepared according to Nunes and Emond (1999). An aliquot of tomato juice was extracted from three tomatoes with a Kenwood JE 550 juice extractor. The fruit slurry was filtered through cheesecloth and 100 ml was centrifuged for 15 minutes at 5000 x g at 4°C. The decanted clear juice was used for the analysis. The pH value of tomato juice was measured with a Metrohm 691 pH meter. The TTA of tomatoes were measured by the methods as described by Maul *et al.* (2000). The titratable acidity expressed as % citric acid, was obtained by titrating 10 ml of tomato juice to pH 8.2 with 0.1N NaOH with an automatic titrator (665 Dosimat, Metrohm).

4.2.6.2. Total soluble solids (TSS)

The TSS, expressed as °Brix, during storage was measured as described in chapter 3, section 3.2.6.1.

4.2.6.3. Free sugar (sucrose, glucose, fructose, sucrose equivalent, SH)

The content of soluble sugars (sucrose, glucose and fructose) of tomatoes was analysed as described in chapter 3, section 3.2.6.2. For sweetness calculation of the hexoses, glucose and fructose, their concentrations were converted mathematically to sucrose equivalents (Koehler and Kays, 1991; Maul *et al.*, 2000). The glucose and fructose concentrations were multiplied by correction factors 0.74 and 1.73, respectively, and the sum was considered as sucrose equivalent. The sucrose-hexose ratios were calculated by dividing sucrose content into the total sum of fructose and glucose. The sugar-acid ratios were obtained by dividing total sugars by titratable acidity.

4.2.6.4. Ascorbic acid analysis

The tomato samples were frozen at –20°C on each sampling interval until analysis was possible within 6 weeks (Maul *et al.* 2000). The ascorbic acid content of tomatoes was estimated according to the procedures as described in chapter 3, section 3.2.6.4.

4.2.6.5. Peroxidase activity (POX)

The tomato samples were frozen at –20°C on each sampling interval until analysis was possible within 6 weeks (El-Zoghbi, 1994, Kato-Noguchi, 1997). The POX in tomato pericarp tissue (12.5 g) was extracted and the activities were assayed according to the methods described in chapter 3, section 3.2.5.5.

4.2.6.6. Polygalacturonase activity (PG)

The tomato samples were frozen at –20°C on each sampling interval until analysis was possible within 6 weeks (El-Zoghbi, 1994, Kato-Noguchi, 1997). Enzyme extraction was done according to Yoshida et al (1984). Frozen tomato pericarp tissue (50g) was homogenised for 2 minutes in 100 ml chilled distilled water in a blender. After the homogenate was centrifuged at 10,000 X g for 15 min, the supernatant was discarded. The remaining pellet was re-suspended in 25 ml of 0.2 M Tris-HCL buffer (pH 9.0) that contained 5% NaCl and stirred for 2hr at 4°C. The ensuing slurry was centrifuged at 15,000 X g for 20 min and its supernatant dialyzed against 3 litres of 20 mM sodium acetate buffer (pH 6.0) for 3 hr.

In order to precipitate protein, 80% $(NH_4)_2SO_4$ was added to the dialysate. Protein was collected by centrifugation at 15,000 x g for 20 min and dissolved in a small amount of water. The protein solution was then dialyzed against 3 l of 20 mM acetate buffer for 3 hr. All procedures used during the extraction processes were performed at 4°C.

The PG activity was measured by the method of Marangoni et al (1995). The total PG was analysed in a 1 ml reaction mixture containing 0.1 ml of an aliquot of tomato extract, 0.2 ml of 0.4 M sodium acetate buffer (pH 4.5), 0.2 ml of 0.4 M NaCl and 0.5

ml of 0.5% polygalacturonic acid. The mixture was incubated at 37°C for 30 min, and the reaction terminated by suspending the mixture in a boiling water bath for 5 min. Blanks were prepared by heating duplicate samples for 5 min at 100°C prior to the assay. The reducing groups formed during the 30 min of incubation at 37°C were estimated by the arsenomolybdate method of Nelson (1944). The PG activity was expressed as nmole reducing groups min^{-1}. g^{-1} fresh weight.

4.2.7. Microbiological analysis

The estimation of the population of total aerobic bacteria, moulds and yeasts, and total coliforms were accomplished as described in chapter 3, section 3.2.7. Microbial populations were not analysed immediately after disinfection, as they were assumed to be around 0 $\log_{10}$ CUF. g^{-1} as was shown by Seyoum et al. (2003).

4.2.8. Subjective quality analysis

The marketable quality was subjectively assessed according to Mohammed *et al.* (1999). On each sampling time a package of tomatoes containing 5 fruits was randomly selected from each treatment group. The number of marketable fruits was used as measure to calculate the percentage marketable fruits during storage. A 1 - 9 rating with 1 = unusable, 3 = unsaleable 5 = fair, 7 = good and 9 = excellent was used to evaluate the fruit quality. The color, shininess, surface defects, sign of mould growth and dehydration were visual parameters for the rating. Tomatoes that received a rating of 5 or above were considered marketable, while those rated less than 5 were considered unmarketable.

4.2.9. Experimental design and data analysis

The experimental design and data analyses were according to the methods as described in chapter 3, section 3.2.8.

4.3. Results

4.3.1. Headspace gas concentration

The changes in O_2 concentration in tomato packages sealed with the Xtend® film are shown in Figure 4.1. The storage temperature highly ($P \leq 0.001$) affected O_2 levels in the headspace of packages of tomatoes during storage. The results presented in Figure 4.1 show that ambient temperature storage increased O_2 utilisation as a result of higher respiration rates of fruit. A rapid decrease in O_2 concentration in packages of tomatoes stored at room temperature to below 18.6% was evident after the first five days of storage, followed by an equilibration of the O_2 concentration. During the first five days of storage the O_2 concentration in packages of tomatoes stored at 13°C remained above 19%, after which it dropped up to 12 days, slightly increased up to 20 days and then stabilised at approximately 19% thereafter.

The disinfecting treatment also had a significant ($P \leq 0.05$) effect on the changes in O_2 concentrations in packages. After 12 days at 13°C, the O_2 concentration decreased to 18.5% in packages of ComCat® treated as well as control tomatoes dipped in chlorinated water, while that of the anolyte treated tomatoes remained above 19%. After 19 days at 13°C the O_2 concentrations of the chlorine disinfected tomatoes equilibrated at approximately 19%.

There was a difference in the O_2 concentrations in packages of ComCat® treated tomatoes dipped in anolyte after five days at room temperature, being above 19%, while all other tomato packages stored at room temperature had O_2 concentrations below 18.5%. In general, the O_2 concentration in packages of ComCat® treated tomatoes dipped in anolyte water was found to follow O_2 content pattern of tomatoes stored at 13°C.

The two-way interaction between preharvest treatment and storage temperature was highly significant ($P \leq 0.001$) on the changes of O_2 levels in tomato packages. The three-way interaction between preharvest, disinfecting and storage temperature factors

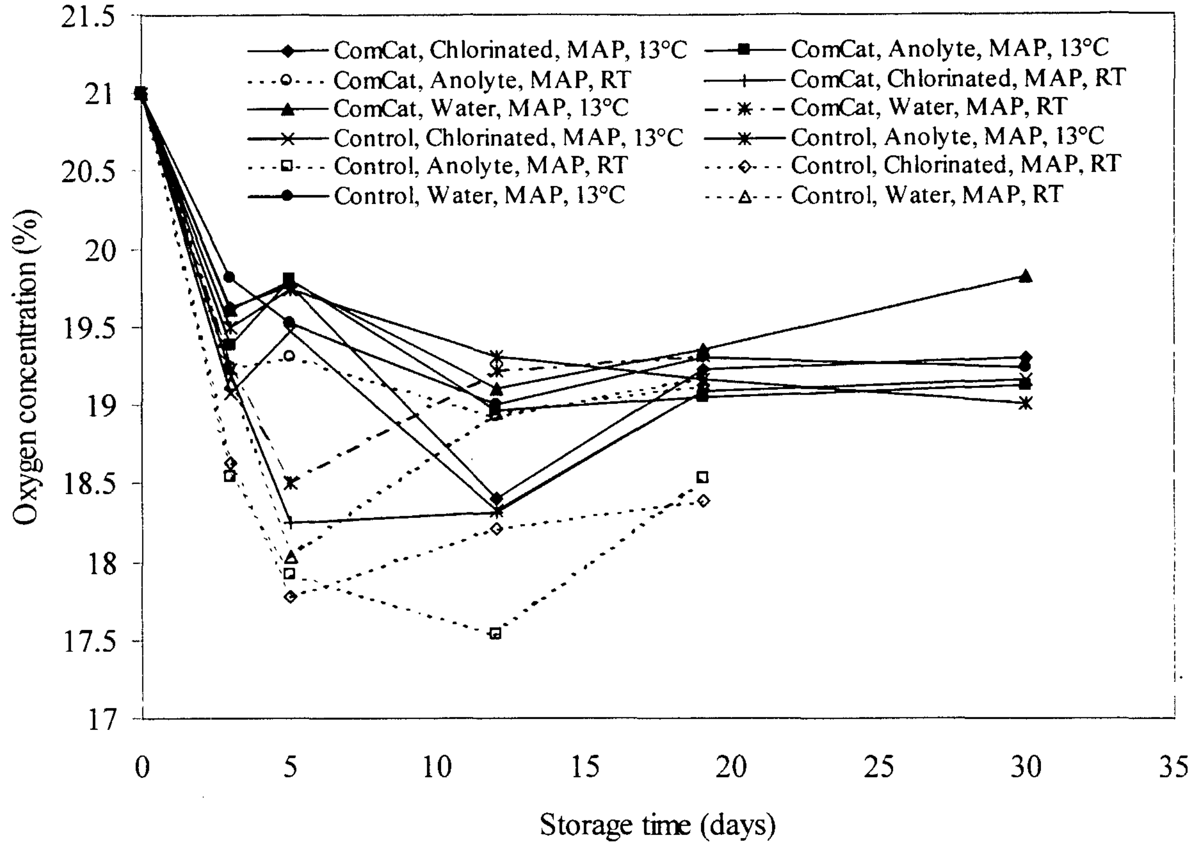


Figure 4.1. Changes in O_2 content (%) in packages of tomatoes in Xtend® film stored at 13°C and room temperature (RT) for 30 days (n = 3 over five storage times).

Significance level showing the effect of pre- and postharvest treatment on O_2 concentrations	
Significance	
Preharvest treatment (A)	**
Disinfecting (B)	**
Storage temperature (C)	***
A X B	NS
A X C	***
B X C	NS
A X B X C	***

NS, *, **, *** Nonsignificant or significant at $P \leq 0.01$, 0.05 or 0.001 respectively. $LSD_{0.05}$ Value = 0.563, S.E. = 0.034 and C.V. = 0.018.

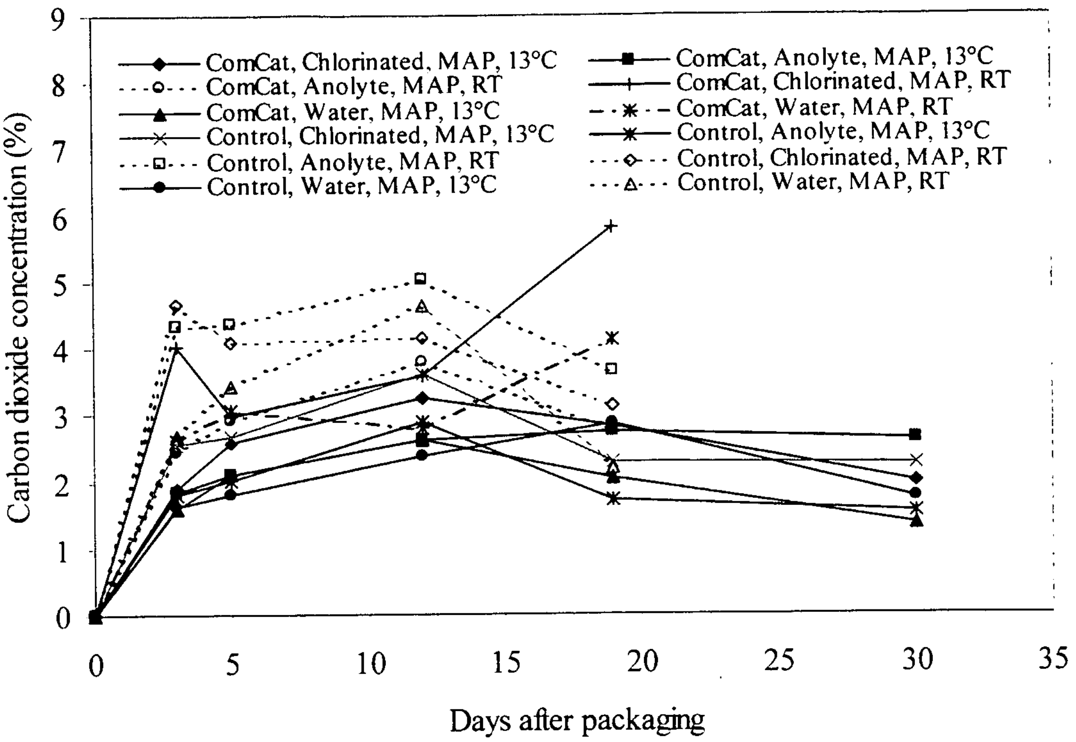


Figure 4.2. Changes in CO_2 content (%) in packages of tomatoes in Xtend® film stored at 13°C and room temperature (RT) for 30 days (n = 3 over five storage time).

Significance level showing the effect of pre- and postharvest treatment on CO_2 concentrations	
Significance	
Preharvest treatment (A)	NS
Disinfecting + MAP (B)	*
Storage temperature (C)	***
A X B	NS
A X C	NS
B X C	NS
A X B X C	***

NS, *, *** Nonsignificant or significant at $P \leq 0.05$ or 0.001 respectively. $LSD_{0.05}$ Value = 1.303, S.E. = 0.064 and C.V. = 0.278.

were also found to be highly significant ($P \leq 0.001$) on the changes in O_2 level in tomato packages during 30 days of storage at 13°C and 19 days of storage at room temperature.

The storage temperature was the dominant factor affecting CO_2 concentrations in the headspace of tomato packages during storage, and was highly significant ($P \leq 0.001$). The CO_2 concentrations in packages of tomatoes subjected to pre- and postharvest treatments showed differences at room temperature, compared to those stored at 13°C. The CO_2 level increased slowly up to 12 days of storage to between 2.0% and 3.5% and seemed to equilibrate thereafter in packages stored at 13°C (Figure 4.2). In tomato packages stored at room temperature, CO_2 levels of 3.5 to 4.5% were reached after 12 days, after which these levels dropped to between 2.0% and 3.5%.

A CO_2 level of 4% was reached quickly, after 3 days, in packages of control fruits, compared to the ComCat® treated tomatoes, and stayed higher up to 12 days of storage. After 12 days of storage the CO_2 level continued to increase in packages of ComCat® treated fruits except in the case of anolyte water treated tomatoes stored at room temperature. These differences, however, were not significant ($P > 0.05$).

The CO_2 concentration was significantly ($P \leq 0.05$) affected by the prepackaging disinfecting treatments. The CO_2 concentrations increased in all packages of ComCat® treated tomatoes stored at 13°C, and which were subjected to different disinfectant and water treatments, up to day 12, declined and stabilised thereafter. At room temperature storage, the chlorine washed ComCat® treated tomatoes showed a CO_2 level of 4% after 3 days, which was in the same order of magnitude as for the controls. The CO_2 level dropped between days 5 and 12, but then increased sharply at 19 days to above 5.5%. At 19 days storage the CO_2 levels of packages of water washed ComCat® treated tomatoes also increased sharply, but to 4%. The CO_2 levels of anolyte washed ComCat® treated tomatoes stored at room temperature, on the contrary, were much lower and followed the CO_2 pattern of tomatoes stored at 13°C.

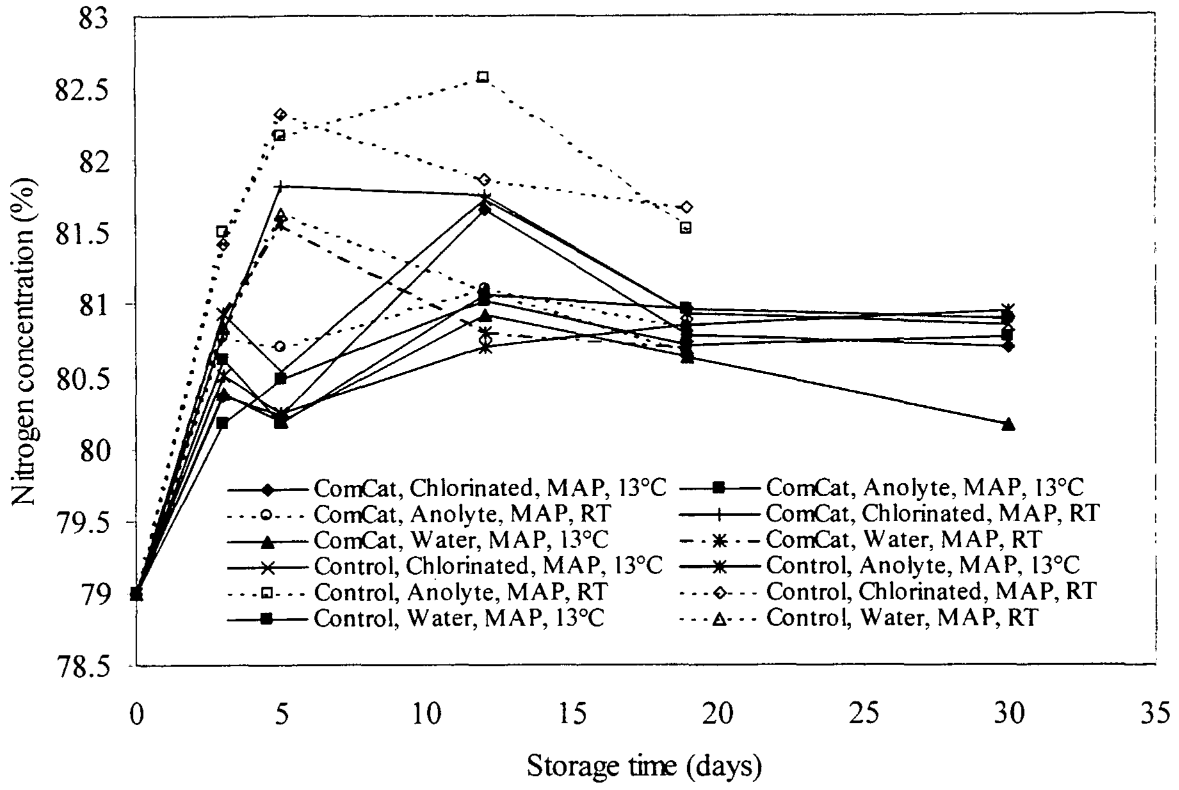


Figure 4.3. Changes in N_2 content (%) in packages of tomatoes in Xtend® film stored at 13°C and ambient temperature (RT) for 30 days (n = 3 over five storage time).

Significance level showing the effect of pre- and postharvest treatment on N_2 concentrations	
Significance	
Preharvest treatment (A)	**
Disinfecting (B)	**
Storage temperature (C)	***
A X B	NS
A X C	***
B X C	NS
A X B X C	***

NS, *, *** Nonsignificant or significant at P ≤ 0.05 or 0.001 respectively. $LSD_{0.05}$ Value = 0.618, S.E. = 0.0175 and C.V. = 0.005.

There was a highly significant ($P \leq 0.001$) interaction between preharvest ComCat® treatment, disinfecting treatments and storage temperature on the response of the N_2 contents in packages of tomatoes during the 30 days of storage. The N_2 concentrations increased up to 5 and 12 days storage in packages of tomatoes stored at room temperature and 13°C respectively. This increase in N_2 was followed by an equilibration between 80.5% and 81% during the rest of the storage period at 13°C. The storage temperature had a highly significant ($P \leq 0.001$) effect on the N_2 concentration in packages of tomatoes during storage. The N_2 concentrations remained higher in the packages of tomatoes stored at room temperature i.e. above 81.3%, especially during the first five days of storage. At day 5 of storage at room temperature, the N_2 levels of control tomatoes, which were disinfected with chlorine or anolyte, increased to above 82%, and stayed higher than the water washed ones throughout storage. The N_2 levels of the ComCat® treated and chlorine washed packaged tomatoes increased to above 81.5% from day 5 to day 12, but dropped to 81% at day 19. The water washed ComCat® treated tomato packages also had approximately 81.5% of N_2 content at day 5, but dropped to 81% at day 12, while the anolyte treated ones stayed below 81%, following approximately the same pattern as the tomatoes stored at 13°C.

The two-way interaction between the preharvest ComCat® treatment and storage temperature had a significant ($P \leq 0.001$) effect on the changes in the N_2 concentration in packages of tomatoes during storage. The N_2 concentrations were also significantly ($P \leq 0.001$) influenced by the three-way interaction between preharvest ComCat® treatment, disinfecting treatments and storage temperature.

4.3.2. Physiological weight Loss (PWL)

MAP plays an important role in reducing loss of moisture from tomatoes during storage (Table 4.1). MAP had a significant ($P < 0.05$) effect on reducing the PWL during storage at both 13°C as well as room temperature. During 24 days of storage, the PWL was found to be 6.3% and 6.6% more in unpackaged preharvest ComCat® treated tomatoes and controls respectively, than in packaged fruit stored at room temperature.

Table 4.1. Changes in physiological weight loss (%) of tomatoes packaged in Xtend® film or unpackaged and stored at 13°C and room temperature (RT) for 30 days (n = 3 over four storage times).

Treatment	Physiological loss in weight (%)			
	Day 8	Day 16	Day 24	Day 30
ComCat®, Cl_2, MAP, 13°C	1.232^{efg}	2.496^{fgh}	5.017^{gh}	5.546^{b}
ComCat®, Anolyte, MAP, 13°C	0.749^{g}	2.461^{fghi}	3.728^{ghi}	4.428^{bcd}
ComCat®, Cl_2, MAP, RT	5.467^{ab}	5.768^{cd}	11.597^{bcd}	
ComCat®, Anolyte, MAP, RT	4.303^{abc}	6.118^{c}	11.415^{bcd}	
ComCat®, H_2O, MAP, 13°C	0.799^{g}	1.713^{ghi}	2.813^{ijk}	5.100^{bc}
ComCat®, H_2O, MAP, RT	3.938^{abcd}	4.781^{cdef}	10.390^{cdef}	
ComCat®, H_2O, 13°C	2.930^{bcdef}	4.524^{cdefg}	5.925^{def}	8.261^{a}
ComCat®, H_2O, RT	9.819^{a}	13.452^{a}	16.712^{a}	
Control, Cl_2, MAP, 13°C	0.905^{fg}	2.150^{fghi}	2.938^{ijk}	4.132^{cde}
Control, Anolyte, MAP, 13°C	0.256^{g}	1.752^{ghi}	2.239^{jk}	4.057^{cde}
Control, Cl_2, MAP, RT	3.953^{abcd}	5.287^{cde}	12.754^{bc}	
Control, Anolyte, MAP, RT	3.538^{bcde}	4.990^{de}	10.743^{de}	
Control, H_2O, MAP, 13°C	0.255^{g}	1.544^{ghi}	4.435^{gh}	4.826^{bc}
Control, H_2O, MAP, RT	3.291^{bcdef}	5.074^{cde}	9.240^{def}	
Control, H_2O, 13°C	2.261^{defgh}	4.522^{cdefg}	5.920^{g}	8.252^{a}
Control, H_2O, RT	8.688^{a}	12.488^{ab}	15.863^{a}	

Significance

Preharvest treatment (A)	*
Disinfecting + MAP (B)	***
Storage temperature (C)	***
A X B	NS
A X C	NS
B X C	***
A X B X C	NS

NS, *, *** Nonsignificant or significant at $P \leq 0.05$ or 0.001 respectively. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). LSD Value = 2.004, S. E. = 0.079, MSE = 1.535 and C.V. = 0.195.

At 13°C, the PWL was 3.2% and 3.4% more in unpackaged ComCat® treated and control tomatoes respectively for 30 days, compared to packaged ones. Packaging in microperforated Xtend® film not only offered normal respiration of the fruit, but also significantly ($P < 0.05$) reduced PWL.

The PWL was highly affected ($P \leq 0.001$) by the storage temperature during 30 days of storage at 13°C and 24 days at room temperature. There was a direct correlation between storage temperature and PWL. The PWL was significantly reduced with MAP and lower storage temperature. For example, storage of ComCat® treated tomatoes in MAP for 24 days at 13°C reduced PWL from 11.597 to 5.017, which is a by 56.7% reduction, compared to tomatoes stored at RT which reduced PWL from 11.415 to 3.728, which is a 67.3% reduction. Similarly, at 13°C, PWL was reduced by 52.0% to 79.2% in controls compared to storage at room temperature. During 24 days of storage, due to the coupled effect of packaging and storage temperature, PWL was reduced by 83% and 72% in ComCat® treated and untreated tomatoes respectively. In general, MAP and proper low temperature storage are critical factors for increasing shelf life of tomatoes without excessive loss of moisture.

The PWL was affected by the prepackaging disinfecting + MAP treatments (Table 4.1). However, the multiple comparison of the treatment mean showed that the disinfecting treatment had no significant effect on the PWL, suggesting the importance of MAP in order to reduce moisture loss.

Losses were slightly higher in packages of fruits dipped in chlorine supplemented water compared to those dipped in anolyte water and stored at 13°C, however, not significantly. The differences in PWL of tomatoes dipped in chlorinated and anolyte water observed after 24 days storage at room temperature was significantly ($P \leq 0.001$) affected by the interaction between disinfecting treatment, MAP and storage temperature. During the first 24 days of storage, the PWL was less in water washed ComCat® treated and control fruits compared to those dipped in chlorine supplemented, or anolyte water, stored at 13°C. However, the PWL became faster in water washed fruits stored at 13°C after 24 days of storage. The preharvest ComCat® treatment had a significant ($P \leq 0.05$) effect on the PWL of tomatoes during 30 days of storage (Table 4.1). The PWL remained below 6% during 30 days of storage for both ComCat® treated as well as control tomatoes packaged and stored at 13°C. There was no interaction between the preharvest and postharvest treatments on the changes in PWL of the fruit, but the interaction between disinfecting treatment, MAP and storage temperature was significant ($P \leq 0.001$).

4.3.3. Chemical and biochemical changes

4.3.3.1. pH and Total Titratable Acidity (TTA)

Table 4.2 shows the changes in pH of tomatoes during storage at 13°C and room temperature. The changes in pH varied between 3.9 and 4.4. The data show that the pH values of tomatoes generally increased with the ripening process. The packaged control tomatoes stored at 13°C displayed a decrease in pH during the first 8 days of storage, followed by an increase. After the fruit became red and overripe, the pH values seemed to drop, increasing the fruit acidity.

A highly significant ($P \leq 0.001$) difference in pH values of groups of tomatoes stored under different storage temperature was obtained during the storage period as shown in Table 4.2. Throughout the 30 days storage, the pH of stored fruit was distinctively higher in both packaged, as well as unpackaged, tomatoes stored at room temperature, compared to storage at 13°C. The pH also increased faster during storage at room temperature, indicating a faster ripening process. These data also showed that the combination of postharvest treatments had a significant effect on the pH value of tomatoes during storage at 13°C and room temperature. In general, storage at room temperature resulted in an increase in pH in both chlorine and anolyte treated tomatoes.

The pH values of ComCat® treated fruits dipped in chlorinated water were significantly ($P \leq 0.001$) higher than the values in fruit subjected to anolyte water dipping treatment during the first 16 days of storage at 13°C, while after 16 days, the

Table 4.2. Changes in pH value of tomatoes packaged in Xtend® film or unpackaged and stored at 13°C and room temperature (RT) for 30 days (n = 3 over four storage times).

Treatment	pH				
	Day 0	Day 8	Day 16	Day 24	Day 30
ComCat®, Cl_2, MAP, 13°C	4.062^{b}	4.150^{bcde}	4.177^{de}	4.183^{ef}	4.107^{de}
ComCat®, Anolyte, MAP, 13°C	4.062^{b}	4.103^{ef}	4.107^{fgh}	4.190^{ef}	4.143^{bc}
ComCat®, Cl_2, MAP, RT	4.062^{b}	4.107^{ef}	4.213^{bc}	4.350^{a}	
ComCat®, Anolyte, MAP, RT	4.062^{b}	4.083^{f}	4.187^{de}	4.220^{de}	
ComCat®, H_2O, MAP, 13°C	4.062^{b}	4.093^{ef}	4.133^{efgh}	4.123^{gh}	4.130^{bc}
ComCat®, H_2O, MAP, RT	4.062^{b}	4.227^{a}	4.210^{bcd}	4.353^{a}	
ComCat®, H_2O, 13°C	4.062^{b}	3.977^{g}	4.153^{defg}	4.147^{efg}	4.130^{bc}
ComCat®, H_2O, RT	4.062^{b}	4.097^{ef}	4.280^{a}	4.267^{bcd}	
Control, Cl_2, MAP, 13°C	4.120^{a}	4.090^{ef}	4.097^{cd}	4.117^{ghi}	4.170^{ab}
Control, Anolyte, MAP, 13°C	4.120^{a}	4.073^{f}	4.120^{fgh}	4.170^{ef}	4.183^{a}
Control, Cl_2, MAP, RT	4.120^{a}	4.207^{ab}	4.227^{ab}	4.293^{ab}	
Control, Anolyte, MAP, RT	4.120^{a}	4.187^{bc}	4.197^{cd}	4.310^{ab}	
Control, H_2O, MAP, 13°C	4.120^{a}	4.190^{bc}	4.157^{def}	4.083^{i}	4.170^{ab}
Control, H_2O, MAP, RT	4.120^{a}	4.167^{bcd}	4.213^{bc}	4.343^{a}	
Control, H_2O, 13°C	4.120^{a}	4.000^{g}	4.163^{ef}	4.110^{hi}	4.070^{de}
Control, H_2O, RT	4.120^{a}	4.027^{fg}	4.203^{cd}	4.303^{ab}	
Significance					
Preharvest Treatment (A)		***			
Disinfecting + MAP (B)		***			
Storage Temperature (C)		***			
A X B		***			
A X C		***			
B X C		***			
A X B X C		***			

*** Significant at $P \leq 0.001$ respectively. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The LSD Value = 0.041, S.E. = 0.002, MSE = 0.001 and C.V. = 0.012

pH of anolyte disinfected tomatoes was higher. At room temperature, the pH value remained higher in control fruit dipped in chlorinated water during the first 8 days of storage.

At harvest, the pH value of ComCat® treated tomatoes was significantly ($P \leq 0.05$) lower than in controls. During storage at 13°C the pH in disinfected control tomatoes dropped after 8 days of storage, and then increased up to 30 days. However, under the same circumstances, the pH changes in ComCat® treated tomatoes followed a different pattern, i.e. an increase up to day 24, followed by a decrease up to day 30. At day 30 the pH was significantly lower than that of the control tomatoes.

The two-way interaction between disinfecting + MAP treatment and storage temperature was highly significant ($P \leq 0.001$) on the changes in pH of fruits during storage, suggesting the importance of combining proper postharvest treatments to improve the shelf life of tomatoes. Similarly, the three-way interaction between pre-and postharvest treatments, i.e. preharvest ComCat® treatment, disinfecting + MAP and storage temperature, was highly significant ($P \leq 0.001$) on the changes in the pH values of the fruit during 30 days of storage.

Table 4.3 displays the changes in TTA of green mature tomatoes during ripening and storage at 13°C and room temperature, which varied between 0.22% and 0.49%. The TTA of tomatoes decreased during ripening in storage, both at 13°C as well as room temperature. Storage temperature had a highly significant ($P \leq 0.001$) effect on the changes in TTA, when storage at 13°C is compared with storage at room temperature. Table 4.3 shows that the higher storage temperature resulted in a faster decline in TTA of tomatoes. After 24 days of storage, the TTA was lower in tomatoes stored at room temperature compared to storage at 13°C.

Packaging affected the change in TTA significantly. The TTA of tomatoes was lower during the first 8 days of storage in packaged tomatoes compared to the unpackaged ones. During the first 24 days storage at room temperature, packaged control tomatoes

Table 4.3. Changes in titratable acidity of tomatoes packaged in Xtend® film or unpackaged and stored at 13°C and room temperature (RT) for 30 days (n = 3 over four storage times).

Treatment	TTA (% acetic acid)				
	Day 0	Day 8	Day 16	Day 24	Day 30
ComCat®, Cl_2, MAP, 13°C	0.472^{a}	0.365^{cde}	0.416^{ab}	0.336^{bcd}	0.368^{abc}
ComCat®, Anolyte, MAP, 13°C	0.472^{a}	0.385^{def}	0.411^{abc}	0.328^{bcd}	0.336^{bcd}
ComCat®, Cl_2, MAP, RT	0.472^{a}	0.402^{de}	0.331^{de}	0.261^{ef}	
ComCat®, Anolyte, MAP, RT	0.472^{a}	0.393^{de}	0.337^{de}	0.326^{bcd}	
ComCat®, H_2O, MAP, 13°C	0.472^{a}	0.363^{def}	0.417^{ab}	0.390^{ab}	0.288^{cd}
ComCat®, H_2O, MAP, RT	0.472^{a}	0.325^{ef}	0.317^{def}	0.225^{f}	
ComCat®, H_2O, 13°C	0.472^{a}	0.509^{a}	0.399^{bc}	0.358^{abc}	0.351^{bcd}
ComCat®, H_2O, RT	0.472^{a}	0.409^{de}	0.318^{ef}	0.333^{bcd}	
Control, Cl_2, MAP, 13°C	0.387^{b}	0.473^{abc}	0.429^{ab}	0.394^{a}	0.386^{ab}
Control, Anolyte, MAP, 13°C	0.387^{b}	0.435^{bcd}	0.442^{a}	0.339^{abc}	0.381^{abc}
Control, Cl_2, MAP, RT	0.387^{b}	0.342^{def}	0.374^{cde}	0.291^{de}	
Control, Anolyte, MAP, RT	0.387^{b}	0.396^{de}	0.374^{bcde}	0.283^{de}	
Control, H_2O, MAP, 13°C	0.387^{b}	0.418^{cd}	0.392^{bcd}	0.387^{ab}	0.337^{bcd}
Control, H_2O, MAP, RT	0.387^{b}	0.356^{def}	0.348^{cde}	0.282^{de}	
Control, H_2O, 13°C	0.387^{b}	0.487^{ab}	0.375^{bcde}	0.375^{abc}	0.402^{a}
Control, H_2O, RT	0.387^{b}	0.468^{bcd}	0.387^{bcd}	0.320^{cde}	

Significance

Preharvest Treatment (A)	NS
Disinfecting + MAP (B)	***
Storage Temperature (C)	***
A X B	***
A X C	***
B X C	***
A X B X C	***

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01 or 0.001 respectively. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). LSD Value = 0.051, S.E. = 0.017, MSE = 0.0018 and C.V. = 0.019.

maintained a lower TTA, compared to unpackaged controls. In general, the disinfecting treatment did not affect the changes in TTA, except that a significantly higher TTA was noted for chlorine washed ComCat® treated tomatoes after 24 days of storage at room temperature.

