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I am submitting herewith a thesis written by Jianjin Hou entitled "Growth of Spoilage organisms and *Yersinia enterocolitica* in osmotically dehydrated carrot slices under modified atmospheres held at 4° and 10°C." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

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GROWTH OF SPOILAGE ORGANISMS AND *YERSINIA ENTEROCOLITICA* IN OSMOTICALLY DEHYDRATED CARROT SLICES UNDER MODIFIED ATMOSPHERES HELD AT 4° AND 10°C

A Thesis

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Degree

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Jianjin Hou

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DEDICATION

This thesis is dedicated with love to my parents,

Mrs. Kewen Wang and Mr. Zhibang Hou,

and my dearest wife, Xueying Zhou.

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ABSTRACT

The effects of the combination of osmotic dehydration and modified atmosphere on growth of spoilage organisms and pathogenic *Yersinia enterocolitica* on carrot slices stored at 4° and 10°C were determined in this study. Microbiological analyses showed that during 21 days storage at 4°C, the initial aerobic plate count (APC) and psychrotrophic counts on carrots ranged from 3.5-4.4 log CFU/g and significantly increased to maximum levels of 7.4-8.5 log CFU/g, respectively; a range of 2.7-3.8 log CFU/g initial number of coliforms increased to 6.7-7.7 log CFU/g; lactic acid bacterial counts of 2.1-2.9 log CFU/g increased to 4.6-8.6 log CFU/g, mesophilic sporeforming counts of 1.7 log CFU/g increased to 3.7 log CFU/g; pectinolytic bacteria increased approximately 3.7 log CFU/g; initial numbers of yeasts were 2.0-3.0 log CFU/g increased approximately 3 log CFU/g; and molds did not grow well in packages with either treatment. A statistical comparison of growth between dehydrated and non-dehydrated carrot samples showed that osmotic dehydration significantly slowed growth of aerobes, psychrotrophic, coliforms, pectinolitics, and yeast before 9 days storage, but reached a higher maximum population than that of non-dehydrated carrots after 21 days at 4°C; mesophilic anaerobic sporeforming bacteria did not significantly increase at 4°C. Salt treatment significantly stimulated growth of lactic acid bacteria which reached significantly higher maximum numbers in osmotically treated carrots than that in non-treated samples. A storage temperature of 10°C caused a faster increase and higher maximum populations of organisms on carrots within 15 days compared to that at 4°C. The APC and

psychrotrophic counts for carrots with salt treatment increased from approximately 4.0 and 3.4 log CFU/g to 9.2 and 9.1 log CFU/g. The counts for non-dehydrated carrots were increased from initially 4.4 and 4.1 log CFU/g to 7.5 and 7.4 log CFU/g, respectively; the maximum coliform counts reached 8.6-9.0 log CFU/g for dehydrated carrots; significantly better growth and higher maximum counts of lactic acid bacteria occurred in osmotically treated carrots (7.8 CFU/g for dehydrated and 3.6-4.9 log CFU/g for non-dehydrated samples); the storage time, temperature of 10°C, and osmotic dehydration significantly increased the levels of mesophilic anaerobic sporeforming bacteria; pectinolytic bacteria increased approximately 3.7 log CFU/g, but were not significantly affected by salt treatment; the number of yeasts was increased approximately 2.4 log CFU/g at 10°C after 15 days, and osmotic dehydration significantly increased their growth on carrots; carrots packaged in air or vacuum did not support the growth of molds at 10°C. Packaging atmosphere did not significantly affect growth of most bacteria, but significantly greater growth of lactic acid bacteria at 4°C and mesophilic anaerobic sporeforming bacteria at 10°C occurred under vacuum condition.

The numbers of *Yersinia enterocolitica* rapidly grew up from the range of 3.9-4.6 to 6.6-8.2 log CFU/g for the carrots at 4°C after 21 days, and from 3.2-3.8 to 6.8-8.9 log CFU/g at 10°C after 15 days storage. Osmotic dehydration did not significantly affect the growth of *Y. enterocolitica* at 4°C, but significantly increased its growth on carrots at 10°C. The increased storage temperature greatly reduced the lag phase and increased maximum counts, especially, for the osmotically dehydrated carrots. Atmosphere conditions had less effect on the growth of *Y. enterocolitica.* Congo red assay and salicin

fermentation test found *Y. enterocolitica* retained their virulent plasmid. Minimally processed vegetables are ready-to-eat foods and the cut surfaces and high moisture level could provide *Y. enterocolitica* with good conditions for growth. High CO_2 levels in packages and inadequate temperature control during refrigeration storage may favor the growth of *Y. enterocolitica*. From these data, modified atmosphere combined with osmotic dehydration does not seem to be an effective way to reduce growth of *Y. enterocolitica* in minimally processed carrots. An additional barrier (s) is necessary to prevent growth of *Y. enterocolitica* during storage of the products.

The increased temperature and osmotically dehydration caused an increased respiration rate of plant tissue and microbial metabolism resulting in high CO_2 concentrations in packaging and reduced shelf life of products due to the physiological damage of plant tissue and rapid growth of lactic acid bacteria and yeasts. Although osmotic dehydration increased the spoilage of carrots, it provided a way to prevent moisture loss and discoloration of carrot surfaces during storage. This might be a new way for improving appearance of carrots during cold storage.

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Chapter I

INTRODUCTION

Consumption of minimally processed fresh vegetables (MPV) has increased dramatically in recent years. These are fresh vegetables which are washed, sliced, chopped, or grated. Unfortunately, the shelf life of MPV may be greatly reduced because the respiration rate of the damaged vegetable tissues will increase, and the increased surface area and water content of MPV can increase growth of spoilage and pathogenic bacteria. Maintenance of high quality and safety of MPV is a formidable challenge.

Modified atmosphere packaging (MAP) is a popular technique used in recent years for maintaining food quality and extending the useful marketing life of MPV (Bolin and Huxsoll, 1989; Brecht, 1980; Shewfelt, 1987). MAP means that atmospheres in a package surrounding produce are modified by elevating the CO_2 level, reducing O_2 level, or both. Growth of spoilage organisms and the respiration rate of vegetables is affected by low O_2 or high CO_2 concentrations in packages and low storage temperature (El-Goorani and Sommer, 1981; Wang, 1990; Zagory and Kader, 1988). However, the high relative humidity (RH) in a package, low storage temperature and low oxygen environment, may change the microbial profile of MPV (Brackett, 1987), creating conditions favorable for pathogen growth, especially, for psychrotrophic pathogens, such as *Yersinia enterocolitica* (Hanna et al, 1976; Hotchkiss, 1988; Silliker and Wolfe, 1988; Zagory and Kader, 1988). However, the visual qualities of produce are improved by MAP whether or not the difference in microbial population is observed (Berrang et al.,

1990; Brackett, 1987; Hao and Brackett, 1993; and Makhlouf et al., 1989). There is a need for development of additional barriers to the growth of pathogenic organisms on MPV under MAP.

Osmotic dehydration is another important method for preservation of vegetables. Osmotic dehydration removes water from vegetables with the aim of lowing the water activity of vegetable material by immersing vegetable tissue in concentrated solution of osmoactive substances (Biswal and Maguer, 1989; Biswal et al., 1991; Lenart and Lewicki, 1990). Lower water activity of vegetable tissue reduces its respiration rate, and thus may produce better overall quality and storage stability (Speck et al., 1977) and provide a hurdle to the growth of spoilage and pathogenic organisms (Torreggiani, 1993). The combination of osmotic dehydration of MPV with MAP has been introduced to increase the shelf life of green beans (Biswal et al., 1990). But an important aspect, so far unknown, is the effects on growth of microorganisms and the microbial safety of the process.

Yersinia enterocolitica is a Gram-negative, nonspore-forming, facultatively anaerobic, pathogenic bacterium which has the capacity of surviving and multiplying at refrigeration temperatures and thus presents a potential public health concern (Stern, 1979). *Y. enterocolitica* has been isolated from a variety of food sources, such as milk, beef, pork, chicken, and vegetables (Aulisio et al., 1983; Black et al., 1978; DeBoer et al., 1982; Moustafa et al., 1983; Stern, 1981; and Stern and Pierson, 1979). Hanna et al. (1976) and Manu-Tawiah et al. (1993) reported that *Y. enterocolitica* can grow in vacuum-packaged beef and lamb, and modified gas packaged pork chops. These results

show that some modified atmospheres provide an environment in packages that allows growth of *Y. enterocolitica.* The post-harvest contamination of MPV may create a potential source of infection particularly in MAP.

Osmotic dehydration in combination with MAP under refrigeration temperatures could provide a method to reduce the growth of spoilage and pathogenic organisms in MPV. Since the safety of osmotically dehydrated, MPV packaged with MA Pand stored under refrigeration temperatures had not been previously studied, the objective of our study was to determine the effects of the combination of osmotic dehydration and MAP on the survival and growth of spoilage microorganisms and *Y. enterocolitica* in carrot slices stored at 4 and 10°C.

Chapter II

REVIEW OF LITERATURE

Minimally processed vegetables (MPV)

Minimally processed vegetables (MPV) are fresh, raw vegetables processed to supply a ready-to-eat or ready-to-use product. The vegetables are usually trimmed, peeled, sliced or cut if necessary, washed and sometimes disinfected. The products are packaged in sealed pouches, or in plastic films. A shelf-life of several days is necessary for transport and retail of final products. The ready-to-eat vegetables can be used as ingredients for cooked dishes, but in many instances they are consumed raw. A wide range of vegetables are offered as minimally processed products such as cut or sliced carrots, cut celery sticks, leek, celeriac, chopped lettuce, cauliflower and broccoli florets, sliced or chopped onions and other salad constituents (Garg et al., 1990; Nguyen-the and Carlin, 1994).

Microbiology and safety of minimally processed vegetables

The main features of MPV, cut surfaces or damaged plant tissues, high respiration rate and active metabolism of plant tissue, and high moisture content of surfaces, may increase the risk of microbial spoilage and growth of pathogenic bacteria (Nguyen-the and Carlin, 1994). Raw vegetables are already heavily contaminated when they enter the processing lines. The fresh cut vegetables are not generally exposed to a critical control step to eliminate many of the contaminating microorganisms and the microorganisms on the packaged products may reflect the microflora of the vegetables in the growing field as

well as contamination during processing (Garg et al., 1990). Several authors have listed the numbers of microorganisms on samples of MPV (Table 1). Garg et al. (1990) believed that different processing steps affect microbial populations of samples. Their investigation showed that the inner leaves of lettuce and cabbage contained relatively low populations, usually 10^4 cells/g, but counts in the packaged products were considerably higher (10^5-10^6/g) due to contamination contributed by the shredder. The amount of soil adhering to the surface of the carrot determined the initial level of the contamination. Field contamination could be reduced by the removal of the outer leaves from lettuce, cabbage, and cauliflower, and by the peeling step that was given to carrots and onions. Sprays and water baths reduced microbial counts to some extent. A chilled chlorinated dip treatment was effective under laboratory conditions, but counts were not always reduced in the processing plants (Garg et al., 1990). Washing with chlorinated water of 20 ppm did not change the aerobic colony counts of ready-to-use sliced carrots (Torriani and Massa, 1994).

Many of the vegetables contained significant numbers of psychrotrophic bacteria and sometimes the psychrotrophic and mesophilic counts were comparable. Gram-negative rods were the predominant microorganisms. The most numerous species were members of genus *Pseudomonas*; it is likely that many were psychrotrophic (Garg et al., 1990). This is unfortunate because the initial population of psychrotrophs affects the shelf life of these vegetables. They are usually shipped and stored under refrigeration conditions. Garg et al. (1990) reported that lactic acid bacteria did not yield high counts on lettuce, cabbage, cauliflower floret and carrot sticks, but may predominate in vegetable

Table 1. Number of microorganisms in minimally processed vegetables samples

Products	Mesophilic microflora	Lactic acid bacteria	Coliforms	Psychrotrophic microflora	Yeasts & molds	Ref.
Shredded cabbage	10^4-10^7	$2.5x10^2$		$1.8x10^5$	$1.7x10^5$	Garg et al.,1990
Carrot sticks	10^5-10^6	10^3		$9.5x10^4$	$1.8x10^4$	Garg et al.,1990
Carrot slices	10^4-10^7		10^2-10^5			Torriani & Massa, 1994
Cauliflower florets	$5x10^5$-$5x10^6$	< 10		$5.5x10^3$	$3.5x10^2$	Garg et al., 1990
Mixed vegetables from retail outlet (lettuce, cabbage, carrot, onion, pepper)	10^5	10^3	10^4			Manvell & Ackland, 1986
Shredded carrots	10^6	10^4			< 10^2	Carlin et al., 1989

salads when held at an abuse temperature of 30°C (Manvell and Ackland, 1986). Potential foodborne pathogens in minimally processed vegetables have been detected in various vegetables. *Listeria monocytogenes,* has been isolated in lettuce heads, potato, radish and various other vegetables (25.8 to 30.3% of samples contaminated) (Heisick et al., 1989; Tarrago and Ferrer, 1992). *Staphylococcus aureus, Shigella* spp, and *Salmonella* spp. were found in vegetables and salads at a level of 10^3 CFU/g in Egypt (Sadik et al., 1985). *Yersinia enterocolitica* has frequently been isolated from vegetables, but the strains were not identified as pathogenic to human (Nguyen-the and Carlin, 1994). Vegetables were found in the U.S. to carry 10^2 to 10^4 CFU/g cytotoxic and hemolytic *Aeromonas* spp. (Callister and Agger, 1987). Contamination of vegetables by foodborne pathogens frequently occurs through agricultural practices such as irrigation with polluted water or fertilization with manure or sewage sludge. The risk of contamination during harvesting and processing is also a source of pathogens (Nguyen-the and Carlin, 1994).

Carrot and its storage

The carrot, with a content of carotene of 12 mg/100 g, is by far the most important source of pro-vitamin A, contributing about half the requirement assuming a RDA of 3 mg/day of carotene (Burton, 1982). Fresh carrots are minimally processed to produce carrot sticks, slices, baby peeled carrots, and grated carrots. Observations (Beuchat and Brackett, 1990) indicate that carrot root tissue fluid is antilisterial. Beuchat et al. (1994) thought that the broad spectrum of antimicrobial activity of phytoalexins elicited or naturally present in carrots was particularly attractive in the terms of their use as secondary barriers to kill or prevent the growth of *Listeria monocytogenes* and

perhaps other foodborne pathogens and spoilage organisms in foods. Research (Beuchat et al., 1994) showed that the antilisterial effect of carrot juice was highest at a somewhat lower pH range (5.0-6.4). But the total population of aerobic mesophilies was not effected by pH or juice concentration. Carrot roots grow in a near anaerobic environment because oxygen tension of the soil is low. When the root is harvested it is exposed to the high oxygen tension of the atmosphere which brings about numerous metabolic changes. Pyruvic acid contributes about 30% of the total acids in the growing carrot. However,24 h after harvesting pyruvic acid is about 0.1% of the total acids and malic and isocitric acids increase to 95% from about 25% of total acids (Phan, 1987). In addition, enhanced aerobic respiration promotes depletion of soluble sugars. When the carrot is processed into carrot sticks the respiration rate abruptly but temporarily increase 10-fold. The monosaccharides are metabolized depleting the soluble sugar (Laties, 1978). Shelf life and acceptability of minimally processed carrots are limited because of microbial deterioration and the consequences of slicing and exposure of the cut surfaces to air. Accelerated senescence is a major consequence of slicing carrot sticks, which results in loss of flavor, discoloration, and change in texture (Bruememer, 1987). Respiratory CO_2 formation is an index of general metabolism. Agents that affect CO_2 formation are potential regulators for controlling senescence. A conventional approach to retard respiration and metabolic rates is to lower temperature of the product and modify the gaseous atmosphere. A less conventional approach would be to supply metabolites or growth regulators to the product (Bruemmer, 1988). Bruemmer (1987) showed that carrot sticks vacuum infused with sodium pyruvate, lecithin, or glucose and stored two weeks at 2°C retained flavor as

well as freshly prepared carrot sticks. But another report by Bruemmer (1988) showed that carrot flavor was lost under several modified atmospheres and with infused metabolites. He concluded that harvested carrot is physiologically too mature for senescence control. Carlin et al. (1989 and 1990) reported that spoilage of retail packs of fresh, "ready-to-use," grated carrots was the results of lactic acid fermentation, associated with high counts of lactic acid bacteria, and a high content of ethanol and lactic acid. In gas tight containers with controlled atmospheres, high CO_2 (over 30%) or low O_2 (below 2%) resulted in anaerobic respiration and induced spoilage, whereas controlled atmospheres of 15-20% CO_2 and about 5% O_2 which caused aerobic respiration provided satisfactory preservation. It was unlikely that pectinolytic yeasts played an important role in the spoilage of the products because they were isolated from pouches of grated carrots at low frequency and only at the beginning of storage. All the yeasts isolated at the end of storage, in pouches that contained 20% or more CO_2, were fermentative and produced a large amount of CO_2 in vitro. In this respect, CO_2 production by yeasts may have contributed to spoilage (Babic et al., 1992). Buik and Damoglou (1987) found that the microbial development on vacuum packaged carrot slices was slower than that of non-vacuum packaged material. Vacuum packaging significantly extended the shelf-life of the product when stored at 4°C from 5 to 8 days.

Modified atmosphere packaging (MAP) of vegetables

Mechanism of action. Modified atmosphere packaging is a type of packaging in which food products are stored in something other than air. This includes vacuum-packaging, gas-flushing, naturally-respiring products that use special permeable films and

controlled atmosphere packaging. Farber (1991) reviewed the possible mechanism of this action. These include: (1) O_2 generally stimulates the growth of aerobic bacteria and can inhibit the growth of strictly anaerobic bacteria; (2) N_2 is an inert tasteless gas which displays little or no antimicrobial activity on its own. It can replace oxygen in the pack and also inhibit the growth of aerobic bacteria; and (3) CO_2 is mainly responsible for the bacteriostatic effect seen on microorganisms grown in MAP environment and has an inhibitory effect on product respiration. In most foods, gaseous CO_2 applied to a biological tissue would exit in the liquid phase of the tissue primarily as dissolved CO_2 gas and carbonic acid. Some theories may explain the way in which CO_2 exerts its influence on a bacterial cell: a) alteration of cell membrane function including effects on nutrient uptake and absorption; b) direct inhibition of enzymes or decreases in the rate of enzyme reactions; c) penetration of bacterial membranes, leading to intracellular pH changes; and d) direct changes to the physico-chemical properties of proteins. For maximum antimicrobial effect, the storage temperature of a MAP product should be kept as low as possible, because the solubility of CO_2 decreases dramatically with increasing temperature. Thus, improper temperature control will usually eliminate the beneficial effects of elevated CO_2 (Daniel et al., 1985).

Effects of MAP on vegetable quality and safety. The benefit of using MAP for extending shelf life of vegetables is that it not only preserves the organoleptic qualities, but also prolongs the shelf life of vegetables (Zagory and Kader, 1988). However, MAP may increase microbial risks in three ways: 1) the increased storage life of vegetables increases the time to allow pathogens to develop to significant numbers or produce toxin. 2) MAP

may retard the development of competing spoilage organisms and enhance pathogen growth. 3) packaging respiring vegetables could alter the atmosphere such that the growth of pathogens is stimulated, such as CO_2 can enhance toxigenesis by *C. botulinum* (Hotchkiss, and Banco, 1992). In Brackett's research (1990), fresh bell peppers were individually shrink-wrapped in film, sealed in gas-flushed film pouches or unpackaged as control, and stored at 13°C. Wrapped peppers developed higher populations of total aerobic organisms, yeasts and molds, and Enterobacteriaceae than did controls. Color and surface pH of peppers did not differ in any of the treatments. Shrink-wrapped and gas-flushed peppers remained unspoiled for 6 weeks, whereas control peppers spoiled in 3 weeks. Ballantyne et al. reported (1988) that initial flushing with low O_2 and high CO_2 gas provided a important extension to storage life. An equilibrated modified atmosphere containing 1-3% O_2 and 5-6% CO_2 was established with 35 μm low density polyethylene film after flushing with 5% O_2, 5% CO_2 in N_2. This delayed the onset of browning and off-odors to approximately 14 days at 5°C, almost double that of the controls. MAP did not significantly inhibit spoilage bacteria tested (Hao and Brackett, 1993). Growth of *Erwinia, Pseudomonas, Xanthomonas* and pectinolytic *Pseudomonas* at 10 and 20°C was not reduced by gas mixtures. Modified atmosphere containing 10% CO_2, 5% O_2 did not reduce the ability of bacteria tested to grow at elevated concentrations of sodium chloride. In addition, MAP composition did not change the percentage of bell peppers spoiled by tested bacteria inoculated. However, they found the overall visual quality was enhanced by MAP. *Listeria monocytogenes* was found to grow on lettuce subjected to commonly used packaging and distribution procedures used in food industry (Beuchat and Brackett,

1990). The population of *L. monocytogenes* was not significantly changed during the first 8 days of incubation at 5°C, but significantly increased between 8 and 15 days. Significant increases were observed within 3 days when lettuce was stored at 10°C. The populations reached 10^8-10^9 CFU/g. Chlorine treatment, modified atmosphere (3% O_2 and 97% N_2) did not affect growth of *L. monocytogenes*.

Osmotic dehydration (OD) of vegetables

Mechanism of action. Osmotic dehydration can be used to remove water from food with the aim of lowering water activity of the materials and improving the finished products durability (Lenart and Lewicki, 1990). It is a useful technique for the concentration of vegetables, realized by placing the solid vegetables in sugar, or salt aqueous solutions of high osmotic pressure. During osmotic dehydration, there are two simultaneous counter-current flows: water flows out of the vegetables into the solution and simultaneously solute transfers from the solution into the vegetables. Movement of both is due to the water and solute activity gradients across the cell's membrane (Torreggiani, 1993). The cell wall membranes of vegetable freely allow solvent to pass through and also allow , to a lesser degree, the passage of some of the solute molecules. Therefore, osmotic dehydration cause a twofold transformation of vegetable, by both a decrease in water content and a solute incorporation, which can cause a weight loss of vegetables (Torreggiani, 1993). Addition of NaCl to osmotic solutions increases the driving force for drying due to the water activity lowering capacity of the salt.

Application of OD in vegetables. According to Torreggiani (1993), the effects of osmotic dehydration as a pre-treatment are mainly related to the improvement of some

nutritional, organoleptic and functional properties of the product. Air drying following osmotic dipping is commonly used for the production of 'semi-candied' dried fruits. The organoleptic qualities of the end products could be improved because some of the acids are removed from the fruit during the osmotic bath (Ponting et al., 1966). Compared to simple air dehydration, a softer dried product could be obtained, more pleasant to eat out of the hands as a snack item or to incorporate in such products as pastry (Torreggiani et al., 1991). Osmotic dehydration could be used instead of air drying to obtain an energy saving or a quality improvement especially for fruit and vegetables sensitive to air drying (Biswal et al., 1991). When air dried carrots were treated with sodium chloride prior to dehydration, salt-treated carrots reached a higher degree of reconstitution and were of softer texture after reconstitution than non-treated carrots. Salt treatment not only improved color and texture, but also flavor, and thus produced a better overall quality and storage stability than water blanched and sulfite treated samples (Speck et al., 1977). Research also showed that treatment of sliced vegetables with sodium chloride prior to dehydration improved rehydration, color, texture and flavor of the finished product (Mazza, 1983). Green beans were dipped in 10% NaCl solution at 20°C for 30 min and then were processed further by freezing in an air blast freezer. The color, hardness, texture, taste, and overall acceptability of the products were organoleptically acceptable (Biswal et al., 1991). The combination of osmotic dehydration with modified atmosphere has been introduced to increase the shelf life of green bean (Biswal et al., 1990). There is no further research related to the storage of fresh vegetables with salt treatment. It should be investigated before further marketing usage.

Yersinia enterocolitica

Microbiological Characteristics. *Yersinia enterocolitica* is classified in the family of Enterobacteriaceae by virtue of its common antigens and its biochemical and core DNA relatedness (Brenner et al., 1976). It is a Gram-negative, nonsporeforming, facultatively anaerobic rod. It is somewhat unique in that it is psychrotrophic as a pathogen, is motile at temperatures below 30°C and nonmotile at 37°C (Nilehn, 1969), is oxidase negative, ferments glucose with little or no gas, is usually urease positive and reduces nitrate to nitrite. In Bergey's 8th edition, the temperature growth range was given as -2 to 45°C with optimum growth at 30-37°C (Mollaret and Thal, 1974). The addition of salt to growth medium raises the minimum growth temperature. In brain heart infusion (BHI) broth containing 7% NaCl, growth did not occur at 3° or 25°C after 10 days. At pH 7.2, growth of one strain was observed at 3°C in BHI broth and very slow growth at pH 9.0 at the same temperature, while no growth at pH 4.6 and 9.6 (Stern et al., 1980). *Y. enterocolitica* produces a heat-stable (ST) enterotoxin that survives 100°C for 2 min. It is not affected by proteases and lipases (Okamoto et al., 1982). Schiemann (1981) demonstrated that the ST was not critical to virulence. Although the determinants responsible for the pathogenicity of *Y. erterocolitica* have not been fully identified, it has been established that virulence is associated with the presence of plasmids with masses of 40-82 megadaltons (Md) (Gemski et al., 1980; Kay et al., 1982). Properties determined by these plasmids include autoagglutination (Laird and Cavanaugh, 1980), calcium dependence (Gemski et al., 1980), and pathogenicity for mice (Sciemann et al., 1981).