At harvest, the TTA of ComCat® treated, mature green tomatoes was higher and significantly different from the TTA of control tomatoes (Table 4.3). However, the statistical analysis on the overall data during the 30 days of storage showed no significant differences between the ComCat® treated and controls.

The two-way interaction between preharvest treatment and disinfecting + MAP was highly significant ($P \leq 0.001$) on the changes in TTA of tomatoes. Similarly, there was a significant ($P \leq 0.001$) interaction between preharvest ComCat® treatment and storage temperature on the changes in TTA content of fruit during the 30 days of storage. There was also a significant ($P \leq 0.001$) interaction between disinfecting + MAP and storage temperature on the response to TTA content of tomatoes, and the three way interaction between pre- and postahrvest treatments was also highly significant ($P \leq 0.001$).

4.3.3.2. Total soluble solids (TSS)

The TSS of tomatoes varied between 4.0 and 5.0 °Brix in this study (Table 4.4). In general, the TSS of tomatoes increased during the first 8 days of storage and dropped thereafter. MAP had a significant ($P \leq 0.05$) effect on the TSS of tomatoes during ripening in storage. At room temperature, unpackaged control tomatoes displayed a higher TSS from 8 to 16 days of storage than packaged ones. At 13°C storage, unpackaged tomatoes showed the lowest TSS's for ComCat® treated tomatoes from day 16 to day 30. In the control tomatoes the TSS of unpackaged tomatoes was distinctly different from all the packaged ones at any time interval, however, a specific pattern was not visible.

Storage temperature was one of the main factors governing the changes in TSS content of tomatoes. Tomatoes that were disinfected before packaging, showed a

Table 4.4. Changes in total soluble solid (TSS) of tomatoes packaged in Xtend® film or unpackaged and stored at 13°C and room temperature (RT) for 30 days (n = 3 over four storage times).

Treatment	Total soluble solid TSS (°Brix)				
	Day 0	Day 8	Day 16	Day 24	Day 30
ComCat®, Cl_2, MAP, 13°C	4.360 ^{a}	4.767 ^{bc}	4.500 ^{bcdef}	4.403 ^{c}	4.267 ^{bcd}
ComCat®, Anolyte, MAP, 13°C	4.360 ^{a}	4.502 ^{efg}	4.697 ^{a}	4.797 ^{a}	4.337 ^{ab}
ComCat®, Cl_2, MAP, RT	4.360 ^{a}	4.760 ^{bc}	4.487 ^{cdef}	4.344 ^{d}	
ComCat®, Anolyte, MAP, RT	4.360 ^{a}	4.393 ^{hi}	4.523 ^{bcde}	4.597 ^{b}	
ComCat®, H_2O, MAP, 13°C	4.360 ^{a}	4.627 ^{d}	4.613 ^{abc}	4.240 ^{def}	3.997 ^{f}
ComCat®, H_2O, MAP, RT	4.360 ^{a}	4.327 ^{ij}	4.593 ^{bc}	4.003 ^{h}	
ComCat®, H_2O, 13°C	4.360 ^{a}	4.593 ^{def}	4.033 ^{i}	4.033 ^{h}	4.050 ^{ef}
ComCat®, H_2O, RT	4.360 ^{a}	4.917 ^{a}	4.537 ^{bcde}	4.410 ^{c}	
Control, Cl_2, MAP, 13°C	4.248 ^{ab}	4.750 ^{bc}	4.190 ^{jk}	4.390 ^{cd}	4.393 ^{ab}
Control, Anolyte, MAP, 13°C	4.248 ^{ab}	4.417 ^{hi}	4.410 ^{gh}	4.421 ^{bc}	4.400 ^{f}
Control, Cl_2, MAP, RT	4.248 ^{ab}	4.433 ^{ghi}	4.423 ^{gh}	4.333 ^{de}	
Control, Anolyte, MAP, RT	4.248 ^{ab}	4.817 ^{ab}	4.493 ^{bcdef}	4.200 ^{fg}	
Control, H_2O, MAP, 13°C	4.248 ^{ab}	4.583 ^{def}	4.190 ^{jk}	4.197 ^{g}	4.033 ^{f}
Control, H_2O, MAP, RT	4.248 ^{ab}	4.483 ^{fgh}	4.593 ^{bcd}	4.397 ^{c}	
Control, H_2O, 13°C	4.248 ^{ab}	4.433 ^{ghi}	4.493 ^{cdef}	4.197 ^{g}	4.147 ^{dc}
Control, H_2O, RT	4.248 ^{ab}	4.833 ^{ab}	4.677 ^{a}	4.033 ^{h}	

Significance

Preharvest Treatment (A)	**
Disinfecting + MAP (B)	**
Storage Temperature (C)	***
A X B	*
A X C	***
B X C	***
A X B X C	**

, * Significant at $P \leq 0.01$ or 0.001 respectively. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($p<0.05$). The LSD Value = 0.072, S.E. = 0.003, MSE = 0.002 and C.V. = 0.011.

significantly different profile of TSS changes during storage at 13°C, with water washed tomatoes having the lowest TSS content from day 24 on. There was also a difference between the disinfecting treatments, in that the anolyte dipped tomatoes contained more TSS than the chlorine dipped ones, from day 16 onwards.

At room temperature storage the ComCat® treated tomatoes had higher TSS contents after disinfecting. The highest TSS content, from day 16 onwards, was observed for ComCat® treated tomatoes disinfected with anolyte. Water washed control samples had the lowest TSS content at day 8 of storage, but they reached higher contents from day 16 on, compared to the disinfected ones. Disinfecting + MAP in general resulted in significantly ($P \leq 0.01$) different TSS changes during storage. Storage temperature also had a significant ($P \leq 0.001$) influence on the TSS content. The TSS content of unpackaged tomatoes was higher when stored at room temperature in ComCat® treated tomatoes throughout storage. In unpackaged control tomatoes, the TSS content was significantly ($P < 0.05$) higher during room temperature storage up to day 16. Other comparisons did not result in a specific pattern regarding storage temperature.

ComCat® treatment resulted in a higher TSS content at harvest, however, not significantly ($P > 0.05$) higher. During storage, significant differences were observed ($P \leq 0.001$). ComCat® treated tomatoes had higher trends of TSS contents during storage after disinfection than the control, while the TSS contents were observed to be lower when not disinfected. These results suggested that coupled effects exist, which was confirmed by the statistical analysis. Significant two-way interactions ($P > 0.001$) and a three-way interaction ($P > 0.01$), were obtained between all the treatments.

4.3.3.3. Free sugar

The free sugars consist of glucose and fructose, present in approximately equal amounts. In this study, the sucrose content remained below 0.1 g. 100 g^{-1} during the 30 days of storage, both at 13°C and room temperature (Table 4.5). The sucrose content increased during the first 8 days of storage, but declined thereafter during storage at both temperatures.

Disinfecting treatments and packaging showed no significant effect on the sucrose content at both storage temperatures.

Storage temperature had a significant ($P \leq 0.001$) effect on the sucrose content. At 13°C the sucrose content increased up to 8 days, from 0.03 $g.100g^{-1}$ to above 0.04 $g.100g^{-1}$, followed by a slow return thereafter, to around the initial values after 24 days. At room temperature, a decrease to below the initial values is observed in most of the tomato samples subjected to different treatments after day 16 of storage.

The preharvest ComCat® treatment had a significant ($P \leq 0.05$) effect on the changes in sucrose content of tomatoes. In general, the sucrose content was found to be slightly lower in ComCat® treated fruits during the 30 days of storage. The effect was mainly expressed in tomatoes stored at room temperature, where the sucrose content of the ComCat® treated tomatoes was observed to drop to lower levels, i.e. below 0.027 $g.100g^{-1}$, from day 16 onwards, as compared to the controls.

The sucrose content of tomatoes was not significantly affected by the interaction of preharvest ComCat® treatment and prepackaging disinfectant + MAP. However, sucrose content was influenced by the interaction of preharvest ComCat® treatment and storage temperature. These results confirm that, in order to maintain the sucrose content of ComCat® treated tomatoes, the proper storage temperature should be maintained during storage. The interaction between different postharvest treatments (disinfecting, MAP and storage temperature) had a slight influence on the changes in sucrose content of tomatoes stored at 13°C as well as at room temperature.

The range of the values of glucose (between 0.077 $g.100\ g^{-1}$ and 1.24 $g.100\ g^{-1}$) and fructose (between 1.01 $g.100\ g^{-1}$ and 1.58 $g.100\ g^{-1}$) in tomatoes found in this study was similar to those reported by Maul *et al.* (2000). MAP slightly reduced the rate of glucose utilisation during storage (Table 4.5 (b)). Throughout storage the glucose content was found to be higher in packaged than in unpackaged fruits. Disinfecting treatment also resulted in different glucose dynamics. In general, the glucose levels were lower after 16 days of storage at 13°C in water washed tomatoes, compared to the

Table 4.5 (a). Changes in sucrose content of tomatoes packaged in Xtend® film or unpackaged and stored at 13°C and room temperature (RT) for 30 days (n = 3 over four storage times).

Treatment	Sucrose (g.100g^{-1})				
	day 0	day 8	Day 16	day 24	day 30
ComCat®, Cl_2, MAP, 13°C	0.030^{a}	0.042^{abcd}	0.033^{abcd}	0.030^{abc}	0.032^{a}
ComCat®, Anolyte, MAP, 13°C	0.030^{a}	0.044^{abc}	0.028^{bcde}	0.025^{bcde}	0.029^{ab}
ComCat®, Cl_2, MAP, RT	0.030^{a}	0.039^{bcdefg}	0.027^{cdef}	0.020^{de}	
ComCat®, Anolyte, MAP, RT	0.030^{a}	0.038^{bcdefg}	0.028^{cdef}	0.022^{de}	
ComCat®, H_2O, MAP, 13°C	0.030^{a}	0.050^{a}	0.026^{cdef}	0.024^{cde}	0.028^{abc}
ComCat®, H_2O, MAP, RT	0.030^{a}	0.043^{abc}	0.026^{cdef}	0.020^{de}	
ComCat®, H_2O, 13°C	0.030^{a}	0.040^{bcdef}	0.039^{a}	0.033^{ab}	0.030^{a}
ComCat®, H_2O, RT	0.030^{a}	0.036^{cdefg}	0.024^{def}	0.019^{e}	
Control, Cl_2, MAP, 13°C	0.031^{a}	0.043^{abc}	0.035^{abc}	0.033^{ab}	0.033^{a}
Control, Anolyte, MAP, 13°C	0.031^{a}	0.041^{bcde}	0.037^{ab}	0.034^{a}	0.028^{abc}
Control, Cl_2, MAP, RT	0.031^{a}	0.047^{ab}	0.034^{abc}	0.031^{abc}	
Control, Anolyte, MAP, RT	0.031^{a}	0.040^{bcdef}	0.034^{abc}	0.028^{bcd}	
Control, H_2O, MAP, 13°C	0.03^{a}	0.042^{abcd}	0.029^{bcde}	0.032^{abc}	0.032^{a}
Control, H_2O, MAP, RT	0.03^{a}	0.041^{bcd}	0.031^{abcd}	0.031^{abc}	
Control, H_2O, 13°C	0.03^{a}	0.047^{ab}	0.033^{abcd}	0.033^{ab}	0.031^{a}
Control, H_2O, RT	0.03^{a}	0.041^{bcd}	0.027^{cdef}	0.024^{cde}	

Significance	
Preharvest treatment (A)	*
Disinfecting + MAP (B)	NS
Storage temperature (C)	***
A X B	NS
A X C	***
B X C	*
A X B X C	NS

NS, *, *** Nonsignificant or significant at $P \leq 0.05$ or 0.001 respectively. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The LSD Value = 0.007, S.E. = 0.003, MSE = 0.001 and C.V. = 0.139.

chlorine and anolyte washed ones. At room temperature, the glucose content was higher at days 16 in water washed tomatoes, before dropping to lower values.

Although the difference was not significant ($P > 0.05$), anolyte dipping resulted in a higher glucose content after 24 days of storage at 13°C in ComCat® treated tomatoes, and higher contents at days 8 and 16 at room temperature, compared to the chlorine dipped tomatoes. ComCat® treatment was shown to have a significant ($P \leq 0.05$) effect on the glucose content of tomatoes during storage, and was, in general, lower from harvest throughout storage. The changes in glucose content were not affected by the interaction of preharvest treatment and disinfecting + MAP, but was highly influenced by the interaction of preharvest treatment and storage temperature. Table 4.5 (c) shows the changes in fructose in tomatoes stored at 13°C as well as room temperature.

Unlike changes in glucose, the fructose content increased during the active period of the ripening process, until the fruit were close to the climacteric peak. Only from the full ripe and overripe stages of the fruit, did the fructose content decrease.

In general, the packaged tomatoes showed higher levels of fructose at both storage temperatures. Disinfection did not have a very obvious effect on the fructose content, however, lower fructose contents were noted in chlorine and anolyte dipped samples at 8 days of storage at 13°C and 8 and 16 days storage at room temperature, compared to the water washed ones. The fructose content of ComCat® treated tomatoes was lower at harvest and generally had a slightly higher fructose content in tomatoes dipped in chlorinated and anolyte water during storage at 13°C.

The fructose content was not affected by the interaction between ComCat® treatment and disinfecting + MAP, but was influenced by the interaction between preharvest ComCat® treatment and storage temperature. The three-way interaction between preharvest ComCat® and postharvest treatments i.e. disinfecting, MAP and storage temperature influenced the changes in fructose concentration at $p \leq 0.05$ significance level.

Table 4.5 (b). Changes in glucose content of tomatoes packaged in Xtend® film or unpackaged and stored at 13°C and room temperature (RT) for 30 days (n = 3 over four storage times).

Treatment	Glucose ($g.100g^{-1}$)				
	day 0	Day 8	day 16	day 24	day 30
ComCat®, Cl_2, MAP, 13°C	1.374^{ab}	1.193^{abc}	1.177^{abc}	0.994^{abc}	1.026^{ab}
ComCat®, Anolyte, MAP, 13°C	1.374^{ab}	1.017^{cdef}	1.017^{cd}	1.157^{a}	1.120^{a}
ComCat®, Cl_2, MAP, RT	1.374^{ab}	1.013^{cdef}	0.978^{cde}	0.952^{abcd}	
ComCat®, Anolyte, MAP, RT	1.374^{ab}	1.023^{cdef}	1.018^{cd}	0.852^{cde}	
ComCat®, H_2O, MAP, 13°C	1.374^{ab}	1.213^{ab}	1.171^{abc}	0.967^{abcd}	1.030^{ab}
ComCat®, H_2O, MAP, RT	1.374^{ab}	1.138^{abc}	1.134^{bcd}	0.890^{bcde}	
ComCat®, H_2O, 13°C	1.374^{ab}	0.872^{efg}	1.010^{cd}	0.972^{abcd}	0.929^{abc}
ComCat®, H_2O, RT	1.374^{ab}	0.959^{defg}	1.042^{cde}	0.751^{e}	
Control, Cl_2, MAP, 13°C	1.394^{a}	1.279^{a}	1.270^{a}	1.037^{abc}	1.077^{a}
Control, Anolyte, MAP, 13°C	1.394^{a}	1.268^{a}	1.243^{ab}	1.021^{abc}	1.044^{ab}
Control, Cl_2, MAP, RT	1.394^{a}	1.099^{bcde}	1.203^{abc}	1.017^{abc}	
Control, Anolyte, MAP, RT	1.394^{a}	1.220^{ab}	1.219^{ab}	1.016^{abc}	
Control, H2O, MAP, 13°C	1.394^{a}	1.176^{abc}	1.168^{abcd}	0.984^{abcd}	1.015^{ab}
Control, H2O, MAP, RT	1.394^{a}	1.170^{abc}	1.302^{a}	1.042^{abc}	
Control, Water, 13°C	1.394^{a}	1.150^{abcd}	1.118^{abcd}	0.965^{abcd}	0.901^{abc}
Control, water, RT	1.394^{a}	1.027^{cdef}	0.897^{ef}	0.776^{abc}	

Significance

Preharvest treatment (A)	*
Disinfecting + MAP (B)	***
Storage temperature (C)	***
A X B	NS
A X C	***
B X C	*
A X B X C	NS

NS, *, ** Nonsignificant or significant at $P \leq 0.05$ or 0.01 respectively. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The LSD Value = 0.169, S. E. = 0.019, MSE = 0.011 and C.V. = 0.095.

Table 4.5 (c). Changes in fructose content of tomatoes packaged in Xtend® film or unpackaged and stored at 13°C and room temperature (RT) for 30 days (n = 3 over four storage times).

Treatment	Fructose ($g.100g^{-1}$)				
	day 0	day 8	day 16	day 24	day 30
ComCat®, Cl_2, MAP, 13°C	1.287^{ab}	1.359^{abcde}	1.499^{ab}	1.431^{ab}	1.290^{a}
ComCat®, Anolyte, MAP, 13°C	1.287^{ab}	1.272^{bcde}	1.377^{abc}	1.535^{a}	1.348^{a}
ComCat®, Cl_2, MAP, RT	1.287^{ab}	1.161^{de}	1.226^{cdef}	1.223^{bcd}	
ComCat®, Anolyte, MAP, RT	1.287^{ab}	1.167^{de}	1.269^{bcde}	1.131^{cd}	
ComCat®, H_2O, MAP, 13°C	1.287^{ab}	1.527^{ab}	1.486^{abc}	1.305^{abc}	1.326^{a}
ComCat®, H_2O, MAP, RT	1.287^{ab}	1.155^{de}	1.324^{abcd}	1.181^{cd}	
ComCat®, H_2O, 13°C	1.287^{ab}	1.141^{e}	1.320^{abcd}	1.354^{ab}	1.221^{ab}
ComCat®, H_2O, RT	1.287^{ab}	1.164^{de}	1.142^{f}	1.049^{d}	
Control, Cl_2, MAP, 13°C	1.371^{a}	1.556^{a}	1.387^{abcd}	1.310^{abc}	1.264^{ab}
Control, Anolyte, MAP, 13°C	1.371^{a}	1.393^{abcde}	1.552^{a}	1.258^{bcd}	1.141^{b}
Control, Cl_2, MAP, RT	1.371^{a}	1.322^{abcde}	1.489	1.221^{bcd}	
Control, Anolyte, MAP, RT	1.371^{a}	1.464^{ab}	1.472^{abc}	1.304^{abc}	
Control, H_2O, MAP, 13°C	1.371^{a}	1.449^{abc}	1.462^{abc}	1.328^{ab}	1.269^{ab}
Control, H_2O, MAP, RT	1.371^{a}	1.593^{a}	1.577^{a}	1.266^{abc}	
Control, H_2O, 13°C	1.371^{a}	1.418^{abcde}	1.411^{abcd}	1.349^{ab}	1.212^{ab}
Control, H_2O, RT	1.371^{a}	1.445^{abc}	1.176^{ef}	1.013^{d}	

Significance

Preharvest treatment (A)	*
Disinfecting + MAP (B)	*
Storage temperature (C)	***
A X B	NS
A X C	**
B X C	NS
A X B X C	*

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01 or 0.001 respectively. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The LSD Value = 0.210, S.E. = 0.016, MSE = 0.017 and C.V. = 0.100.

4.3.3.4. Total soluble sugar

Table 4.6 displays the total soluble sugar content calculated from the summation of sucrose, glucose and fructose content of tomatoes during the storage, which varied between 1.8 $g.100^{-1}g$ and 2.96 $g.100^{-1}$. The total soluble sugar decreased faster in

Table 4.6. Changes in total sugar content of tomatoes packaged in Xtend® film or unpackaged and stored at 13°C and room temperature (RT) for 30 days (n = 3 over four storage times).

Treatment	Total soluble sugar ($g.100g^{-1}$ FW)				
	Day 0	Day 8	Day 16	Day 24	Day 30
ComCat®, Cl_2, MAP, 13°C	2.691^{ab}	2.595^{abcd}	2.802^{ab}	2.456^{abc}	2.348^{a}
ComCat®, Anolyte, MAP, 13°C	2.691^{ab}	2.536^{abcde}	2.422^{bcdef}	2.717^{a}	2.497^{a}
ComCat®, Cl_2, MAP, RT	2.691^{ab}	2.214^{defg}	2.230^{cdefg}	2.195^{bcd}	
ComCat®, Anolyte, MAP, RT	2.691^{ab}	2.229^{defg}	2.313^{cdefg}	2.003^{cd}	
ComCat®, H_2O, MAP, 13°C	2.691^{ab}	2.791^{abc}	2.683^{abcd}	2.296^{abcd}	2.384^{a}
ComCat®, H_2O, MAP, RT	2.691^{ab}	2.368^{cdef}	2.484^{bcde}	2.091^{cd}	
ComCat®, H_2O, 13°C	2.691^{ab}	2.204^{defg}	2.369^{bcdef}	2.359^{abc}	2.181^{ab}
ComCat®, H_2O, RT	2.691^{ab}	2.160^{efg}	2.064^{egf}	1.819^{d}	
Control, Cl_2, MAP, 13°C	2.796^{a}	2.879^{ab}	2.465^{bcde}	2.380^{abc}	2.374^{a}
Control, Anolyte, MAP, 13°C	2.796^{a}	2.701^{abc}	2.832^{ab}	2.314^{abcd}	2.213^{ab}
Control, Cl_2, MAP, RT	2.796^{a}	2.469^{abcde}	2.726^{abc}	2.269^{bcd}	
Control, Anolyte, MAP, RT	2.796^{a}	2.754^{abc}	2.725^{abc}	2.348^{abc}	
Control, H_2O, MAP, 13°C	2.796^{a}	2.667^{abcd}	2.659^{abcd}	2.344^{abc}	2.317^{b}
Control, H_2O, MAP, RT	2.796^{a}	2.954^{a}	2.910^{a}	2.339^{abc}	
Control, H_2O, 13°C	2.796^{a}	2.615^{abcd}	2.562^{abcde}	2.347^{abc}	2.144^{ab}
Control, H_2O, RT	2.796^{a}	2.647^{abcd}	2.159^{defg}	1.818^{d}	

Significance

Preharvest treatment (A)	*
Disinfecting + MAP (B)	*
Storage temperature (C)	***
A X B	NS
A X C	***
B X C	NS
A X B X C	NS

NS, *, *** Nonsignificant or significant at $P \le 0.05$ or 0.001 respectively. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The LSD Value = 0.042, S.E.= 0.042, MSE = 0.064 and C.V. = 0.103.

unpackaged tomatoes stored at room temperature, as well as at 13°C. No conclusion could be drawn from the disinfecting treatment in terms of the total soluble sugar content, when compared with each other, or with the water washed samples.

Storage temperature had a significant ($P \leq 0.001$) effect on the total soluble sugar of packaged stored tomatoes. Storage at room temperature generally resulted in lower total soluble sugar levels than that of tomatoes stored at 13°C from day 16 onwards.

ComCat® treatment resulted in tomatoes having a lower total soluble sugar than the controls at harvest, although not significantly ($P > 0.05$). This difference was continued up to 8 days at both temperatures. From day 24 onwards, the ComCat® treated tomatoes stored at 13°C had a higher total soluble sugar than the controls, except for the chlorine washed samples.

The interaction between ComCat® treatment and storage temperature had an influence on the changes in total soluble sugar of tomatoes during storage at 13°C and room temperature. In general, the results showed that the overall sugar content of tomatoes was maintained better when preharvest ComCat® treatment is combined with a proper disinfecting treatment, MAP and low storage temperature.

4.3.3.5. Sucrose equivalent

Table 4.7 displays the sucrose equivalent ($g.100^{-1}$ g) in tomato fruits subjected to different pre-and postharvest treatments and stored either at 13°C or room temperature. The values were calculated from experimentally obtained glucose and fructose values, as being between 2.32 $g.100^{-1}$ and 3.73 $g.100^{-1}$, and is an indication of the sweetening power of these sugars (Maul *et al.*, 2000). The general trend of the sucrose equivalent was to drop to lower values during storage, from above 3.2 $g.100^{-1}$ to below 3.0 $g.100^{-1}$, except for anolyte washed ComCat® treated tomatoes.

Packaging affected the sucrose equivalent such that sucrose equivalent values dropped to lower values in unpackaged tomatoes. Disinfecting + MAP had a significant ($P \leq 0.05$) effect on the sucrose equivalent of tomatoes during storage. Anolyte washed

ComCat® treated tomatoes had higher sucrose equivalent values from 24 days of storage on, compared to water washed and chlorine disinfected ones. However, the control

Table 4.7. Changes in sucrose equivalent (g.100^{-1} g fresh weight) of tomatoes packaged in Xtend® film or unpackaged and stored at 13°C and room temperature (RT) for 30 days (n = 3 over four storage times).

Treatment	Sucrose Equivalent (g.100g^{-1} FW)				
	Day 0	Day 8	Day 16	Day 24	Day 30
ComCat®, Cl_2, MAP, 13°C	3.244^{ab}	3.137^{abcde}	3.465^{abc}	2.357^{ef}	2.990^{a}
ComCat®, Anolyte, MAP, 13°C	3.244^{ab}	2.954^{cdef}	2.970^{cdef}	3.511^{a}	3.161^{a}
ComCat®, Cl_2, MAP, RT	3.244^{ab}	2.759^{def}	2.845^{defgh}	2.820^{cdef}	
ComCat®, Anolyte, MAP, RT	3.244^{ab}	2.776^{cdef}	2.948^{cdef}	2.588^{ef}	
ComCat®, H_2O, MAP, 13°C	3.244^{ab}	3.540^{ab}	3.437^{abc}	2.974^{bcde}	3.055^{a}
ComCat®, H_2O, MAP, RT	3.244^{ab}	3.580^{a}	3.130^{abcdef}	2.702^{def}	
ComCat®, H_2O, 13°C	3.244^{ab}	2.618^{ef}	3.031^{bcdef}	3.062^{abcd}	2.800^{abc}
ComCat®, H_2O, RT	3.244^{ab}	2.724^{def}	3.105^{abcdef}	2.371^{ef}	
Control, Cl_2, MAP, 13°C	3.403^{a}	3.639^{a}	3.171^{abcdef}	3.033^{abcd}	2.984^{ab}
Control, Anolyte, MAP, 13°C	3.403^{a}	3.274^{ab}	3.605^{ab}	2.933^{bcde}	2.747^{bc}
Control, Cl_2, MAP, RT	3.403^{a}	3.101^{abcde}	3.467^{abc}	2.864^{cdef}	
Control, Anolyte, MAP, RT	3.403^{a}	3.458^{abcd}	3.448^{abc}	3.007^{abcd}	
Control, H_2O, MAP, 13°C	3.403^{a}	3.377^{abc}	3.393^{abcd}	3.026^{abcd}	2.981^{ab}
Control, H_2O, MAP, RT	3.403^{a}	3.732^{a}	3.691^{a}	2.961^{bcde}	
Control, H_2O, 13°C	3.403^{a}	3.304^{abcd}	3.269^{abcde}	3.048^{abcd}	2.764^{bc}
Control, H_2O, RT	3.403^{a}	3.359^{abc}	2.743^{efgh}	2.327^{f}	
Significance					
Preharvest treatment (A)		*			
Disinfecting + MAP (B)		*			
Storage temperature (C)		***			
A X B		NS			
A X C		***			
B X C		NS			
A X B X C		*			

NS, *, *** Nonsignificant or significant at $P \leq 0.05$ or 0.001 respectively. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The LSD Value = 0.492, S.E. =0.040, MSE = 0.093 and C.V. = 0.099.

tomatoes disinfected with anolyte showed lower sucrose equivalent values during the same storage period. No conclusive deductions could therefore be made from comparisons of the disinfecting treatments amongst each other, or with the water washed tomatoes.

The storage temperature was highly significant ($P \leq 0.001$) on the sucrose equivalent, in that the lowest levels were reached after 24 days of storage at room temperature, compared to 30 days storage at 13°C.

The sucrose equivalent was lower in ComCat® treated tomatoes at harvest, although not significant, and remained lower in most of the tomato samples throughout storage when compared to the controls ($P \leq 0.05$), except for anolyte washed ComCat® treated tomatoes. It was also not influenced by the interaction between the preharvest treatment with disinfecting + MAP. The interaction between the preharvest ComCat® treatment and storage temperature had a significant effect on the sucrose equivalent ($P \leq 0.001$). The three-way interaction between the pre-and postharvest treatment also had a significant ($P \leq 0.05$) effect on the sucrose equivalent.

4.3.3.6. Sucrose-hexose ratio

The sucrose-hexose (SH) ratio varied between 0.009 and 0.021, and increased during the first 8 days of storage (Table 4.8), followed by a general decrease from day 16 on. The SH ratios were slightly affected by storage temperature, with tomatoes stored at 13°C showing higher values.

The SH ratio was slightly influenced with the disinfecting + MAP treatment, but the difference here was not distinct between the SH ratio values for tomatoes disinfected by chlorinated or anolyte water, nor water wash, during storage. The SH ratio was slightly affected by the preharvest ComCat® treatment during storage at 13°C as well as room temperature. Only during the early stage of storage (day 8) was the sucrose-hexose ratio significantly higher in preharvest ComCat® treated tomatoes at both storage temperatures. None of the two–way interactions between pre- and postharvest treatments had an influence on the changes in SH ratio during storage.

Table 4.8. Changes in SH ratio of tomatoes packaged in Xtend® film or unpackaged and stored at 13°C and room temperature (RT) for 30 days (n = 3 over four storage times).

Treatment	Sucrose-hexose ratio				
	Day 0	Day 8	Day 16	Day 24	Day 30
ComCat®, Cl_2, MAP, 13°C	0.011^{a}	0.017^{cde}	0.012^{cd}	0.012^{de}	0.014^{abc}
ComCat®, Anolyte, MAP, 13°C	0.011^{a}	0.018^{bc}	0.011^{de}	0.009^{g}	0.012^{cde}
ComCat®, Cl_2, MAP, RT	0.011^{a}	0.019^{ab}	0.012^{cd}	0.009^{g}	
ComCat®, Anolyte, MAP, RT	0.011^{a}	0.018^{bc}	0.012^{cd}	0.010^{fg}	
ComCat®, H_2O, MAP, 13°C	0.011^{a}	0.019^{ab}	0.010^{ef}	0.011^{ef}	0.012^{cde}
ComCat®, H_2O, MAP, RT	0.011^{a}	0.019^{ab}	0.010^{ef}	0.010^{fg}	
ComCat®, H_2O, 13°C	0.011^{a}	0.019^{ab}	0.017^{a}	0.014^{bc}	0.014^{abc}
ComCat®, H_2O, RT	0.011^{a}	0.017^{cde}	0.012^{cd}	0.010^{fg}	
Control, Cl_2, MAP, 13°C	0.011^{a}	0.016^{ef}	0.014^{b}	0.014^{bc}	0.014^{abc}
Control, Anolyte, MAP, 13°C	0.011^{a}	0.016^{ef}	0.013^{bc}	0.015^{ab}	0.013^{bcd}
Control, Cl_2, MAP, RT	0.011^{a}	0.021^{a}	0.013^{bc}	0.014^{bc}	
Control, Anolyte, MAP, RT	0.011^{a}	0.015^{fg}	0.013^{bc}	0.012^{de}	
Control, H_2O, MAP, 13°C	0.011^{a}	0.016^{ef}	0.011^{de}	0.014^{bc}	0.014^{abc}
Control, H_2O, MAP, RT	0.011^{a}	0.014^{gh}	0.011^{de}	0.013^{cd}	
Control, H_2O, 13°C	0.011^{a}	0.018^{bc}	0.013^{bc}	0.014^{bc}	0.015^{a}
Control, H_2O, RT	0.011^{a}	0.016^{ef}	0.013^{bc}	0.016^{a}	
Significance					
Preharvest treatment (A)		**			
Disinfecting + MAP (B)		*			
Storage temperature (C)		*			
A X B		NS			
A X C		NS			
B X C		NS			
A X B X C		NS			

NS, * Nonsignificant or significant at $P \leq 0.05$. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The LSD Value = 0.002, S.E. = 0.001, MSE = 0.001 and C.V. = 0.206.

4.3.3.7. Sugar-acid ratio

Table 4.9 shows the sugar-acid (SA) ratio in treated tomatoes varying between 4.34 and 8.41. MAP had a significant effect on the SA ratio. The SA was significantly ($P \leq 0.001$) lower for unpackaged tomatoes stored at room temperature. The packaged

Table 4.9. Changes in sugar-acid ratio of tomatoes packaged in Xtend® film or unpackaged and stored at 13°C and room temperature (RT) for 30 days (n = 3 over four storage times).

Treatment	Sugar-acid ratio				
	Day 0	Day 8	Day 16	Day 24	Day 30
ComCat®, Cl_2, MAP, 13°C	5.545^{b}	7.118^{abc}	6.741^{cde}	7.310^{bcde}	6.388^{cde}
ComCat®, Anolyte, MAP, 13°C	5.545^{b}	6.623^{bcde}	5.893^{ef}	8.287^{abc}	7.437^{ab}
ComCat®, Cl_2, MAP, RT	5.545^{b}	5.510^{ef}	6.785^{cde}	8.407^{ab}	
ComCat®, Anolyte, MAP, RT	5.545^{b}	5.662^{def}	6.870^{cde}	6.146^{efg}	
ComCat®, H_2O, MAP, 13°C	5.545^{b}	7.695^{ab}	6.432^{def}	5.893^{fgh}	8.279^{a}
ComCat®, H_2O, MAP, RT	5.545^{b}	7.282^{abc}	7.828^{abc}	9.306^{a}	
ComCat®, H_2O, 13°C	5.545^{b}	4.342^{f}	5.936^{def}	6.596^{cdef}	6.220^{cde}
ComCat®, H_2O, RT	5.545^{b}	5.283^{f}	6.509^{cdef}	5.468^{gh}	
Control, Cl_2, MAP, 13°C	7.226^{a}	6.089^{cde}	5.745^{ef}	6.045^{efg}	6.155^{cde}
Control, Anolyte, MAP, 13°C	7.226^{a}	6.222^{cde}	6.407^{def}	6.830^{defg}	5.813^{de}
Control, Cl_2, MAP, RT	7.226^{a}	7.258^{abc}	7.292^{abcd}	7.799^{bcd}	
Control, Anolyte, MAP, RT	7.226^{a}	6.965^{bcd}	7.299^{abcd}	8.287^{abc}	
Control, H_2O, MAP, 13°C	7.226^{a}	6.391^{cde}	6.792^{def}	6.055^{efg}	6.865^{abc}
Control, H_2O, MAP, RT	7.226^{a}	8.288^{a}	8.379^{a}	8.280^{abc}	
Control, H_2O, 13°C	7.226^{a}	5.364^{ef}	6.854^{cde}	6.264^{efg}	5.332^{ef}
Control, H_2O, RT	7.226^{a}	5.647^{def}	5.586^{efg}	5.681^{fgh}	
Significance					
Preharvest treatment (A)		*			
Disinfecting + MAP (B)		***			
Storage temperature (C)		***			
A X B		NS			
A X C		***			
B X C		***			
A X B X C		***			

NS, *** Nonsignificant or Significant at $P \leq 0.001$. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The LSD Value = 1.101, S.E. = 0.101, MSE = 0.465 and C.V. = 0.102.

tomatoes stored at 13°C had a higher SA ratio at days 8 and 30 only, compared to unpackaged ones. The disinfecting + MAP had a significant ($P \leq 0.001$) effect on the SA ratio, although differences between the disinfecting treatments show no real conclusive differences.

Storage temperature also had a significant influence on the SA ratio. After 24 days of storage, the SA ratio was found to be significantly ($P \leq 0.001$) lower in packaged tomatoes stored at 13°C compared to those stored at room temperature, except in ComCat® treated tomatoes dipped in anolyte water.

At harvest, green-mature ComCat® treated tomatoes had a significantly ($P \leq 0.05$) lower SA ratio, when compared to the controls. During the ripening time the SA ratio generally increased more in ComCat® treated tomatoes, while a general tendency of a decrease was observed in ripening control tomatoes.

The SA ratio was significantly ($P \leq 0.001$) affected by the interaction between ComCat® treatment and storage temperature. The two-way interaction between postharvest treatments (disinfecting, MAP and storage temperature) had a significant ($P \leq 0.001$) influence on the SA ratio. Similarly, the three-way interaction between the pre-and postharvest treatment was also highly significant ($P \leq 0.001$) on the changes in the SA ratio during storage of green mature tomatoes.

4.3.3.8. Ascorbic acid

Table 4.10 displays the changes in ascorbic acid content in ripening tomatoes, subjected to different pre- and postharvest treatments, as between 11.10 $g.100^{-1}$ and 23.23 $g.100^{-1}$. The general tendency observed was an increase in AA during days 8 to 16, followed by a decrease towards the end of storage. MAP seemed to maintain higher AA contents in tomatoes during storage than unpackaged fruit. Disinfecting treatments also affected the AA content of tomatoes. Anolyte disinfecting resulted in higher AA content throughout ripening at both storage temperatures when compared to chlorine disinfecting and water washing.

Table 4.10. Changes in ascorbic acid of tomatoes packaged in Xtend® film or unpackaged and stored at 13°C and room temperature (RT) for 30 days (n = 3 over four storage times).

Treatment	Ascorbic acid ($g.100g^{-1}$ FW)				
	Day 0	Day 8	Day 16	Day 24	Day 30
ComCat®, Cl_2, MAP, 13°C	11.580^{ab}	12.529^{hij}	14.387^{def}	16.677^{bc}	16.445^{bcd}
ComCat®, Anolyte, MAP, 13°C	11.580^{ab}	16.116^{defg}	17.520^{ab}	19.356^{a}	23.223^{a}
ComCat®, Cl_2, MAP, RT	11.580^{ab}	17.218^{cde}	12.733^{efg}	8.679^{l}	
ComCat®, Anolyte, MAP, RT	11.580^{ab}	15.598^{efg}	16.963^{abc}	16.603^{bc}	
ComCat®, H_2O, MAP, 13°C	11.580^{ab}	16.826^{cde}	15.933^{bcd}	12.696^{fgh}	15.716^{de}
ComCat®, H_2O, MAP, RT	11.580^{ab}	22.629^{a}	18.582^{a}	14.966^{cde}	
ComCat®, H_2O, 13°C	11.580^{ab}	19.817^{b}	11.841^{fg}	12.324^{gh}	13.181^{efgh}
ComCat®, H_2O, RT	11.580^{ab}	15.427^{efg}	13.832^{def}	12.715^{fgh}	
Control, Cl_2, MAP, 13°C	13.868^{a}	13.895^{ghi}	14.883^{cde}	15.635^{cde}	15.017^{def}
Control, Anolyte, MAP, 13°C	13.868^{a}	14.617^{fgh}	15.598^{cd}	18.649^{ab}	18.782^{b}
Control, Cl_2, MAP, RT	13.868^{a}	13.329^{hij}	10.911^{g}	11.022^{h}	
Control, Anolyte, MAP, RT	13.868^{a}	11.107^{j}	13.515^{defg}	16.454^{bc}	
Control, H_2O, MAP, 13°C	13.868^{a}	13.626^{ghi}	12.696^{efg}	16.112^{bcd}	18.404^{bc}
Control, H_2O, MAP, RT	13.868^{a}	11.692^{ij}	12.869^{efg}	15.970^{cd}	
Control, H_2O, 13°C	13.868^{a}	16.951^{cde}	12.762^{efg}	13.292^{efgh}	11.645^{h}
Control, H_2O, RT	13.868^{a}	18.388^{bcd}	14.852^{cde}	11.748^{h}	

Significance	
Preharvest treatment (A)	*
Disinfecting + MAP (B)	***
Storage temperature (C)	***
A X B	*
A X C	NS
B X C	***
A X B X C	***

Nonsignificant, *** Significant at $P \leq 0.05$ or 0.001 respectively. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The LSD Value = 2.069, S.E. = 0.085, MSE = 1.644 and C.V. = 0.089.

Storage temperature significantly ($P \leq 0.001$) affected the AA content of tomatoes. In general, higher levels of AA were observed in tomatoes stored at 13°C than at room temperature. The AA content of tomatoes rapidly increased during the first 8 days, the fast ripening period, at room temperature, but drops thereafter. At 13°C the AA content of ripening packaged tomatoes continued to increase up to 24 or 30 days.

The AA content of tomatoes was significantly ($P \leq 0.05$) affected by the preharvest ComCat® treatment during storage at 13°C and room temperature. The AA content in ComCat® treated tomatoes was higher at harvest, although not significantly ($P > 0.05$), but remained significantly ($P \leq 0.05$) higher after most of the postharvest treatments during storage.

The AA content was significantly ($P \leq 0.05$) influenced by the interaction between ComCat® treated and disinfecting + MAP treatments. The AA content of the fruits was highly ($P \leq 0.001$) affected by the interaction between the pre- and postharvest treatments. It was shown that AA content was better maintained in tomatoes during ripening in low temperature storage, use of packaging and disinfecting treatment as well as preharvest ComCat® treatments.

4.3.3.9. Peroxidase activity (POX)

The activities of POX were highly ($P \leq 0.001$) influenced with storage temperature (Figure 4.4). The general tendency was a decrease in POX up to 8 days of storage, followed by an increase. In general, a greater level of the activity of POX was observed for tomatoes stored at room temperature at days 16 to 24, compared to those stored at 13°C. From these data, it seemed as if ripening is delayed by approximately one week in tomatoes stored at 13°C, compared to those stored at room temperature. The disinfecting treatment had a highly significant ($P \leq 0.001$) effect on the POX in tomatoes, but only the ComCat® treated ones. The activities of POX in ComCat® treated tomatoes dipped in anolyte water was significantly lower at 16 and 24 days of storage at 13°C, when compared to tomatoes dipped in chlorinated water.

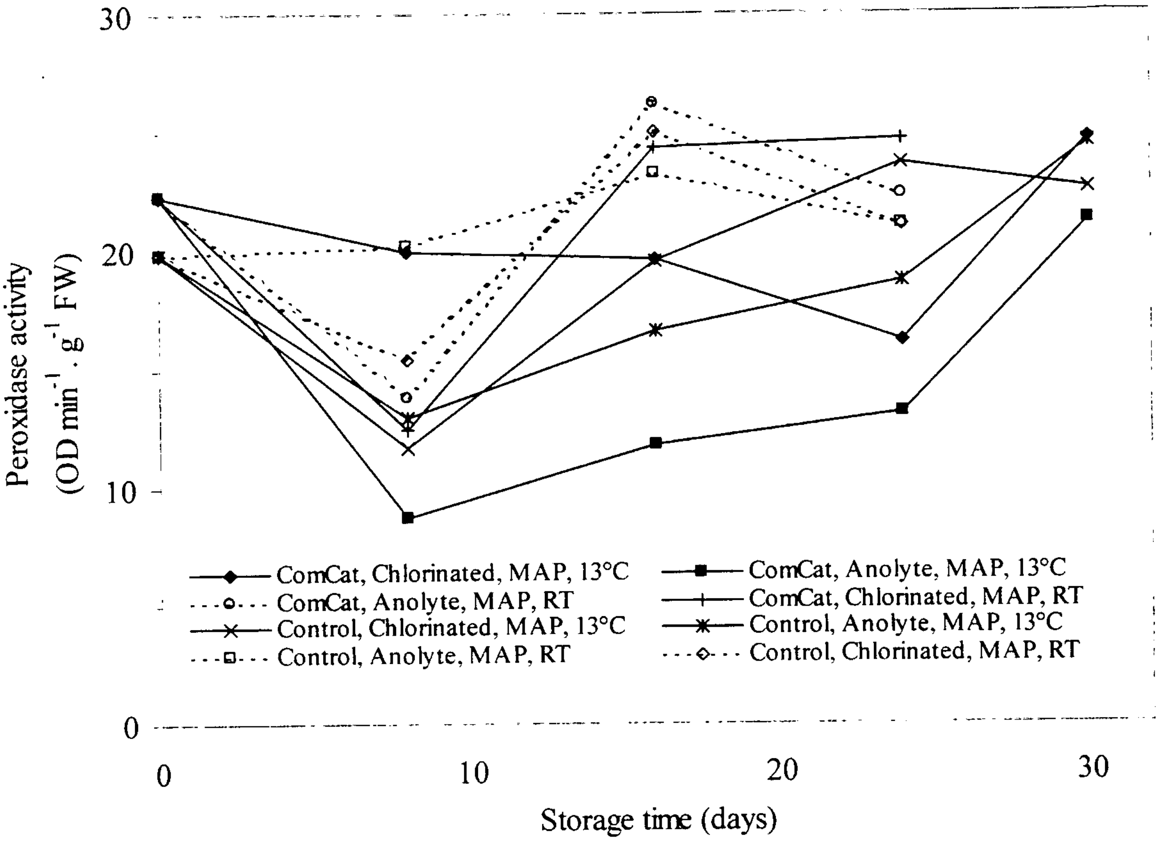


Figure 4.4. Changes in peroxidase activity of tomatoes packaged in Xtend® film or unpackaged and stored at 13°C and room temperature (RT) for 30 days (n = 3 over four storage times).

Significance level showing the effect of pre- and postharvest treatment on changes in peroxidase activity	
Significance	
Preharvest treatment (A)	***
Disinfecting (B)	***
Storage temperature (C)	***
A X B	***
A X C	*
B X C	***
A X B X C	**

NS, *, **, *** Nonsignificant or significant at $P \leq 0.01$, 0.05 or 0.001 respectively. $LSD_{0.05}$ Value = 1.446, S.E. = 0.027 and C.V. = 0.047.

Preharvest ComCat® treatment had a highly significant ($P \leq 0.001$) effect on the levels of POX activities during 30 days of storage at 13°C or room temperature. POX activity was higher in ComCat® treated fruits at harvest. The activity of POX was significantly ($P \leq 0.001$) influenced by the interaction between preharvest ComCat® treatment and disinfecting treatment.

The interaction between storage temperature and disinfecting treatment also had a highly significant ($P \leq 0.001$) effect on the changes in levels of POX activity in tomato fruits. The two-way interaction between preharvest ComCat® treatment and storage temperature was significant ($P \leq 0.05$) on the changes in POX activities in fruits during 30 days at 13°C or room temperature. In general, the activity of POX in ripening tomato fruits in storage was significantly different ($P \leq 0.01$) due to the three-way interaction between all the pre-and postharvest treatments.

4.3.3.10. Polygalacturonase activity (PG)

Development of polygalacturonase activities in ComCat® treated and control tomatoes, stored under different conditions, is shown in Figure 4.5. No PG activity was detected in green mature tomatoes at harvest, but it changed from 0 to as high as 0.78 nmole $min^{-1}.g^{-1}$. Storage temperature had a highly significant ($P \leq 0.001$) effect on the PG activity in tomatoes. The PG activity was significantly ($P \leq 0.001$) higher in tomatoes stored at room temperature up to 24 days of storage.

The PG activity was slightly influenced by the prepackaging disinfecting treatment during 24 days storage at 13°C or room temperatures. The differences were more pronounced at 30 days storage at 13°C, than during the earlier periods, where the activities of PG were higher in tomatoes dipped in chlorinated water than in tomatoes dipped in anolyte water. The interactive effect of preharvest ComCat® treatment and storage temperature had a significant ($P \leq 0.001$) influence on the changes in PG activity of tomatoes. Similarly, the two-way interaction between disinfecting and storage temperature had a significant ($P \leq 0.05$) effect on the PG activity of the tomatoes.

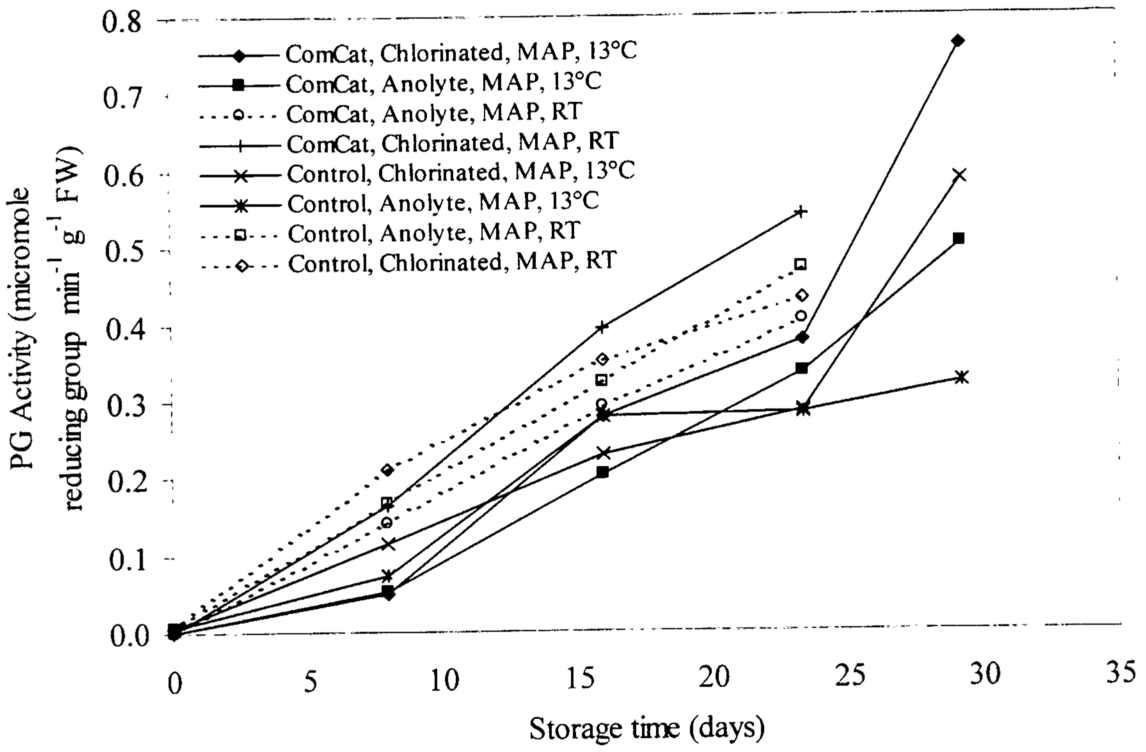


Figure 4.5. Changes in polygalacturonase activities of tomatoes packaged in Xtend® film or unpackaged and stored at 13°C and room temperature (RT) for 30 days (n = 3 over four storage times).

Significance level showing the effect of pre- and postharvest treatment on changes in peroxidase activity

Significance	
Preharvest treatment (A)	NS
Disinfecting (B)	*
Storage temperature (C)	***
A X B	NS
A X C	***
B X C	*
A X B X C	NS

NS, *, *** Nonsignificant or significant at $P \leq 0.05$ or 0.001 respectively. $LSD_{0.05}$ Value = 0.073, S.E. = 0.012 and C.V. = 0.171.

PG activities in tomatoes were not significantly affected by preharvest ComCat® treatment (Figure 4.5). During the later weeks of storage i. e. at day 30, the PG activities increased faster in ComCat® treated tomatoes dipped in chlorine supplemented water and stored at 13°C compared with the PG activities in normal tomatoes subjected to the same treatments. It was also lower in tomatoes dipped in anolyte water than in those dipped in chlorinated water.

PG activity remained to be higher in normal tomatoes dipped in anolyte water, during 20 days at 13°C, compared to the PG activities in ComCat® treated tomatoes subjected to the same treatments. At 30 days at 13°C, PG activities became slightly different between ComCat® treated and control tomatoes dipped in anolyte and chlorinated water.

4.3.4. Microbiological changes

Table 4.11 displays the change in total aerobic bacteria in preharvest ComCat® treated and control tomatoes subjected to different postharvest treatments. Populations of aerobic bacteria did not exceed 8.5 $\log_{10}$CFU g^{-1} during the storage time of 30 days at either temperature. MAP had a significant effect on the growth of microorganisms on tomatoes during storage. After 30 days at 13°C, the populations of total aerobic bacteria were higher in unpackaged treated tomatoes than in packaged ones.

Disinfecting treatments reduced the number of aerobic bacteria significantly ($P \leq 0.001$). During storage, there was a significant difference between the water washed tomatoes, which had higher aerobic bacteria populations than the disinfected ones. The populations of aerobic bacteria did not exceed 3.3 $\log_{10}$CFU g^{-1} in both ComCat® treated and control tomatoes disinfected with chlorinated and anolyte water for 16 days at 13°C. However, there was no significant difference between the chlorine and anolyte water dipping treatment.

Table 4.11. Changes in total aerobic bacteria populations in tomatoes packaged in Xtend® film or unpackaged and stored at 13°C and room temperature (RT) for 30 days (n = 3 over four storage times).

	Total aerobic bacteria ($\log_{10}$cfu g^{-1} FW)				
Treatment	day 0	Day 8	day 16	day 24	day 30
ComCat®, Cl_2, MAP, 13°C	3.076^{b}	$<1^{kl}$	1.480^{ij}	2.154^{lmn}	$<1^{fg}$
ComCat®, Anolyte, MAP, 13°C	3.076^{b}	1.434^{hij}	1.031^{j}	2.319^{klmn}	2.699^{gh}
ComCat®, Cl_2, MAP, RT	3.076^{b}	3.261^{fg}	3.425^{fgh}	3.713^{hijk}	
ComCat®, Anolyte, MAP, RT	3.076^{b}	2.300^{ghi}	2.660^{ghi}	3.713^{hijk}	
ComCat®, H_2O, MAP, 13°C	3.076^{b}	2.538^{ghi}	4.672^{cde}	3.956^{hijk}	4.628^{cdef}
ComCat®, H_2O, MAP, RT	3.076^{b}	4.092^{def}	4.009^{fgh}	6.294^{cde}	
ComCat®, H_2O, 13°C	3.076^{b}	3.066^{fgh}	5.690^{abc}	5.900^{def}	6.994^{ab}
ComCat®, H_2O, RT	3.076^{b}	5.322^{cde}	6.357^{a}	5.539^{defg}	
Control, Cl_2, MAP, 13°C	5.187^{a}	$<1^{k}$	1.284^{ij}	3.261^{ijk}	1.305^{hi}
Control, Anolyte, MAP, 13°C	5.187^{a}	$<1^{ghi}$	2.322^{hij}	2.920^{jklm}	3.149^{cd}
Control, Cl_2, MAP, RT	5.187^{a}	3.483^{efg}	5.866^{abc}	5.155^{efgh}	
Control, Anolyte, MAP, RT	5.187^{a}	3.661^{efg}	5.554^{abcd}	4.325^{hijk}	
Control, H_2O, MAP, 13°C	5.187^{a}	3.818^{efg}	4.814^{bcd}	5.200^{efgh}	5.746^{bc}
Control, H_2O, MAP, RT	5.187^{a}	3.644^{efg}	5.671^{abcd}	8.705^{a}	
Control, H_2O, 13°C	5.187^{a}	6.350^{a}	5.464^{abcd}	4.447^{fghij}	7.551^{a}
Control, H_2O, RT	5.187^{a}	6.021^{ab}	6.156^{ab}	8.402^{b}	

Significance

Preharvest treatment (A)	*
Disinfecting + MAP (B)	***
Storage temperature (C)	***
A X B	NS
A X C	***
B X C	***
A X B X C	NS

Nonsignificant, *, *** Significant at $P \leq 0.05$ or 0.001 respectively. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The LSD Value = 1.362, S.E. = 0.161, MSE = 0.712 and C.V. = 0.199.

The total aerobic bacteria population was affected by the preharvest ComCat® treatment ($P \leq 0.05$). At harvest the population of aerobic bacteria was significantly lower in ComCat® treated tomatoes, when compared with the controls. Highly significant interactions ($P \leq 0.001$) were obtained between the ComCat® treated and storage temperature, as well as between the disinfection treatments + MAP and the storage temperature ($P \leq 0.001$).

Population of moulds and yeasts on tomatoes, as influenced by treatment and time of storage at 13°C or room temperature, are shown in Table 4.12. Populations of viable fungi did not exceed 5 $\log_{10}$CFU g^{-1} during the storage time of 30 days at either temperature.

MAP suppressed the growth of moulds and yeasts during the first few weeks of storage. The population of these microorganisms was generally lower in packaged tomatoes, compared to unpackaged ones during storage at both temperatures. At room temperature storage, the number of viable moulds and yeasts was significantly lower in packaged tomatoes than in unpackaged ones during 16 days of storage. However, the populations in packaged tomatoes started to exceed that of unpackaged fruits after 16 days at room temperature.

The number of viable moulds and yeasts was significantly lower in tomatoes disinfected in chlorinated and anolyte water, compared to water washed ones, and did not exceed 1 $\log_{10}$cfu g^{-1} in both disinfected ComCat® and controls for 16 days at 13°C. The results showed that disinfecting with anolyte water seemed to be more effective than chlorinated water, as lower counts of moulds and yeasts were observed, although significant differences were only observed in the packaged control tomatoes stored at room temperature.

As a general trend, ComCat® treatment resulted in lower yeast and mould populations at harvest, a few significant differences were observed during storage, where this trend was continued. The populations were also significantly ($P \leq 0.05$)

Table 4.12. Changes in yeast and moulds populations in tomatoes packaged in Xtend® film or unpackaged and stored at 13°C and room temperature (RT) for 30 days (n = 3 over four storage times).

	Total Fungi Count ($\log_{10}$cfu g^{-1} FW)				
Treatment	day 0	day 8	day 16	day 24	day 30
ComCat®, Cl_2, MAP, 13°C	1.778^{ab}	$<1^{i}$	$<1^{h}$	1.366^{efgh}	1.630^{cdef}
ComCat®, Anolyte, MAP, 13°C	1.778^{ab}	$<1^{i}$	$<1^{h}$	$<1^{gh}$	1.007^{fg}
ComCat®, Cl_2, MAP, RT	1.778^{ab}	$<1^{i}$	1.952^{cdef}	2.111^{def}	
ComCat®, Anolyte, MAP, RT	1.778^{ab}	1.404^{efghi}	1.842^{cdef}	1.551^{efgh}	
ComCat®, H_2O, MAP, 13°C	1.778^{ab}	$<1^{ghi}$	3.268^{abc}	2.491^{cde}	3.059^{abc}
ComCat®, H_2O, MAP, RT	1.778^{ab}	$<1^{i}$	$<1^{fgh}$	4.105^{ab}	
ComCat®, H_2O, 13°C	1.778^{ab}	$<1^{ghi}$	3.241^{abc}	2.836^{bcde}	4.214^{a}
ComCat®, H_2O, RT	1.778^{ab}	3.051^{abcd}	3.717^{ab}	3.551^{abc}	
Control, Cl_2, MAP, 13°C	2.342^{a}	$<1^{hi}$	$<1^{gh}$	1.438^{efgh}	1.270^{efg}
Control, Anolyte, MAP, 13°C	2.342^{a}	$<1^{hi}$	$<1^{gh}$	$<1^{gh}$	$<1^{g}$
Control, Cl_2, MAP, RT	2.342^{a}	$<1^{i}$	4.152^{a}	3.439^{abc}	
Control, Anolyte, MAP, RT	2.342^{a}	$<1^{i}$	2.853^{bcd}	1.813^{defg}	
Control, H_2O, MAP, 13°C	2.342^{a}	1.032^{fghi}	3.443^{ab}	3.127^{abcd}	2.928^{abcd}
Control, H_2O, MAP, RT	2.342^{a}	1.777^{defgh}	3.270^{abc}	4.755^{a}	
Control, H_2O, 13°C	2.342^{a}	2.172^{cdefg}	3.453^{ab}	3.808^{abc}	4.314^{a}
Control, H_2O, RT	2.342^{a}	4.255^{a}	4.135^{a}	4.500^{ab}	
Significance					
Preharvest treatment (A)		*			
Disinfecting + MAP (B)		***			
Storage temperature (C)		***			
A X B		NS			
A X C		*			
B X C		*			
A X B X C		NS			

NS, *, *** Nonsignificant or significant at $P \leq 0.05$ or 0.001 respectively. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The LSD Value = 1.270, S.E. = 0.080, MSE = 0.619 and C.V. = 0.3594.

influenced by the interaction between storage temperature and ComCat® treatment, as well as disinfecting + MAP treatments and storage temperature.

The effect of ComCat® treatment and different postharvest treatments on the growth of total coliform bacteria is shown in Table 4.13. Populations of coliform bacteria did not exceed 8.3 $\log_{10}$CFU g^{-1} during the storage time of 30 days at either temperature, and *E. coli* was not detected.

The other very important factor affecting microorganism growth and multiplication is storage temperature. Populations of coliform bacteria in tomatoes were significantly ($P \leq 0.001$) affected by the storage temperature. The number of viable coliforms rapidly increased in tomatoes stored at room temperature. Reducing storage temperature to the minimum temperature specific for tomatoes, i.e. 13°C, had a positive effect on controlling them.

Disinfecting treatments reduced the number of coliform bacteria. During storage, there was a significant difference between the disinfected tomatoes, which had lower coliform populations than the water washed ones. There was also a difference between the disinfecting treatments, as chlorine treatment resulted in less coliform development compared to anolyte treated tomatoes.

The number of total coliforms was influenced ($P \leq 0.05$) by ComCat® treatment. At harvest, the number of viable coliforms were significantly lower in ComCat® treated tomatoes compared to the controls. As a general trend, the population of total coliform bacteria was found to be lower in ComCat® treated tomatoes, stored at 13°C or room temperature, than in control tomatoes. However, this difference was only significant for the storage of disinfected tomatoes at room temperature. The population of coliform bacteria was influenced significantly ($P \leq 0.001$) by the interaction between preharvest treatment and storage temperature. Similarly, there was a significant ($P \leq 0.01$) influence of the interaction between disinfecting + MAP and storage temperature on coliforms. The three-way interaction between pre-and postharvest treatments was also significant ($P \leq 0.01$).

Table 4.13. Changes in total coliform populations in tomatoes packaged in Xtend® film or unpackaged and stored at 13°C and room temperature (RT) for 30 days (n = 3 over four storage times).

	Total coliform bacteria ($\log_{10}$cfu g^{-1} FW)				
Treatment	day 0	day 8	day 16	Day 24	day 30
ComCat®, Cl_2, MAP, 13°C	2.279^{b}	$<1^{g}$	1.459^{ijk}	1.699^{ghij}	1.100^{g}
ComCat®, Anolyte, MAP, 13°C	2.279^{b}	1.176^{efg}	1.185^{ijk}	2.001^{gh}	2.112^{ef}
ComCat®, Cl_2, MAP, RT	2.279^{b}	$<1^{fg}$	1.233^{ijk}	2.871^{fgh}	
ComCat®, Anolyte, MAP, RT	2.279^{b}	2.026^{defg}	3.299^{efgh}	3.443^{efgh}	
ComCat®, H2O, MAP, 13°C	2.279^{b}	1.394^{efg}	3.875^{cdef}	3.988^{ef}	5.801^{abcd}
ComCat®, H2O, MAP, RT	2.279^{b}	3.912^{abcd}	3.513^{defg}	5.677^{cd}	
ComCat®, H_2O, 13°C	2.279^{b}	2.693^{cde}	3.867^{cde}	3.878^{ef}	6.711^{ab}
ComCat®, H_2O, RT	2.279^{b}	4.339^{abc}	6.602^{a}	7.860^{ab}	
Control, Cl_2, MAP, 13°C	5.293^{a}	$<1^{g}$	$<1^{k}$	1.688^{ghi}	1.295^{g}
Control, Anolyte, MAP, 13°C	5.293^{a}	$<1^{g}$	2.682^{fghi}	2.211^{fghi}	2.653^{f}
Control, Cl_2, MAP, RT	5.293^{a}	3.649^{abcd}	5.190^{abcd}	4.893^{cde}	
Control, Anolyte, MAP, RT	5.293^{a}	1.477^{efg}	5.274^{abcd}	4.047^{def}	
Control, H2O, MAP, 13°C	5.293^{a}	2.921^{bcde}	3.882^{cdef}	5.563^{cd}	6.223^{abc}
Control, H2O, MAP, RT	5.293^{a}	3.695^{abcd}	5.396^{abc}	8.025^{a}	
Control, H_2O, 13°C	5.293^{a}	2.496^{cdef}	4.673^{bcde}	4.940^{cde}	6.743^{a}
Control, H_2O, RT	5.293^{a}	5.251^{a}	5.871^{ab}	8.248^{a}	

Significance

Preharvest treatment (A)	*
Disinfecting + MAP (B)	***
Storage temperature (C)	***
A X B	NS
A X C	***
B X C	**
A X B X C	**

NS, **, *** Nonsignificant or significant at $P \leq 0.001$ respectively. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The LSD Value = 1.475, S.E. = 0.1114, MSE = 0.838 and C.V. = 0.238.

4.3.5. Subjective quality analysis

The percentage marketable fruits of tomatoes subjected to different treatments are shown in Table 4.14. The marketability of tomatoes was significantly higher for packaged tomatoes than for unpackaged ones stored at both temperatures. Percentage marketable fruits were significantly affected by disinfecting + MAP treatment. MAP resulted in 52.4% and 23.8% more marketable control and ComCat® treated tomatoes during the 16 days of storage at room temperature respectively. Similarly, there were 29.7% and 40.3% more marketable ComCat® treated and control tomatoes after 30 days at 13°C respectively. Disinfecting helped to protect the tomatoes, as water washed tomatoes started to deteriorate, from 8 days on, at room temperature, and 16 days at 13°C, compared to disinfected ones.

Storage temperature had a strong influence on the marketability. Marketability of tomatoes stored at room temperature started to decrease from 16 days on, while at 13°C, the tomatoes lasted at least for 24 days.

ComCat® treatment did not seem to have much of an effect on the marketability of tomatoes, when compared to the controls. However, it could be interpreted that the onset of senescence is postponed in ComCat® treated tomatoes, as unpackaged control tomatoes, that were not disinfected, were only 50% and 33.3% marketable after 8 and 16 days storage at room temperature, compared to the 74.6% and 64.8% of the ComCat® treated ones. An almost similar trend is observed for packaged, water washed ComCat® treated tomatoes stored at room temperature.

The two-way interaction between preharvest treatment and disinfecting + MAP had a significant ($P \leq 0.01$) influence on the changes in percentage marketable fruit during 30 days storage at 13°C or room temperature. The percentage marketable fruits were significantly ($P \leq 0.01$) affected by the interaction between preharvest treatment and storage temperature.

Table.4.14. Changes in percentage marketability of tomatoes packaged in Xtend® film or unpackaged and stored at 13°C and room temperature (RT) for 30 days (n = 3 over four storage times).

Treatment	Percentage marketable fruits				
	Day 0	Day 8	Day 16	Day 24	Day 30
ComCat®, Cl_2, MAP, 13°C	100^{a}	100^{a}	100^{a}	91.7^{c}	91.5^{b}
ComCat®, Anolyte, MAP, 13°C	100^{a}	100^{a}	100^{a}	95.8^{b}	95.8^{a}
ComCat®, Cl_2, MAP, RT	100^{a}	100^{a}	94^{b}	72.2^{f}	-
ComCat®, Anolyte, MAP, RT	100^{a}	100^{a}	95.2^{b}	77.9^{e}	-
ComCat®, H_2O, MAP, 13°C	100^{a}	100^{a}	100^{a}	90.5^{cd}	81.0^{g}
ComCat®, H_2O, MAP, RT	100^{a}	90.5^{cd}	88.6^{e}	64.4^{h}	-
ComCat®, H_2O, 13°C	100^{a}	100^{a}	82.2^{g}	55.0^{i}	51.3^{e}
ComCat®, H_2O, RT	100^{a}	74.6^{f}	64.8^{h}	-	-
Control, Cl_2, MAP, 13°C	100^{a}	100^{a}	100^{a}	100^{a}	89.9^{c}
Control, Anolyte, MAP, 13°C	100^{a}	100^{a}	100^{a}	100^{a}	91.7^{b}
Control, Cl_2, MAP, RT	100^{a}	95.8^{b}	90.6^{cd}	68.6^{g}	-
Control, Anolyte, MAP, RT	100^{a}	100^{a}	95.8^{b}	72.6^{e}	-
Control, H_2O, MAP, 13°C	100^{a}	100^{a}	100^{a}	90.4^{cd}	81.4^{d}
Control, H_2O, MAP, RT	100^{a}	84.9^{e}	85.7^{f}	53.6^{k}	-
Control, H_2O, 13°C	100^{a}	100^{a}	63.3^{i}	54.0^{j}	41.1^{f}
Control, H_2O, RT	100^{a}	50^{g}	33.3^{j}	-	-

Significance	
Preharvest treatment (A)	*
Disinfecting + MAP (B)	***
Storage temperature (C)	***
A X B	**
A X C	**
B X C	NS
A X B X C	NS

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01 or 0.001 respectively. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The LSD Value = 1.332, S.E. = 1.011, MSE = 60.111 and C.V. = 0.099.

4.4. Discussion

The quality that fresh produce has at harvest can be extended by a variety of postharvest processes, such as MAP (Ballantyne *et al.*, 1988a and b; Zagory and Kader, 1988; Geeson, 1988; Salunkhe et al., 1991; Exama *et al.*, 1993; Jeon and Lee, 1999; Seyoum *et al.*, 2001). The results of this chapter show that MAP with microperforated Xtend® film not only resulted in normal respiration of tomatoes, but also reduced moisture loss from the produce, and therefore effectively reduced ($P \leq 0.001$) PWL (Table 4.1). This is in agreement with previous findings that the medium or least permeable film maintains the moisture in packages at a proper level during storage (Howard and Hernandez-Brenes, 1997; Jeon and Lee, 1999). Condensation is also prevented by this material, thus preventing microbial growth due to high relative humidity (Zagory and Kader, 1988).