Yersinia enterocolitica ***as a foodborne pathogen***. *Y. enterocolitica* has been implicated in causing water and foodborne enteritis. The frequently reported symptoms include diarrhea, fever, vomiting, abdominal pain, nausea, and headache. In addition, patients may develop an acute form of lymphadenitis that may be mistaken for acute appendicitis (Black et al., 1978; Leino and Kalliomaki, 1974; Randall and Bannatyne, 1975; Schiemann, 1980). The isolates from varieties of food sources, such as milk, beef, lamb, pork, and chicken (Black et al., 1978; DeBoer, et al., 1982; Hanna et al., 1976; Schiemann, 1980) have demonstrated that contamination of food products may be a possible source of infection. A outbreak of illness from *Y. enterocolitica* for which foodborne transmission was proven occurred in September, 1976, in New York. Thirty-six children were hospitalized. Serotype O:8 was isolated from the clinical specimens of the infected children and an unopened carton of the suspect chocolate milk (Black et al, 1978). *Y. enterocolitica* are frequently isolated from vegetables (up to 76% of samples), but the strains were identified as nonpathogenic on the basis of their serovar (Nguyen-the and Carlin, 1994), whereas pathogenic *Y. enterocolitica* are commonly found on meat (Andersen et al., 1991).

Growth of **Yersinia enterocolitica** ***in modified atmospheres***. In the past, the major concerns in MAP packaged vegetables have been the anaerobic pathogens, especially the psychrotrophic anaerobic pathogens since the extended shelf life of many MAP products may allow extra time for these pathogens to reach dangerously high levels in a food (Farber, 1991). Only a few reports are related to growth of *Y. enterocolitica* in modified atmosphere, and there are no reports regarding its growth in modified

atmosphere packaged vegetables. Hudson et al. reported the growth of *Y. enterocolitica* on sliced roast beef packaged under vacuum or saturated CO_2 controlled atmosphere conditions. At -1.5°C, the pathogen declined under saturated CO_2, but grew under vacuum packaging and reached maximum numbers of 10^8 CFU/g after 8 weeks storage. At 3°C, the pathogen increased 4 log after 3 weeks under vacuum, and 3 log after 10 weeks under saturated CO_2. Zee et al.(1984) examined the effect of different MAP on the growth of *Y. enterocolitica* in trypticase soy broth at 25°C. While 10% CO_2 appeared to be stimulatory to the growth of *Yersinia* as compared to growth in air. 40% CO_2 increased the lag phase and 100% CO_2 both increased the lag phase and decreased the growth rate during the logarithmic period. *Y. enterocolitica* from vacuum-packaged pork were inoculated onto fresh pork chops, and survival and growth were determined in different atmospheres at 4°C during 35-days by Manu-Tawiah et al.(1993). Atmospheres were gas mixtures [20/0/80, 40/0/60, and 40/10/50 ($CO_2/O_2/N_2$)], vacuum and air. In air *Yersinia* grew slower than psychtotrophic spoilage flora. In gas atmospheres, *Yersinia* grew at the same rate as psychrotrophic flora. When 10% O_2 was included in the 40% CO_2 mixture, growth was reduced. Vacuum packaging was no more effective than gas mixtures in retarding growth. However, Gill and Reichel (1989) found *Y. enterocolitica* to be capable of growing on high pH beef packaged under 100% CO_2 at both 5 and 10°C. This CO_2-packaged beef stored at 0, 2, or -2°C was incapable of supporting the growth of *Y. enterocolitica*. They believed that modified atmospheres provide an environment in the package that would allow growth of *Y. enterocolitica*. Exposure of plasmid-bearing virulent cells both in growing and stationary phases to anaerobic atmospheres consisting of

80% N_2 plus 20% H_2; 94% CO_2 plus 6% H_2 or under vacuum for 24 h at 28°C did not lead to the loss of the virulence plasmid from the cells (Bhaduri and Turner, 1993).

***Isolation and identification methods for* Y. enterocolitica.** The methods for the isolation of *Y. enterocolitica* from foods are described in the "Compendium of Methods for the Microbiological Examination of Foods" (Schiemann and Wauters, 1992), and the "Bacteriological Analytical Manual" (Aulisio and Stanfield, 1984). The general methods for detection of *Yersinia* in foods include primary enrichment, alkali treatment, selective enrichment, isolation agar, biochemical identification, and virulent testing. Cefsulodin-irgasan-novobiodin (CIN) agar performs better with inoculation at 25 °C among isolation agars due to the distinctive colonial morphology (Schiemann and Wauters, 1992).

The ability of *Y. enterocolitica* to absorb Congo red from agar media is correlated with virulence (Prpic et al., 1983). Their studies showed that Congo red positive strains bore plasmids of between 40-50 megadaltons and were positive in several tests of *Y. enterocolitica* virulence, including autoagglutination and lethality for iron-overloaded mice. Congo red negative strains were without plasmids and were negative in all these assays. The Congo red reaction provides a simple and efficient means of screening *Y. enterocolitica* for virulence and is the best method for identifying individual plasmid-bearing colonies (Prpic et al., 1983).

Chapter III

MATERIALS AND METHODS

Carrot slice samples

Whole carrots were purchased in 50-lb bags from a local vegetable wholesale store and stored at 4°C until processed but were used within one day of storage. The carrots were peeled by hand and washed with 200 ppm chlorine solution before treatment to partially remove microorganisms. Then the carrots were sliced into 3 - 5 mm thick slices with a Hobart vegetable chopper (The Hobart Manufacturing Company, Troy, Ohio). The carrots were treated and packaged within 24 hours of arrival. All treatments were designed as described in Table 2. Each treatment design had eight packages for a series of 3-day interval sampling for 21 days at 4°C and six packages for the 15 days at 10°C storage temperature. For each replication only one temperature was studied due to limited storage space. The packaged bags were distributed in incubators to permit air flow and the doors of incubators were opened twice a day for fresh air exchange in incubators. Packages for each treatment were tested at each sampling day for each storage temperature, consisting of an uninoculated and inoculated, osmotically dehydrated and non-osmotically dehydrated, packaged under air or vacuum treatments. All treatments were repeated three times.

Dehydration of carrots

Sliced carrots were dipped in 10% salt solution (carrots:water = 1:4 W/V) for 5 min at room temperature (24 - 26°C) and then drained to remove excess moisture for two

Table 2. Treatment design of samples

Treatment	Dehydration	Inoculation	Packaging
1	ND	IN	Air
2	ND	IN	Vacuum
3	ND	NI	Air
4	ND	NI	Vacuum
5	DE	IN	Air
6	DE	IN	Vacuum
7	DE	NI	Air
8	DE	NI	Vacuum

Note: Dehydration treatment: ND=no osmotic dehydration; DE=10% salt solution dehydration for 5 min. Inoculation level: NI=no inoculation; IN=inoculation with 10^3 cells/g *Yersinia enterocolitica.* Atmosphere condition: Air or vacuum packaging.

h at 24-26°C. Water activity and salt content of carrot slices were analyzed on dehydrated carrot slices. Non-dehydrated carrot samples were used as controls. All the samples were dipped in 200 ppm chlorine solution for 10 seconds before packaging. Salt content of carrots was analyzed using 0.1 N $AgNO_3$ solution titration as AOAC procedures 971.27 and 971.29D (AOAC, 1990). The water activity of carrot slices was tested by using a CX-2 water activity detector (Decagon Devices, Inc., Pullman, Washington).

***Yersinia enterocolitica* inoculum**

Four pathogenic serotypes of *Yersinia enterocolitica*, (YE 288) O:3, (YE 133) O:8, (ATCC 55075) O:9, and (YE 321) O:20 were obtained from lab stock of the Department of Food Science and Technology, University of Tennessee and maintained on tryptic soy slants held at 4°C. All strains were confirmed by Schiemann and Wauters' procedures (1992), negative Gram stain, KIA slants of alkaline/acid/without gas at 35°C, positive urease test, positive motility at 25°C and negative at 37°C, positive sucrose fermentation at 25°C. A positive Congo red binding assay at 25°C and negative salicin fermentation test at 35°C were performed for determining each pathogenicity before inoculation. Each strain was grown in 50 mL brain heart infusion broth (BHI) (Difco, Detroit, MI) at 25°C for 48 h. Each culture was centrifuged at 4000 x g for 20 min at 4°C in a Biofuge 17R refrigerated centrifuge (Baxter Sci., Atlanta, GA.). Resulting pellets were suspended in 10 mL sterile Butterfield's buffer and then mixed to yield approximately 10^8 CFU/mL (OD of 0.5 at 640 nm) for inoculation of carrot slices. One group of dehydrated and one group of non-dehydrated carrot slice samples were immersed in a suspension of 10^5 cells/mL *Y. enterocolitica* at a 1:4 (W/V) ratio for 10 seconds for

inoculation (final bacterial concentration at approximately 10^3 cells/per gram carrots). Two groups of non-inoculated carrots with and without dehydration were used as controls.

Sample packaging

Dehydrated carrot slices and non-dehydrated carrot slices with or without *Yersinia* inoculation were packaged in 250 g portions in 24 x 30 cm semi-permeable PD961EZ film bags (oxygen transmission rate = 6,000 - 8,000cc/sq.M./ 24hrs, carbon dioxide transmission rate = 19,000 - 22,000cc/ sq.M./24hrs, water vapor transmission rate = 0.9 - 1.1g/ 100sq.in/24hrs) (Cryovac, Duncan, SC). The packages were sealed and flushed with air or vacuum using a Multivac A300 packaging machine (Wolfertschwenden, W. Germany) and then incubated at 4 or 10°C.

Microbial analysis and sampling of carrot slices

To obtain microbial counts, 25 g of the carrot sample were added to 225 mL Butterfield's diluent to yield a 1:10 dilution and mixed for 2 min. in a Stomacher Lab Blender, Model 400 (Steward; London, England). Serial dilutions were then performed to monitor aerobic mesophilic flora and aerobic psychrotrophic flora counts using APC agar (Difco, Detroit, MI) incubated at 32°C for 48 h and 7°C for 5 days, respectively. Coliform count were performed using Violet Red Bile agar (VRB) (Difco, Detroit, MI) inoculated at 32°C for 24 h, lactic acid bacteria counts using MRS lactobacilli agar (Becton Dickinson Microbiology System, Cockeysville, MD). Pectinolytic bacteria were counted using Pectin agar (Bowen and Kominos, 1979). Anaerobic agar (Difco, Detroit, MI) was used for mesophilic anaerobic sporeformer counts by pouring hot agar (60°C)

into Fung's Double Tube (Mohammed et al, 1991) and incubating at 35°C for 3 days. Rose Bengal agar (Oxoid, Columbia, MD) was used for yeast and mold counts. Three replications of all analyses were conducted for each treatment tested.

***Y. enterocolitica* detection and numeration**

Y. enterocolitica enumeration for inoculated and control samples was performed by directly spreading the appropriate dilution from Butterfield's diluent onto Cefsulodine-Irgasan-Novobiocin agar (CIN) (Difco, Detroit, MI) and incubated at 32°C for 48 h. Two presumptive *Yersinia* colonies of each random CIN agar plate were picked after incubation at 32°C for 48hr, and confirmed by Schiemann and Wauters' methods (1992). The virulence assays were performed using negative reaction of salicin fermentation at 35°C for 24 h (Sciemann and Wauters, 1992) and using positive reaction of Congo red binding assay at 25°C for 48 h (Prpic et al., 1983).

Gas Analysis

The headspace gases (CO_2 and O_2)of each package were analyzed on each sampling day. A small surface area of plastic bag was sealed with a piece of rubber tape. A 5 mL sample of the headspace gas was withdrawn using a 5 cc syringe through the sealing rubber. The gas sample was then injected into a Hewlett Packard Model 5890A Series II Gas Chromatograph equipped with a TCD at 25°C. The gases were separated using a stainless steel CTRI column (Alltech Associates, Inc., Deerfield, IL) at an oven temperature of 25°C. Helium was used as the carrier gas at a flow rate of 40 mL/min. The percentages of oxygen, carbon dioxide, and nitrogen were determined from a standard curve obtained using Scotty I analyzed calibration gases (Scott Specialty Gases,

Wakefield, MA).