The O_2 utilisation by the tomatoes was faster during the first 3 days of storage at 13°C as well as room temperature. This pattern was similar to that previously described for many packaged fruits and vegetables (Henig and Gilbert, 1975; Rizvi, 1981; Smith *et al.*, 1987; Carlin *et al.*, 1990). During MAP, O_2 and CO_2 concentrations remained above 15% and below 6% in microperforated Xtend® film during storage, respectively (Fig. 4.1 and 4.2), suggesting that there was normal respiration of tomatoes during the storage period (Exama *et al.*, 1993; Jeon and Lee, 1999). Although, the modified atmosphere created by microperforated Xtend® film did not excessively reduce O_2 and increase CO_2 levels, the data clearly show that the metabolic activity was retarded due to MAP when compared to unpackaged fruit. The greater level of free sugars (sucrose, glucose and fructose) showed this in packaged tomatoes stored at 13°C, as well as room temperature (Tables 4.5 (a - c), 4.6 and 4.7). Since free sugars are substrates for respiration, MAP had a significant effect on reducing the glucose utilisation by up to 25.5% and 11.2%, during storage at room temperature and 13°C, respectively, when compared to the glucose content of unpackaged tomatoes. The fructose and total soluble sugar contents were also maintained better in both packaged, ComCat® treated and control tomatoes stored at these storage temperatures (Tables 4.5 (c) and 4.6).

MAP also resulted in the preservation of quality attributes such as AA content. After 24 days at room temperature the AA was 15.0% and 26.4% more in packaged ComCat® treated and control tomatoes respectively (Table 4.10). Similarly, the AA retention was 16.1% and 36.7% more in packaged ComCat® treated and control tomatoes stored at 13°C for 30 days, respectively. The modified atmospheres created by Xtend® film delayed the ripening of tomatoes, and the AA increased with ripening during storage. This is in agreement with similar findings reported by Geeson *et al.* (1985), Howard and Hernandez-Brenes, (1997), Batu and Thompson (1998) and Saito *et al.* (2000).

Although least permeable packaging might be preferred to reduce microbial growth (Batu and Thompson, 1994), MAP with high permeability was reported to be significantly protective (Exama *et al.*, 1993; Batu and Thompson, 1997; Jeon and Lee, 1999; Seyoum *et al.*, 2001). The current results showed that the population of total aerobic bacteria, molds, yeasts and total coliforms were generally found to be less in packaged tomatoes than in unpackaged tomatoes stored at 13°C, as well as room temperature (Tables 4.11 - 4.13), although some discrepancies were observed during some storage intervals.

Storage temperature was the most important factor affecting the postharvest quality and shelf life of tomatoes. All quality parameters tested, except SH ratio, were significantly ($P \leq 0.001$) influenced by the storage temperature. The changes in SH ratio was generally not an important parameter of the quality characteristics of tomatoes (Table 4.8), but changes in SA ratio was more important in explaining the changes in quality characteristics in tomatoes subjected to different postharvest treatments (Table 4.9). With increasing temperature, the headspace concentrations of oxygen decreased, while carbon dioxide increased rapidly (Fig. 4.1 and 4.2). Before equilibration, the concentrations of O_2 decreased up to 5 and 12 days in packages of tomatoes stored at room temperature and 13°C, respectively. Similarly, the CO_2 concentrations increased up to 5 and 12 days in the headspace of packages of tomatoes stored at room temperature and 13°C, respectively, after which equilibrium was attained. This would indicate that at higher temperature the metabolism in tomatoes was enhanced and the utilisation of O_2 increased with subsequent production of CO_2. Varoquaux and Wiley (1994) reported similar findings. Lowering the storage temperature therefore reduces the respiration, and delays senescence, while high temperature storage hastened the senescence of tomatoes (Table 4.1 - 4.14).

The changes in moisture content, TTA, pH, TSS, free sugars, AA, microbiological and enzyme activity occurred at substantially faster rates in tomatoes stored at room temperature than in tomatoes stored at 13°C. Storage at 13°C resulted in a substantial decrease in PWL of tomatoes compared with fruits stored at room temperature (Table 4.1). High temperature increases the vapour pressure difference between the product and the surrounding air, which is the driving potential for faster moisture transfer from tomatoes to the surrounding air (Ryall and Lipton, 1979; Ryall and Pentzer, 1982; Kader *et al.*, 1985; Salunkhe *et al.*, 1991; Cantwell *et al.*, 1992).

There was a faster increase in the pH and a decrease in TTA of tomatoes stored at room temperature, compared to fruits stored at 13°C (Table 4.2 and 4.3). The increase in pH of tomatoes at room temperature with increased storage time was in agreement with the previous findings by Mohammed et al (1999), but also contrary to the findings reported by Carne and Zilva (1949). After 24 days of storage, the AA content was better retained in tomatoes stored at 13°C compared to those stored at room temperature (Table 4.10). The AA content and pH values of tomatoes were increasing while the TTA content was decreasing in tomatoes during ripening and storage. Mohammed *et al.* (1999) reported a similar relationship in the changes in AA content, pH values and TTA of tomatoes during ripening in storage at 20°C. In general, the rapid increase in pH, decrease in TTA and depletion of AA in tomatoes stored at room temperature could be attributed to the enhanced metabolism, ripening and senescence of these tomatoes compared to those stored at 13°C. Higher nutritional values in terms of higher AA content of tomatoes stored at 13°C for 30 days was evident from these data (Table 4.10). The AA content generally increased with ripening in tomatoes during 30 days of storage at 13°C. However, at room temperature the AA rapidly increased within 8 to 16 days of ripening and showed a decline after full ripeness of tomatoes. This trend was in agreement with previous data that AA content increased with ripeness (Brecht *et al.*, 1976; Mohammed *et al.*, 1999), however, Watada *et al.* (1976) observed no change in AA content with ripeness.

The TSS of tomatoes was better maintained at 13°C than in tomatoes stored at room temperature (Table 4.4). The higher rate of increase in TSS of some of the tomato samples stored at room temperature was due to excessive moisture loss (Table 4.1) as well as the hydrolysis of carbohydrate to soluble sugars.

The utilization of free sugars was faster in tomatoes stored at room temperature, compared to those stored at 13°C (Tables 4.5 (a - c) and 4.6), which could be ascribed to the reduced respiration and metabolism at 13°C. Reducing sugars, such as glucose, are the main substrates in the respiration process to produce energy required in the metabolism of fruit. It was calculated that in green mature tomatoes, 73% of glucose degradation took place through the Embden-Meyerhof processes and 27% through the alternative oxidative path way, which functions mainly as a mechanism for the conversion of glucose to various intermediates for biosynthesis and possibly, to provide $NADPH_2$ for this process (Wang *et al.*, 1962, in Hobson and Davies, 1971). The observed changes in gas compositions therefore match this relationship to metabolic activity.

Changes in metabolic activity can also be monitored by enzyme activities, since enzyme activities govern most of the chemical and physical effects that occur during ripening of fruit. For instance, the loss of firmness of tomatoes during ripening and storage is due to the increased activity of PG in tomatoes (Hobson, 1965; Brady *et al.*, 1987). The activities of POX and PG were higher ($P \leq 0.001$) in ripening tomatoes stored at room temperature than in tomatoes stored at 13°C (Fig. 4.4 and 4.5). Similar results were reported by Mattoo *et al.*, 1975 and Salunkhe *et al.*, 1991.

Storage at room temperature, in part, created conditions that are favourable for the growth of microorganisms especially during early stages of storage (Table 4.11 - 4.13). The physical or biochemical characteristics of the fruit may influence the type and populations of microorganisms, which develop on fresh produce (Brackett, 1990), but this was not investigated in the current study. The activity of PG increased in tomatoes stored at room temperature, compared to those in tomatoes stored at 13°C (Fig. 4.5), and resulted in a loss of firmness of the fruit. Thus, higher storage temperature, the availability of water-soluble, free sugar, and moisture creates conditions favourable for the proliferation of microorganisms in tomatoes. The rapid decrease in TTA in tomatoes stored at room temperature (Table 4.3) could also partially contribute to the greater growth of microorganisms in tomatoes stored at room temperature. Gould (1983) considered fruit acidity as a major postharvest factor affecting the keeping quality of tomatoes. Mohammed *et al.* (1999) supported this view that the higher TTA could protect fruit from fungal infection. In this study, the fact that the data showed that TTA of tomatoes stored at room temperature decreased faster (Table 4.3), could in part, contribute to the greater growth of microorganism in tomatoes stored at room temperature.

The percentage marketable fruit were lower ($P \leq 0.001$) for tomatoes stored at room temperature compared to those stored at 13°C (Table 4.14). The percentage marketable tomatoes was 33.3% and 64.8% for unpackaged control and ComCat® treated tomatoes stored at room temperature for 16 days respectively. After 24 days at room temperature the percentage marketable fruits varied from 53.6% - 77.9% for both ComCat® treated and control tomatoes. However, the percentage marketable fruit ranged from 81.0 - 95.8% in packaged tomatoes stored at 13°C for 30 days. These results thus demonstrated the overall effect of temperature on the quality of tomatoes during ripening and storage. The observed differences between control and ComCat® treated tomatoes is discussed separately.

Chlorine solution and anolyte water are disinfecting agents and were used to control microorganisms. The use of chlorine is well studied and described. It was reported that chlorine treatment at 200 ppm reduced the population of pathogens by 0.35 to 3 log CFU cm $^{-2}$ in tomatoes (Beuchat *et al.*, 1998 and Ukuku and Sapers, 2001). In the current study, chlorinated water dipping treatment for 20 minutes significantly reduced the populations of total aerobic bacteria, moulds and yeasts, and total coliforms, compared to the populations in water washed tomatoes (Tables 4.11 - 4.13). However, the effectiveness of chlorine treatment decreased when tomatoes were stored at room temperature. These findings were similar to those reported by Ukuku and Sapers (2001), that the effectiveness of chlorine treatment diminished with increase in temperature and storage time. The anolyte water reduced ($P \leq 0.001$) and suppressed the growth of total aerobic bacteria, molds, yeasts and total coliform bacteria in tomatoes during storage as effectively as chlorinated water (Tables 4.11 - 4.13). It was also shown that 5 minutes dipping in anolyte water was sufficient for desirable control of microorganisms. The main reason for this is that the anolyte water contains freely available radicals (see Chapter 2), which are immediately available for action on the microorganisms, whereas in the case of chlorinated water these radicals are not freely available, and are formed slowly. As a result, anolyte water was found to be an effective method in controlling postharvest decay through killing and suppressing growth of total aerobic bacteria, moulds and yeasts, and total coliform bacteria during tomato storage for 30 days at 13°C as well as 24 days at room temperature.

The advantages of anolyte water above chlorine treatment were not only restricted to control of microorganisms. During the early active ripening period of tomatoes, the lowest O_2 and highest CO_2 concentrations were found in packages of tomatoes dipped in chlorinated water, compared to those dipped in anolyte, and those washed with water (Figures 4.1 and 4.2). These results thus suggested that the tomatoes dipped in chlorinated water had higher rates of respiration and metabolism than tomatoes dipped in anolyte water, or those washed with water. The loss of moisture was generally higher in tomatoes dipped in chlorinated water than in anolyte water (Table 4.1). This could be attributed to the effect of chlorine solution on the skin of tomatoes and surface tissue. It was observed that chlorinated water dipping treatment generally left a taint on the surface of tomatoes, while the surface of tomatoes dipped in anolyte water remained to be very shiny during the 30 days of storage at 13°C. These results thus implied that chlorinated water might not be the optimum treatment for tomatoes, because it affects tomatoes by causing some stress condition.

Other differences in quality parameters between chlorine and anolyte water disinfected tomatoes are as follows. The TSS content was generally maintained better in tomatoes dipped in anolyte water than in tomatoes dipped in chlorinated water (Table 4.4). The other interesting point was that the fructose content increased continuously in tomatoes dipped in anolyte water for up to 24 days while it increased only up to 16 days in tomatoes dipped in chlorinated water during storage at 13°C (Table 4.5 (c)). The ComCat® treated tomatoes dipped in anolyte water also performed well in maintaining the total sugar content. The total sugar content was 9.6% and 6.0% more after 24 and 30 days storage at 13°C respectively, although the total soluble sugar was lower during early ripening due to delayed ripeness. It seemed that the overall physical and chemical characteristics of tomatoes dipped in anolyte water had an effect on the microbiological flora during storage at 13°C. At this optimum storage temperature for tomatoes, the population of microorganism was suppressed better in tomatoes dipped in anolyte water (Tables 4.11 -4.13).

The level of activity of POX seemed to be slightly higher in tomatoes dipped in chlorinated water than in those dipped in anolyte water. The POX activity level in tomatoes dipped in anolyte water generally remained lower up to days 16 at 13°C. The overall effect of these quality parameters on the tomatoes dipped in anolyte water improved the percentage marketable fruit to the same extent as chlorinated water, and better than those washed in water (Table 4.14).

The combined effect of disinfecting and packaging is discussed next. Disinfecting + MAP had a significant effect on the postharvest quality parameters tested (Tables 4.1 - 4.6 and 4.14), except on the sucrose content, which is actually very low in tomatoes (Table 4.5 (a)). The effectiveness of microperforated packaging film increased when combined with a prepackaging disinfecting treatment. The main advantage of microperforations is that it allows gas dynamics to create optimum gas composition specific to each fruit and vegetables. However, at relatively higher O_2 and lower CO_2 level, the growth of microorganisms could not be suppressed, due to low CO_2 concentration. In this study, the prepackaging disinfecting treatment seemed to solve the problem of microorganisms and maximise the effectiveness of the modified atmosphere, created by the microperforated film during storage of tomatoes. These results thus showed that the coupled effect of disinfecting treatment and MAP was highly significant ($P \leq 0.001$) on the population of total aerobic bacteria, moulds and yeasts, and total coliforms in tomatoes (Tables 4.11 - 4.13).

Similarly, the interaction between disinfecting treatment + MAP and storage temperature had a significant effect on gas concentrations, PWL, pH, TTA, TSS, sucrose and glucose contents, SA ratio, AA content, and POX and PG activity of tomatoes (Fig. 4.1 - 4.5, Tables 4.2 - 4.5 (b), 4.9 and 4.10).

The effect of ComCat® on postharvest quality and shelf life of tomatoes was investigated under different postharvest treatment practices. Directly after harvest the ComCat® treated tomatoes differed from the control tomatoes in having a lower pH, lower contents of AA, glucose and fructose, total soluble sugar, sucrose equivalent, SA ratio, natural microbial populations and higher TTA, TSS, POX activity and sucrose content.

This treatment also had an effect on some of the quality parameters during storage. The O_2 and N_2 concentrations, PWL, pH of tomatoes, TSS, fructose, glucose, sucrose and total sugar contents, sucrose equivalent, SH ratio, SA ratio, AA content, peroxidase activity, populations of total aerobic bacteria, moulds and yeasts, total coliform bacteria, and marketability were significantly affected by the ComCat® treatment. However, the preharvest ComCat® treatment had no significant effect on titratable acidity and PG activity in tomatoes.

Although the differences in gas concentrations (O_2, CO_2 and N_2) in the headspace of packages were not clear for storage at optimum temperature (13°C), the differences were highly significant in packages of ComCat® treated and control tomatoes stored at room temperature (Fig. 4.1 - 4.3). The small difference in O_2, CO_2, and N_2 concentrations in packages of ComCat® treated and control tomatoes stored at 13°C suggest that at this storage temperature, both products had similar respiration and metabolism rates. However, during storage at room temperature, the air in the headspace of ComCat® treated tomatoes had higher O_2, lower CO_2 and lower N_2 concentrations than the microatmosphere in the packages of control tomatoes. This indicates that control tomatoes had a higher rate of respiration, utilising more O_2 and releasing more CO_2 at room temperature than the ComCat® treated ones.

The pH of tomatoes was highly influenced by preharvest treatments. Directly after harvest, the pH of green mature ComCat® treated tomatoes was lower than in normal tomatoes. The pH of tomatoes increased with increasing ripeness, supporting the results of Mohammed *et al.*, (1999). The changes in pH of tomatoes were highly dependent on the storage temperature and stage of ripeness. There was a greater increase in pH in control tomatoes stored at room temperature than in ComCat® treated tomatoes (Table 4.2), which could be due to a different rate of metabolism.

The TTA of ComCat® treated tomatoes was greater ($P \leq 0.001$) than that of control tomatoes at harvest (Table 4.3). According to Mohammed *et al.* (1999) a higher fruit acidity is an advantage, which causes a lower incidence of fungal infection. The effect of ComCat® treatment was not significant on the TTA during storage.

Although not significant at $P < 0.001$, the TSS content was slightly higher in ComCat® treated tomatoes at harvest, but in general, the TSS seemed to be maintained better in the ComCat® treated tomatoes during storage at both temperatures (Table 4.4). After 24 days at room temperature the TSS was 8.55% higher in unpackaged ComCat® treated tomatoes than in unpackaged controls. This would also be an indication that, at room temperature, ComCat® treated tomatoes had a lower respiration rate, and hence experienced a delayed senescence. Although the AA content was slightly less in ComCat® treated tomatoes at harvest (Table 4.10), it increased more in ComCat® treated tomatoes during storage at 13°C up to 30 days. The AA content was 8.7% and 19.1% more in ComCat® treated tomatoes disinfected in chlorinated and anolyte water after 30 days at 13°C respectively, when compared with controls.

The contents of TSS, glucose, fructose, total sugar and sucrose equivalent were not significantly affected at $P < 0.001$ by the preharvest ComCat® treatment. At harvest, the ComCat® treated tomatoes had slightly higher sucrose concentrations, but it remained lower in ComCat® treated tomatoes during storage (Table 4.5 (a)). The fructose content was also lower in ComCat® treated tomatoes at harvest and during the early stages of storage, but increased with ripeness to maximum levels at days 16, 24 and 8 in ComCat® treated tomatoes dipped in chlorinated, anolyte and distilled water respectively. Maximum levels of control tomatoes were respectively 8 days earlier for the disinfected ones, but the same for the water washed tomatoes. This may indicate that the ripening process (climacteric peak) was delayed in ComCat® treated tomatoes by almost one week. The fructose content decreased after these mentioned days, but remained to be higher in ComCat® treated tomatoes than in controls. At room temperature storage, the fructose content consistently remained lower in ComCat® treated tomatoes than in controls. The lower free sugar content associated with ComCat® treated tomatoes could also be an indication of lower respiration and metabolism rates, supporting the same deduction made for the headspace gas dynamics (Fig. 4.1 - 4.3). However, during the later stages of ripening, and in ripe ComCat®

treated tomatoes, these sugars began to accumulate. Normally, free sugars decrease faster if there is a high respiration rate, since respiration in plants involves the oxidative metabolism of sugars and organic acids to end products, CO_2 and H_2O with the simultaneous production of energy (Varoquaux and Wiley, 1994). The decline in reducing sugar does not continue until the end of storage in ComCat® treated tomatoes and there was no correlation with the headspace gas dynamics, which would suggests that the lower free sugar content in ComCat® treated tomatoes may not be related to the respiration process, but could be attributed to the effect of the plant growth hormones (i.e. gibberellin) in ComCat® that delayed the ripening of the fruit. Such correlations were reported by Salunkhe *et al.* (1975), Stallknecht *et al.* (1983) and Bartholomew *et al.* (1983).

The activity of POX was higher in ComCat® treated tomatoes at harvest. In this context, increased POX activity in tomatoes is thought to be responsible for increasing resistance to pathogenic infection, at least in part (Khripach *et al.*, 1997). This could support the results in Table 4.11 - 4.13 where lower counts in microorganisms are shown to occur on ComCat® treated tomatoes. The proposed mechanism for higher resistance against microorganisms is explained by the findings of previous researchers, who showed that ComCat® treatment induces POX, chitinase and β1-3 gluconase, which are enzymes that protect cell walls and prevent infection (Schnabl *et al.*, 2001; Hüster, 2001). However, during storage and ripening, the activities of POX in ComCat® treated tomatoes were found to remain significantly ($P \leq 0.001$) lower for 30 days at 13°C, compared to the activities of POX in control tomatoes. Previous findings indicated that decompartmentation, a consequence of senescence due to chilling (Parkin *et al.*, 1989; Palma *et al.*, 1995), may lead to the increase in extractable POX activity. Therefore, the lower levels of POX activity in ComCat® treated tomatoes may indicate that this product has a better postharvest quality with lower oxidation and ripening related processes during ripening and storage (Thomas *et al.*, 1981). It could be that differences in fruit ripeness, as indicated by the sugar contents (Table 4.5 and 4.6), resulted in differences in POX activity between ComCat® treated and control tomatoes.

The PG activity in tomatoes has a positive correlation with the firmness of fruits (Foda, 1957; Hobson, 1965; Brady *et al.*, 1987). High PG activity in tomatoes would therefore imply loss of firmness. This enzyme activity increased in all tomatoes during storage, which was in agreement with results of other researchers (Tucker *et al.*, 1980; Brady *et al.*, 1982; Tucker and Grierson, 1982; Yoshida *et al.*, 1984). The preharvest ComCat® treatment had no effect on PG activity during the first three weeks of storage (Fig. 4.5). Although not significant ($P > 0.05$), PG activities were slightly higher in control tomatoes during the first week of ripening, compared to ComCat® treated tomatoes, when stored at 13°C. The greatest difference between the ComCat® treated and control tomatoes in PG activity was only visible after 30 days at 13°C. This is in agreement with the work of Lampe (1971), who showed that PG was low during the first two or three weeks of storage of tomatoes, but not in the later stages, and ascribed this inhibition to indol-acetic-acid, which is one of the major constituents of ComCat®.

At harvest, the population of aerobic bacteria, moulds and yeasts, and total coliform bacteria were significantly lower in ComCat® treated tomatoes. These results were in agreement with the previous hypothesis on the preharvest performance of ComCat® treated products in terms of levels of photogenic infections (Schnabl *et al.*, 2001). A reduction of plant disease symptoms up to 45% in comparison to untreated control plants has been reported by these researchers. It seems as if the low pH, low soluble sugar content, higher titratable acidity and higher POX activity of ComCat® treated tomatoes may therefore contribute to the lower microbial populations at harvest.

During the early stages of tomato ripening in storage at 13°C, the population of total aerobic bacteria, moulds and yeasts seemed to be lower in ComCat® treated tomatoes disinfected in chlorinated and anolyte water than in control tomatoes. The populations of total aerobic bacteria were significantly higher in unpackaged control tomatoes than in unpackaged ComCat® treated ones, both stored at room temperature. The lower soluble sugar content (Tables 4.5 and 4.6), lower pH (Table 4.2), higher TTA (Table 4.3) and higher POX activity (Fig. 4.4) in ComCat® treated tomatoes during the early ripening period could be partially the reason for the reduced population of microorganisms. The importance of fruit acidity, as a major factor affecting the keeping quality of tomatoes, has been examined by Gould (1983). The results in the current study support these findings, since the higher titratable acidity of ComCat® treated tomatoes at harvest could explain the lower incidence of microbial infection, compared to the untreated controls. Thome and Alvarez (1982), as presented by Mohammed *et al.* (1999), have examined the importance of titratable acidity of tomato cultivars, and suggested that higher TTA protect fruit from microbial infection. The availability of more free sugars (Tables 4.5 and 4.6) and higher POX activity in control tomatoes created, in part, conditions more favourable for microbiological flora. However, the populations of microorganisms seemed to increase in some of the ComCat® treated tomatoes during the later stages of storage. This is in positive correlation with the free sugar content in ComCat® treated tomatoes. For example, the fructose concentrations increased more in ComCat® treated tomatoes after two weeks of storage and may be responsible for the proliferation of microorganisms in some samples of ComCat® treated tomatoes. In general, there was no significant difference between ComCat® treated and control tomatoes in populations of moulds and yeasts, and total coliform after 30 days at 13°C. The total aerobic bacteria, however, were lower ($P \leq 0.05$) in ComCat® treated tomatoes.

The high quality attributes of ComCat® treated tomatoes during storage were shown by the percentage marketable fruits (Table 4.14). The overall visual appearance of ComCat® treated tomatoes was better than the control during storage, even at room temperature.

The results that ComCat® treated tomatoes were superior to the controls, in many cases at room temperature storage, was indicated by the two-way interaction between preharvest ComCat® treatment and storage temperature which was highly significant (P

$\leq$ 0.001) on gas concentrations, changes in pH, sucrose content, TSS, AA content, microbiological flora and marketability of tomatoes during storage. Lower metabolism at room temperature in ComCat® treated tomatoes contributed to the conservation of better quality at room temperature storage. Otherwise, storage at 13°C was the optimum storage temperature for both ComCat® treated and control tomatoes, and made the differences in quality attributes more complex.

To summarise, the results in this chapter showed that chlorine treatment may not be the optimum disinfectant, as is generally accepted. Other alternatives should be investigated. The anolyte water used here showed to be a promising candidate in saving time, having less effect on the fruits and being environmentally friendly. Preharvest ComCat® treatment not only improved crop yield, as was found in previous work by other researchers, but resulted in better keeping quality of fruit e.g. tomatoes. Benefits at 13°C storage were:

1) offers a new strategy for preharvest plant protection and strong produce at harvest
2) better microbiological quality in terms of less population of total aerobic bacteria, molds, yeasts and total coliform
3) better nutritional quality in terms of higher AA content at full ripeness
4) better shelf life at 13°C due to a delayed senescence

Further benefits on the shelf life at room temperature storage, being:

1) lower respiration (high O_2, low CO_2, and low N_2) and delayed senescence
2) better chemical quality in terms of higher TSS content, and lower pH
3) accumulation of fructose towards the last two weeks of storage
4) higher total soluble sugar and SH ratio
5) better microbiological quality in terms of lower total aerobic bacteria, molds, and yeast populations

The findings that ComCat® treated tomatoes showed better storage performance than untreated controls, not just at 13°C, but also at room temperature, opens the possibility to investigate the storage performance of these tomatoes at temperatures higher than the optimum storage temperature of 13°C, but under controlled conditions. Such controlled temperatures can be achieved in an evaporative cooling chamber. The results of such research are presented in Chapter 6.

CHAPTER 5

EFFECT of PREHARVEST TREATMENT and FORCED VENTILATION EVAPORATIVE COOLING on CARROTS

Abstract

A forced ventilation evaporative cooling (EC) system was designed and developed in which the temperature could be reduced by 8.4 - 13.4°C below ambient temperature, with a rise of relative humidity up to 91%. Storage in this EC resulted in increased shelf lives of both ComCat® treated and control carrots from 4 to 24 days, compared to storage at ambient conditions. Preharvest ComCat® treatment significantly ($P \leq 0.05$) affected pH, total sugar content and the population of moulds and yeasts in carrots during storage at EC. MAP significantly ($P \leq 0.001$) reduced physiological weight loss, moisture, and juice content of carrots stored inside EC. MAP coupled with evaporative cooling reduced the rate of sugar utilization for metabolic activities, compared to unpackaged carrots stored at ambient conditions. The populations of aerobic bacteria and fungi were significantly ($P \leq 0.001$) affected by MAP coupled with EC temperature. Disinfecting with chlorinated helped additionally to limit microbial growth during EC storage.

5.1. Introduction

Ethiopia has a wide variety of climatic and soil types that can enable it to produce crops for both home consumption and the export market (Agonafir, 1991). Agriculture is the mainstay of the economy, with vegetable production around 2.86 million tons. Ninety five per cent of the total volume of horticultural products is fresh vegetables. There is a need for high crop yield in Ethiopia to feed its increasing population, but due to a lack of agricultural input, such as fertilizers, the production of vegetables is low and fully dependent on traditional farming systems. Postharvest handling of vegetables is also poor.

Presently, small plots with farmers do not have the capacity to consistently buy agrochemicals for boosting their production. ComCat® has been developed and used to increase agricultural production of crops by more than 20% (Schnabl *et al.*, 2001; Hüster, 2001). It could be an alternative to other agrochemicals, as it is required in low doses. It is also environmentally and ecologically friendly.

There are very few modern means of transportation, storage, and processing of fresh vegetables in the Ethiopia. This is one of the reasons for high postharvest losses that the country is experiencing. Wolde (1991) pointed out the necessity of government involvement in the improvement of packaging, cold storage, grading and transportation facilities, in order to maintain quality of vegetables for markets both local and abroad.

In chapter 3 and 4 it was shown that ComCat® treated vegetables performed better after harvest than untreated controls, even at temperatures above that of the recommended storage temperatures of specific vegetables. It was therefore decided to investigate the postharvest performance of ComCat® treated vegetables at a controlled temperature that is higher than the optimum storage temperature. A storage facility, which is suited for use in a developing country, would be used for this purpose. The aim of the work in this chapter is, therefore, to investigate the postharvest qualities of ComCat® treated carrots stored in a low-cost evaporative cooling chamber at temperatures between 16°C and 22°C, and relative humidity of 78%-91%.

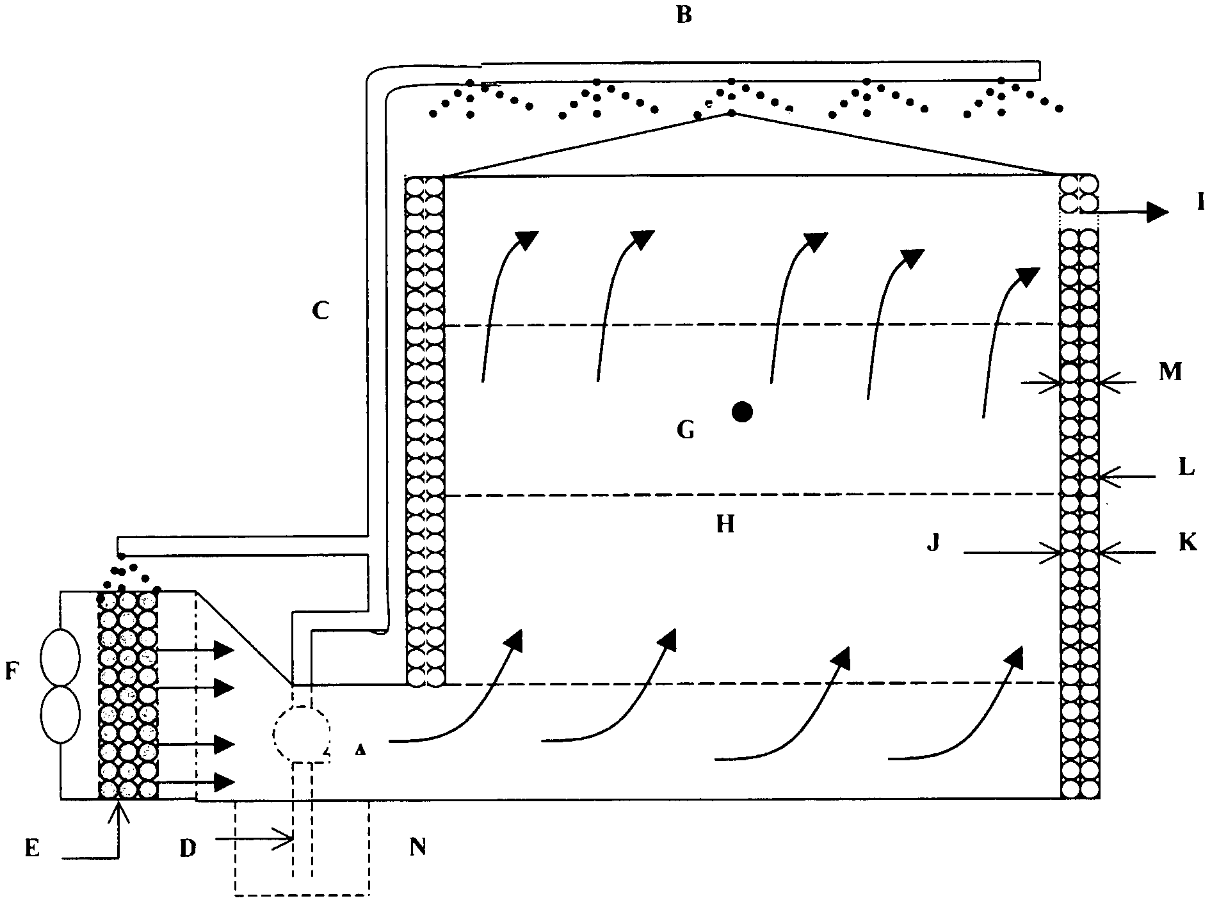
The objectives of the study are:

1. to investigate the effectiveness of forced ventilation EC in reducing temperature and increasing relative humidity during storage of carrots;
2. to investigate the storability of preharvest ComCat® treated carrots using EC methods;
3. to explore the synergistic effect of pre-packaging treatments (disinfecting in chlorinated water) when combined with MAP and EC storage.

5.2. Materials and methods

5.2.1. Evaporative cooling chamber

In earlier work, a naturally ventilated EC was designed and constructed using locally available and low cost materials (Seyoum and W/Tsadik, 2000). That cooler was able to reduce the storage temperature relative to the surrounding air temperature at the study site of Alemaya University, Ethiopia. Using these results as a starting point, and with the objective to further reduce the temperature as well as increase the relative humidity of the air, a forced ventilation evaporative cooler was designed and constructed for vegetable storage. Fig. 5.1 shows a schematic diagram of the EC. The cooler consists of three major units: the air conditioning unit, watering pipe systems and cool storage chamber. The water distribution system was designed so that the water is continuously pumped to the top surfaces of the cooler and to the cooling pad. The top surface of the structure was designed to enable water to run down all the four lateral surfaces by gravity, back into a holding tank. The inner dimensions of the unit were 2 X 2 X 1.3 m, to hold a capacity 0.5 ton vegetables. The frame was constructed from 25 X 25 X 4 mm angle iron. All the five faces of the cooler were covered by using sheet metal (1 mm in thickness), welded against each vertical and horizontal angle iron. In order to obtain good strength the sheet metals were properly welded, both along the vertical and horizontal angle irons. The water tank was placed below ground level beneath the water pump (N) and a vertical water pipe was installed to withdraw water from the tank during operation (D). During operation, water is sprinkled by a small 0.186 KW water pump (A) over the top surface, to wet the top surface and all four sides of cooling pad layers by a horizontal perforated pipe (B). The hose was connected from a vertical pipe to sprinkle water continuously on the cooling pad filled with charcoal (E) from the top (C). An in-built fan (F) blew air through the cooling pad (E) into the evaporative cooling chamber, to effectively increase the relative humidity, while the temperature is decreased. In Fig. 5.1. the directions of arrows show the airflow pattern after passing through the cooling pad. To minimize bruising of perishable produce, and improve airflow, three equally spaced shelves (H) were inserted.



A = Water pump (0.5 HP)
B = perforated horizontal water pipe
C = hose
D = Vertical pipe connected to water pump
E= Cooling pad (Charcoal)
F = Fan
G = Location of thermocouple
& hygrometer

H = Storage chamber
I = Ventilation port
J = Sheet metal
K = Meshed wire holding surface cooling pad
L = mesh wire
M = Wet jutty sack layer
N = water tank

Figure 5.1. Schematic diagram of an experimental evaporative cooler for vegetable storage under hot arid and semi-arid conditions.

The dry-bulb air temperature inside the EC was monitored by thermocouples at the center of the middle shelf (G). An air vent was inserted in the top of the cooler (I). The three side surfaces and the door were covered with a thin-layer pad of 5-mm jutty sack (M), which was sandwiched between sheet metal, on the inside (J), and mesh wire (L), on the outside, facing the ambient air, to allow evaporation (K). In this way the maximum surface area from which evaporation of water can take place, was exposed. Reduction in surface temperature would therefore directly be related to the rate of evaporation.

The can easily be adopted for use by peasants and small-scale farmers from the sub sector. It can be adapted for use without an electric power source using natural ventilation for cooling, however, the efficiency of the cooler in reducing temperature and increasing relative humidity would not be as high as with forced ventilation. The structure could also be modified to cool vegetables and fruits during local transportation by truck or long distances by train.

5.2.2. Temperature and relative humidity measurement

The ambient air temperature and relative humidity were measured by using a Jenway-digital psychrometer 5105, UK. The psychrometer recorded dry bulb temperature, wet bulb temperature, dew point temperature and relative humidity. The dry bulb air temperature inside the EC was monitored using a thermocouple installed at the center of the chamber (G), and was connected to a digital temperature control. Simultaneously, a hygrometer (0 - 50°C) with dry and wet bulb thermometers was used to monitor the dry and wet bulb temperature of air inside the EC.

5.2.3. Vegetable production

Carrots (var. Nantes) were grown during the summer season of 2001 at the experimental fields of the Alemaya University, in Eastern Ethiopia. The agro climatic condition of this location, which is at an altitude range of 1500-2300m, is classified as

"Dry Weyna Dega". The climatic conditions of the study site during the carrot production period from December to May 2001 is shown in Table 5.1.

Table 5.1. The climatic conditions of the study site during sample carrot production (2001).

Month	Temperature (°C)		Rain fall (mm)	Relative Humidity (%)	Wind	Sun shine intensity
	Min	Max				
December	3.0	22.3	4.8	39	1.53	9.3
January	5.0	21.8	4.9	39	2.07	9.8
February	6.4	24.4	18.8	38	1.79	10.6
March	12.8	25.0	56.6	51	1.72	6.7
April	11.7	25.4	94.0	41	1.59	8.6
May	13.9	25.2	146.0	55	1.77	8.6

During the growing period, carrots were treated twice with ComCat®. Experimental plants were treated with 10 g ha^{-1} ComCat® in 350 l of water (see section 3.2.1), and control plants 0 g ha^{-1}. Carrots were sprayed once at the three leaves unfolding stage, and a second time at a vegetative stage. All other agricultural practices were the same as those normally practiced by the Farm Management Department of the Alemaya University during carrot production. At a proper maturity stage, carrots were harvested, topped in the field, and were immediately transported from the farm to the Dire Dawa University Fruit and Vegetable Research Station, which is 30 km away. The topping, harvesting and transportation of carrots were made early in the morning before the temperature was too high. For protection against mechanical injury during transportation, carrots were packed in plastic crates.

5.2.4. Postharvest treatment

The procedures described in chapter 3, section 3.2.2. were followed. Following washing, both ComCat® treated and untreated carrots were subjected to one of the following postharvest treatments according to the procedures:

- dipping in chlorinated water (100μg. ml^{-1} chlorine, made with 5% NaOCl) at ambient temperature for 20 minutes, packaged in Xtend® Film (see Chapter 3, section 3.2.2) and stored in EC;
- dipping in chlorine supplemented water, unpackaged and stored in EC
- water washed, packaged in Xtend® Film and stored in EC;
- water washed, unpackaged and stored in EC;
- water washed, packaged in Xtend® Film and stored at ambient conditions, to serve as a postharvest control; and
- water washed and stored under ambient conditions without packaging, to serve as a postharvest control.

These treatments were performed in three different containers, each as a replication for each treatment group. After washing and dipping treatments, the surfaces of carrots were drip dried to avoid the occurrence of condensation inside the packages. Carrots were packed in 2 kg packages or unpackaged groups. Randomly, 2 kg samples were subjected to physiological, microbiological and chemical analyses. Due to limited facilities, not all analyses could be carried out at the same sampling times. PWL and percentage marketability were determined on days 0, 4, 8, 12, 16, 20 and 24. Changes in moisture content, juice content, pH, TSS, populations of total aerobic bacteria and fungi were determined on days 0, 8 and 16, and sugars were determined on days 0 and 16.

5.2.5. Physiological weight loss (PWL)

The analysis of PWL was conducted as described in Chapter 3, section 3.2.4.

5.2.6. Moisture content

The moisture contents of carrots were determined by drying approximately 20 g of sample at 105°C for 24 hours (Mohammed et al., 1999). Drying was continued until the change in carrot sample weight became constant (approaching equilibrium moisture content). The moisture content was calculated per wet material as follows:

$$MC = \frac{(W_w - W_d)}{W_w}$$

Where

MC = Moisture Content

W_w = weight of wet sample

W_d = weight of dry sample.

5.2.7. Juice content

The carrot juice percentage was obtained according to the methods described by Waskar et al (1999). The juice content of carrots was extracted using a juice extractor (Kenwood). The intact carrot weight was recorded prior to juice extraction. After extraction, the puree was filtered through a double-layer cheesecloth, and the weight of clear carrot juice was recorded. The juice percentage was expressed as percentage mass per initial carrot mass used.

5.2.8. Chemical analysis

5.2.8.1. pH and total soluble solids (TSS)

The pH of carrots was measured with a TOA pH meter (model HM-20E, Ogawa Seiki Co., Ltd., Japan). The procedure for TSS determination was as described in Chapter 3, section 3.2.5.1. The TSS was determined with a bench top 60/70 ABBE (No A90067, Bellingham & Stanley Ltd, England) refractometer with a reading range of 0 to 32 °Brix and RI range of 1.3 to 1.7 °Brix, with a precision of ± 0.0003. Between readings, the prism of the referactrometer was cleaned with tissue paper and methanol, rinsed with distilled water and dried before use. The refractometer was standardized against distilled water (0% TSS).

5.2.8.2. Sugar analysis

Reducing and total sugars were estimated by using the techniques of Somogyi *et al.* (1945). The same procedure was used to estimate reducing and total sugars in carrots during storage (Phan *et al.*, 1973). Clear juice (10 ml) was added to 15 ml of 80%

ethanol, mixed, and heated in a boiling water bath for 30 minutes. After extraction, 1 ml of saturated $Pb(CH_3COO)_2.3H_2O$ and 1.5 ml of Na_2HPO_4 were added, and the contents mixed by gentle shaking. After filtration, the extract was made up to 50 ml with distilled water. An aliquot of 1 ml extract was diluted to 25 ml with distilled water, of which 0.5 ml was mixed with 1 ml copper reagent (see below) in a test tube and heated for 20 minutes in a boiling water bath. After heating, the contents were cooled under running tap water without shaking. Arsenomolybdate color reagent (1 ml) (see below) was added, mixed, made up to 10 ml, and left for about 10 minutes to allow color development, after which the absorbancy was determined at 540 nm in a Jenway model 6100 spectrophotometer. For total sugar determination, sugar was first hydrolyzed with 1N HCl by heating at 70°C for 30 minutes. After hydrolysis, the determination of total sugar was made by following the same procedure employed for the reducing sugar determination. Blanks were prepared using distilled water instead of extract. The difference between the total and the reducing sugar values correspond to the nonreducing sugar content of carrots.

Copper reagent was prepared by dissolving 25 g Na_2CO_3, 25 g $C_4H_4KNaO_6.H_2O$, 20 g $NaHCO_3$ and 200 g Na_2SO_4 in 800 ml of distilled water, and finally made up to 1000 ml in a volumetric flask.

The arsenomolybdate color reagent was prepared from 25 g $(NH_4)_6Mo_7O_{24}.4H_2O$, 21 ml of concentrated H_2SO_4 with 3 g $NaAsSO_4.7H_2O$, all dissolved in 25 ml distilled water and kept in an incubator at 37°C for 24 hours before use in the analysis.

5.2.9. Microbiological analysis

5.2.9.1. Total aerobic bacteria and fungi

The microbiological analysis was conducted as described in Chapter 3, section 3.2.6. Three carrots were cut into pieces with a sterile knife and homogenized using a sterile mortar and pestle. The procedure used for estimating the microbial populations was the same as that of Brackett (1990). Samples of 25 g were blended with 225 ml 0.1%

peptone water (pH 7.0) in a stomacher bag for 3 minutes. The slurries were serially diluted in 9 ml 0.1% peptone water. To determine populations of total aerobic microorganisms, duplicate samples were plated on plate count agar (PCA, Oxoid CM463, and pH 7.0 ± 0.2) and incubated at 30°C for 2 days. For the determination of moulds and yeasts, duplicate samples were plated on Rose-Bengal Chlorampehnicol Agar Base (Oxoid CM549) and incubated at room temperature for 3 to 5 days. In all the cases pour plate methods were used. The mean $\log_{10}$ of viable counts from duplicate plates were determined.

5.2.10. Subjective quality analysis

The percentage marketable quality of carrots was evaluated according to the procedures described in chapter 3, section 3.2.7.

5.2.11. Data analysis

The experimental design and data analyses were according to the methods as described in chapter 3, section 3.2.8.

5.3. Results and Discussion

Due to limited facilities at the Alemaya University, Ethiopia, it was not possible to carry out the same analyses as reported in Chapter 3. Some different analyses, such as moisture content and juice content were carried out, as well as alternative analyses such as reducing sugars. It was also not possible to collect samples or carry out all the analyses on the same day, as was managed in Chapter 3.

5.3.1. Temperature and relative humidity

The ambient dry bulb environmental air temperature and relative humidity varied from 25.0°C - 36.0°C and 25.4% - 53.0% during the storage period, respectively. Inside the evaporative cooler, the dry bulb temperature and relative humidity were between 16.4°C - 22.6°C and 78.0% - 91.0%, respectively. Fig. 5.2. displays mean dry bulb temperatures (°C) and mean relative humidities (%) at a particular time ranging from 6 a.m. up to 6 p.m. of each day against storage time. The differences in dry bulb temperature between environmental air and the air inside the cooler ranged from 8.6°C-13.4°C.

During the storage of 24 days, the differences in temperature were found to be at a minimum (8.6°C) at 6 a.m., whereas the maximum difference (13.4°C) was recorded at 12 am (Fig. 5.3). This was because the rate of evaporation of water from the wet cooling pad increases with increasing outside temperature. Earlier reports also indicated that evaporative cooling is more efficient under hot and dry conditions (Seyoum and W/Tsadik, 2000), as are found in arid or semi-arid regions where the problem of postharvest losses of vegetables and fruit is severe due to the effect of high temperature and low relative humidity.

Rama and Narasimham (1991) showed that a temperature drop from 38.0°C to 22.1°C (a drop of 15.9°C), and heat removal of 2658kJ against a wet bulb temperature of 21.1°C was possible. Rama and Narasimham (1991) and Nadre *et al* (1999) also reported reductions in temperature of 10-12°C, and relative humidity increases from 23% to 91% during peak summer. However, if the ambient temperature is very high, this decrease in temperature may not be sufficient to suppress microbial development at high relative humidity.

From the data presented (Fig. 5.2 and 5.3), it was evident that at 12 am, vegetables stored under environmental conditions are exposed to harsh conditions, due to a coupled effect of both high temperature and low relative humidity. The EC was therefore effective in minimizing these extremes.

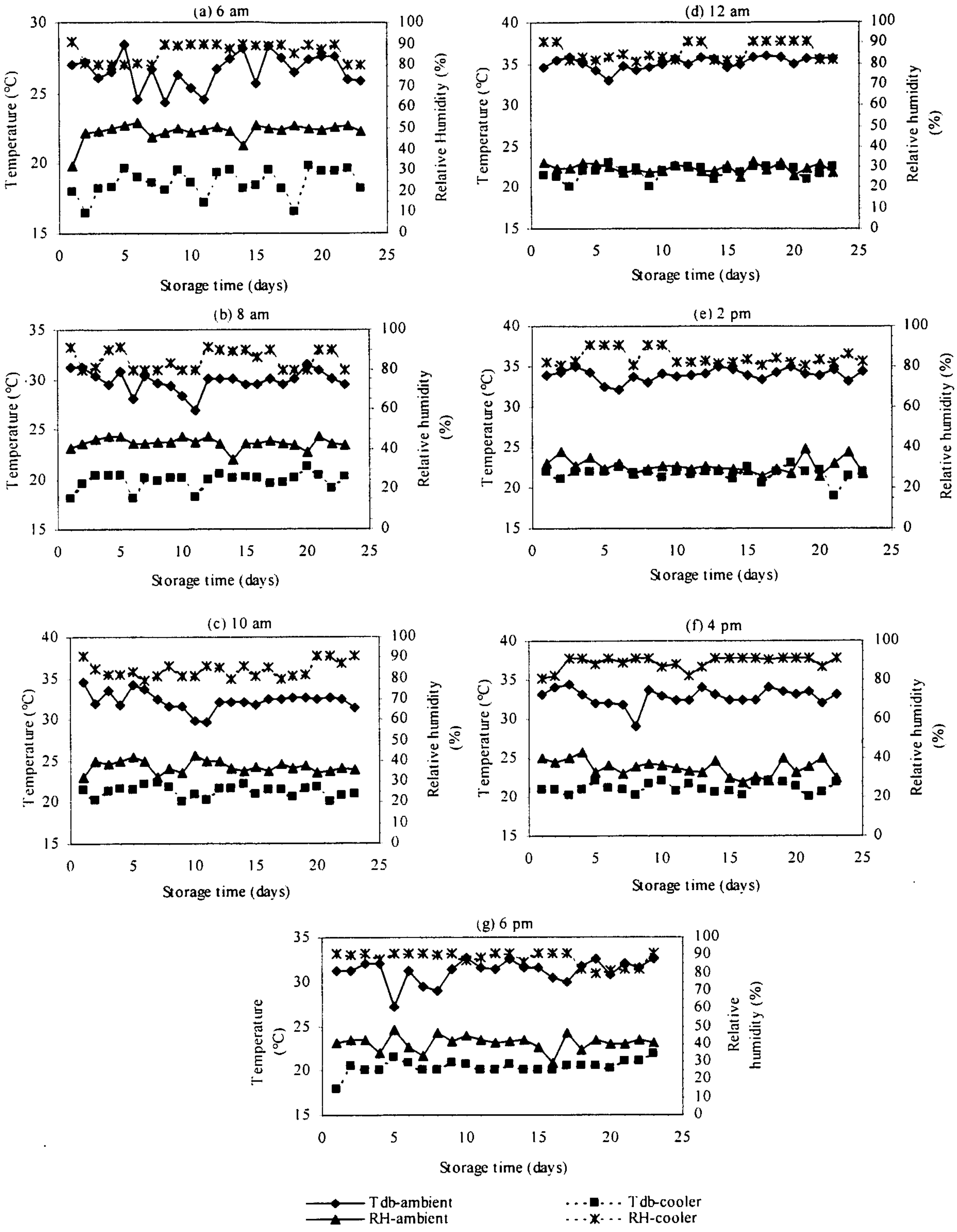


Figure 5.2. Temperature (°C) and relative humidity (%) of the environmental air and the air inside the EC during carrot storage at 6 am (a), 8 am (b), 10 am (c), 12 am (d), 2 pm (e), 4 pm (f) and 6 pm (g).

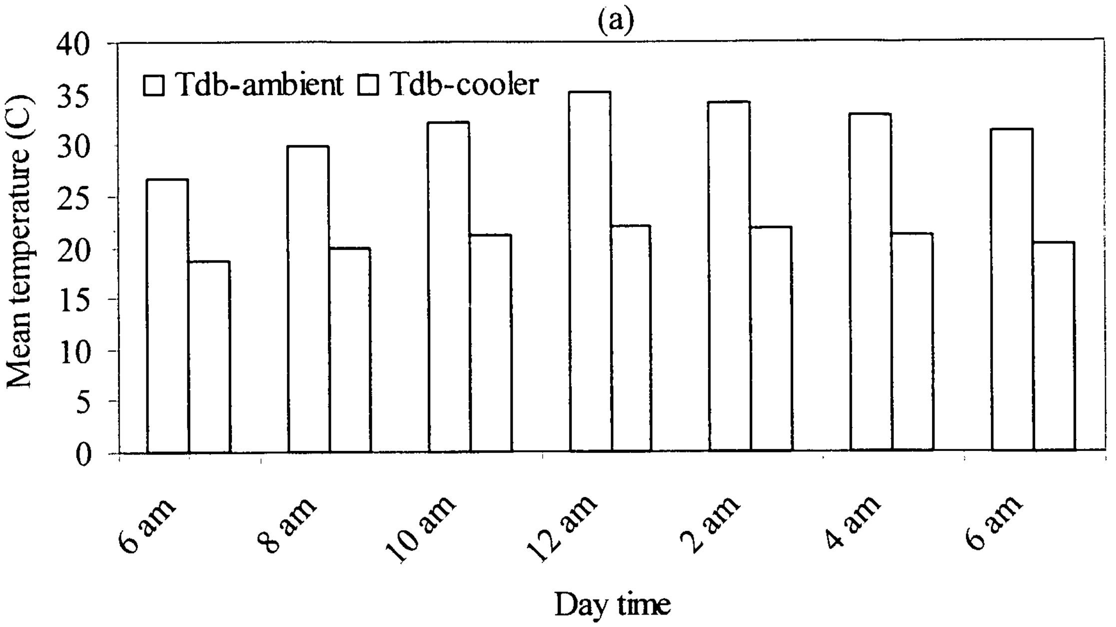


Figure 5.3. (a). The effect of daytime on the average environmental and evaporative cooler temperatures (°C) during storage of carrots.

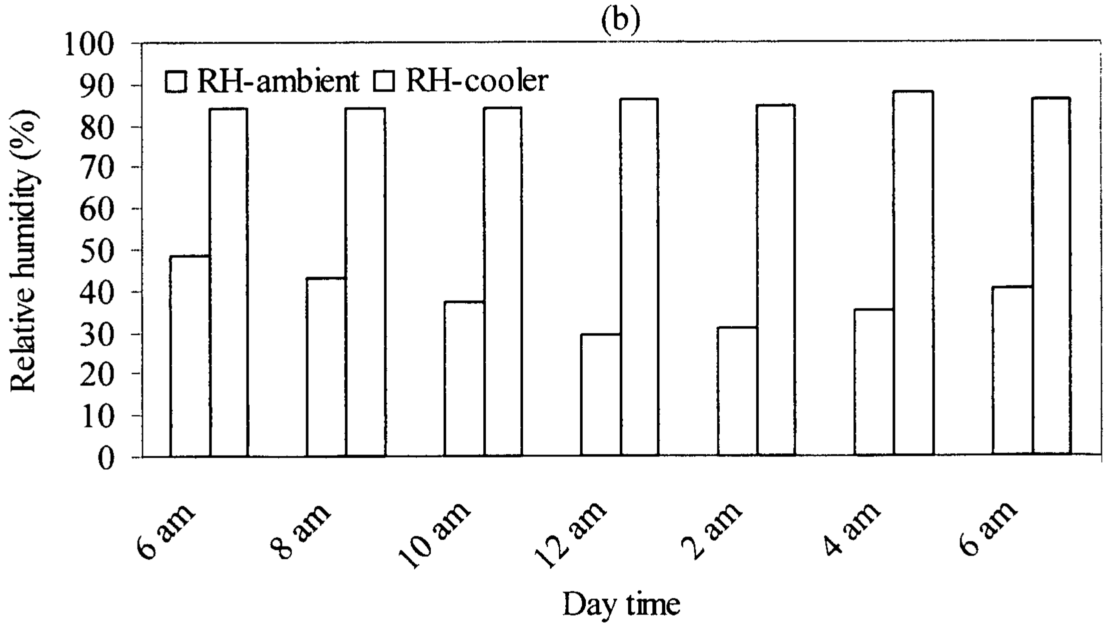


Figure 5.3. (b). The effect of daytime on the average environmental and evaporative cooler relative humidity (%) during storage of carrots.

5.3.2. Physiological weight loss, moisture and juice content

Dipping carrots in chlorine supplemented water seemed to have an effect on the PWL during storage (Table 5.2), although only significant at $P \leq 0.09$. The PWL was higher in carrots dipped in chlorinated water, compared to those dipped in tap water.

Table 5.2. Changes in physiological weight loss of carrots stored in evaporative cooling chamber and ambient temperature (RT) for 24 days.

	Physiological loss in weight (%)					
Treatment	Day 4	Day 8	Day 12	Day 16	Day 20	Day 24
ComCat®, Cl_2, MAP, EC	0.568^{d}	4.970^{ef}	10.291^{d}	12.807^{d}	17.100^{b}	20.649^{b}
Control, Cl_2, MAP, EC	0.877^{d}	6.752^{e}	12.056^{d}	15.093^{c}	19.485^{b}	23.108^{b}
ComCat®, Cl_2, EC	1.286^{cd}	10.718^{cde}	20.361^{bc}	25.947^{b}	36.272^{a}	45.084^{a}
Control, Cl_2, EC	6.667^{bcd}	13.867^{cd}	24.603^{bc}	30.545^{b}	41.626^{a}	50.107^{a}
ComCat®, H_2O, MAP, EC	0.688^{d}	5.097^{e}	7.907^{e}	10.645^{de}	14.841^{c}	18.625^{c}
Control, H_2O, MAP, EC	0.700^{d}	5.363^{e}	8.628^{e}	11.429^{d}	15.858^{bc}	20.246^{bc}
ComCat®, Cl_2, MAP, RT	8.724^{abc}	16.137^{bc}	22.275^{bc}	26.323^{b}	33.553^{a}	40.600^{a}
Control, Cl_2, MAP, RT	12.096^{ab}	20.298^{b}	26.867^{b}	31.580^{b}	38.016^{a}	44.598^{a}
ComCat®, H_2O, EC	0.506^{d}	9.246^{de}	16.595^{cd}	22.598^{bc}	34.424^{a}	43.479^{a}
Control, H_2O, EC	2.043^{cd}	10.621^{de}	20.653^{bc}	26.768^{b}	33.968^{a}	41.986^{a}
ComCat®, H_2O, RT	9.993^{ab}	47.452^{a}	70.810^{a}	88.604^{a}	-	-
Control, H_2O, RT	14.034^{a}	44.656^{a}	70.254^{a}	84.399^{a}	-	-

Significance

Preharvest treatment (A)	NS
Disinfecting treatment (B)	*
Packaging + Storage temperature (C)	***
A X B	NS
A X C	NS
B X C	***
A X B X C	*

NS, *, *** Nonsignificant or significant at $P \leq 0.09$ or 0.001 respectively. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The coefficient of variation and standard error were 0.150 and 1.196 respectively. LSD Value = 6.508.

The combined effect of MAP and storage temperature was found to be highly significant ($P \leq 0.001$) in reducing the PWL during storage. The loss in PWL was higher from unpackaged than packaged carrots stored in the EC.

The results in Table 5.2 show the importance of MAP for use in combination with EC to maintain freshness and increase shelf life. The PWL from unpackaged carrots stored at ambient temperature was above 85% at the end of 16 days of storage respectively, whereas for carrots packaged and stored at ambient temperature, the PWL was found to be below 32% during the same storage time. While the unpackaged carrots were dried out after 16 days, the packaged ones lasted up to 24 days storage, with a PWL of 40 - 45%. The effect of EC on the PWL was also significant ($P \leq 0.05$) during the storage period. Due to the lower temperature and higher relative humidity, the PWL was significantly reduced ($P \leq 0.05$), which was in agreement with previous studies by Roy and Pal (1994) and Waskar *et al* (1999). The PWL in packaged carrots stored in EC was only 18-23%, while unpackaged carrots stored in EC showed a PWL of 41-50%. Disinfecting with chlorinated water had no significant effect on PWL. The PWL was higher in control carrots, however not significant ($P > 0.05$), perhaps because the sizes of the control carrots were smaller than the ComCat® treated ones. Similar results were observed in Chapter 3, Table 3.1. In addition to the effect of size on the PWL, plant growth hormones (gibberilin and auxin) play an important role in reducing the PWL during storage (Salunkhe *et al*, 1991; Rodrigues and Subramanyam, 1996). These hormones are the major constituent of ComCat® and hence could have contributed to the reduction in PWL during storage of carrots. Compared to the storage at room temperature in Chapter 3, which was at the same temperature as ion EC, but with a lower relative humidity, the PWL was less. The higher humidity therefore prevented moisture loss through evaporation.

Table 5.3. Changes in moisture content of carrots stored in evaporative cooling chamber and ambient temperature (RT) for 24 days.

Treatment	Moisture content (% w.b.)			
	Day 0	Day 8	Day 16	Day 24
ComCat®, Cl_2, MAP, EC	88.205^{a}	88.079^{a}	87.711^{a}	86.819^{a}
Control, Cl_2, MAP, EC	88.002^{a}	87.828^{a}	87.060^{ab}	85.806^{ab}
ComCat®, Cl_2, EC	88.205^{a}	87.335^{ab}	86.096^{ab}	84.312^{ab}
Control, Cl_2, EC	88.002^{a}	87.703^{a}	86.656^{ab}	83.771^{bc}
ComCat®, H_2O, MAP, EC	88.205^{a}	87.362^{a}	87.174^{ab}	85.685^{ab}
Control, H_2O, MAP, EC	88.002^{a}	87.508^{ab}	86.533^{a}	84.763^{ab}
ComCat®, Cl_2, MAP, RT	88.205^{a}	87.585^{a}	85.408^{b}	84.296^{b}
Control, Cl_2, MAP, RT	88.002^{a}	85.469^{bc}	84.953^{bc}	83.681^{c}
ComCat®, H_2O , EC	88.205^{a}	85.878^{bc}	84.917^{abc}	82.989^{c}
Control, H_2O, EC	88.002^{a}	85.278^{bc}	83.007^{cd}	81.747^{d}
ComCat®, H_2O, RT	88.205^{a}	79.117^{d}	72.700^{e}	-
Control, H_2O, RT	88.002^{a}	83.936^{b}	75.144^{e}	-

Significance

Preharvest treatment (A)	NS
Disinfecting treatment (B)	*
Packaging + Storage temperature (C)	***
A X B	NS
A X C	NS
B X C	***
A X B X C	*

NS, *, *** Nonsignificant or significant at $P \leq 0.07$ or 0.001 respectively. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The coefficient of variation and standard error were 0.027 and 0.303 respectively. LSD Value = 3.643.

Table 5.3 shows moisture loss of carrots during EC storage. General trends of higher moisture content in packaged ComCat® treated carrots dipped in chlorinated water and stored in EC were noticed, however not significant ($P > 0.05$). The conservation or loss of moisture was due to MAP and storage at EC temperature, or the lack thereof, respectively. The moisture content of carrots was therefore significantly affected with packaging and storage temperature ($P \leq 0.001$). Chlorine disinfecting did not affect ($P > 0.05$) moisture loss, although the loss was significant at $P \leq 0.07$. Since these results basically are the same as that of PWL (Table 5.1), it can be concluded that the PWL observed, is mainly due to the loss of moisture, and not due to metabolic activity in the form of CO_2.

Table 5.4. Changes in juice content of carrots stored in evaporative cooling chamber and ambient temperature (RT) for 24 days.

	Juice Content (%)			
Treatment	Day 0	Day 8	Day 16	Day24
ComCat®, Cl_2, MAP, EC	64.467 ^{a}	62.193 ^{a}	60.194 ^{a}	59.062 ^{a}
Control, Cl_2, MAP, EC	60.421 ^{ab}	60.051 ^{ab}	58.625 ^{ab}	57.793 ^{ab}
ComCat®, Cl_2, EC	64.467 ^{a}	54.413 ^{bcd}	53.870 ^{bc}	51.208 ^{b}
Control, Cl_2, EC	60.421 ^{ab}	55.383 ^{bcd}	53.428 ^{abc}	52.547 ^{b}
ComCat®, H_2O, MAP, EC	64.467 ^{a}	60.509 ^{ab}	57.051 ^{abc}	57.030 ^{ab}
Control, H_2O, MAP, EC	60.421 ^{ab}	58.208 ^{abc}	56.125 ^{abc}	55.156 ^{ab}
ComCat®, Cl_2, MAP, RT	64.467 ^{a}	59.604 ^{bc}	52.516 ^{c}	51.058 ^{b}
Control, Cl_2, MAP, RT	60.421 ^{ab}	53.786 ^{cde}	49.346 ^{c}	46.698 ^{cd}
ComCat®, H_2O, EC	64.467 ^{a}	56.827 ^{bcd}	54.879 ^{bc}	52.728 ^{b}
Control, H_2O, EC	60.421 ^{ab}	54.247 ^{bcd}	51.047 ^{bc}	48.575 ^{bcd}
ComCat®, H_2O, RT	64.467 ^{a}	39.107 ^{e}	27.472 ^{d}	-
Control, H_2O, RT	60.421 ^{ab}	46.596 ^{de}	29.610 ^{d}	-
Significance				
Preharvest treatment (A)		NS		
Disinfecting treatment (B)		*		
Packaging + Storage temperature (C)		***		
A X B		NS		
A X C		NS		
B X C		**		
A X B X C		*		

NS, *, **, *** Nonsignificant or significant at $P \leq 0.09$ 0.01 or 0.001 respectively. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The coefficient of variation and standard error were 0.103 and 1.010 respectively. LSD Value = 9.089.

The juice content (Table 5.4) explains nothing more than PWL and moisture loss. However, this analysis was included, mainly to investigate differences between ComCat® treated and control carrots, as a measurement of water binding, due to possible differences in structural components such as cellulose and other fibers. There are thus no differences of that kind.

5.3.3. Chemical changes

5.3.3.1. Total soluble solids (TSS)

The results presented in Table 5.5 showed that there was a general trend of an increase in TSS of carrots during storage from around 8.3 to maximum 13.67 °Brix at EC after 24 days, and as high as 18 °Brix for carrots stored unpackaged at ambient temperature after 16 days. The changes in TSS also fall within the same limits as that observed in Chapter 3. Lingaiah and Huddar (1991), Waskar et al. (1999) and Jitender-Kumar et al. (1999) also showed an increasing trend of TSS in carrots, but at lower numbers, i.e. from 1.9-2.9 °Brix, for packaged carrots stored in EC.

At harvest the TSS content of ComCat® treated carrots was slightly lower than that of the control, and remained that way during storage, however, these differences were not significant ($P > 0.05$), although the two-way interaction between preharvest and prepackaging treatments was significant at $P \leq 0.09$ on the changes in TSS of carrots. The effect of disinfecting carrots in chlorinated water was found to be not significant (($P > 0.05$) on the changes of TSS of carrots during storage.

The combined effect of packaging and storage at environmental conditions were highly significant ($P \leq 0.001$) on the TSS content of carrots. The result also showed that there is an overall interaction ($P \leq 0.001$) between prepackaging disinfecting of carrots, MAP and EC temperatures during storage. In general, the TSS of carrots was better maintained in the packaged carrots stored inside the EC. The TSS of carrots was affected by the interaction between the pre- and postharvest treatments during storage, however, only at a significance level of $P \leq 0.09$. These result thus indicate that the quality of carrots may be maintained better by applying the combinations of pre- and postharvest treatments such as ComCat® treatment, disinfecting, MAP and followed by EC during storage.

Table 5.5. Changes in TSS of carrots stored in evaporative cooling chamber and ambient temperature (RT) for 24 days.

	Total soluble solid (°Brix)			
Treatment	Day 0	Day 8	Day 16	Day 24
ComCat®, Cl_2, MAP, EC	8.30 ^{ab}	10.77 ^{cd}	9.97 ^{c}	10.20 ^{d}
Control, Cl_2, MAP, EC	8.60 ^{a}	10.77 ^{cd}	10.20 ^{c}	11.40 ^{cd}
ComCat®, Cl_2, EC	8.30 ^{ab}	10.53 ^{cd}	11.30 ^{bc}	11.38 ^{cd}
Control, Cl_2, EC	8.60 ^{a}	11.43 ^{bc}	10.87 ^{bc}	13.67 ^{a}
ComCat®, H_2O, MAP, EC	8.30 ^{ab}	10.48 ^{cd}	9.96 ^{c}	10.67 ^{d}
Control, H_2O, MAP, EC	8.60 ^{a}	10.55 ^{cd}	9.25 ^{c}	11.50 ^{bcd}
ComCat®, Cl_2, MAP, RT	8.30 ^{ab}	10.50 ^{cd}	11.17 ^{bc}	12.73 ^{abc}
Control, Cl_2, MAP, RT	8.60 ^{a}	10.33 ^{cd}	10.97 ^{bc}	12.87 ^{ab}
ComCat®, H_2O, EC	8.30 ^{ab}	9.78 ^{d}	12.70 ^{b}	12.70 ^{abc}
Control, H_2O, EC	8.60 ^{a}	10.75 ^{cd}	11.23 ^{bc}	13.10 ^{a}
ComCat®, H_2O, RT	8.30 ^{ab}	14.87 ^{a}	18.15 ^{a}	-
Control, H_2O, RT	8.60 ^{a}	14.62 ^{a}	17.00 ^{a}	-
Significance				
Preharvest treatment (A)		*		
Disinfecting treatment (B)		NS		
Packaging + Storage temperature (C)		***		
A X B		*		
A X C		NS		
B X C		***		
A X B X C		*		

NS, *, *** Nonsignificant or significant at $P \leq 0.09$ or 0.001 respectively. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The coefficient of variation and standard error were 0.077 and 0.084 respectively. LSD Value = 1.397.

5.3.3.2. pH

The initial pH values of ComCat® and control carrots were 6.01 and 5.98 respectively, but the difference was not significant (Table 5.6). The pH of ComCat® treated carrots were maintained around 6.00 during the 24 days of storage in the EC. The pH of ComCat® treated carrots significantly ($P \leq 0.05$) differed from the pH of control carrots after 8 days of storage at room temperature.

Table 5.6. Changes in pH of carrots stored in evaporative cooling chamber and ambient temperature (RT) for 24 days.

	pH			
Treatment	Day 0	Day 8	Day 16	Day 24
ComCat®, Cl_2, MAP, EC	6.01^{a}	6.08^{bc}	6.08^{ab}	6.06^{a}
Control, Cl_2, MAP, EC	5.98^{ab}	6.02^{cde}	6.02^{bc}	6.06^{a}
ComCat®, Cl_2, EC	6.01^{a}	6.26^{a}	6.12^{a}	6.08^{a}
Control, Cl_2, EC	5.98^{ab}	6.04^{bcd}	6.06^{bc}	5.99^{bc}
ComCat®, H_2O, MAP, EC	6.01^{a}	6.08^{bc}	6.01^{bc}	5.98^{abc}
Control, H_2O, MAP, EC	5.98^{ab}	5.95^{de}	5.99^{cd}	6.03^{a}
ComCat®, Cl_2, MAP, RT	6.01^{a}	5.93^{de}	6.00^{bc}	5.87^{bc}
Control, Cl_2, MAP, RT	5.98^{ab}	5.91^{c}	5.91^{de}	5.64^{d}
ComCat®, H_2O, EC	6.01^{a}	6.11^{b}	6.02^{bc}	6.00^{ab}
Control, H_2O, EC	5.98^{ab}	5.98^{cde}	6.01^{bc}	6.03^{a}
ComCat®, H_2O, RT	6.01^{a}	6.12^{b}	5.84^{e}	5.84^{c}
Control, H_2O, RT	5.98^{ab}	5.98^{cde}	5.97^{cd}	5.97^{abc}

Significance

Preharvest treatment (A)	**
Disinfecting treatment (B)	NS
Packaging + Storage temperature (C)	***
A X B	*
A X C	*
B X C	***
A X B X C	**

NS, *, **, *** Nonsignificant or significant at $P \leq 0.09$, 0.05 or 0.001 respectively. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The coefficient of variation and standard error were 0.010 and 0.004 respectively. LSD Value = 0.103.