Statistical analysis

The microbial data were averaged over the three replications at each treatment to obtain mean scores and standard deviations. The General Linear Model procedure of SAS (1990) was used to evaluate the main effects of storage temperature, storage time, osmotic dehydration, packaging, and interactions. Mean separations for the data were performed using the Student-Newman-Keuls analysis of SAS to evaluate the effects at a significance level of $P < 0.05$.

Chapter IV

RESULTS AND DISCUSSION

Microbiological measurements

Mesophilic and psychrotrophic aerobic bacteria. The initial APC (aerobic plate count) and psychrotrophic counts on the carrots ranged from 3.6 to 4.4 and from 3.5 to 4.2 log CFU/g (Figure 1), respectively. During 21 days storage of samples held at 4°C, the APC and psychrotrophic counts increased to maximium levels of 7.4 to 8.5 log CFU/g. Osmotic dehydration slowed the growth of bacteria during the initial 9 days storage, but by 12 days the counts were similar. However, the final populations (8.3 - 8.5 log CFU/g) of bacteria of salt-treated carrots were higher than that (7.4 - 7.6 log CFU/g) of the non-dehydrated samples held at 4°C (Figures 1 and 2). The growth of mesophilic aerobic bacteria on carrots held at 4°C closely resembled that of psychrotrophic bacteria on carrots held at the same conditions (Figure 1). This is a common finding because vegetables contain significant numbers of psychrotrophic bacteria and sometimes the psychrotrophic and mesophilic counts are comparable (Garg et al., 1990). The overall mean APC and psychrotrophic counts for osmotically dehydrated carrots were 6.1 and 6.0 log CFU/g at 4°C. The mean APC and psychrotrophic counts for carrots without salt treatment were 6.6 and 6.5 log CFU/g at 4°C, respectively (Table A11). A statistical comparison of growth between dehydrated and non-dehydrated carrot samples, showed that osmotic dehydration significantly ($P < 0.05$) reduced APC and psychrotrophs at 4°C (Tables A1-A2).

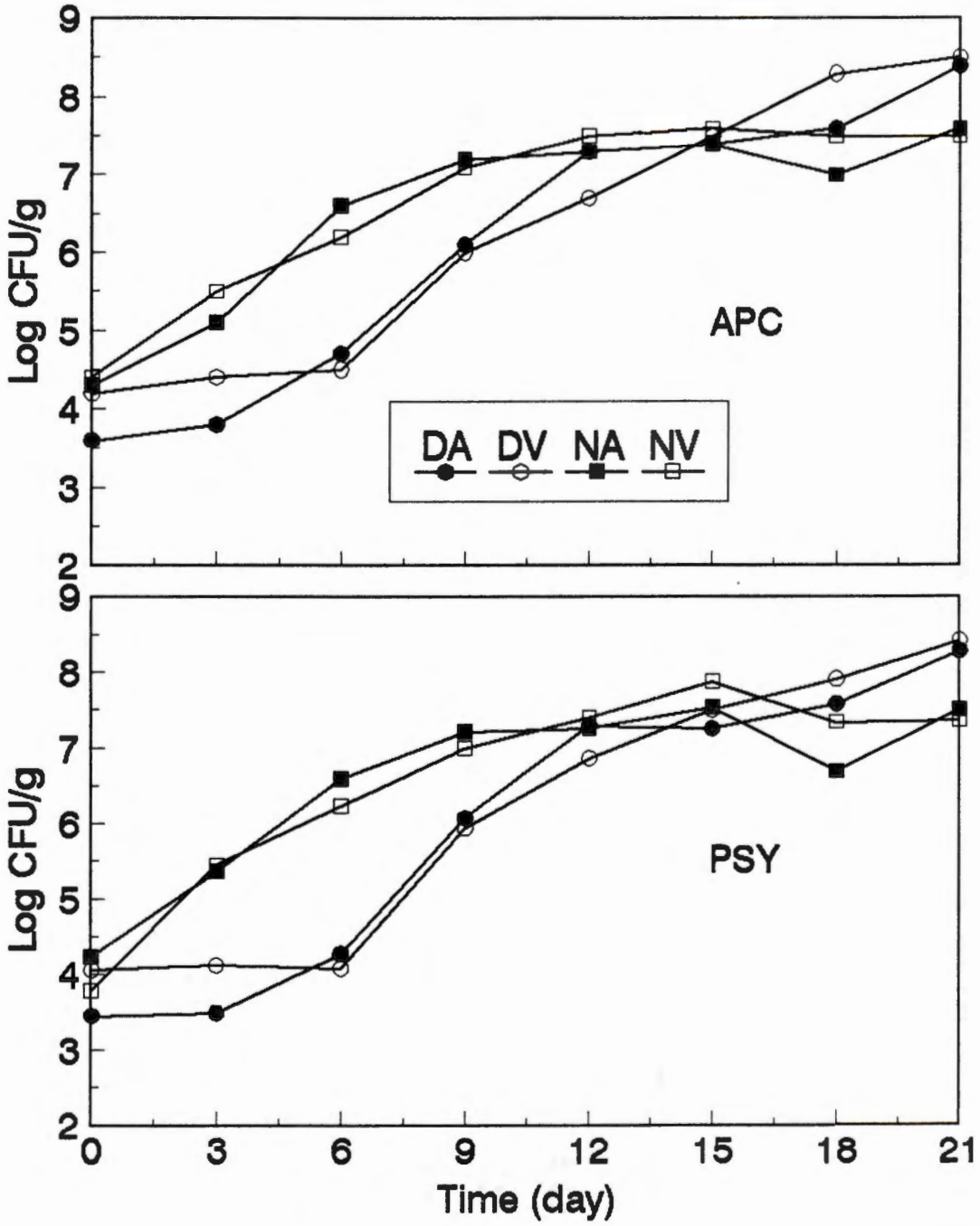

Fig. 1. Growth of mesophilic (APC) and psychrotrophic (PSY) aerobic flora in osmotically dehydrated or non-dehydrated carrot slices held at 4°C under air or vacuum packaging. [DA(V)=dehydrated under air(vacuum); NA(V)=non-dehydrated under air(vacuum).]

At 10°C, the mean APC and psychrotrophic counts for carrot samples with osmotic dehydration were 7.2 and 7.0 log CFU/g, and the mean APC and psychrotrophic counts for carrots without salt treatment were 6.8 and 6.6 log CFU/g, respectively (Figure 2; Table A22). The packaging atmosphere (air or vacuum) did not significantly affect ($P > 0.05$) APC or psychrotrophic counts or the bacterial profile at either 4 or 10°C (Figures 1-2; Tables A1-A2, A11-A22).

A storage temperature of 10°C caused a faster increase in the APC and psychrotrophic populations compared to that at 4°C. The APC and psychrotrophic counts for carrots with salt treatment increased from approximately 4.0 and 3.4 log CFU/g to 9.2 and 9.1 log CFU/g after 15 days, respectively. Whereas, the counts for non-osmotically treated carrots were increased from initially 4.4 and 4.1 log CFU/g to 7.5 and 7.4 log CFU/g after 15 days storage (Figure). Garg et al. (1990) showed that Gram-negative rods were the predominant microorganisms, making up over 86% of the isolates of fresh-cut vegetables. The most numerous species were members of the genus *Pseudomonas*, of which many were psychrotrophic. The high numbers of psychrotrophic bacteria are unfortunate because the higher population of psychrotrophs affects the shelf life of the refrigerated foods (Garg et al., 1990). Nguyen-the and Carlin (1994) reported that increasing CO_2 concentration significantly reduced development of mesophilic bacteria at 2°C and 6°C on cut chicory leaves but had no effect at 10°C. They suggested that storage temperature affected the influence of MA since the inhibitory effects on bacterial growth decrease as the temperature rises and follows the decrease of solubility of CO_2. Although some carrot materials were reported to have antimicrobial activity (Babic et al., 1994;

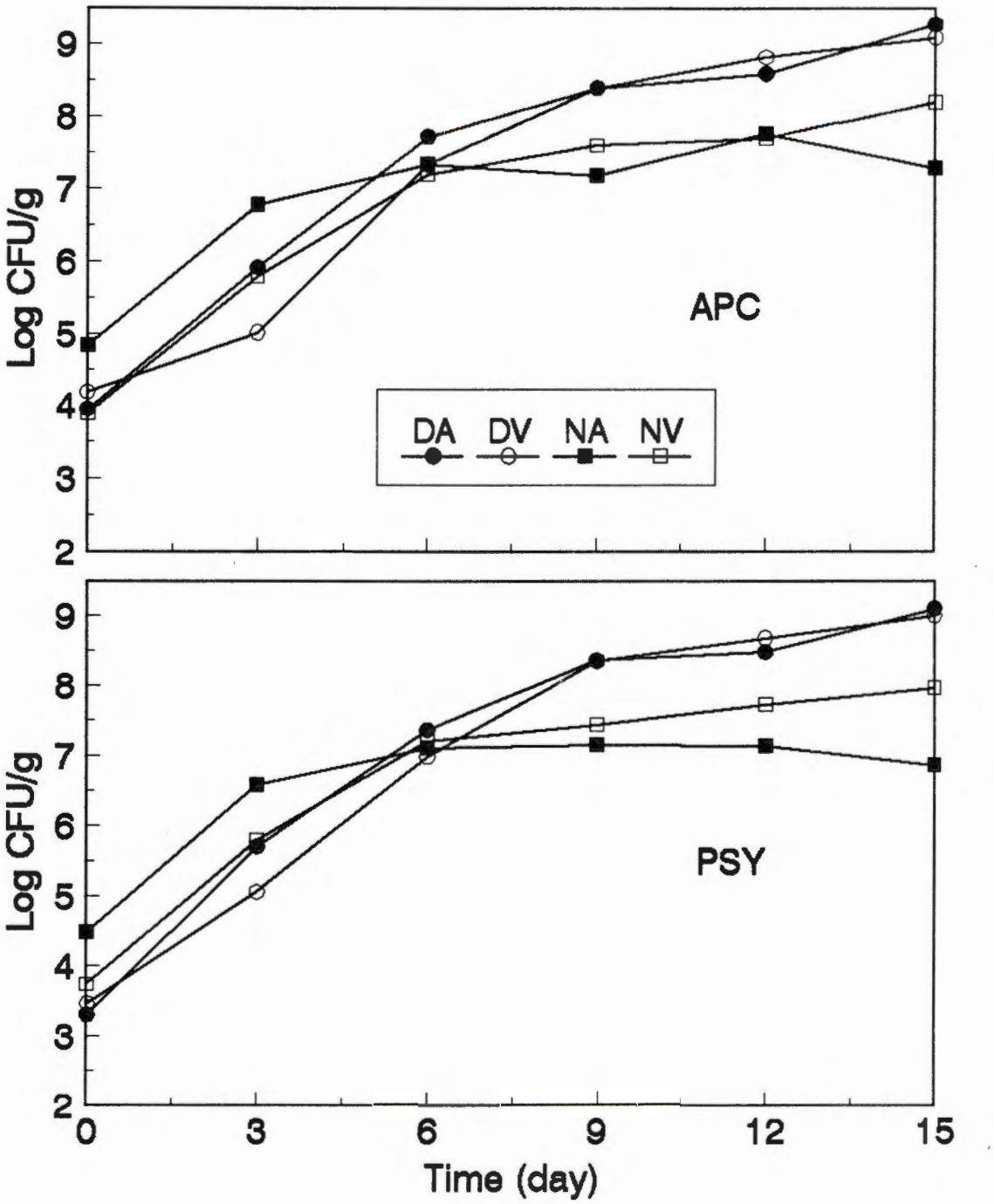

Fig. 2. Growth of mesophilic (APC) and psychrotrophic (PSY) aerobic flora in osmotically dehydrated or non-dehydrated carrot slices held at 10°C under air or vacuum packaging. [DA(V)=dehydrated under air(vacuum); NA(V)=non-dehydrated under air(vacuum).]

Beuchat et al., 1994;), the microflora naturally present on carrot roots may indeed owe their presence to an ability to resist antimicrobial activity associated with carrot. The total population of aerobic mesophiles was largely unaffected by pH or carrot juice concentration (Beuchat et al., 1994). Nguyen-the and Carlin (1994) concluded that the benefits of modified atmosphere for the quality of vegetables are not systematically related to a reduction in growth of mesophilic flora. The rapid growth of aerobic and psychrotrophic bacteria and the same profiles of bacterial growth under either packaging condition, and greater increase of APC and psychrotrophic counts at 10°C in this study, further supported these conclusions. Instead of reducing microbial growth, salt treatment may add a nutrient and moisture condition favorable to growth of microorganisms.