The changes in pH of the packaged ComCat® treated carrots dipped in chlorinated water showed significant ($P \leq 0.05$) differences from that of the packaged control carrots treated with chlorine during storage at ambient temperature, although there was no significant difference in those stored in EC. MAP had a slight effect on the pH of carrots. As a general trend the rate at which the pH was increasing in packaged ComCat® treated carrots was lower compared to unpackaged ones. Similarly, general trends of rapid increase in pH of control carrots were noticed during storage. The storage temperature also affected the pH significantly ($P \leq 0.05$) during storage. As a general trend, a drop in pH of carrots was observed during storage at ambient temperature, which could be associated with higher rates of respiration, since acids are formed due to the catabolism of carbohydrates (Hao et al., 1999). However, the results of Hao et al. (1999) do not agree that packaging material and storage temperature significantly affected the pH of carrot ($P \leq 0.05$).

Chlorine treatment had no significant effect on the pH of stored carrots, but the two-way interaction between postharvest treatment i.e. disinfecting treatment, MAP and storage temperature was highly significant. The combined effect of preharvest and disinfecting treatment was significant, but only at $P \leq 0.09$. There was also a significant two-way interaction between preharvest and MAP + storage temperature during storage at $P \leq 0.09$ level. The three-way interaction between ComCat® treatment, disinfecting treatment, MAP and storage temperature was found to be significant at $P \leq 0.05$ level.

5.3.3.3. Sugar analysis

The sugar dynamics during storage are shown in Table 5.7. The non-reducing and total sugars of carrots decreased consistently during the 16 days of storage, while the reducing sugar contents seemed to increase in some carrot samples subjected to different treatments during 16 days, confirming the findings of Phan et al. (1973) and Nilsson (1987). In Chapter 3 an increase in glucose content during storage was also noted, which also support these results obtained during EC storage.

Table 5.7. Changes in reducing, non-reducing and total sugar contents of carrots stored in evaporative cooling chamber and ambient temperature (RT) for 24 days.

Treatment	Total Sugar (g/100g)		Reducing sugar (g/100g)		Non-reducing Sugar (g/100g)	
	Day 0	Day 16	Day 0	Day 16	Day 0	Day 16
ComCat®, Cl_2, MAP, EC	6.910 ^{a}	7.187 ^{a}	2.670 ^{a}	4.784 ^{a}	4.240 ^{ab}	2.978 ^{bc}
Control, Cl_2, MAP, EC	7.412 ^{a}	6.917 ^{a}	2.694 ^{a}	2.763 ^{b}	4.981 ^{a}	4.154 ^{ab}
ComCat®, Cl_2, EC	6.910 ^{a}	4.934 ^{b}	2.670 ^{a}	2.245 ^{bc}	4.240 ^{ab}	2.689 ^{c}
Control, Cl_2, EC	7.412 ^{a}	5.315 ^{b}	2.694 ^{a}	0.971 ^{d}	4.981 ^{a}	4.344 ^{a}
ComCat®, H_2O, MAP, EC	6.910 ^{a}	5.127 ^{b}	2.670 ^{a}	2.586 ^{bc}	4.240 ^{ab}	2.541 ^{c}
Control, H_2O, MAP, EC	7.412 ^{a}	3.782 ^{c}	2.694 ^{a}	1.948 ^{c}	4.981 ^{a}	1.835 ^{cd}
ComCat®, H_2O, EC	6.910 ^{a}	3.906 ^{c}	2.670 ^{a}	2.969 ^{b}	4.240 ^{ab}	0.936 ^{d}
Control, H_2O, RT	7.412 ^{a}	1.924 ^{d}	2.694 ^{a}	0.979 ^{d}	4.981 ^{a}	0.945 ^{d}

Significance	Total sugar	Reducing sugar	Non-reducing sugar
Preharvest treatment (A)	NS	*	NS
Disinfecting treatment (B)	***	**	***
Packaging + storage temperature (C)	****	****	*
A X B	*	NS	*
A X C	NS	NS	NS
B X C	*	**	NS
A X B X C	**	*	NS

NS, *, **, ***, **** Nonsignificant or significant at $P \leq 0.09$, 0.05, 0.01 or 0.001 respectively. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The coefficient of variation and standard error were 0.195 and 0.180 for reducing sugar, 0.192 and 0.306 for nonreducing sugar and 0.086 and 0.217 for total sugar respectively. LSD Value = 0.836, 1.158 and 0.872 for reducing, nonreducing and total sugar respectively.

In Chapter 3 it was shown that ComCat® treatment significantly affected the changes in glucose and fructose content, while the total sugar content was not affected. Here ComCat® treatment significantly affected the changes in reducing sugars at $p \leq 0.09$), which would therefore repeat the results observed for glucose content in Chapter 3. It shows that ComCat® in general has an effect on sugars. At harvest, there was no significant difference ($P > 0.05$) in total and non-reducing sugar content between ComCat® treated and untreated control carrots, however at $p \leq 0.09$, ComCat® treated carrots had a lower reducing sugar content compared to the controls. Similarly, the ComCat® treated carrots had a lower glucose content at harvest in the carrots investigated in chapter 3. The results in Chapter 3 showed that even though the free sugars were higher in controls, the total available carbohydrates (TAC) were found to be higher in ComCat® treated carrots, than in the control carrots. It should be mentioned that a concentration effect could play a role in the results of the current Chapter, as it was noted that ComCat® treated carrots contain slightly more juice than the control carrots (Table 5.4). After 16 days of storage, the highest total sugar content was maintained in disinfected and packaged carrots stored in EC. This result shows that the postharvest treatments such as disinfecting, MAP and EC, can maintain the chemical quality, regarding the sugars, better during short storage periods.

A considerable decrease in total sugar content was found in carrots stored at ambient temperature compared to EC. There was a 74% percent depletion in total sugar content in unpackaged carrots stored at ambient conditions. The reason for this could be associated with a high respiration rate of carrots stored at relatively higher temperature. High temperature increases the metabolic activity, and therefore also the activity of enzymes, responsible for biochemical reactions, in carrots during storage. Faster utilization of freely available sugars by microorganisms could also contribute to the reduction of sugars.

The interaction between disinfecting, MAP and storage temperature had a significant ($P \leq 0.05$) effect on the changes in reducing sugar in carrots during 16 days at EC temperature. The reducing sugars were significantly higher in both ComCat® treated carrots and controls that were disinfected in chlorinated water, packaged and stored in EC. The two-way interaction between the preharvest ComCat® treatment and disinfecting had a significant ($P \leq 0.05$) influence on the changes of non-reducing sugar content. The three-way interaction between the pre- and postharvest treatment on the changes of reducing sugar content of carrots was significant ($P \leq 0.09$) during storage.

These results thus demonstrated that ComCat® treatment in general has an effect on the sugar content.

5.3.4. Microbiological changes

5.3.4.1. Total aerobic bacteria

Table 5.8 displays the estimated populations of total aerobic bacteria in stored carrots. The populations of total aerobic bacteria on carrots were significantly ($P \leq 0.01$) affected by disinfecting in chlorinated water, which significantly reduced microbial populations for up to 16 days.

MAP had a significant effect ($P \leq 0.01$) on the population of the total aerobic bacteria. The populations of total aerobic bacteria was lower in packaged carrots at the end of 16 days in the EC compared to those in unpackaged carrots.

Table 5.8 shows that the estimated population of total aerobic bacteria was higher in carrots stored at ambient conditions, than in carrots stored in the EC for 8 days. A sharp increase in the populations of aerobic bacteria in carrots stored at ambient temperature during the first few days were observed, followed by a slight drop thereafter. The reason for this was due to the availability of free moisture in carrots during the first few days, after which they tended to dry out, leaving less moisture for microbial development. The lower sugar contents in these carrots (Table 5.3, 5.4 and 5.7) could also contribute to this observation.

The preharvest ComCat® treatment had an effect on the estimated populations of total aerobic bacteria at $p \leq 0.09$. Similar results were also found in the experiment in Chapter 3. The preharvest ComCat® treatment was shown to have a significant effect on the pH of carrots during storage, which could be the reason for the effect of the preharvest treatment on microbial growth and their population during storage. The populations of aerobic bacteria also remained lower throughout storage, but only at a significance level of $P \leq 0.08$. The two- and three-way interactions between the preharvest treatment and postharvest treatments were not significant for the total aerobic microorganisms in carrots stored in EC.

Table 5.8. Populations of total aerobic bacteria in carrots packaged or unpackaged and stored in evaporative cooling chamber or at ambient temperature (RT) for 16 days.

	Total aerobic bacteria (Log CFU/g)		
Treatment	Day 0	Day 8	Day 16
ComCat®, Cl_2, MAP, EC	4.845 ^{ab}	4.823 ^{d}	4.712 ^{c}
ComCat®, Cl_2, EC	4.845 ^{ab}	5.125 ^{cd}	5.273 ^{bc}
ComCat®, H_2O, MAP, EC	4.845 ^{ab}	5.996 ^{abc}	5.823 ^{b}
ComCat®, H_2O, EC	4.845 ^{ab}	6.378 ^{ab}	7.022 ^{a}
Control, Cl_2, MAP, EC	5.463 ^{a}	5.120 ^{cd}	5.038 ^{c}
Control, Cl_2, EC	5.463 ^{a}	5.697 ^{bcd}	5.822 ^{b}
Control, H_2O, MAP, EC	5.463 ^{a}	6.230 ^{abc}	6.664 ^{a}
Control, H_2O, RT	5.463 ^{a}	7.174 ^{a}	6.500 ^{a}

Significance

Preharvest treatment (A)	*
Disinfecting treatment (B)	**
Packaging + storage temperature (C)	**
A X B	NS
A X C	NS
B X C	NS
A X B X C	NS

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01 or 0.001 respectively. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The coefficient of variation and standard error were 0.093 and 0.268 respectively. LSD Value = 0.854.

5.3.4.2. Total moulds and yeasts

The populations of moulds and yeasts remained lower throughout the storage period of 16 days in ComCat® treated, packaged carrots dipped in chlorinated water, and stored in EC ($P \leq 0.05$) (Table 5.9). Disinfecting carrots in chlorinated water also highly affected ($P \leq 0.001$) the populations of moulds and yeasts during 16 days of storage. This indicated that the use of EC combined with disinfecting treatments significantly reduced decay during storage.

Two-way interactions were observed between packaging + storage temperature and both preharvest treatment ($P \leq 0.05$) and disinfecting treatment ($P \leq 0.001$). With EC, the minimum temperature attained was only 16°C, which could mean that disinfecting carrots in chlorinated water would have little effect in reducing and limiting the growth of aerobic bacteria during storage, compared to storage at 1°C shown in chapter 3. The other possible reason for the presence of higher microbial populations after disinfecting with chlorine, could be associated with the effect of the chlorine solution on the surface tissue of carrots (Seyoum et al., 2003), which could make conditions favorable for microorganisms to grow again. Work of other researchers on the EC of fruits and vegetables focused more on the physiological and chemical changes during evaporative cooling storage, without emphasis on the hazard of postharvest microbiological aspects (Roy and Pal, 1993; Pal and Roy, 1988; Waskar *et al.*, 1999).

The relative humidity in this study was also high, aiding to the proliferation of microorganisms associated with fruits and vegetables. Compared to the storage at room temperature in Chapter 3, which was the same as in the EC, however, with a lower relative humidity, the counts of total bacteria, as well as moulds and yeasts, are in the same order. However, in both cases of storage at higher temperatures, the microbial populations were not as low as observed during storage at 1°C. In general, the preharvest ComCat® treatment had a slight effect on the improvement of the microbiological quality of carrots in terms of less total aerobic bacteria, and mould and yeast populations during storage.

The result demonstrated the importance of combining effective disinfecting treatments with packaging and EC of carrots, particularly under hot and arid conditions. The combined effect of pre- and postharvest treatments, including EC, could aid to solve the problems identified by several workers in Ethiopia (Wolde, 1991; Storck *et al.*, 1991, Kebede, 1991; Agonifar, 1991).

Table 5.9. Populations of moulds and yeasts in carrots packaged or unpackaged and stored in evaporative cooling chamber or at ambient temperature (RT) for 16 days.

	Moulds and yeasts		
Treatment	Day 0	Day 8	Day 16
ComCat®, Cl_2, MAP, EC	4.521 ^{ab}	3.856 ^{d}	3.693 ^{d}
ComCat®, Cl_2, EC	4.521 ^{ab}	4.682 ^{c}	4.248 ^{d}
ComCat®, H_2O, MAP, EC	4.521 ^{ab}	5.534 ^{b}	5.460 ^{bc}
ComCat®, H_2O, EC	4.521 ^{ab}	5.686 ^{b}	5.655 ^{bc}
Control, Cl_2, MAP, EC	4.841 ^{a}	4.055 ^{d}	3.705 ^{d}
Control, Cl_2, EC	4.841 ^{a}	5.525 ^{b}	5.121 ^{c}
Control, H_2O, MAP, EC	4.841 ^{a}	5.824 ^{ab}	6.026 ^{ab}
Control, H_2O, RT	4.841 ^{a}	6.308 ^{a}	6.268 ^{a}
Significance			
Preharvest treatment (A)		*	
Disinfecting treatment (B)		***	
Packaging + storage temperature (C)		***	
A X B		NS	
A X C		*	
B X C		***	
A X B X C		NS	

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01 or 0.001 respectively. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The coefficient of variation and standard error were 0.063 and 0.082 respectively. LSD Value = 0.514.

5.3.5. Subjective quality analysis

MAP, storage in EC, as well as disinfecting treatment of carrots in chlorinated water, had significant ($P \leq 0.05$) effects on the percentage marketability of carrots (Table 5.10). These results indicated that preharvest disinfecting treatments must be coupled with EC in order to decrease postharvest decay, insure a relatively longer shelf life of vegetables, and maintain a better quality. It seemed as if the marketability of ComCat® treated carrots was, in general, somewhat better than the controls, however, not significantly ($P \leq 0.05$). Marketability was not determined over storage time in Chapter 3, because the deterioration was not as fast. The marketability can therefore not be compared.

Table 5.10. Percentage marketable carrots of different treatments after 4, 8, 12, 16, 20 and 24 days of storage at evaporative cooling and ambient temperatures (RT) for 24 days.

	Marketable carrots (%)						
Treatment	Day 0	Day 4	Day 8	Day 12	Day 16	Day 20	Day 24
ComCat®, Cl$_2$, MAP, EC	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	98.3^{a}
Control, Cl$_2$, MAP, EC	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	96.7^{ab}	95^{ab}
ComCat®, Cl$_2$, EC	100^{a}	100^{a}	100^{a}	96.7^{ab}	96.7^{ab}	86.7^{def}	85de
Control, Cl$_2$, EC	100^{a}	100^{a}	100^{a}	96.7^{ab}	93.3^{abcd}	93.3^{abcd}	91.7^{abcd}
ComCat®, H$_2$O, MAP, EC	100^{a}	100^{a}	93.3^{abcd}	93.3^{abcd}	93.3^{abcd}	93.3^{abcd}	93.3^{abc}
Control, H$_2$O, MAP, EC	100^{a}	100^{a}	100^{a}	100^{a}	91.7^{abcde}	86.7^{def}	83.3^{e}
ComCat®, Cl$_2$, MAP, RT	100^{a}	83.3^{c}	71.7^{e}	55^{f}	43.3^{g}	26.7^{h}	15^{g}
Control, Cl$_2$, MAP, RT	100^{a}	90^{bc}	73.3^{e}	56.7^{f}	43.3^{g}	26.7^{h}	8.3^{g}
ComCat®, H$_2$O, EC	100^{a}	100^{a}	98.3^{ab}	88.3^{cde}	85^{ef}	81.7^{f}	81.7^{e}
Control, H$_2$O, EC	100^{a}	100^{a}	96.7^{ab}	86.7^{de}	83.3^{ef}	78.3^{fg}	78.3^{ef}
ComCat®, H$_2$O, RT	100^{a}	41.7^{d}	-	-	-	-	-
Control, H$_2$O, RT	100^{a}	40^{d}	-	-	-	-	-

Significance	
Preharvest treatment (A)	NS
Disinfecting treatment (B)	*
Packaging + Storage temperature (C)	***
A X B	NS
A X C	NS
B X C	**
A X B X C	NS

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01 or 0.001 respectively. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The coefficient of variation and standard error were 0.052 and 0.826 respectively. LSD Value = 6.826.

The combination of postharvest treatments including MAP and storage conditions, highly affected the marketability of carrots during the 24 days of storage, which was significant at $p \leq 0.001$ level. The percentage marketability of unpackaged carrots stored at ambient conditions dropped to 40% during the first 4 days of storage due to excessive dehydration and visible mould growth. Hardening of texture and visible mould growth were the problems in the case of packaged carrots stored at ambient conditions. No sprouting of packaged carrots stored at ambient conditions was observed due to the high temperature that caused excessive dehydration. In the EC, no sprouting was observed, which is one of the major postharvest problems of carrots during storage at relatively higher temperature (Berg and Lentz, 1966).

5.4. Conclusions

A forced ventilation EC unit was developed, using locally available construction material in Dire Dawa, Ethiopia. The unit was designed for possible use on farms, wholesalers, retailers and exporters to close by export markets. It can be fitted to any storeroom, and construction can be modified, within limits, according to desired capacities. This unit reduced the temperature by 8.4 - 13.4°C below ambient temperature, with a rise of relative humidity to 78 - 91% during storage. The temperature and relative humidity were maintained within constant limits, although the outside ambient temperature and relative humidity varied. The shelf life of carrots kept in the unit was dramatically increased from 4 days to 24 days by 6 fold, compared to storage at ambient conditions, mainly by preventing loss of moisture.

The quality of ComCat® treated carrots stored in the EC remained as good as the quality of the untreated control carrots. The PWL, and loss in moisture and juice of carrots were slightly higher in untreated control carrots than in ComCat® treated carrots, although the differences were not significant ($P > 0.05$). The population of moulds and yeasts, pH value and total sugar content of carrots were affected significantly ($P \leq 0.05$) by the preharvest ComCat® treatment during 16 days of storage in EC. The ComCat® treated carrots had a lower total sugars content at harvest, however, the effect of

ComCat® treatment on reducing and non-reducing sugar was not significant ($P > 0.05$) at harvest as well as during the storage period of 16 days.

Unfortunately, not all the analyses for quality could be carried out as in Chapter 3. Only PWL, TSS and the microbiological analysis could be carried out. The reducing sugar content, determined in this chapter, can be compared with the glucose content in chapter 3. Regarding the comparison between ComCat® treated and controls, both investigations show similar insignificant results for changes in TSS and total sugar content, significant differences in reducing sugar or glucose content, but different changes in PWL. It could therefore be concluded that ComCat® treated carrots also express better quality attributes during EC storage than the untreated controls.

Disinfecting carrots in chlorinated water slightly affected nonreducing and reducing sugar content, population of total aerobic bacteria, moulds and yeasts, and marketability during the storage of carrots in EC. In general, the results demonstrated that disinfecting treatment is an important postharvest handling step to be coupled with EC to maintain microbiological quality of carrots.

MAP + storage temperature improved all quality aspects tested. The physiology, biochemistry and microbiology of the root part of plants differ from the other physiological parts, such as fruit. The data obtained on the storability characteristics of ComCat® treated, (and control carrots) may not be appropriate to predict the storability of fruit by EC. Based on this view, and information obtained in Chapter 4, the study on the storability of ComCat® treated (and control tomatoes) in forced ventilation EC was investigated and presented in the next chapter.

CHAPTER 6

EFFECT of PREHARVEST TREATMENT and FORCED VENTILATION EVAPORATIVE COOLING on TOMATOES

Abstract

An evaporative cooler (EC) unit, which allowed an average drop of 11.5°C in temperature and a rise of 43.93% in relative humidity relative to environmental conditions, was used to store tomatoes. The quality of tomatoes stored in this cooler was maintained better, compared to tomatoes stored at ambient conditions, with more than 70% shelf life extension. Preharvest ComCat® treated tomatoes contained lower TSS, reducing sugars and total sugars at harvest, and showed better keeping quality in terms of PWL, juice content, TSS and sugars, compared to untreated controls. No distinct effect of ComCat® treatment on microbial populations was found. Disinfecting with chlorinated water controlled total aerobic bacteria, moulds and yeasts during storage in the EC. MAP and EC temperatures helped to control weight loss, improve juice content, total aerobic bacteria, moulds and yeasts, and resulted in lower pH of stored tomatoes. Microperforated MAP film prevented condensation inside packages and resulted in better marketability when combined with EC. The benefits from the combined effect of pre- and postharvest treatment on tomatoes include: reduction of PWL and loss of fruit juice, better keeping quality in terms of TSS, pH, non-reducing sugar content, total sugar content, microbiological quality, and marketability.

6.1. Introduction

The problems experienced with postharvest vegetable handling in developing countries such as Ethiopia have been reviewed in Chapter 2 section 2.5.3. and were again mentioned in Chapter 5 section 5.1. It was mentioned that ComCat® treatment of vegetables could increase yield, and the postharvest performance of ComCat® treated tomatoes was investigated in Chapter 4. It was found that these tomatoes performed better than untreated controls, not just at the recommended optimum storage temperature of 13°C, but also at higher temperatures. From those results, it was suggested that the postharvest performance of ComCat® treated tomatoes be carried out at a controlled temperature that is higher than the recommended optimum storage temperature, and using storage facilities which are suited for use in developing countries. The aim of the work in this Chapter is, therefore, to investigate the postharvest quality of ComCat® treated tomatoes in a low-cost EC at temperatures between 16°C and 24°C and relative humidity of 73-92%.

The objectives of the study are:

1. to investigate the effectiveness of forced ventilation EC in reducing temperature and increasing relative humidity during storage of tomatoes;
2. to investigate the storability of preharvest ComCat® treated tomatoes using EC methods;
3. to explore the synergistic effect of pre-packaging treatments (chlorine supplemented water) when combined with modified atmosphere packaging and EC storage.

6.2. Materials and methods

6.2.1. Vegetable production

Tomatoes (*Leucopersicon esculentum*, cultivar malgrove) were grown during the summer season of 2001 at the experimental fields of the Alemaya University, in Eastern Ethiopia. The agro climatic conditions of the site and its location were described in Chapter 5, section 5.2.3. ComCat® was administered by spraying tomato seedlings just before transplantation, and a second time at the start of flowering, with 10 g. ha^{-1} in 350 l water and, control plants with 0 g. ha^{-1}. All other agricultural practices were the same as those normally practiced by the University Farm Management Department during tomato production. Tomatoes were manually harvested at a green mature stage and immediately transported from the Alemaya University Campus to the Dire Dawa University Fruit and Vegetable Research Center, which is 30 km away. To protect the tomatoes against mechanical injury during transportation, tomatoes were packed in plastic crates.

6.2.2. Postharvest treatment

The postharvest treatments, such as washing, disinfecting and packaging, were according to the procedures described in Chapter 3, section 3.2.2. Tomatoes with visible defects were discarded. After washing, both ComCat® treated and untreated tomatoes were subjected to one of the following postharvest dipping treatments discussed in Chapter 4:

- dipping in chlorinated water (100µg. ml^{-1} chlorine, made with 5% NaOCl) at ambient temperature for 20 minutes, packaged in Xtend® Film (see Chapter 3, section 3.2.2) and stored in;
- dipping in chlorine supplemented water, unpackaged and stored in EC;
- water washed, packaged in Xtend® Film and stored in EC;
- water washed, unpackaged and stored in EC;
- water washed, packaged in Xtend® Film and stored at ambient conditions, to serve as a postharvest control;
- water washed and stored under ambient conditions without packaging, to serve as a postharvest control.

These treatments were performed in three different containers, each as a replication for each treatment group. After washing and dipping treatments, the surfaces of tomatoes were drip dried to avoid the occurrence of condensation inside the packages. Tomatoes were packed in 2 kg packages or unpackaged groups. Randomly, 2 kg samples of packaged or unpackaged tomatoes were taken from each of the 12 treatment replications and subjected to physiological, microbiological and chemical analyses on each sampling time. Due to limited facilities, not all analyses could be carried out at all sampling times. PWL and percentage marketability were determined on days 0, 4, 8, 12, 16, 20 and 24. Changes in juice content, TSS and pH were determined on days 0, 8, 16 and 24. The TTA, reducing sugar, total sugar, total aerobic bacteria and fungi population were determined on days 0, 8, and 16. Seventeen tomatoes from each treatment and replications were stored separately and used for subjective quality analysis.

6.2.3. Temperature and relative humidity measurement

The storage temperature and relative humidity of air inside the EC as well as of the ambient conditions was monitored according to the methods described in Chapter 5, section 5.2.2.

6.2.4. Physiological weight loss, moisture and juice content

The analysis of PWL was conducted as described in Chapter 4, section 4.2.4. Moisture content and juice content were conducted as described in Chapter 5, sections 5.2.6 and 5.2.7., respectively.

6.2.5. Chemical analysis

6.2.5.1. pH, total titratable acidity (TTA) and total soluble solid (TSS)

The pH and TSS of tomatoes during the storage period were measured as described in Chapter 4, sections 4.2.5.1 and 4.2.5.2, respectively. The TTA was determined according to the procedures described in Chapter 4, section 4.2.6.1.

6.2.5.2. Sugar analysis

Estimation of reducing and total sugars were carried out according to the methods described in Chapter 5, section 5.2.8.

6.2.6. Microbiological analysis

6.2.6.1. Total aerobic bacteria and fungi

The populations of aerobic bacteria and fungi were determined according to the procedures described in Chapter 3, section 3.2.6.

6.2.7. Subjective quality analysis

The subjective quality analyses were made according to the procedure described in Chapter 4, section 4.2.7. Marketable quality of tomatoes was evaluated according to the scoring method used by Mohammed *et al.* (1999). The details of the method were given in Chapter 4, section 4.2.7. In this case, emphasis was given to visually observable decay and loss of firmness.

6.2.8. Data analysis

The experimental design and data analyses were according to the methods described in Chapter 3, section 3.2.7. In Chapter 3 the statistical analysis of the disinfecting treatment was coupled with MAP in order to see the overall effect of these treatments on the quality parameters. During the current investigation, the statistical analysis of MAP was coupled with storage temperature to look at their combined effect on the quality parameters. Here the individual effect of MAP and storage temperature treatments was analyzed, using multiple comparison of each treatment means by mean separation of Duncan's multiple range tests.

6.3. Results and Discussions

Due to limited facilities at Alemaya University, Ethiopia, it was not possible to carry out the same analyses as reported in Chapter 3. Some different analyses, such as moisture content and juice content, were carried out, as well as alternative analyses such as reducing sugars. It was also not possible to collect samples or carry out all the analyses on the same day, as was managed in Chapter 3.

6.3.1. Temperature and relative humidity

Fig. 6.1. shows the dry bulb temperature and relative humidity changes against storage period. The ambient air-dry bulb temperature was 25 - 36.5°C with the average being 32°C during the 24 days of storage. The dry bulb temperature of the air inside the EC was found to be from 14.4 - 23.5°C with the average being 20.5°C during the storage period. An average drop in dry bulb temperature of 11.5°C was observed in this study. The air temperature is an important factor for controlling tomato flesh temperature.

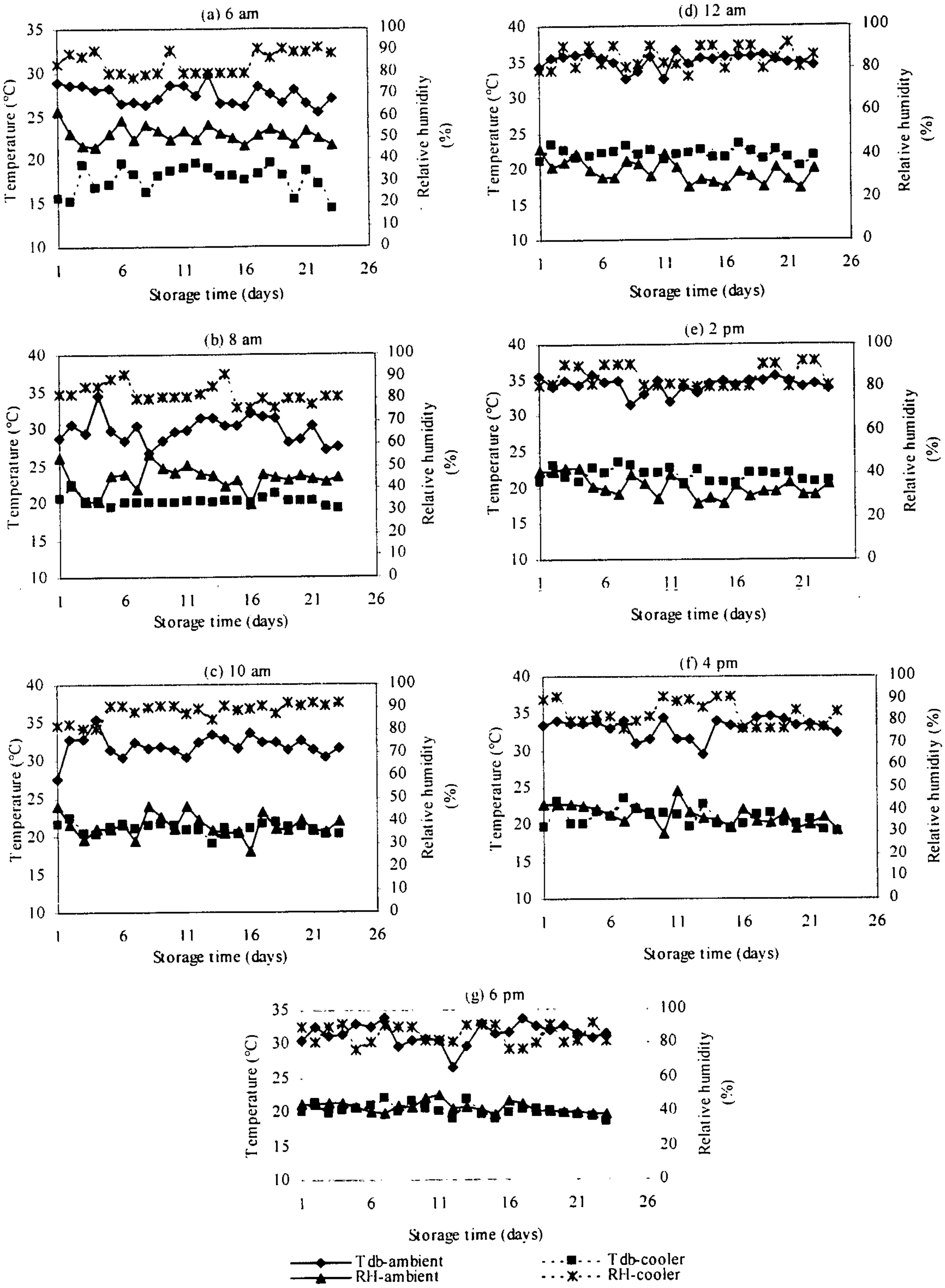


Figure 6.1. Temperature (°C) and relative humidity (%) of the environmental air and the air inside the EC during tomato storage at 6 am (a), 8 am (b), 10 am (c), 12 am (d), 2 pm (e), 4 pm (f) and 6 pm (g).

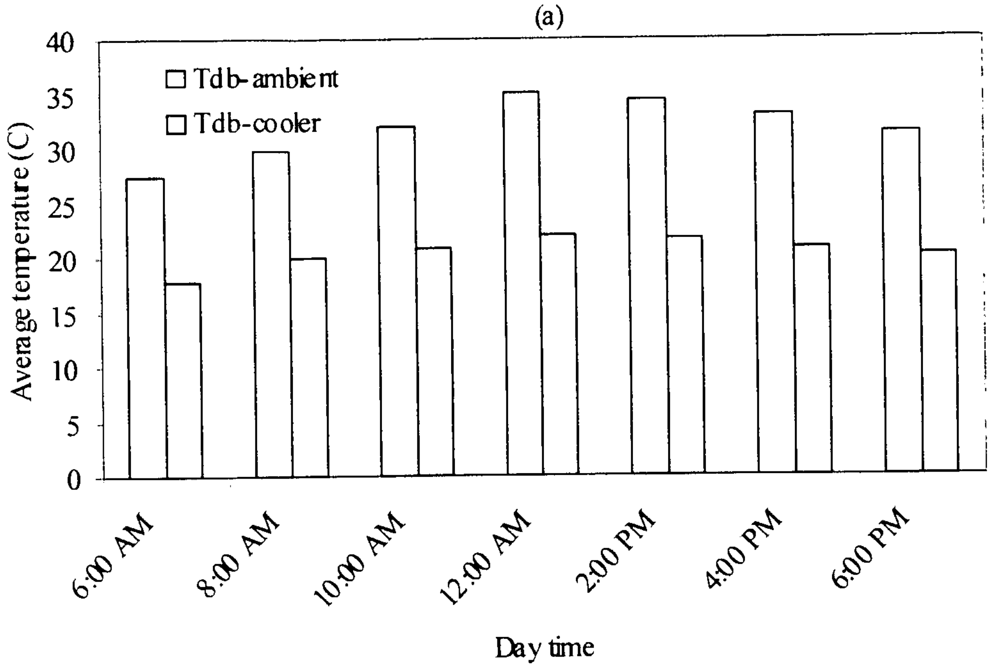


Figure 6.2. (a). The effect of daytime on the average environmental and evaporative cooler temperatures (°C) during storage of tomatoes.

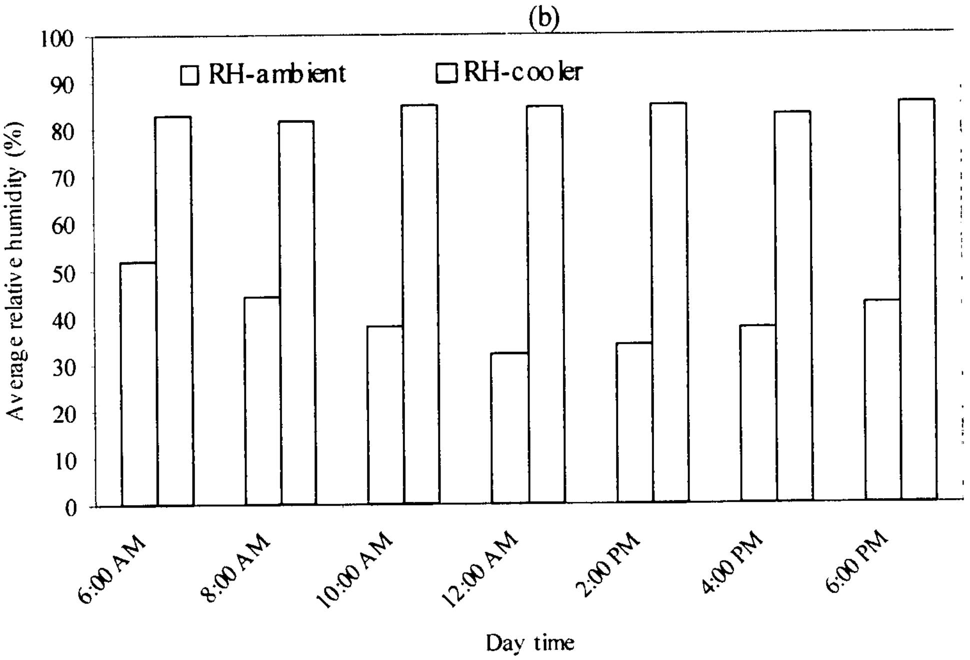


Figure 6.2. (b). The effect of daytime on the average environmental and evaporative cooler relative humidity (%) during storage of tomatoes.