Coliform bacteria. Coliforms grew well on carrots during storage ($P < 0.05$) at 4 and 10°C (Figure 3). A range of 2.7 to 3.8 log CFU/g initial number of coliforms was increased to 6.7 to 7.7 log CFU/g after 21 days storage at 4°C (Figure 3). At 10°C, coliforms increased from a range of 2.9 to 3.9 log CFU/g to 6.2 to 9.0 log CFU/g after 15 days storage (Figure 3). Osmotic dehydration (OD) significantly slowed the growth of coliforms before 9 days ($P < 0.05$) (Figure 3; Table A3), but later, in storage OD carrots had a higher maximum number (7.2 - 7.7 log CFU/g) than non-dehydrated carrot samples (6.7 log CFU/g) when stored at 4°C. The mean coliform counts for carrots with and without osmotic dehydration was 5.4 and 5.7 log CFU/g, respectively (Table A11). Osmotic dehydration significantly increased the growth of coliforms and the maximum bacterial counts reached 8.6 - 9.0 log CFU/g when carrots were held at 10°C (Figure 3). The mean counts of coliforms were 6.8 log CFU/g for carrots dehydrated and 6.0 log

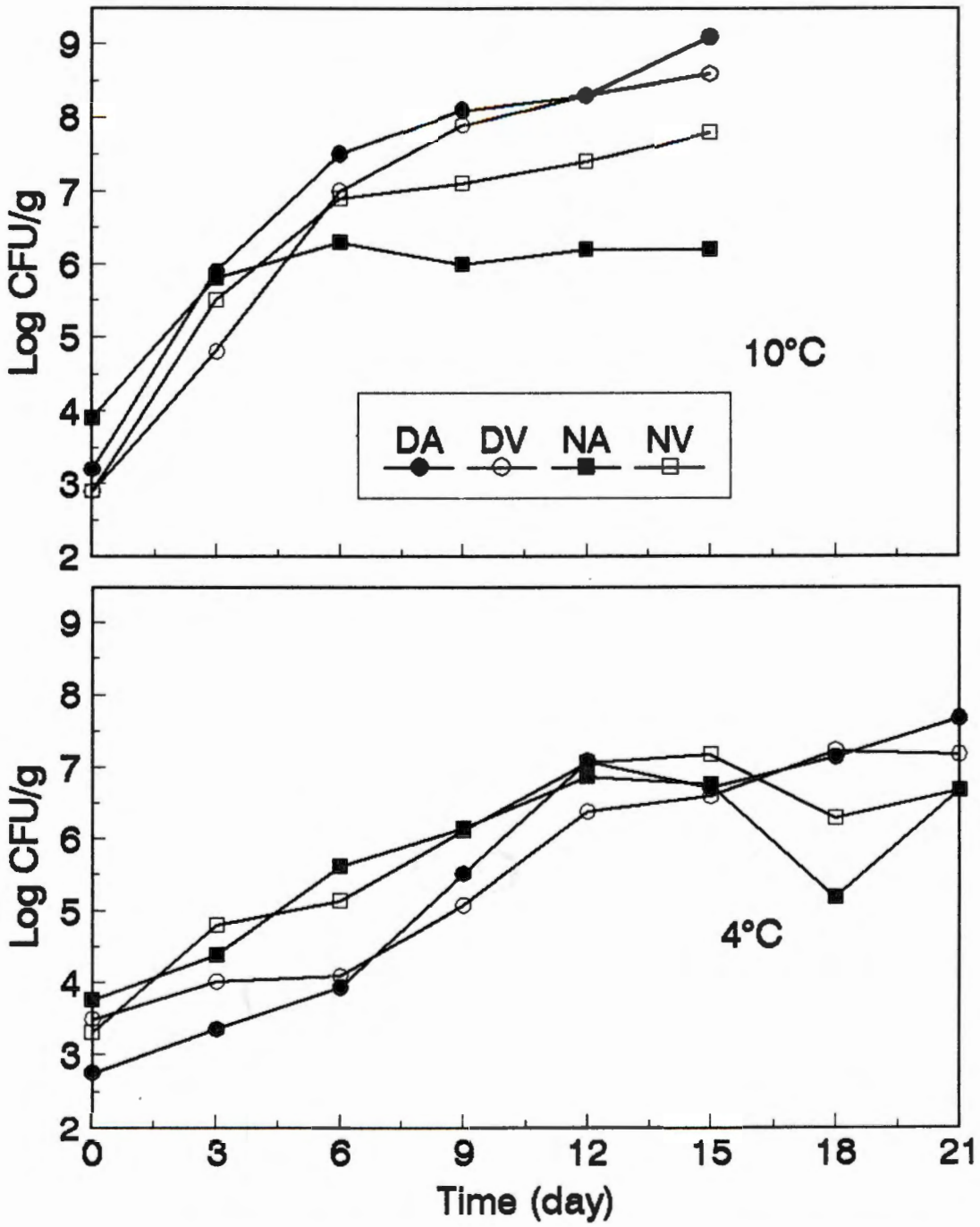

Fig. 3. Growth of coliforms in osmotically dehydrated or non-dehydrated carrot slices held at 4 or 10°C under air or vacuum packaging. [DA(V)=dehydrated under air(vaccum); NA(V)=non-dehydrated under air(vacuum).]

CFU/g for that non-dehydrated (Table A22). Packaging conditions had no significant effect on growth of coliforms when held at 4 or 10°C ($P > 0.05$) (Tables A3, A14, A11 and A22).

The Enterobacteriaceae isolates found in vegetables are common in soil and therefore do not necessarily represent a public health concern (Torriani and Massa, 1994), but outbreaks of *Escherichia coli* O157:H7 in vegetables have been reported in America (Besser et al., 1993; Dadmye and Doyle, 1992; Steele et al., 1982) and it is necessary to pay attention to the rapid growth of coliforms in packaged minimally processed carrots. The need for strict hygienic procedures in all processing phases must be stressed. Washing with chlorine at the beginning of processing did not effectively lower the coliform levels or affect their growth. Other appropriate sanitation methods, such as immersion in a solution of potassium sorbate after chlorination, correct sanitization of instruments, and personal hygiene (Torriani and Massa, 1994) should be added to improve the microbiological quality of carrots.

Lactic acid bacteria. Figure 4 shows that growth of lactic acid bacteria increased significantly with storage time ($P < 0.05$) (Tables A4 and A15). The lactic acid bacterial counts initially ranged from 2.1 to 2.9 log CFU/g on the carrots held at 4°C and increased to 4.6 to 8.6 log CFU/g by 21 days. Lactic acid bacteria initially ranged from 1.5 to 2.2 log CFU/g on carrots held at 10°C and increased to 3.6 to 7.8 log CFU/g by 15 days at 10°C (Figure 4), respectively. Salt treatment significantly stimulated the growth of lactic acid bacteria ($P < 0.05$, Table A11 and A22). Lactic acid bacteria grew faster and reached significantly ($P < 0.05$) higher maximum numbers (8.0 - 8.6 log CFU/g at 4°C and 7.8 log

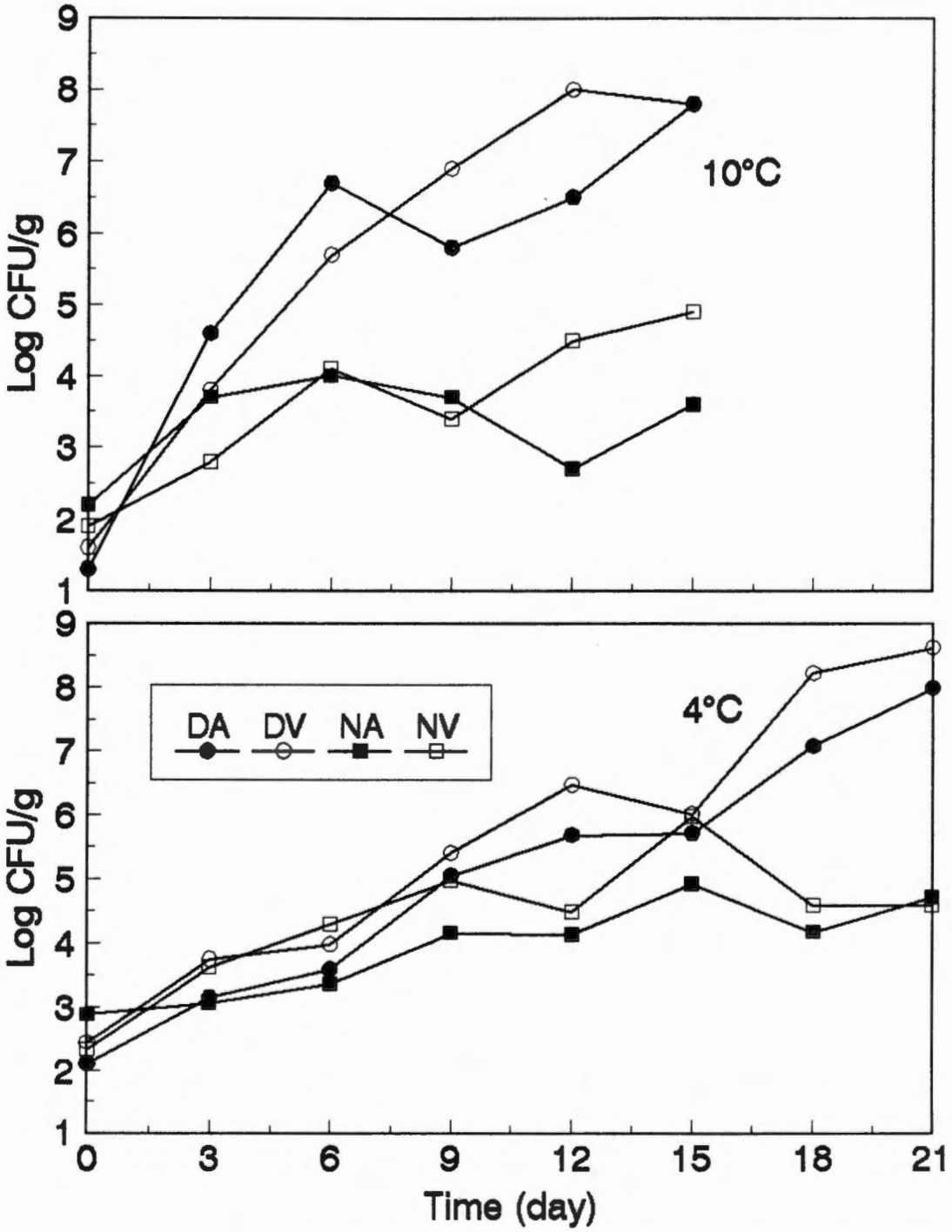

Fig. 4. Growth of lactic acid bacteria in osmotically or non-osmotically dehydrated carrot slices held at 4 or 10°C under air or vacuum packaging. [DA(V)=dehydrated under air(vacuum); NA(V)=non-dehydrated under air(vacuum).]

CFU/g at 10°C) in osmotically treated carrot samples than that in non-salt treated samples (4.6 - 4.7 at 4°C and 3.6 - 4.9 log CFU/g at 10°C) (Figure 4; Tables A4 and A15). The mean counts of lactic acid bacteria for dehydrated carrots were 5.2 log CFU/g at 4°C and 5.6 log CFU/g at 10°C. For non-dehydrated carrots mean counts were 4.1 log CFU/g at 4°C and 3.5 log CFU/g at 10°C (Tables A11 and A22). Packaging conditions did not significantly affect the growth of lactic acid bacteria at 10°C ($P > 0.05$) (Tables A4 and A15). However, significantly greater growth ($P < 0.05$) of lactic acid bacteria occurred in vacuum packaging at 4°C. The mean lactic acid bacterial levels were 4.9 log CFU/g for vacuum packaging and 4.4 log CFU/g for air packaging (Table A11). Garg et al. (1990) reported that lactic acid bacteria yielded low counts on most vegetables including carrot sticks. Over 80% lactic acid bacteria isolated on MRS plates they got were leuconostocs, and the others were equally divided between lactobacilli and streptococci. Carlin et al. (1989) observed that in packages with low oxygen and high carbon dioxide concentrations, microbial spoilage frequently occurred after a few days storage of shredded carrots and was mainly caused by lactic acid fermentation, associated with high counts of lactic acid bacteria, exudation and a high content of ethanol and lactic acid. The CO_2 accounted for 25% or more of the atmosphere of the pouches. The accumulation of those compounds could have been the result of the lactic heterofermentative metabolism of *Leuconostoc mesenteroides*. The addition of sodium chloride to carrots might provide a favorable growth condition to lactic acid bacteria because a moderate salt content (2-3%) is necessary to pull the sugar out of the carrot tissues for the growth of lactic acid bacteria and lactic acid bacteria are more resistant to high CO_2 concentration and appear

more competitive under modified atmosphere than the Gram-negative flora. CO_2 may have direct effects on the Gram-negative bacterial cell or through physiological modification or injury of the plant cell and increase spoilage on commodities sensitive to lactic acid bacteria (Nguyen-the and Carlin, 1994). The lactic acid bacteria may also play a very important role in the rapid deterioration and high CO_2 concentration (over 25%) in the pouches of dehydrated products in our study.