Higher temperatures increase the tissue temperature of tomatoes, which initiate biological and biochemical processes responsible for postharvest quality deterioration. Fresh horticultural commodities respire at rates which double, triple, or even quadruple for every 10°C increase in temperature (Sargent *et al.*, 1991). It was therefore possible to reduce the temperature inside the EC by more than 10°C during storage (Figure 6.1), which in turn should have reduced the respiration rate of the tomatoes during storage.

The relative humidity of the environmental air during the storage period was between 24.0% and 62.2% with an average of 40.0%. The relative humidity of the air inside the EC was 73.0 - 92.0% with an average of 83.9% during the storage period. The average difference between the inside and outside relative humidity during the storage period was 43.9% (Figure 6.1).

During the storage period, the highest mean temperature and minimum relative humidity of ambient air were found to be at 12:00 AM (Figure 6.2 (a) and (b)). As can bee seen in the figure, the average temperature increased with time from 6:00 to 12:00 AM and starts to slightly drop thereafter. The results presented in Chapter 5 gave similar results.

6.3.2. Physiological weight loss (PWL), moisture content and juice content

Table 6.1 shows the PWL of tomatoes stored under evaporative cooling as well as ambient temperature. The PWL was higher in tomatoes stored at ambient temperature. While the PWL was between 10% and 13.5% for MAP tomatoes in EC after 24 days, the same PWL was reached within 16 days at ambient temperature storage.

Preharvest treatment had a significant effect ($p \leq 0.05$) on PWL of the tomatoes. The PWL of ComCat® treated tomatoes seemed to be more during storage at ambient temperature. However, the PWL of disinfected and packaged ComCat® treated tomatoes was more up to 24 days of storage in EC.

Table 6.1. Changes in physiological weight loss of tomatoes stored in evaporative cooling chamber and ambient temperature (RT) for 24 days.

	Physiological loss in weight (%)					
Treatment	Day 4	Day 8	Day 12	Day 16	Day 20	Day 24
ComCat®, Cl_2, MAP, EC	1.660^{b}	3.437^{cde}	5.168^{cd}	7.343^{cde}	9.037^{bc}	10.474^{cde}
Control, Cl_2, MAP, EC	1.260^{b}	2.819^{de}	4.590^{ef}	6.208^{de}	9.641^{bc}	13.414^{ab}
ComCat®, Cl_2, EC	1.823^{b}	3.445^{cde}	4.733^{de}	7.714^{b}	9.942^{b}	12.079^{bcd}
Control, Cl_2, EC	2.436^{b}	4.951^{ab}	6.384^{bc}	8.417^{c}	11.157^{a}	13.744^{ab}
ComCat®, H_2O, MAP, EC	1.078^{b}	2.841^{de}	4.992^{cde}	7.474^{cde}	8.944^{c}	9.717^{def}
Control, H_2O, MAP, EC	1.577^{b}	3.133^{de}	4.873^{cde}	7.086^{cde}	9.796^{b}	12.106^{abc}
ComCat®, Cl_2, MAP, RT	3.473^{a}	6.807^{ab}	10.578^{a}	12.965^{ab}	-	-
Control, Cl_2, MAP, RT	3.059^{ab}	5.720^{ab}	8.828^{b}	11.758^{ab}	-	-
ComCat®, H_2O, EC	1.889^{b}	3.862^{abc}	4.790^{cde}	7.239^{de}	10.804^{ab}	13.790^{a}
Control, H_2O, EC	1.978^{b}	3.563^{bcd}	6.052^{cd}	8.332^{c}	10.294^{b}	12.559^{abc}
ComCat®, H_2O, RT	3.225^{ab}	7.600^{a}	9.531^{ab}	13.244^{a}	-	-
Control, H_2O, RT	3.459^{a}	6.375^{ab}	11.117^{a}	13.172^{ab}	-	-

Significance

Preharvest treatment (A)	*
Disinfecting treatment (B)	NS
Packaging + Storage temperature (C)	***
A X B	NS
A X C	***
B X C	NS
A X B X C	NS

NS, *, *** Nonsignificant or significant at $P \leq 0.05$ or 0.001 respectively. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The coefficient of variation and standard error were 0.166 and 0.791 respectively. LSD Value = 2.213.

During 24 days of storage, the cumulative effect of modified atmosphere packaging and storage conditions were highly significant ($p \leq 0.001$) on the response of tomatoes to PWL. The moisture loss was significantly ($p \leq 0.001$) reduced with the help of MAP, reduced temperature and increased relative humidity during storage. It is known that EC reduces the loss of moisture, PWL and loss of juice content (Lingaiah and Huddar, 1991; Sunil *et al.* 1997; Ashok *et al.*, 1999), which was also confirmed in the present study. Prepackaging dipping treatments of tomatoes, either in chlorinated water or washing in tap water, had no significant ($p > 0.05$) effect on the PWL of tomatoes. The interaction between preharvest treatments, MAP and storage temperature for the PWL, during the 24 days of storage, was significant ($p \leq 0.001$).

Table 6.2. Changes in juice content of tomatoes stored in evaporative cooling chamber and ambient temperature (RT) for 24 days.

	Juice Content (%)			
Treatment	Day 0	Day 8	Day 16	Day 24
ComCat®, Cl_2, MAP, EC	64.698 ^{a}	61.138 ^{ab}	60.178 ^{ab}	59.046 ^{a}
Control, Cl_2, MAP, EC	61.796 ^{ab}	61.007 ^{ab}	56.985 ^{abc}	55.610 ^{abc}
ComCat®, Cl_2, EC	64.698 ^{a}	59.739 ^{bc}	52.674 ^{cd}	43.689 ^{def}
Control, Cl_2, EC	61.796 ^{ab}	54.291 ^{bcd}	48.010 ^{de}	40.259 ^{fg}
ComCat®, H_2O, MAP, EC	64.698 ^{a}	64.310 ^{a}	63.787 ^{a}	57.825 ^{ab}
Control, H_2O, MAP, EC	61.796 ^{ab}	59.961 ^{bc}	53.514 ^{bcd}	52.284 ^{bcde}
ComCat®, Cl_2, MAP, RT	64.698 ^{a}	53.763 ^{bcd}	52.325 ^{cd}	-
Control, Cl_2, MAP, RT	61.796 ^{ab}	47.818 ^{def}	45.761 ^{de}	-
ComCat®, H_2O, EC	64.698 ^{a}	61.499 ^{abc}	59.113 ^{ab}	57.483 ^{ab}
Control, H_2O, EC	61.796 ^{ab}	57.581 ^{abc}	50.682 ^{cde}	50.612 ^{bcde}
ComCat®, H_2O, RT	64.698 ^{a}	48.954 ^{def}	32.184 ^{fgh}	-
Control, H_2O, RT	61.796 ^{ab}	42.736 ^{fg}	35.917 ^{fg}	-

Significance

Preharvest treatment (A)	NS
Disinfecting treatment (B	NS
Packaging + Storage temperature (C)	***
A X B	NS
A X C	NS
B X C	***
A X B X C	*

NS, *, *** Nonsignificant or significant at $P \leq 0.05$ or 0.001 respectively. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The coefficient of variation and standard error were 0.088 and 0.935 respectively. LSD Value = 7.775.

At harvest, the moisture content of tomatoes was found to be 94.42% and 93.98% for ComCat® treated and untreated samples, respectively, and remained above 90% during storage, with not much variation during the 24 days of storage, irrespective of the storage conditions. The recorded data are therefore not shown.

At harvest, the juice content was higher in ComCat® treated than in control tomatoes (Table 6.2), although not significant ($p > 0.05$). Although only significant in some cases, the ComCat® treated tomatoes maintained a higher juice content throughout the storage time in the EC. The packaging and storage temperature were the most important factors affecting the juice content of tomatoes and was highly significant ($p \leq 0.001$).

The interaction between disinfecting, MAP and storage temperature was highly significant ($p \leq 0.001$) on the changes in juice content of tomatoes during the 24 days of storage. During the same interval, the three-way interaction between pre- and postharvest treatments on the changes of juice content of tomatoes, subjected to different pre- and postharvest treatments, was significant ($p \leq 0.05$). In Chapter 5 it was noted that changes in PWL, moisture content and juice content of carrots indicated the same thing, i.e. loss of water, while these parameters show individual changes in tomatoes. The reason could be ascribed to the protective skin of the tomato, which prevents moisture loss, while carrots have an absorbing surface that will also give off moisture easily.

6.3.3. Chemical changes

6.3.3.1. Total soluble solid (TSS)

The ComCat® treatment did not have a significant ($p > 0.05$) effect on the TSS of tomatoes at harvest (Table 6.3), but the effect was significant in a few samples ($p \leq 0.05$) during storage. In general, the TSS increased during storage from around 4.6 °Brix to almost 5.5 °Brix in some cases. The results showed that the TSS content of ComCat® treated tomatoes generally remained higher during 24 days of storage in EC, compared to the control tomatoes. The changes in TSS were found to be faster in fruits stored at ambient conditions, which is supported by the work of Waskar *et al.* (1999) for pomegranate.

Table 6.3. Changes in total soluble solids of tomatoes stored in evaporative cooling chamber and ambient temperature (24) for 24 days.

	Total soluble solid (°Brix)			
Treatment	Day 0	Day 8	Day 16	Day 24
ComCat®, Cl_2, MAP, EC	4.575^{a}	4.867^{abcd}	4.700^{cde}	5.133^{abc}
Control, Cl_2, MAP, EC	4.690^{a}	4.883^{abcd}	4.500^{abcd}	4.767^{cde}
ComCat®, Cl_2, EC	4.575^{a}	5.333^{a}	5.483^{a}	5.483^{a}
Control, Cl_2, EC	4.690^{a}	4.700^{bcd}	4.367^{cde}	4.550^{de}
ComCat®, H_2O, MAP, EC	4.575^{a}	5.417^{a}	5.093^{abc}	4.867^{abcd}
Control, H_2O, MAP, EC	4.690^{a}	4.800^{bcd}	4.933^{de}	4.850^{bcd}
ComCat®, Cl_2, MAP, RT	4.575^{a}	5.100^{ab}	4.917^{ab}	-
Control, Cl_2, MAP, RT	4.690^{a}	4.967^{abcd}	4.533^{bcd}	-
ComCat®, H_2O, EC	4.575^{a}	4.267^{d}	5.000^{abcd}	4.900^{abcd}
Control, H_2O, EC	4.690^{a}	4.717^{bcd}	4.733^{abcd}	4.733^{cde}
ComCat®, H_2O, RT	4.575^{a}	4.800^{bcd}	5.193^{ab}	-
Control, H_2O, RT	4.690^{a}	5.067^{ab}	4.867^{abcd}	-

Significance

Preharvest treatment (A)	*
Disinfecting treatment (B)	NS
Packaging + Storage temperature (C)	NS
A X B	NS
A X C	NS
B X C	*
A X B X C	*

NS, *Nonsignificant or significant at $P \leq 0.05$. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The coefficient of variation and standard error were 0.065 and 0.027 respectively. LSD Value = 0.505.

Surprisingly, the effect of MAP and storage temperature on the TSS content of tomatoes was not significant (p > 0.05). The change in TSS is one of the important postharvest parameters used to evaluate the quality of carrots (see Chapter 3) during storage. Comparing tomatoes with carrots, the rapid change in TSS content of carrots, could be due to the higher PWL of the carrots (see Chapter 3 and 5). Jauregui et al. (1999) have shown that these two parameters are linearly correlated.

The interactive effect of prepackaging chlorinated water disinfecting, MAP and storage temperature on the changes of TSS of tomatoes during storage was significant ($p \leq 0.05$). Similarly, the three-way interaction between pre- and postharvest treatments on the changes of TSS was also significant ($p \leq 0.05$). In general, there was a positive effect of pre- and postharvest treatments on the TSS content of tomatoes stored in EC, when compared to the TSS content of tomatoes stored at ambient conditions.

6.3.3.2. pH

At harvest the pH of ComCat® treated and control tomatoes were not significantly ($p > 0.05$) different. There were also no significant ($p > 0.05$) differences in the changes in pH values of ComCat® treated and control tomatoes during the 24 days of storage (Table 6.4). The pH of tomato juice increased continuously with the progress in storage period, regardless of pre- or postharvest treatments, from around 4.1 to as high as 4.6, which is in agreement with the results reported by Mohammed *et al.* (1999). The prepackaging chlorine disinfecting had a significant effect on the pH values of tomatoes during storage.

After 24 days in EC, the pH of disinfected, packaged tomatoes was significantly ($p \leq 0.05$) higher than the pH of water washed ComCat® treated tomatoes. The pH remained significantly ($p \leq 0.05$) higher in unpackaged, disinfected control tomatoes, compared to the ComCat® treated ones after 24 days in EC. However, the opposite trends were observed in the case of water washed, unpackaged control, as well as ComCat® treated tomatoes stored in EC for 24 days.

During 8 days of storage, the packaged or unpackaged tomatoes stored at ambient temperature, displayed a lower pH, compared to those stored in EC. After 24 days of storage, the pH of tomatoes was significantly ($p \leq 0.05$) higher in packaged samples stored in EC, than that of unpackaged ones stored under similar conditions. This could be associated with the higher rates of respiration at relatively higher storage temperature, since acid is produced from catabolism of sugar, which will be shown later in this Chapter.

Table 6.4. Changes in pH of tomatoes subjected to different pre- and postharvest treatments and stored in evaporative cooling chamber and ambient temperature (RT) for 24 days.

	PH			
Treatment	Day 0	Day 8	Day 16	Day 24
ComCat®, Cl_2, MAP, EC	4.111^{a}	4.253^{ab}	4.347^{bc}	4.625^{a}
Control, Cl_2, MAP, EC	4.109^{a}	4.270^{a}	4.393^{ab}	4.640^{a}
ComCat®, Cl_2, EC	4.111^{a}	4.213^{abc}	4.180^{fgh}	4.180^{ij}
Control, Cl_2, EC	4.109^{a}	4.273^{a}	4.357^{ab}	4.490^{bc}
ComCat®, Cl_2, MAP, RT	4.111^{a}	4.180^{cd}	4.417^{a}	-
Control, Cl_2, MAP, RT	4.109^{a}	4.217^{abc}	4.273^{cd}	-
ComCat®, H_2O, MAP, EC	4.111^{a}	4.220^{abc}	4.360^{ab}	4.477^{bcd}
Control, H_2O, MAP, EC	4.109^{a}	4.260^{ab}	4.407^{a}	4.510^{b}
ComCat®, H_2O, EC	4.111^{a}	4.187^{abc}	4.243^{def}	4.317^{fg}
Control, H_2O, EC	4.109^{a}	4.223^{abc}	4.210^{efg}	4.210^{hi}
ComCat®, H_2O, RT	4.111^{a}	4.177^{bcd}	4.270^{cde}	-
Control, H_2O, RT	4.109^{a}	4.147^{cd}	4.200^{efg}	-

Significance

Preharvest treatment (A)	NS
Disinfecting treatment (B)	*
Packaging + Storage temperature (C)	***
A X B	NS
A X C	***
B X C	NS
A X B X C	***

NS, *, *** Nonsignificant or significant at $P \leq 0.05$ or 0.001 respectively. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The coefficient of variation and standard error were 0.011 and 0.0258 respectively. LSD Value = 0.073.

The interactive effect of preharvest ComCat® treatment, packaging and storage conditions on the pH values of tomatoes was significant ($p \leq 0.001$). Similarly, the three-way interaction between all the pre-and postharvest treatments on the changes of pH values of tomatoes was highly significant ($p \leq 0.001$).

6.3.3.3. Total titratable acidity (TTA)

The TTA decreased dramatically in the tomatoes during ripening from the green mature to the red mature stages during 16 days of storage, from around 0.6 to as low as 0.4 (Table 6.5), confirming the results of Shi *et al.* (1999). However, the TTA-values obtained in the current study were relatively higher than those reported, as well as those observed in Chapter 4, which could be due to the different growing practice or climate and soil type. At harvest, the TTA was higher in ComCat® treated tomatoes than in the control fruit, which is similar to the data presented in Chapter 4, however, not significant ($p > 0.05$) in this case. The effect of higher TTA on microbial populations associated with ComCat® treated tomatoes, compared to the controls, was evident here as well as in Chapter 4, and is confirmed by the work of Mohammed *et al.* (1999). After 16 days storage, the TTA of ripe tomatoes was generally higher in ComCat® treated tomatoes than in the controls, suggesting that ComCat® treatment resulted in more acidity to protect against microbiological proliferation. Microbiological data is shown in section 6.1.2.

The TTA declined at a faster rate in controls than in ComCat® treated tomatoes during storage. The reduction in TTA in ComCat® treated whole tomatoes subjected to different postharvest treatments, and stored inside the EC for 16 days, varied from 22.5% to 27.9%. During the same interval, the reduction in TTA in control tomatoes, subjected to the same postharvest treatments, varied between 26.4% and 34.1%. However, the difference in TTA was not significant ($p > 0.05$). After 16 days of storage, the highest loss of TTA in tomatoes stored at ambient temperature and humidity, was evident from the results presented in Table 6.5. The TTA in packaged ComCat® treated tomatoes dipped in chlorinated water and stored at ambient conditions was reduced by

32.3%, whereas the control tomatoes, subjected to the same postharvest treatment, had lost 34.4%. The TTA in unpackaged ComCat® treated and control tomatoes was reduced by 35.0% and 39.0%, respectively. These results showed that, the higher the storage temperature of fruit, the higher the reduction in the TTA during ripening and storage. This could be associated with rapid ripening and senescence properties of tomatoes when stored at higher temperatures.

Table 6.5. Changes in total titratable acidity of tomatoes subjected to different pre- and postharvest treatments and stored in evaporative cooling chamber and ambient temperature (RT) for 16 days.

	Total titratable acidity (mg citric acid/100 g)		
Treatment	Day 0	Day 8	Day 16
ComCat®, Cl_2, MAP, EC	0.663^{a}	0.628^{a}	0.487^{ab}
Control, Cl_2, MAP, EC	0.637^{ab}	0.621^{a}	0.444^{abc}
ComCat®, Cl_2, EC	0.663^{a}	0.538^{abcd}	0.490^{ab}
Control, Cl_2, EC	0.637^{ab}	0.487^{cde}	0.420^{bc}
ComCat®, H_2O, MAP, EC	0.663^{a}	0.536^{abcd}	0.478^{ab}
Control, H_2O, MAP, EC	0.637^{ab}	0.516^{bcde}	0.465^{ab}
ComCat®, Cl_2, MAP, RT	0. 663^{a}	0.590^{abc}	0.449^{abc}
Control, Cl_2, MAP, RT	0.637^{ab}	0.570^{abc}	0.418^{bc}
ComCat®, H_2O, EC	0.663^{a}	0.505^{bcde}	0.514^{a}
Control, H_2O, RT	0.637^{ab}	0.496^{cde}	0.389^{c}
ComCat®, H_2O, RT	0.663^{a}	0.586^{abc}	0.431^{abc}
Control, H_2O, EC	0.637^{ab}	0.607^{ab}	0.469^{ab}
Significance			
Preharvest treatment (A)	NS		
Disinfecting treatment (B)	NS		
Packaging + Storage temperature (C)	NS		
A X B	NS		
A X C	NS		
B X C	*		
A X B X C	NS		

NS, * Nonsignificant or significant at $P \leq 0.08$ respectively. $^{a, b, c, d, e}$ Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The coefficient of variation and standard error were 0.100 and 0.012 respectively. LSD Value = 0.089.

6.3.3.4. Sugar changes during storage

The total sugars decreased during the storage period (Table 6.6). Similar trends of decreasing total soluble sugar content in tomatoes were observed in Chapter 4. At harvest the total sugar content was significantly higher ($p \leq 0.05$) in ComCat® treated tomatoes, compared to the controls, and remained higher ($p \leq 0.05$) throughout all the postharvest treatments, and during 16 days of storage in EC. Again, this could be an indication of delayed ripening effected by the preharvest ComCat® treatment, while the sugar content was rapidly depleted in control tomatoes, due to faster ripening. The total sugars decreased rapidly in the control tomatoes stored at ambient conditions, compared to those stored in EC. In this case the EC retarded the ripening and senescence process, while the respiration and metabolism of tomatoes stored at ambient temperature was high, and utilized much of the available sugar.

At harvest the control tomatoes contained a higher content ($p \leq 0.05$) of reducing sugars, compared to the ComCat® treated ones, and remained so during storage (Table 6.6). In general, the reducing sugars decreased during 16 days of storage. The preharvest ComCat® treatment had a significant ($p \leq 0.05$) effect on the changes of reducing sugar contents of tomatoes during storage in EC, while the reducing sugar decreased faster in the controls. This could be due to a higher respiration rate of the control tomatoes, and corresponds to the results obtained in Chapter 3, where it was shown that the content of glucose increased more in ComCat® treated tomatoes.

The calculated non-reducing sugar content increased during the first 8 days of storage, and was followed by a faster decline in some of the tomatoes subjected to different postharvest treatments. After 8 days of storage, ComCat® treated tomatoes had a higher content of non-reducing sugars, but after 16 days storage, this trend was not observed for the disinfected samples.

Table 6.6. Changes in reducing, non-reducing and total sugar contents of tomatoes stored in the evaporative cooling chamber and ambient temperature (RT) for 24 days.

Treatment	Total Sugar (g/100g)			Reducing Sugar (g/100g)			Non-reducing Sugar (g/100g)		
	Day 0	Day 8	Day 16	Day 0	Day 8	Day 16	Day 0	Day 8	Day 16
ComCat®, Cl_2, MAP, EC	5.101 ^{a}	4.665 ^{ab}	4.444 ^{ab}	3.867^{ab}	2.517 ^{bc}	3.206 ^{a}	1.233 ^{a}	2.148 ^{abc}	1.238 ^{cd}
Control, Cl_2, MAP, EC	5.227 ^{ab}	3.722 ^{de}	2.535 ^{e}	3.988 ^{a}	2.527 ^{bc}	1.174 ^{e}	1.240 ^{a}	1.195 ^{de}	1.361 ^{bcd}
ComCat®, Cl_2, EC	5.101 ^{a}	4.489 ^{abc}	3.706 ^{c}	3.867 ^{ab}	1.629 ^{d}	3.084 ^{ab}	1.233 ^{a}	2.860 ^{a}	1.380 ^{bc}
Control, Cl_2, EC	5.227 ^{ab}	3.834 ^{cde}	3.027 ^{de}	3.988 ^{a}	1.727 ^{cd}	1.644 ^{de}	1.240 ^{a}	1.584 ^{cd}	1.333 ^{bcd}
ComCat®, H_2O, MAP, EC	5.101 ^{a}	4.902 ^{a}	4.917 ^{a}	3.867 ^{ab}	2.929 ^{a}	2.304 ^{bcd}	1.233 ^{a}	1.973 ^{de}	2.613 ^{a}
Control, H_2O, MAP, EC	5.227 ^{ab}	4.106 ^{bcd}	3.502 ^{cd}	3.988 ^{a}	2.329 ^{bc}	2.075 ^{cd}	1.240 ^{a}	1.777 ^{cd}	1.427 ^{bcd}
ComCat®, H_2O, RT	5.101 ^{a}	4.282 ^{bcd}	4.006 ^{bc}	3.867 ^{ab}	2.755 ^{ab}	2.878 ^{abc}	1.233 ^{a}	1.527 ^{cde}	1.127 ^{cd}
Control, H_2O, RT	5.227 ^{ab}	3.399 ^{ef}	1.665 ^{f}	3.988 ^{a}	1.958 ^{c}	1.504 ^{de}	1.240 ^{a}	1.440 ^{cde}	0.161 ^{def}

Significance	Total Sugar	Reducing Sugar	Non-reducing sugar
Preharvest treatment (A)	*	*	*
Disinfecting treatment (B)	NS	NS	NS
Packaging and storage temperature (C)	***	NS	NS
A X B	NS	NS	*
A X C	NS	NS	NS
B X C	**	NS	**
A X B X C	*	NS	NS

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01 or 0.001 respectively. Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The coefficient of variation and standard error were 0.155 and 0.047 for reducing sugar, 0.322 and 0.266 for nonreducing sugar and 0.088 and 0.218 for total sugar respectively. LSD Value = 0.723, 0.760 and 0.622 for reducing, nonreducing and total sugar respectively.

The two-way interaction between postharvest treatments, i.e. disinfecting and Map + storage temperature, had a significant ($p \leq 0.01$) effect on the changes in total sugar and non-reducing sugar in tomatoes during 16 days at EC temperature. The two-way interaction between the preharvest ComCat® treatment and disinfecting also had a significant ($p \leq 0.05$) influence on the changes in non-reducing sugar. Similarly, the three-way interaction between the preharvest treatment, disinfecting and Map + storage temperature on the changes of total sugar content of tomatoes was significant ($p \leq 0.05$) during storage. These results thus demonstrated that ComCat® treatment in general has an effect on the sugar content of tomatoes.

6.3.4. Microbiological changes

6.3.4.1. Total aerobic bacteria

Table 6.7 shows the changes in population of total aerobic bacteria in tomatoes during storage. The numbers of aerobic bacteria in tomatoes disinfected in chlorinated water were significantly ($p \leq 0.001$) decreased and remained low only up to 8 days, but then increased during storage in EC. Their growth was not suppressed to the same extend as those of low temperature refrigerated storage (see Chapter 5). MAP + storage temperature had a significant ($p \leq 0.001$) effect on the estimated population of total aerobic bacteria during the storage of tomatoes in EC. Compared with the other postharvest treatments, chlorinated water treatment coupled with MAP, were the best for both reducing and limiting the growth of aerobic bacteria during storage.

The population of aerobic bacteria was higher in packaged fruits washed in water than in unpackaged tomatoes subjected to chlorine disinfecting during storage in the EC. This clearly showed the danger of using MAP at higher storage temperatures. Obviously, the humidity in the headspace air of packaged products is high, and creates favourable conditions for the proliferation of microorganisms in packaged fruit and vegetables. The results therefore show that commodities should be disinfected prior to storage in EC. It should, however, be noted that, although water washed packaged tomatoes showed higher total aerobic bacteria populations than disinfected ones, the quality of the fruit remained good, in the sense that no increased spoilage of edible flesh was noted.

Table 6.7. Populations of total aerobic bacteria in tomatoes packaged or unpackaged and stored in evaporative cooling chamber or at ambient temperature (RT) for 16 days.

	Total aerobic bacteria		
Treatment	Day 0	Day 8	Day 16
ComCat®, Cl_2, MAP, EC	4.995^{ab}	3.146^{f}	3.763^{f}
Control, Cl_2, MAP, EC	5.089^{a}	3.646^{ef}	4.115^{de}
ComCat®, Cl_2, EC	4.995^{ab}	4.204^{de}	4.706^{bcd}
Control, Cl_2, EC	5.089^{a}	4.155^{de}	4.528^{cde}
ComCat®, H_2O, MAP, EC	4.995^{ab}	5.617^{abc}	5.227^{ab}
Control, H_2O, MAP, EC	5.089^{a}	5.850^{ab}	5.397^{a}
ComCat®, H_2O, EC	4.995^{ab}	5.945^{ab}	5.037^{abc}
Control, H_2O, RT	5.089^{a}	6.187^{a}	-

Significance	
Preharvest treatment (A)	NS
Pre packaging treatment (B)	***
Packaging + storage temperature (C)	***
A X B	NS
A X C	NS
B X C	NS
A X B X C	*

NS, *, *** Nonsignificant or significant at $P \leq 0.05$ or 0.001 respectively. $^{a, b, c, d, e, f}$ Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The coefficient of variation and standard error were 0.064 and 0.088 respectively. LSD Value = 0.522.

ComCat® had no significant ($p > 0.05$) effect on the aerobic bacteria in this experiment, although the aerobic bacteria population was higher in control than in ComCat® treated fruits at harvest, but not significantly ($0 > 0.05$). This coincides with the TTA being higher in ComCat® treated tomatoes, although also not significant ($p > 0.05$), which was also observed in the work in Chapter 4. The three-way interaction between the preharvest ComCat® treatment and postharvest treatments was significant ($p \leq 0.05$) on the changes in total aerobic bacteria during storage in the EC.

6.3.4.2. Total moulds and yeasts

The populations of moulds and yeasts was less on ComCat® treated tomatoes, although it had no significant ($p > 0.05$) effect on their numbers, both at harvest and during storage (Table 6.8). The number of moulds and yeasts on tomatoes decreased after disinfecting, as well as water washing, and stayed low up to 8 days in EC. The prepackaging disinfecting treatment was highly significant ($p \leq 0.001$) on the populations of moulds and yeasts during storage of tomatoes. These numbers stayed suppressed on the chlorine disinfected tomatoes up to 16 days storage in the EC.

Table 6.8. Populations of moulds and yeasts in tomatoes packaged or unpackaged and stored in the evaporative cooling chamber or at ambient temperature (RT) for 16 days.

	Moulds and yeasts		
Treatment	Day 0	Day 8	Day 16
ComCat®, Cl_2, MAP, EC	4.208^{a}	3.144^{d}	3.326^{d}
Control, Cl_2, MAP, EC	4.186^{a}	3.844^{cd}	3.796^{d}
ComCat®, Cl_2, EC	4.208^{a}	3.398^{d}	4.099^{cd}
Control, Cl_2, EC	4.186^{a}	3.451^{d}	4.350^{bcd}
ComCat®, H_2O, MAP, EC	4.208^{a}	3.787^{d}	5.121^{abc}
Control, H_2O, MAP, EC	4.186^{a}	3.845^{cd}	5.107^{abc}
ComCat®, H_2O, EC	4.208^{a}	4.391^{bcd}	5.107^{abc}
Control, H_2O, RT	4.186^{a}	6.046^{a}	-

Significance	
Preharvest treatment (A)	NS
Prepackaging treatment (B)	***
Packaging (C)	**
A X B	NS
A X C	NS
B X C	NS
A X B X C	NS

NS, **, *** Nonsignificant or significant at $P \leq 0.01$ or 0.001 respectively. $^{a, b, c, d}$ Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The coefficient of variation and standard error were 0.155 and 0.128 respectively. LSD Value = 1.088.

The estimated number of moulds and yeasts highly increased in control tomatoes stored at ambient temperature, indicating the benefit of EC and MAP on the storage quality. The effect of MAP + storage temperature was highly significant ($p \leq 0.001$) on the numbers of moulds and yeasts.

6.3.5. Subjective quality analysis

The percentage marketable tomatoes decreased rapidly during 12 days of storage at ambient conditions (Table 6.9). After 12 days of storage at ambient conditions, the packaged fruit were over 53% unmarketable. During the same interval, unpackaged tomatoes stored at ambient conditions dropped to below 30% marketability, due to excessive moisture loss, as well as decay. Similar findings were reported by Ashok *et al.* (1999) who showed that unwrapped and wrapped tomatoes became unacceptable after 3 and 10 days at ambient temperature respectively. According to this author, the main reason for unacceptability of these tomatoes was PWL. However, in the current study, over-ripening and soft rot were the most serious problems associated with tomatoes stored at ambient temperature and humidity.

Tomatoes stored in the EC fared better, as the temperature inside the store was nearer to the optimum temperature of 13°C for storage of tomatoes, than the ambient temperature. Packaged tomatoes could then be kept for more than 24 days without loss of freshness quality inside the EC. All packaged ComCat® treated tomatoes, as well as controls, disinfected in chlorinated water were 100% marketable up to 12 days in EC storage. After 12 days of storage, the percentage marketability of these fruit remained higher than 96%.

Preharvest ComCat® treatment had no significant effect on the marketability of tomatoes after storage in the EC, but the interactive effect of pre- and postharvest treatments on the marketability of tomatoes was found to be significant at $p \leq 0.05$. The percentage marketable ComCat® treated, packaged tomatoes, disinfected in chlorinated water, was found to be 3.7% higher than the control fruits subjected to the same postharvest treatment. After 24 days at EC, the packaged ComCat® treated tomatoes that were dipped in chlorinated water, showed the highest percentage marketability.

Table 6.9. Percentage marketable ComCat® treated and control tomatoes subjected to different treatments after 4, 8, 12, 16, 20 and 24 days of storage at evaporative cooling and ambient temperatures (RT) for 24 days.

	Marketable tomatoes (%)						
Treatment	Day 0	Day 4	Day 8	Day 12	Day 16	Day 20	Day 24
ComCat®, Cl_2, MAP, EC	100^{a}	100^{a}	100^{a}	100^{a}	96.3^{a}	90.7^{a}	83.3^{a}
Control, Cl_2, MAP, EC	100^{a}	100^{a}	100^{a}	100^{a}	92.6^{ab}	85.2^{abc}	79.6^{ab}
ComCat®, Cl_2, EC	100^{a}	100^{a}	96.3^{ab}	90.7^{abcd}	74.1^{ef}	68.5^{fgh}	68.5^{cdef}
Control, Cl_2, EC	100^{a}	100^{a}	100^{a}	98.5^{ab}	87.0^{abc}	75.9^{cdef}	64.8^{efg}
ComCat®, Cl_2, MAP, RT	100^{a}	96.3^{ab}	75.9^{cd}	46.3^{e}	44.4^{gh}	-	-
Control, Cl_2, MAP, RT	100^{a}	92.6^{ab}	70.4^{de}	46.3^{e}	46.3^{g}	-	-
ComCat®, H_2O, MAP, EC	100^{a}	100^{a}	98.2^{ab}	96.3^{ab}	92.6^{ab}	85.2^{abc}	75.9^{abcd}
Control, H_2O, MAP, EC	100^{a}	100^{a}	100^{a}	98.5^{ab}	98.2^{a}	90.7^{a}	77.8^{abc}
ComCat®, H_2O, EC	100^{a}	100^{a}	98.5^{ab}	92.6^{ab}	85.2^{bcd}	70.4^{efg}	55.6^{g}
Control, H_2O, EC	100^{a}	100^{a}	96.3^{ab}	94.4^{abc}	75.9^{de}	66.7^{fhg}	59.3^{fg}
ComCat®, H_2O, RT	100^{a}	85.2^{c}	64.8^{ef}	35.2^{fg}	27.8^{i}	-	-
Control, H_2O, RT	100^{a}	83.2^{cd}	61.1^{f}	31.5^{g}	29.6^{i}	-	-

Significance

Preharvest treatment (A)	NS
Pre packaging treatment (B)	*
Packaging + Storage temperature (C)	***
A X B	NS
A X C	NS
B X C	***
A X B X C	*

NS, *, *** Nonsignificant or significant at $P \leq 0.05$ or 0.001 respectively. $^{a, b, c, d, e, f, g, h, i}$ Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$). The coefficient of variation and standard error were 0.070 and 1.276 respectively. LSD Value = 9.045.