Mesophilic anaerobic sporeforming bacteria. Growth of anaerobic food poisoning bacteria is a concern with the storage of any neutral or low acid food material under low oxygen atmosphere packaging. In particular, growth of *Clostridium botulinum* and production of botulinal toxin is a public health concern. The average initial mesophilic sporeforming count of 1.7 log CFU/g increased to 3.7 log CFU/g after 21 days for carrots held at 4°C. For carrots held at 10°C, an average mesophilic sporeforming count of 0.76 log CFU/g increased to 4.3 log CFU/g after 15 days (Figure 5). The mean anaerobic counts were significantly higher for osmotically dehydrated carrots (2.9 log CFU/g for carrots held at 4°C and 3.2 log CFU/g held at 10°C) than non-osmotically treated carrots (2.2 log CFU/g for carrots held at 4°C and 2.8 log CFU/g held at 10°C) ($P < 0.05$) (Tables A11 and A22). The mean count of 3.2 log CFU/g in vacuum packaged carrots was higher than that of 2.8 log CFU/g in air packaged carrots at 10°C(Table A22), but there was no significant different at 4°C (Table A11). Mesophilic sporeforming anaerobic bacteria did not increased significantly ($P > 0.05$) at 4°C in carrots packaged under vacuum. Johnston (1979) reported no evidence of botulinum toxin production in vacuum packed fresh celery after eight weeks storage at 7 and 21°C, and suggested nutrient

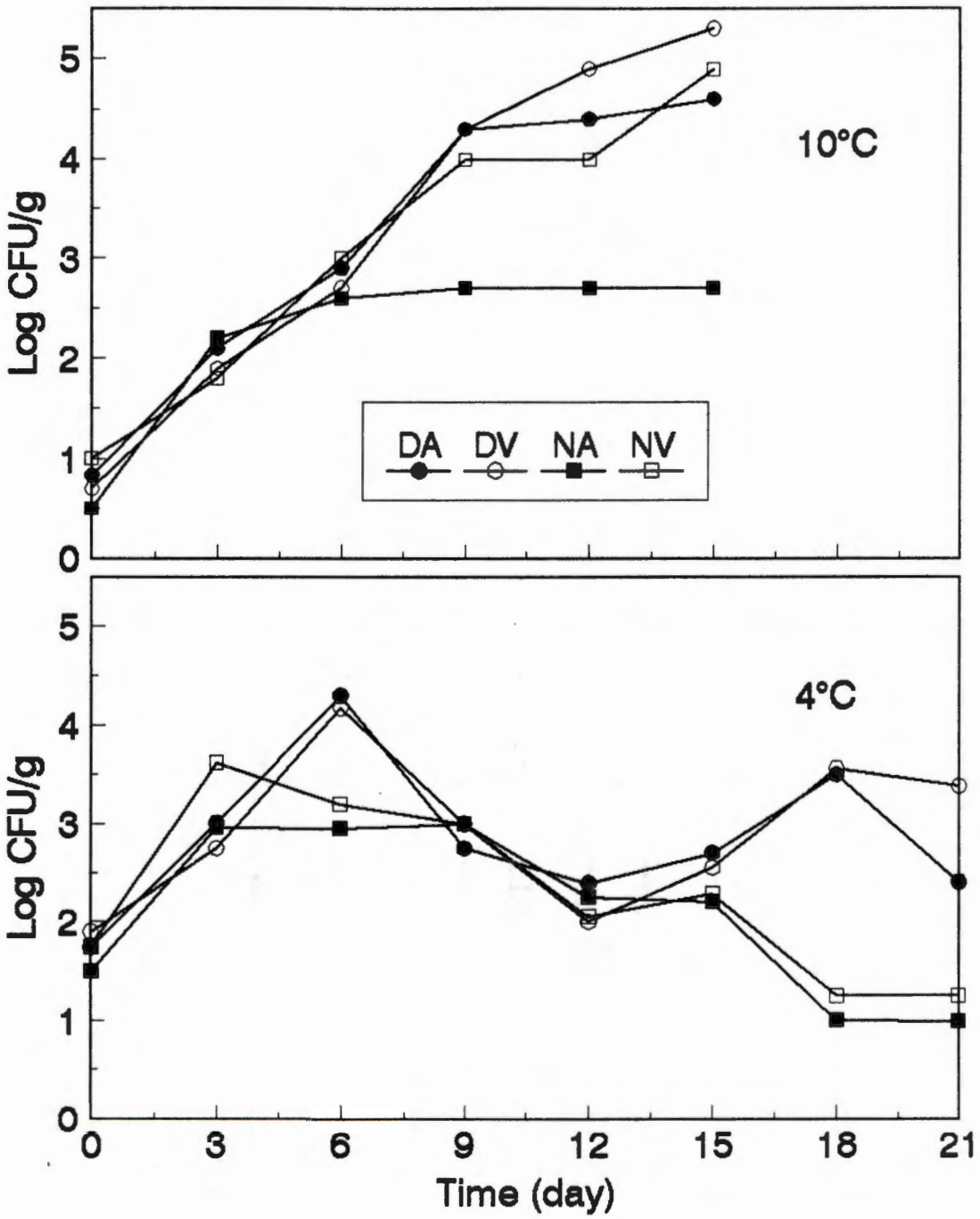

Fig. 5. Growth of mesophilic sporeforming anaerobic bacteria in osmotically dehydrated or non-dehydrated carrot slices held at 4 or 10°C under air or vacuum packaging. [DA(V)=dehydrated under air(vacuum); NA(V)=non-dehydrated under air(vacuum).]

deficiency as a probable cause of the celery being unable to support *C. botulinum* growth. Sugiyama and Yang (1975) demonstated that mushrooms could support botulinum toxin production when heavily inoculated with *C. botulinum* spores at 10^3-10^5 CFU/g, overwrapped with Polyvinyl chloride stretch film and incubated at 20°C. *C. botulinum* spores were also isolated from 13.6% cabbage samples stored at room temperature under modified atmosphere (Solomon et al., 1990). The results of our study showed that storage time and osmotic dehydration significantly increased the levels of anaerobic flora held at 10°C ($P < 0.05$) (Figure 5; Tables A5 and A16). Packaging conditions significantly affected the bacterial counts held at 10°C ($P < 0.05$) (Table A16), but not at 4°C ($P > 0.05$) (Table A5). It would appear that the higher storage temperature of 10°C should be avoided to prevent the growth of *C. botulinum* in low oxygen packs. Temperatures of 4°C and below are suggested for the storage of minimally processed carrots under MA to prevent growth of mesophilic anaerobic sporeforming bacteria.

Pectinolytic organisms. Pectinolytic organisms are very important in the spoilage of fresh vegetables. Initial numbers of pectinolytic organisms on carrots ranged from 1.6 to 3.1 log CFU/g (Figure 6). Osmotic dehydration of carrots significantly reduced growth of pectinolytics on carrots held at 4°C ($P < 0.05$), but had less effect at 10°C ($P > 0.05$) (Tables A6 and A17). The mean counts of pectinolytic bacteria were 5.3 log CFU/g for non-dehydrated samples and 4.1 log CFU/g for dehydrated samples held at 4°C (Table A11). Pectinolytic bacteria increased approximately 2.3 log CFU/g after 21 days storage at 4°C, and 3.7 log after 21 days storage at 10°C (Figure 6). The packaging atmosphere did not significantly affect the growth of pectinolytic bacteria ($P > 0.05$) (Tables A6 and

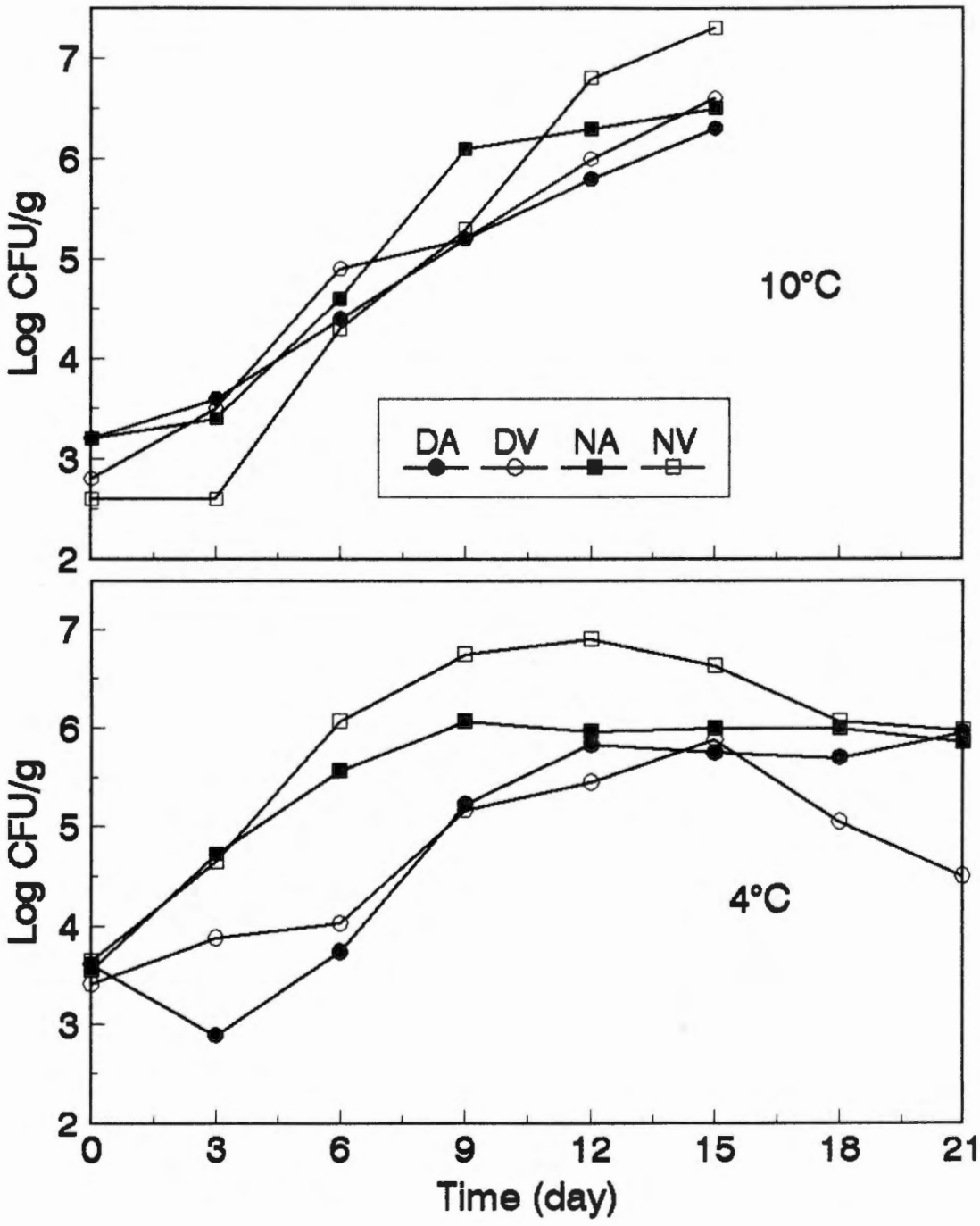

Fig. 6. Growth of pectinolytic organisms in osmotically dehydrated or non-dehydrated carrot slices held at 4 or 10°C under air or vacuum packaging. [DA(V)=dehydrated under air(vacuum); NA(V)=non-dehydrated under air (vacuum).]

A17) at both 4 and 10°C. Pectinolytic microorganisms are numerous in vegetables, but attempts to prove their role in spoilage have not been always successful (Nguyen-the and Carlin, 1994). Carlin et al.(1989) demonstrated that pectinolytic pseudomonads were not the main cause of spoilage of packaged shredded carrots during storage. Pectinolytic yeasts were isolated from pouches of grated carrots at low frequency and only at the beginning of storage. High CO_2 concentration of 15 to 20% can efficiently delay development of pectinolytic organisms and reduce the extent of decay during storage of whole fruits and vegetables (El-Goorani and Sommer, 1981). Therefore, it is unlikely that they played an important role in the spoiled products held under high CO_2 (Babic et al., 1992). They suggested that MA might be applied whenever pectinolytic organisms have a significant role in spoilage. However, the results of our study show that vacuum packaging which contained up to 30% CO_2 did not significantly affect pectinolytic bacteria in cut carrots although a trend was noticed in which higher CO_2 containing samples had lower pectinolytic numbers.

Yeasts and molds. The role of yeasts in spoilage of processed vegetables has received little attention (Nguyen-the and Carlin, 1994). In many foodsuffs, yeasts cause deterioration through fermentative production of off-odors, ethanol and high CO_2 (Babic et al., 1992; Carlin et al., 1990). Initial numbers of yeasts were 2.0-3.0 log CFU/g (Figure 7) increased approximately 3 log CFU/g at 4°C for 21 days and 2.4 log CFU/g at 10°C after 15 days (Figure 7). The osmotic dehydration significantly decreased the growth of yeasts on carrots held at 4°C ($P = 0.13$), but significantly ($P < 0.0001$) increased the growth on carrots held at 10°C (Tables A7 and A18). The mean counts of yeasts was

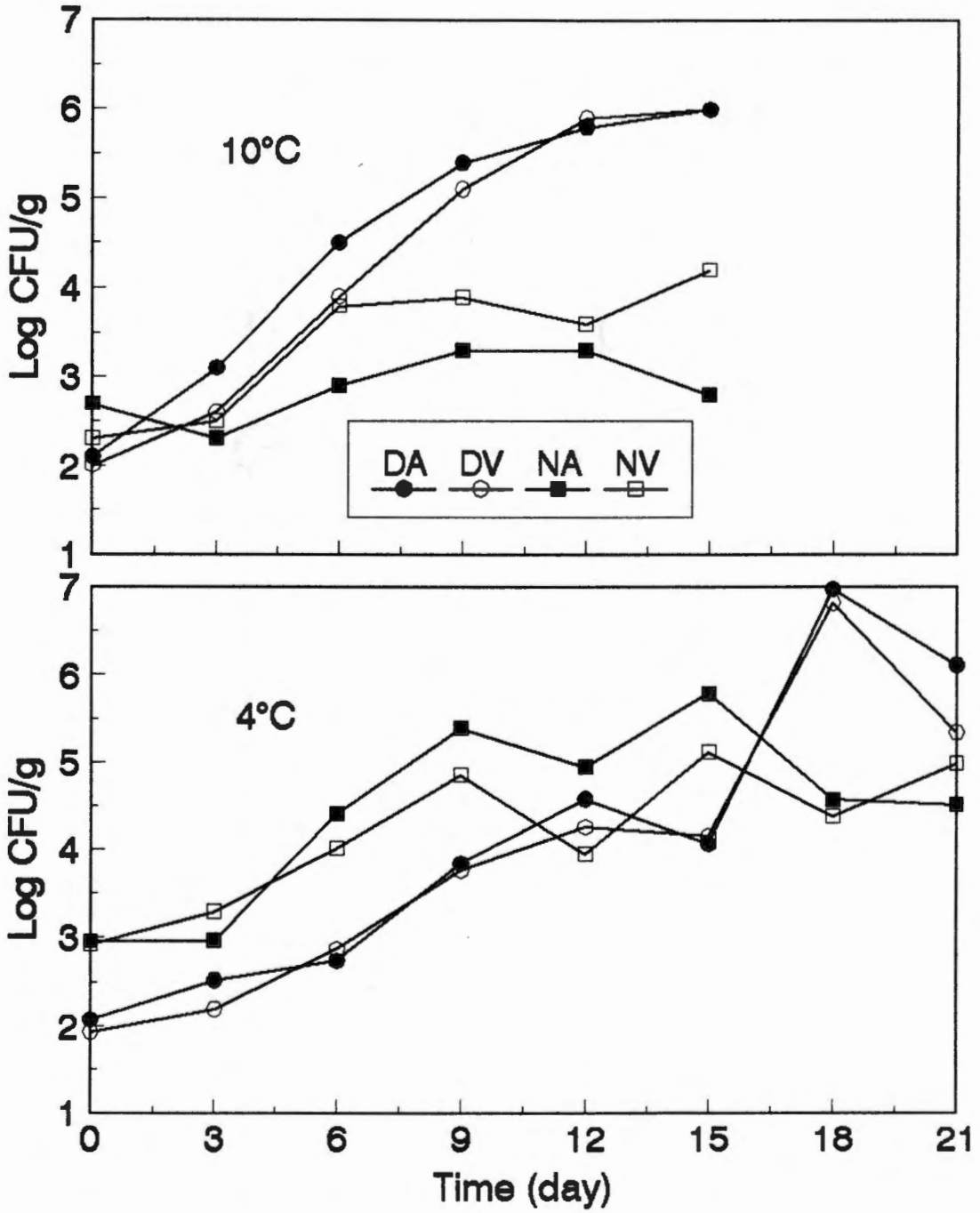

Fig. 7. Growth of yeasts in osmotically dehydrated or non-dehydrated carrot slices held at 4 or 10°C under air or vacuum packaging. [DA(V)=dehydrated under air(vacuum); NA(V)=non-dehydrated under air(vacuum).]

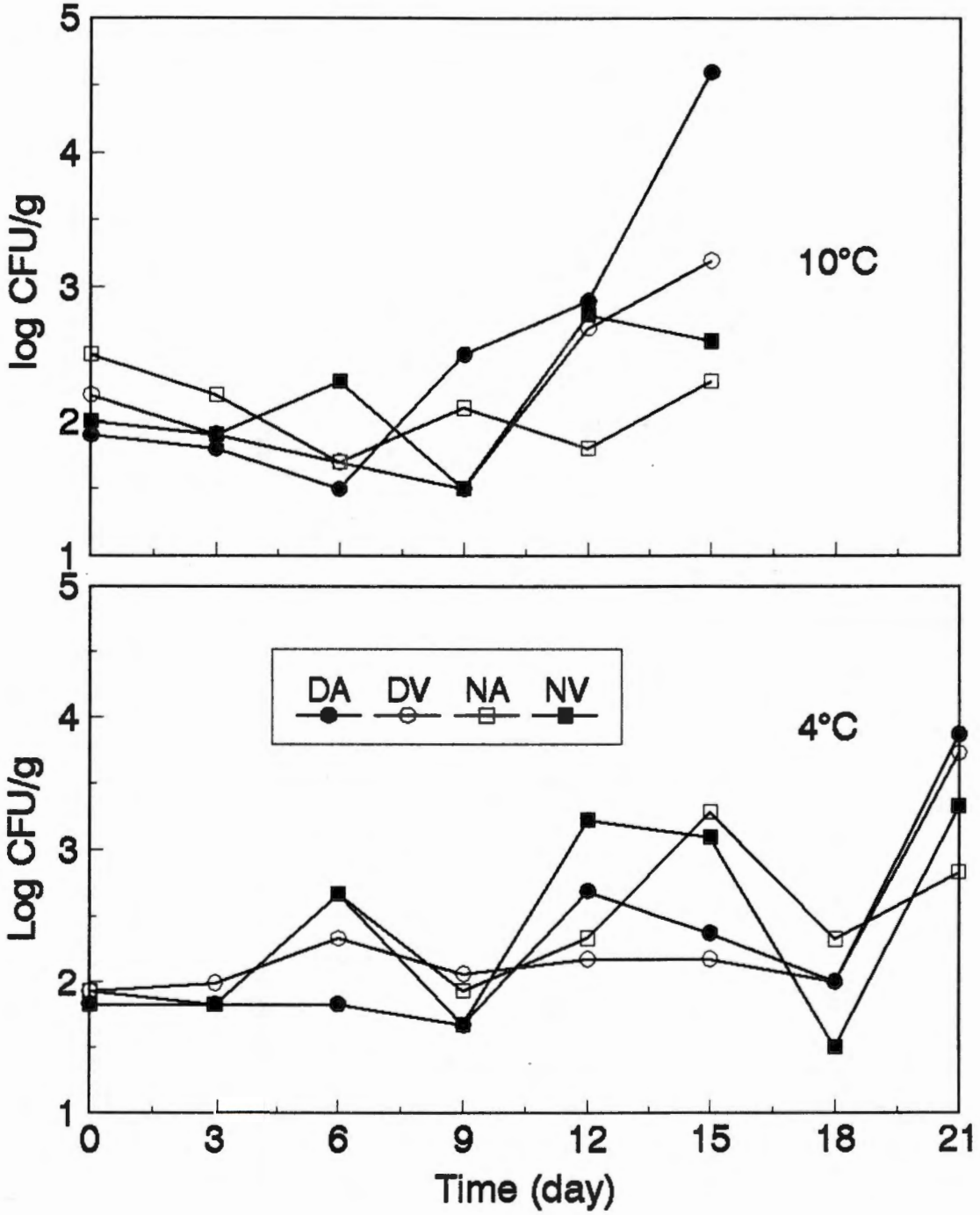

Fig. 8. Growth of molds in osmotically dehydrated or non-dehydrated carrot slices held at 4 or 10°C under air or vacuum packaging. [DA(V)=dehydrated under air(vacuum); NA(V)=non-dehydrated under air(vacuum).]

4.3 log CFU/g for carrots dehydrated and 3.1 log CFU/g for carrots non-dehydrated at 10°C (Tables A11 and A22). Packaging atmosphere did not significantly affect the growth of yeasts held at 4 and 10°C (Tables A7 and A18). Yeasts on minimally processed vegetables are generally unaffected by modified atmospheres, and no differences in yeast counts were found among grated carrots stored under modified atmospheres containing from 20 to 50% CO_2 and from 10 to 2% O_2 at 10°C (Babic et al., 1992). Carlin et al. (1990) reported that CO_2 accumulation in pouches is an important factor in deterioration of grated carrots. In the research of Babic et al. (1992), all yeast isolates at the end of storage of ready-to-use grated raw carrots packaged in polymeric films and stored at 10°C were fermentative species, but neither the number of yeasts nor the composition of the yeast flora were related to the deterioration of the product. In our study, oxygen concentration was very low and CO_2 accumulated rapidly after a few days. In this aspect, CO_2 production by yeasts might have contributed to the spoilage.

Carrots packaged in air or vacuum did not support the growth of molds at 4 ro 10°C until late in storage (Figure 8). Osmotic dehydration and different packaging conditions did not significantly affect the growth of molds ($P > 0.05$) (Tables A8 and A19). Modified atmosphere with high CO_2 and low O_2 concentrations should inhibit mold growth because they are aerobic microorganisms (El-Goorani and Sommer, 1981). The fluctuation of the mold counts is probably due to the uneven distribution of molds on carrot samples.

Yersinia enterocolitica. The numbers of *Yersinia enterocolitica* rapidly grew up from the ranges of 3.9 - 4.6 to 6.6 - 8.2 log CFU/g for the carrots held at 4°C after 21

days (Figure 9). Initial numbers of *Y. enterocolitica* ranged from 3.2 - 3.8 log CFU/g and increased to 6.8 - 8.9 log CFU/g held at 10°C after 15 days storage (Figure 9). Osmotic dehydration and packaging atmosphere did not significantly affect the growth of *Y. enterocolitica* at 4°C ($P > 0.05$) (Table A9). The mean counts of *Y. enterocolitica* were 6.2 log CFU/g for osmotically treated or not treated carrots, and 6.2 log CFU/g for air or vacuum packaged carrots held at 4°C (Table A11). Osmotic dehydration significantly increased the growth of *Y. enterocolitica* for carrots held at 10°C ($P < 0.05$) (Table A20 and A28), and the mean counts were 7.0 log CFU/g for carrots dehydrated and 6.4 log CFU/g for carrots which were non-dehydrated (Tables A22). But packaging conditions had less effect on the growth of *Y. enterocolitica* ($P > 0.05$) (Tables A9) on carrots held at 10°C. Increased storage temperature greatly reduced the lag phase and increased maximum counts, especially, for the osmotically dehydrated carrots (Figure 10). The mean counts were 7.0 for corrots held at 10°C and 6.2 log CFU/g for carrots held at 4°C (Tables A29). Congo red assay and salicin fermentation test found that these bacteria were still pathogen and retained their plasmids. *Y. enterocolitica* has been isolated from vegetables, but the strains were recognized as nonpathogenic on the basis of their serovar (Nguyen-the and Carlin, 1994). However, Brocklehurst et al. (1987) reported a pathogenic strain was isolated from packed salad vegetables. Vegetables are probably minor sources of foodborne diseases when compared with other foods. From 1973 to 1987 in the U.S., vegetables were implicated in only 2% of cases of identified origin (Bean and Griffin, 1990). However, consumption of minimally processed vegetables has increased dramatically in the last five years. Minimally processed vegetables are