Previous studies by Acedo-AL Jr (1997) also showed that the pre-storage treatment delayed the ripening and reduced incidence of decay, supporting the results obtained in this study. It was noticed that a colour change from green, at harvest, to red was most retarded in packaged tomatoes stored in the EC. These results therefore demonstrated the importance of combining preharvest treatments aimed at increasing yield and quality, with proper postharvest treatments, to improve the shelf life and maintain the quality of perishable vegetables, such as tomatoes, under hot and dry climatic conditions.

The results in Chapter 4 showed that the onset of senescence in unpackaged ComCat® treated tomatoes that were not disinfected, was delayed, compared to the controls. However, this was not the case in the hot dry climate of Dire Dawa, Ethiopia. The higher relative humidity in the EC, which was higher than that of the room temperature studies of Chapter 4, helped to improve the marketability. In the EC, tomatoes were only 83% and 79% marketable after 24 days storage for the ComCat® treated and control tomatoes, respectively, compared to the 75% and 77% at the room temperature conditions of Chapter 4. The water washed samples showed a similar difference.

6.4. Conclusions

An EC unit, that maintained a temperature between 14.4°C and 23.5°C and relative humidity between 73.0% and 92% during storage, was used to store tomatoes. This temperature was somewhat lower than the room temperature reported in Chapter 4, which was between 16.9°C and 25.2°C, while the relative humidity was higher than the 62.2% recorded in Chapter 4. The higher humidity was advantageous for maintaining some of the chemical quality characteristics, such as moisture content and TSS, but seemed to favour microbial growth due to a combined effect with high temperature. The quality parameters tested were maintained better in EC, and resulted in an extension of over 70% in shelf life of tomatoes. This method has been shown to have great potential for application in the study site in Ethiopia to reduce huge postharvest vegetable losses. It is suggested that the EC air temperature can be reduced further, until it falls in the range of 8-13°C, which is the optimum for tomato storage, by installing a multi-stage EC pad connected in series.

At harvest, the green mature ComCat® treated tomatoes contained lower TSS, reducing sugar, non-reducing sugar, and total sugar. These parameters also remained higher in ComCat® treated tomatoes during storage in EC. The PWL and content of TSS, total sugars, reducing sugars and non-reducing sugars were significantly ($p \leq 0.05$) affected by the preharvest ComCat® treatment during storage in EC. Preharvest ComCat® treatment of tomato plants can therefore contribute to improve the quality of tomatoes during storage in an EC.

Disinfecting treatment of tomatoes did not have any effect on the chemical parameters during storage in the EC, although, the pH of tomatoes was significantly ($p \leq 0.05$) influenced by this treatment resulting in an increase in pH. The disinfecting treatment significantly affected the postharvest microbial populations associated with tomatoes stored in EC. Protection against microorganisms resulted in the marketability of tomatoes being significantly higher ($p \leq 0.001$). These results showed that the postharvest disinfecting treatment was important to control decay, although not as effective as at low temperature storage (Chapter 4). It is possible that the high humidity of the EC contributed to the re-establishment of microorganisms.

MAP in microperforated Xtend® film, together with the storage temperature in the EC, had a highly significant ($p \leq 0.001$) effect on the PWL, juice content and pH of stored tomatoes, but had no significant effect on the total, reducing and non-reducing sugars, and TTA of the tomatoes. It had a highly significant ($p \leq 0.001$) effect on the total aerobic bacteria and moulds and yeasts during storage of tomatoes in the EC. MAP + storage temperature in EC reduced the rate of ripening of tomatoes and PWL during storage, and resulted in significant ($p \leq 0.001$) improvement of the marketability of tomatoes. The microperforations associated with Xtend® film had several benefits, such as preventing condensation and maintaining optimum gas levels for normal respiration.

The combinations of preharvest ComCat® treatment of tomato plants, disinfecting, and MAP storage in EC, was shown to benefit the storage quality of tomatoes. A higher juice content was obtained, higher contents of TSS and total sugars, lower pH and lower growth of aerobic bacteria and better marketability, compared to the controls. However, these parameters were not as good as was observed during low temperature storage of tomatoes, as well as room temperature storage, reported in Chapter 4. Since the latter room temperatures and the temperature in EC were almost the same, it seems as if the high relative humidity when combined with relatively high EC temperature, used in this chapter, may contribute to faster deterioration of tomatoes. Adaptation of the EC to lower temperatures should therefore also incorporate alterations to address the relative humidity, otherwise higher relative humidity ranging from 85% up to 90% are desirable for tomato storage.

CHAPTER 7

GENERAL DISCUSSION and CONCLUSIONS

Preharvest treatment of plants with ComCat® was previously shown to increase the yield of vegetables (Schnabl *et al.*, 2001). The question arose how would these complex plant growth regulators and natural metabolites in ComCat® affect postharvest handling and storage of fruit and vegetables. According to the review in chapter 2, the postharvest quality of fruits and vegetables are affected by preharvest conditions, e.g. environmental conditions, fertilization practices, water management, plant growth promoters, and integrated and ecological farming systems (Bialczyk *et al.*, 1996; Gao *et al.*, 1996 and Carmer *et al.*, 2001). These reviews suggested that preharvest practices, which improved yield, should be integrated with its postharvest quality performance.

Postharvest handling includes washing methods, normally with chlorine containing solutions, to reduce microorganisms (Beuchat *et al.*, 1998; Escudero *et al.*, 1999; Beuchat *et al.*, 2001; Ukuku and Sapers, 2001; Prusky *et al.* 2001), and packaging. Although chlorine treatment is effective, alternative disinfecting methods, which are environmentally friendly, are being sought (Wisniewski *et al.*, 1992, Elmer and Gaunt, 1994, Wisniewski *et al.*, 2001; Ben-Yehoshua *et al.* 2000). In this study, anolyte water was investigated as such an alternative (Seyoum *et al.*, 2002). For packaging, MAP was selected for its O_2 and CO_2 regulation properties, and control of moisture loss without occurrence of condensation (Exama *et al.*, 1993; Seyoum *et al.*, 2001).

The aims of this study therefore were: (1) investigation of the effect of preharvest ComCat® treatment on postharvest quality of stored carrots and tomatoes, (2) investigation of the effect of pre-packaging disinfecting by anolyte and chlorinated water on the quality of preharvest ComCat® treated and untreated control carrots and tomatoes, (3) investigation of the storability of preharvest ComCat® treated carrots and tomatoes using MAP and storage temperatures and (4) investigation of the use of evaporative cooling techniques to reduce postharvest losses of both ComCat® treated and untreated carrots and tomatoes in hot climatic regions, in this case, Ethiopia.

This study showed that MAP conserves postharvest physiological, chemical, biochemical and microbiological qualities of carrots and tomatoes, resulting in increased shelf life and marketability, when combined with proper storage conditions. The results generally indicated that MAP highly reduced PWL, and improved contents of TSS, total sugars, sugar/acid ratio and ascorbic acid of carrots and tomatoes. The packaging reduced the rate of respiration and some metabolic processes of commodities, and resulted in less utilisation of sugars as metabolic substrate, confirming the research of Kader (1986) and Zagory and Kader (1988). As a general trend, the microbiological flora of carrots and tomatoes were higher in unpackaged carrots and tomatoes stored at lower temperatures. When MAP was combined with disinfecting treatments and proper storage temperature, the control of microorganisms was successful. At room temperature, MAP seemed to create conditions favourable for pathogens. The marketability of tomatoes was significantly improved with packaging, since MAP reduced the rate of respiration and some biochemical activities responsible for hastened ripening of fruits (Kader, 1986). It was also shown that the benefits already gained from the preharvest ComCat® treatment and anolyte water as a disinfectant can be maximised by using MAP and proper storage temperature.

Storage temperature greatly influenced the concentrations of O_2, CO_2, and N_2 in packages of both tomatoes and carrots. The gas data clearly showed that the respiration and metabolism of carrots and tomatoes were higher when stored at room temperature than in cold storage (1°C or 13°C for carrots and tomatoes respectively). The respiration rate, based on the gas dynamics, seemed to be distinctively different only during the early active ripening stage of tomatoes, which is before the climacteric onset, stored at 13°C and room temperature (16.9 - 25.2°C). After full ripening, the concentrations of gases became approximately equivalent, irrespective of storage temperatures. These results thus suggested that proper temperature management is important for tomato storage during the early stage of ripening of one or two weeks, in order to extend the shelf life. The PWL of both carrots and tomatoes significantly increased with an increase in storage temperature, and the effect was higher for carrots than for tomatoes during storage. The skin and epidermis tissue of tomatoes seemed to be highly resistant to moisture transfer. Because carrots do not have that protection, excessive dehydration and consequent shrinking was the most serious problem associated with carrots stored at room temperature. This confirms that air RH is more crucial for carrots than for tomatoes during storage. The interaction between pre- and postharvest treatments and storage temperature had a significant effect on the PWL, although this interactive effect affected carrot moisture loss more than that of tomatoes. As a general trend, the TAC decreased faster in carrots stored at room temperature, due to a higher rate of hydrolysis of polysaccharides to free sugars, and high utilization of these sugars in the glycolysis process. Similarly, the free sugars decreased faster in tomatoes stored at room temperature than at 13°C.

Storage temperature was shown to be the most important factor that affects the microbiological flora. Room temperature generally allowed significantly greater growth of total aerobic bacteria, molds, yeasts and total coliforms, in both tomatoes and carrots, than storage at lower temperatures. This study also showed that the lower optimum storage temperatures, 1°C for carrots and 13°C for green mature tomatoes, maintained the chemical, biochemical and biological qualities better, resulting in prolonged shelf life with better marketability. At room temperature storage, ComCat® treated tomatoes performed better in maintaining postharvest quality, compared to control tomatoes.

Regarding disinfecting treatments, the anolyte water was found to be as effective as chlorinated water in reducing the microbial populations in carrots and tomatoes during storage. A dipping time as short as 5 minutes in anolyte water was shown to be as effective as 20 minutes in chlorinated water (Seyoum *et al.*, 2002). It was noticed that visible mold growth and decay was higher in carrots dipped in chlorinated water towards the end of storage at room temperature, while the anolyte water treated carrots showed no sign of visible mold development and decay. The effectiveness of disinfecting treatment was maximized with MAP, and their coupled effect significantly decreased the natural microbiological flora of tomatoes and carrots during storage. The disinfecting treatment, when combined with MAP and optimum temperature storage of carrots and tomatoes, further improved the microbiological quality. The statistical levels of interactions between these postharvest treatments and storage time on shelf life and quality improvement of carrots and tomatoes are shown in Appendix A.1.- A.4.

These disinfecting treatments also affected several quality parameters of tomatoes and carrots. Carrots lost more moisture when treated with chlorine solution than with anolyte and distilled water, at both low and high storage temperatures. A general trend of higher chemical quality such as TSS, TAC, glucose, fructose and total soluble sugar contents, were found in carrots dipped in anolyte water. This would imply that anolyte water least affected the chemical quality of carrots, when compared to chlorinated water, suggesting less influence on physiological and biochemical activities. However, the ComCat® treated carrots dipped in chlorinated water, had slightly higher glucose and fructose contents at the end of 28 days of storage. The higher concentrations of these free sugars (glucose and fructose) in ComCat® treated carrots dipped in chlorinated water could indicate higher rates of hydrolysis of the available carbohydrates.

Gas concentrations were also affected by the disinfectants. The O_2 concentrations decreased slightly faster in packages of ripening tomatoes dipped in chlorinated water than in packages of tomatoes treated with anolyte water. Similarly, the CO_2 concentrations increased slightly faster in packages of ripening tomatoes treated with chlorinated water, than those treated with anolyte water, especially during the early active ripening periods. These results thus showed that chlorine treatment caused the rate of respiration and metabolism of tomatoes to increase, which is not desirable for the extension of shelf life. This effect on gas concentrations was greater for tomatoes than for carrots. The PWL and concentrations of free sugars were slightly lower in tomatoes dipped in anolyte water than in chlorinated water. After 30 days at room temperature and 13°C, the TSS and AA content was significantly higher in tomatoes dipped in anolyte water than in chlorinated water. This was because anolyte water decreased the ripening and senescence processes of tomatoes, compared to chlorine disinfected ones. Similarly, the PG and POX activities were slightly lower in tomatoes disinfected in anolyte water, supporting the above-derived explanation of lower respiration and metabolism rates. Anolyte water therefore resulted in a better marketability of tomatoes.

Sprouting of carrots was inhibited more by chlorine solution than by anolyte water during storage at room temperature. Anolyte water treated carrots started sprouting after 1 week during storage at ambient conditions. Although sprouting is not a desirable quality during storage, carrots dipped in anolyte water seemed to have normal physiological activities, such as respiration and metabolic activities, and therefore will sprout. It therefore seems as if the normal physiological state of carrots at harvest were not affected by anolyte water, compared to that of carrots dipped in chlorinated water. These differences in metabolic activity were not clearly noticeable in the case of tomatoes that were disinfected in chlorinated or anolyte water and stored at room temperature. The reason for this could be that the skin and epidermis of tomatoes seemed not to be permeable to external materials such as chlorine, and therefore protect the tissue from damage. Carrots have absorbing tissue, while tomato epidermis, and the outer wall of the pericarp tissue, is more resistant to diffusion of exogenous material to the internal parts. This could be the reason why the surface skin and tissue of carrots showed signs of etching by the chlorine solution. The subsequent effect of this damage on storage quality was more evident at room temperature storage. Thus, anolyte water dipping treatment, when combined with optimum storage temperature, improves the shelf life of carrots.

These results therefore provide evidence that anolyte water disinfecting of vegetables is not just equal to that of chlorinated water, as the microbiological analyses indicate, but even better, as derived from the chemical and biochemical analyses.

The main aim of this study was the integration of preharvest ComCat® treatment with postharvest quality changes of carrots and tomatoes. The preharvest ComCat® treated carrots and tomatoes were investigated on a wide variety of variables. The TAC, glucose and fructose content, SH ratio, and population of total aerobic bacteria of carrots during storage, were significantly affected by the preharvest ComCat® treatment. This is confirmed by the work of Nilsson (1987), who reported that the growing conditions of carrots influenced the sucrose content in the roots. The ComCat® treatment had no significant effect on the concentrations of gases, PWL, TSS, sucrose content, populations of yeasts, molds and coliforms in carrots during storage. As a general trend, the free sugar contents seemed to be lower in ComCat® treated carrots during storage. The reason for this could be a higher metabolism rate, due to increased utilization of sugar for normal respiration and metabolism, or lower hydrolysis of carbohydrates to sucrose, glucose and fructose. Apart from sucrose content, the free sugars remained lower in ComCat® treated tomatoes, although differences were not significantly different from the controls. It also seemed as if the sucrose contents of tomatoes were more sensitive to ComCat® treatment, when compared to the hexose sugars. The calculated sucrose equivalent was not significantly affected by ComCat® treatment and should therefore also not affect the sweetness. The TSS, hexose content and PG activity in tomatoes during storage were not significantly affected by the ComCat® treatment. The two-way interaction between ComCat® treatment and disinfecting + MAP treatments had a significant influence on the sucrose and fructose content, SH ratio, and population of total aerobic bacteria of carrots during storage. Likewise, the two-way interaction between the ComCat® treatment and storage temperature had a significant effect on PWL, TSS, TAC, fructose content, and populations of total aerobic bacteria, and moulds and yeasts. The PWL, populations of aerobic bacteria, moulds and yeasts, and coliforms were significantly influenced by the three-way interaction between the pre- and postharvest treatments. In general, the data showed the existence of strong relationships between the ComCat® treatment, and quality changes of carrots during storage. The high fresh harvest yield of ComCat®

treated carrots can be best maintained by subjecting to disinfecting, MAP and low temperature storage. The statistical levels of interactions between these pre- and postharvest treatments, and storage time on shelf life and quality improvement of carrots and tomatoes are shown in Appendix A.1.- A.4.

In chapter 4 the effect of preharvest ComCat® treatment on the postharvest quality of tomatoes was explored. In this study, several advantages of ComCat® treatment on the storage of tomatoes were identified. ComCat® treatment had a significant effect on several parameters including PWL, pH, sucrose content, AA content, POX activity, total aerobic bacteria, molds and yeasts, total coliforms and marketability. The low pH, high TTA, low concentrations of free sugars and high POX activity in green mature tomatoes at harvest, could be responsible for better microbiological quality of ComCat® treated tomatoes. It is known that pathogenesis resistant proteins, such as POX, are antimicrobial enzymes, since these enzymes protect cell walls from microbial infection (Khripach *et al.* 1997; Schnabl *et al.*, 2001).

At room temperature storage, ComCat® treated tomatoes performed better than control tomatoes. ComCat® treatment significantly reduced the rates of consumption of O_2 and liberation of CO_2 in packaged tomatoes stored at room temperature. These suggest lower respiration rates, compared to the controls. Further benefits of ComCat® treatment on the shelf life of tomatoes at room temperature include better chemical quality in terms of higher TSS content, lower pH and higher TTA, accumulation of fructose towards the end of storage, higher total soluble sugar and sugar-acid ratio, better microbiological quality and marketability.

The effect of ComCat® treatment on the postharvest quality of vegetables seemed to vary with the type of produce. The preharvest ComCat® treatment had a significant effect on the O_2 and N_2 concentrations in packages of tomatoes, while it had no significant effect on the gas concentrations in packages of carrots during storage. None of the interactions between the preharvest ComCat® and postharvest treatments had a significant influence on the concentrations of O_2, CO_2 and N_2 in packages of carrots during storage. However, the CO_2, O_2, and N_2 concentrations in the headspace of tomato packages were highly affected due to the interactive effect of pre- and postharvest treatments. These results therefore support the findings that preharvest ComCat® treatment affected the respiration and metabolism of tomatoes more than that of carrots.

The interactive effect of pre- and postharvest treatments had a significant influence on the changes in TSS of tomatoes during storage, while only the interaction between ComCat® treatment and storage temperature had an effect on changes in TSS of carrots. This analysis therefore implied that ComCat® treatment, when combined with postharvest treatments, seemed to have a greater influence on the changes in TSS of tomatoes than on carrots. The sugar turnover was also differently affected by ComCat® treatment in stored carrots and tomatoes. The changes in sucrose content of carrots, as well as tomatoes, was influenced by the ComCat® treatment, while TAC, glucose and fructose content and SH ratio was only affected in carrots. The interactive effect of ComCat® treatment and storage temperature on the changes in free sugars and total soluble sugar affected tomatoes more than carrots. AA content and POX activity was only affected in ComCat® treated tomatoes.

At harvest, the natural microbial flora were lower in both ComCat® treated vegetables studied. During the early storage period, the populations of microorganisms in tomatoes were significantly decreased with the ComCat® treatment, compared to the numbers of microorganisms in control tomatoes, while the microorganisms were not significantly affected with the ComCat® treatment alone in carrots. The interactive effect of ComCat® treatment and storage temperature was higher on the changes in microbiological population in tomatoes than in carrots during storage. The reason for this could have been due to the effect of ComCat® treatment on the TSS, sucrose, glucose, fructose and total soluble sugar contents and POX activity observed in tomatoes. This is in agreement with the view of Brackett (1987 and 1990) that the type and population of natural microbiological flora of fresh produces could be dependent on the chemical content of fruits and vegetables. It could also be possible that ComCat® treated tomatoes and carrots have tissue that is more resistant to microbial growth than the controls.

In summary, it seemed as if the ComCat® treatment had a higher significant effect on the changes of the postharvest quality of the fruit part of the plant, or the vegetable above ground, compared to the root part of the plant, or subsurface vegetable. Although the keeping quality of ComCat® treated carrots and tomatoes were not markedly different from the qualities of control vegetables when stored at the recommended optimal temperatures, the postharvest quality was distinctly different. However, at room temperature storage, ComCat® treated vegetables did not only display distinctively better qualities, but also showed improved shelf life and better marketability.

The superior storage quality of ComCat® treated carrots and tomatoes encouraged further investigations on the potential use of low-cost evaporative cooling, where relatively lower temperatures and higher relative humidities could be maintained, compared to ambient conditions. This part of the research was conducted in Ethiopia.

In chapter 5 and 6 the shelf life of ComCat® treated and control carrots and tomatoes stored under evaporative cooling conditions was investigated, for the potential application to reduce postharvest losses of. A forced ventilation experimental EC unit was developed for use in dry and hot conditions. The evaporative cooling reduced the temperature by about 10°C (average) from the day temperature (25.0 - 36.0°C) to 16 - 25°C, with a rise in relative humidity of more than 38% above that of ambient air. The advantages of this unit were, that temperature and relative humidity could be maintained within limits during storage, to protect vegetables from environmental conditions, resulting in increased shelf life.

Due to very high ambient air temperature and very low relative humidity, it was not possible to store carrots and tomatoes even for 1 week in the study site at Alemaya, Ethiopia. A rapid moisture loss in unpackaged carrots lead to the excessive dehydration of within 5 days of storage at ambient temperature, while the ripening process was hastened in unpackaged tomatoes, which was immediately followed by the collapse of tissue. This then created conditions favourable for microbial proliferation and progression of decay. MAP, with microperforated Xtend® film, significantly reduced the PWL and juice content of carrots and tomatoes stored in EC and at ambient temperature, delaying the senescence process, and improved the marketability of carrots and tomatoes.

The quality of the ComCat® treated carrots and tomatoes stored in EC remained as good as the quality of the untreated controls. However, the PWL, moisture loss, and loss of juice content of carrots were slightly lower in ComCat® treated carrots than in control carrots, confirming the previous data on these parameters (Chapter 3 and 4). At harvest, ComCat® treated green mature tomatoes contained lower TSS, reducing sugar, non-reducing sugar, total sugar and total aerobic bacteria. The TSS, reducing sugar, non-reducing sugar and total sugars remained higher in ComCat® treated tomatoes during storage in the EC. These results also confirmed that ComCat® treated tomatoes seemed to have a better keeping quality when stored under evaporative cooling conditions, which was slightly higher than the optimum storage temperature of 13°C. Although a slight effect on microbial populations was observed, the difference between the microbiological flora of ComCat® treated and control carrots and tomatoes was not clear, due to the higher storage temperature as well as higher relative humidity in the EC unit.

Despite the relatively higher minimum temperature levels, that can be achieved by the EC, no sign of sprouting of the carrots was noticed during the 3 weeks of storage in the evaporative cooling chamber.

These results therefore showed that the integration of pre- and postharvest practices is important to increase the potential benefits that can be gained from preharvest treatments. The statistical levels of interactions between these pre- and postharvest treatments and storage time on shelf life and quality improvement of carrots and tomatoes are shown in Appendix A.1.- A.4.

Future Research

Further research on the effect of ComCat® treatment and anolyte water treatment on other fresh vegetables and minimally processed "fresh-like" vegetables should be carried out. The integrated pre- and postharvest treatment approaches, to improve productivity as well as storage performance, used in these studies, should be extended to other fresh fruits and vegetables. The most resistant types of natural microorganisms of vegetables to anolyte water disinfecting treatment should be identified. Regarding EC, a multi-stage-cooling pad should be developed in order to reduce the storage temperature down to a minimum temperature possible. Practical applications for EC units, e.g. in markets, should be investigated. More work will be necessary on combining MAP in nested packaging systems with EC in order to look at the interactive effect on biological and biochemical changes of stored vegetables.

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SUMMARY

The improvement of the shelf life of carrots and tomatoes through integrated pre- and postharvest treatments was investigated. Preharvest ComCat® treatment of carrot and tomato plants was combined with postharvest disinfecting using chlorinated or anolyte water, modified atmosphere packaging in Xtend® film, and different storage temperatures.

The physiological, chemical, biochemical and microbiological quality of carrots and tomatoes were effectively improved by the combined effect of modified atmosphere packaging in Xtend® film and the optimum storage temperatures of carrots, at 1°C, and tomatoes, at 13°C. The shelf life of carrots and tomatoes was effectively improved, while the quality characteristics were maintained.

Disinfecting vegetables with anolyte water gave better results than chlorinated water. Chlorinated water treatment decreased ascorbic acid content, and increased physiological weight loss and peroxidase activity. The surface tissue of carrots was etched by chlorine, which created penetration zones for microorganism, and disadvantaged the shelf life. Disinfecting with anolyte water, in combination with modified atmosphere packaging and optimum storage temperatures, supported quality characteristics, and therefore contributed to improving shelf life of carrots and tomatoes.

Preharvest spraying of plants with ComCat®, improved the quality of carrots. At harvest a slightly higher ascorbic acid, total soluble solids, total available carbohydrates and sucrose content was noted. The sucrose to hexose ratio at harvest was also slightly higher, and the microbial populations were slightly lower. Preharvest ComCat® treated tomatoes had a lower pH, fructose and glucose content, and higher titratable acidity and peroxidase activity at harvest, which resulted in lower populations of natural microorganisms.

During storage at 13°C and room temperature, ComCat® treated tomatoes displayed better maintenance of total soluble solids and ascorbic acid, and lower peroxidase activity. These tomatoes also maintained better microbiological and marketable quality. The free sugar content remained lower in ComCat® treated tomatoes as well as carrots during storage. It seems as if the fruit part of a plant is more affected by the ComCat® treatment, e.g. tomato, than the root part, e.g. carrots.

The interactive effect of preharvest ComCat® spraying, disinfecting with chlorinated or anolyte water, modified atmosphere packaging in Xtend® film and different storage temperatures was significant on several postharvest quality parameters of carrots and tomatoes. The postharvest quality of ComCat® treated carrots and tomatoes in the storage atmosphere, created by forced ventilation evaporative cooling, was also better than that of the controls.

OPSOMMING

Die verebetering van rakleeftyd van geelwortels en tamaties gedurende ge-ïntegreerde voor- en na-oes behandelings is ondersoek. Voor-oes ComCat® behandeling van geelwortel- en tamatieplantjies is gekombineer met na-oes ontsmetting met gechlorineerde of anolyte water, gemodifieerde atmosfeer verpakking met Xtend® film en verskillende bergingstemperature.

Die fisiologiese, chemiese, biochemiese en mikrobiologiese gehalte van geelwortels en tamaties is effektief verbeter deur die gekombineerde effek van gemodifieerde atmosfeer verpakking in Xtend® film en optimum bergingstemperature van geelwortels, by 1°C, en tamaties, by 13°C. Die rakleeftyd van geelwortels en tamaties is effektief verbeter terwyl die gehalte-eienskappe behou is.

Ontsmetting van groente met anolyte water het beter resultate gelewer as met gechlorineerde water. Gechlorineerde water behandeling het askorbiensuurinhoud verlaag, en fisiologiese massaverlies en peroksidase aktiwiteit verhoog. Die oppervlakweefsel van geelwortels is deur chloor geëts, wat indringingsones vir mikroörganismes geskep het , en die rakleeftyd benadeel het. Ontsmetting met anolyte water, in kombinasie met gemodifieerde atmosfeer verpakking en optimum bergingstemperature, het gehalte-eienskappe ondersteun, en so bygedra tot verbetering van rakleeftyd van geelwortels en tamaties.

Voor-oes bespuiting van plantjies met ComCat®, het die gehalte van geelwortels verbeter. Tydens oes is 'n effens hoër askorbiensuur-, totale oplosbare soliede-, totale

beskikbare koolhidrate- en sukrose inhoud waargeneem. Die sukrose tot heksose verhouding was ook effens hoër, en die mikrobiese populasies was effens laer. Voor-oes ComCat® behandelde tamaties het by oes 'n laer pH, fructose- en glukose inhoud, en hoër titreerbare suur en peroksidase aktiwiteit gehad, wat tot laer populasies mikroörganismes gelei het.

Gedurnede berging by 13°C en kamertemperatuur, het ComCat® behandelde tamaties 'n beter behoud van totale oplosbare soliede en askorbiensuur, en laer peroksidase aktiwiteit getoon. Hierdie tamaties het ook beter mikrobiologiese- en bemarkbare kwaliteit behou. Die vrye suikerinhoud het laer gebly in ComCat® behandelde geelwortels sowel as tamaties gedurende berging. Dit blyk dat die vruggedeelte van 'n plant, bv tamatie, meer deur die ComCat® behandeling beïnvloed word as die wortelgedeelte, bv geelwortel.

Die interaktiewe effek van voor-oes ComCat® bespuiting, na-oes ontsmetting met gechlorineerde- of anolyte water, gemodifieerde atmosfeer verpakking met Xtend® film en verskillende bergingstemperature was beduidend op verskeie na-oes gehalteparameters van geelwortels en tamaties. Die na-oes gehalte van ComCat® behandelde geelwortels en tamaties in die bergingsatmosfeer, geskep deur forseerde ventilasie verdampingsverkoeling, was ook beter as die van die kontroles.

Appendix A

Appendix A.1. Analysis of variance for the effects of storage time and the interactive effects of storage time with pre- and postharvest treatments on the qualities of tomatoes during storage at 13°C and room temperature (RT) for 30 days.

Treat-ment	Postharvest quality parameters																		
	O_2	CO_2	N_2	TSS	PWL	pH	Suc.	Gluc.	Fruc.	TSug	SE	S/H	TTA	S/A	AA	TAB	TF	TC	Mar.
D	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
AD	NS	***	NS	***	NS	*	**	NS	**	*	**	***	*	***	***	**	NS	***	NS
BD	**	NS	*	***	NS	**	NS	NS	NS	NS	NS	NS	**	***	***	***	***	***	***
ABD	NS	NS	NS	***	***	*	*	NS	NS	NS	NS	NS	*	NS	***	**	NS	NS	NS
CD	***	NS	***	***	NS	***	**	NS	**	NS	*	NS	***	**	***	***	***	***	***
ACD	NS	**	NS	***	NS	**	NS	NS	NS	NS	NS	NS	**	*	***	**	NS	NS	**
BCD	*	NS	NS	***	NS	***	NS	NS	NS	NS	NS	NS	***	***	***	***	***	*	***
ABCD	NS	*	NS	***	NS	**	NS	NS	NS	NS	NS	NS	**	***	***	***	NS	NS	NS

D = Storage time
A = Preharvest treatment
B = Disinfecting + MAP
C = Storage temperature
PWL = Physiological weight loss
TSS = Total soluble solid
TTA = Titratable acidity

Suc. = Sucrose
Gluc. = Glucose
Fruc. = Fructose
Tsug. = Total soluble sugar
SE = Sucrose equivalent
S/H = Sucrose to hexose ratio
S/A = Sucrose to acid ratio

AA = Ascorbic acid
TAB = Total aerobic bacteria
TF = Total molds and yeasts
TC = Total coliform bacteria
Mark. = Marketability

NS, *, **, *** Nonsignificant or significant at P≤ 0.05, 0.01 or 0.001 respectively.

Appendix A.2. Analysis of variance for the effects of storage time and the interactive effects of storage time with pre- and postharvest treatments on the qualities of carrot during storage at 1°C and room temperature (RT) for 28 days.

Treatment	Postharvest quality parameters															
	O_2	CO_2	N_2	TSS	PWL	TAC	Suc.	Gluc.	Fruc.	Tsug	S/H	AA	POX	TAB	TF	TC
D	NS	NS	NS	***	***	***	***	***	***	***	***	***	***	***	***	***
AD	NS	NS	NS	NS	***	***	*	***	***	*	**	NS	NS	NS	***	NS
BD	NS	*	*	***	***	NS	***	*	**	*	NS	NS	NS	***	***	***
ABD	NS	NS	NS	NS	***	NS	NS	*	*	NS	*	NS	NS	**	NS	NS
CD	NS	***	NS	***	***	***	***	***	***	***	*	***	***	***	***	***
ACD	NS	NS	*	*	NS	*	*	NS	*	NS	NS	NS	NS	*	NS	NS
BCD	*	***	NS	*	***	NS	NS	NS	NS	NS	NS	NS	NS	***	***	***
ABCD	NS	NS	NS	NS	***	NS	*	NS	NS	NS	NS	NS	NS	***	NS	***

D = Storage time
A = Preharvest treatment
B = Disinfecting + MAP
C = Storage temperature
PWL = Physiological weight loss
TSS = Total soluble solid
Suc. = Sucrose
Gluc. = Glucose
Fruc. = Fructose
Tsug. = Total soluble sugar
TTA = Total available carbohydrates
S/H = Sucrose to hexose ratio
AA = Ascorbic acid
POX = Peroxidase activity
TAB = Total aerobic bacteria
TF = Total molds and yeasts
TC = Total coliform bacteria

NS, *, **, *** Nonsignificant or significant at P≤ 0.05, 0.01 or 0.001 respectively.

Appendix A.3. Analysis of variance for the effects of storage time and the interactive effects of storage time with pre- and postharvest treatments on the qualities of tomatoes during storage inside the evaporative cooling chamber and ambient temperature (RT).

Treatment	Postharvest quality parameters										
	TSS	PWL	JC	pH	TTA	RS	NRS	Tsug	TAB	TF	Mark
D	***	***	***	***	***	***	***	***	*	**	***
AD	***	*	NS	NS	***	***	***	*	NS	NS	NS
BD	NS	NS	NS	***	NS	NS	NS	NS	***	***	*
ABD	NS	NS	NS	*	NS	**	NS	***	NS	NS	NS
CD	NS	***	***	***	NS	*	**	**	***	NS	***
ACD	NS	NS	NS	**	NS	NS	NS	NS	NS	NS	NS
BCD	NS	*	***	*	NS	NS	NS	*	NS	*	**
ABCD	NS	NS	NS	***	NS	*	*	NS	NS	NS	*

D = Storage time
A = Preharvest treatment
B = Disinfecting + MAP
C = Storage temperature
PWL = Physiological weight loss
TSS = Total soluble solid
RS = Reducing sugar
NRS = Non-reducing sugar
TS = Total sugar
TAB = Total aerobic bacteria
TF = Total molds and yeasts
TTA = Titratable acidity
JC = Juice content
Mark = Percentage marketability

NS, *, **, *** Nonsignificant or significant at P≤ 0.05, 0.01 or 0.001 respectively.

Appendix A.4. Analysis of variance for the effects of storage time and the interactive effects of storage time with pre- and postharvest treatments on the qualities of carrots during storage at ambient (RT) and inside the evaporative cooler.

Treatment	Postharvest quality parameters										
	pH	PWL	TSS	MC	JC	RS	NRS	TSUG	TAB	TF	Mark
D	***	***	***	***	***	***	***	***	***	***	***
AD	**	NS	*	NS	NS	***	*	***	NS	NS	NS
BD	*	***	***	***	**	*	***	***	***	***	***
ABD	**	NS	NS	NS	NS	NS	*	NS	*	NS	NS
CD	***	***	***	***	***	***	*	***	NS	***	***
ACD	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS
BCD	**	***	***	***	*	*	NS	*	NS	*	*
ABCD	**	*	*	NS	NS	*	NS	*	NS	NS	NS

D = Storage time
A = Preharvest treatment
B = Disinfecting + MAP
C = Storage temperature
PWL = Physiological weight loss
TSS = Total soluble solid
RS = Reducing sugar
NRS = Non-reducing sugar
Tsug. = Total soluble sugar
TAB = Total aerobic bacteria
TF = Total molds and yeasts
Mark = Percentage marketability
JC = Juice content
MC = Moisture content

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01 or 0.001 respectively.