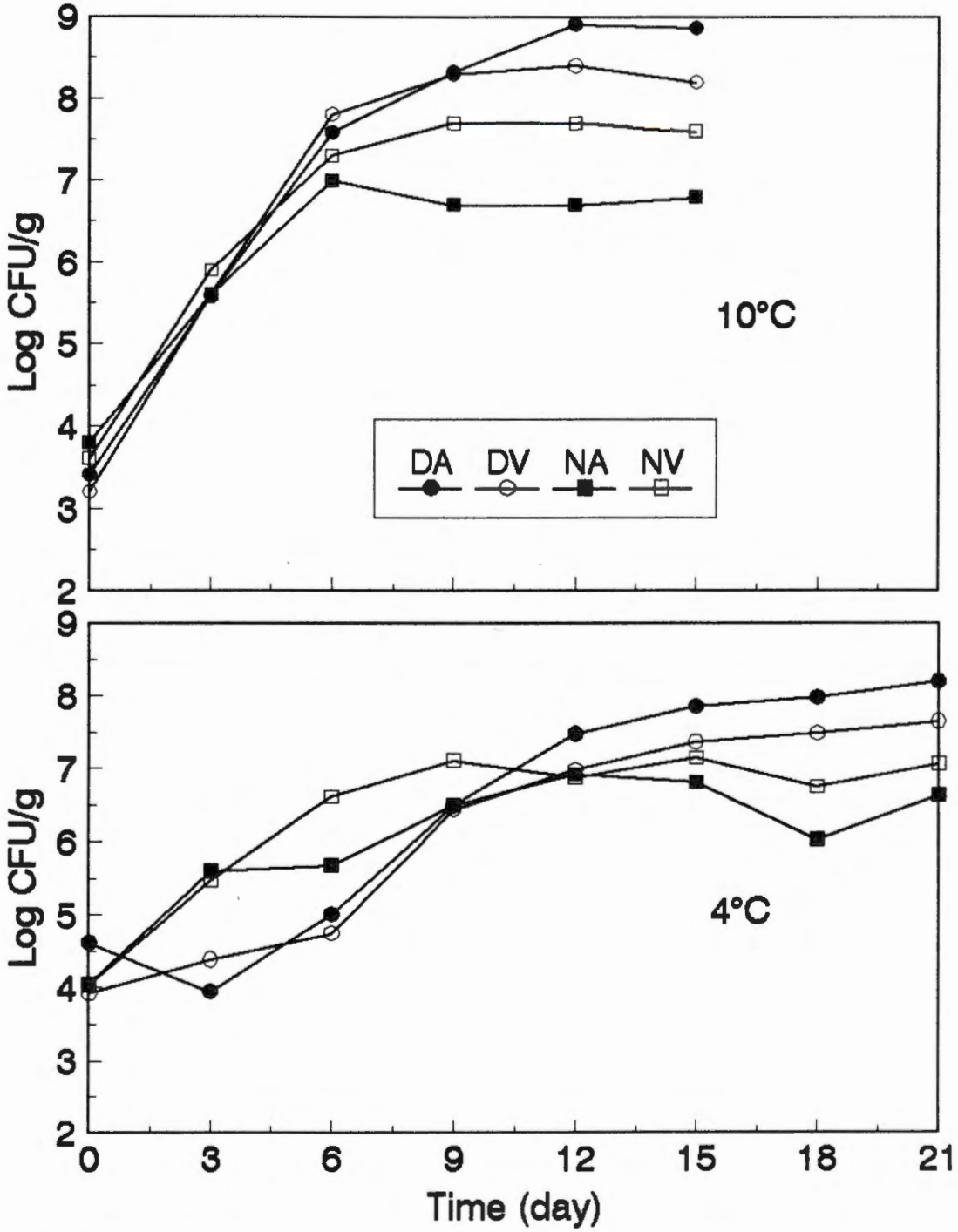

Fig. 9. Growth of *Yersinia enterocolitica* in osmotically dehydrated or non-dehydrated carrot slices held at 4 or 10°C under air or vacuum packaging. [DA(V)=dehydrated under air(vacuum); NA(V)=non-dehydrated under air(vacuum).]

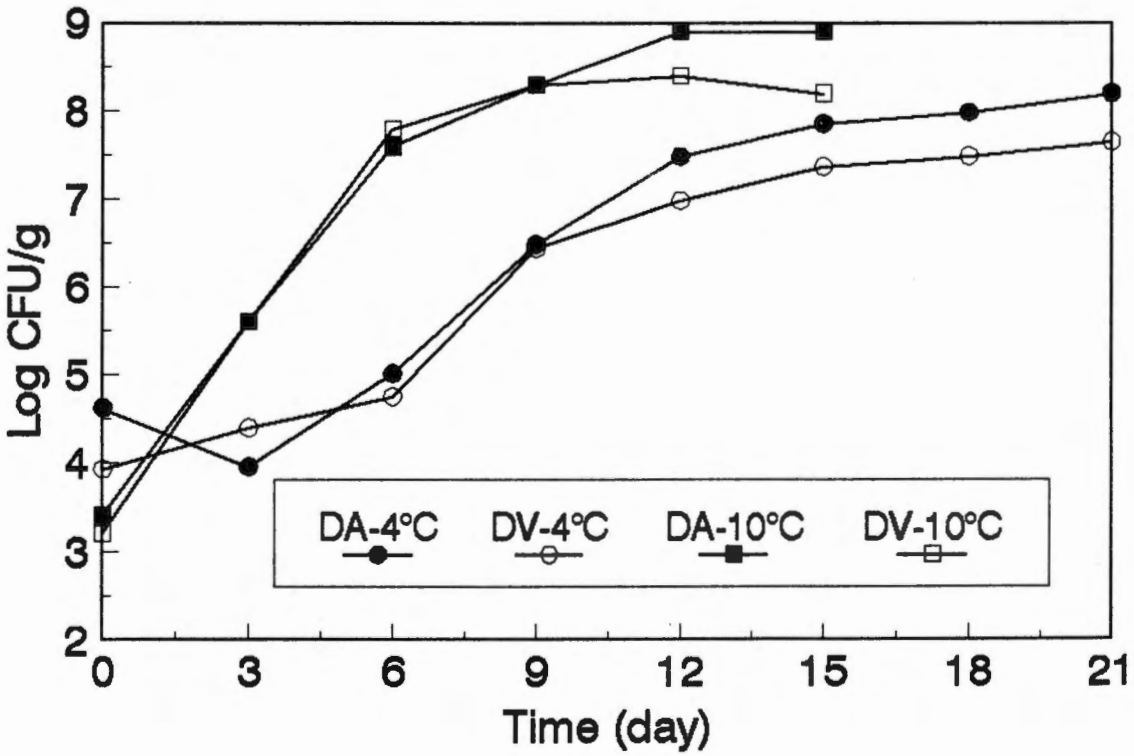

Fig. 10. Growth of *Yersinia enterocolitica* in osmotically dehydrated carrot slices held at different temperatures under air or vacuum. [DA(V)=dehydrated under air(vacuum).]

ready-to-eat foods and the cut surfaces and high moisture level could provide *Y. enterocolitica* with good conditions for growth. High CO_2 levels in packages of carrots and inadequate temperature control during refrigeration storage might favor the growth of *Y. enterocolitica* because it is a facultatively anaerobic and psychrotrophic bacterium. Contamination of vegetables by foodborne pathogens frequently occurs throuth agricultural practices and processing lines. From these data, modified atmosphere combined with osmotic dehydration does not seem to be an effective way to reduce multiplication of *Y. enterocolitica* in minimally processed carrots. Additional methods for control of *Y. enterocolitica* should be studied to find ways to inhibit the growth of this pathogen in minimally processed carrots.

Headspace gas analysis

Sealed polymeric film plastic packaging of minimally processed vegetables creates a modified atmosphere containing a higher CO_2 concentration and lower O_2 concentration than in air. This modified atmosphere can be established by the respiration of the commodity (Kader et al., 1989) or microorganisms. The initial level of 21% O_2 in the air containing packages decreased to approximately 2-3%, respectively, for dehydrated carrots, and to approximately 4-7%, for non-dehydrated carrots at 4 and 10°C, respectively. The initial level 0.6% CO_2 increased to approximately 12% for dehydrated carrots and 3-7% for non-dehydrated carrots at 4 and 10°C. There was a rapid decline in O_2 from 21% to approximately 4-8% along with an increase in CO_2 from 0.3-0.6 to apporoximately 5-6% over the first 6 days storage at 4°C (Figures 11 and 12) due to the

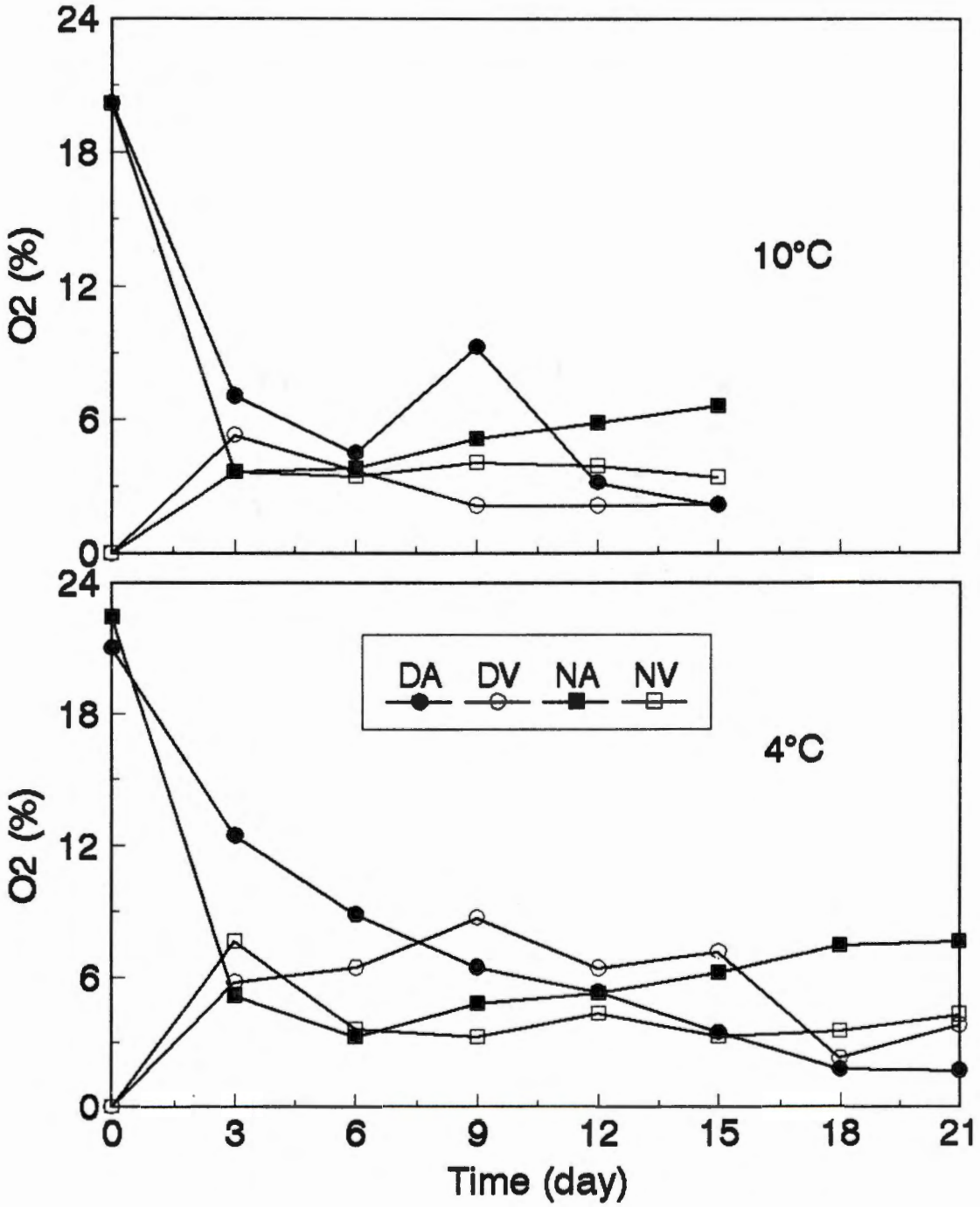

Fig. 11. O_2 content of headspace in air or vacuum packaging of osmotically dehydrated or non-dehydrated carrot slices held at 4 or 10°C. [DA(V)=dehydrated under air(vacuum); NA(V)=non-dehydrated under air or vacuum.]

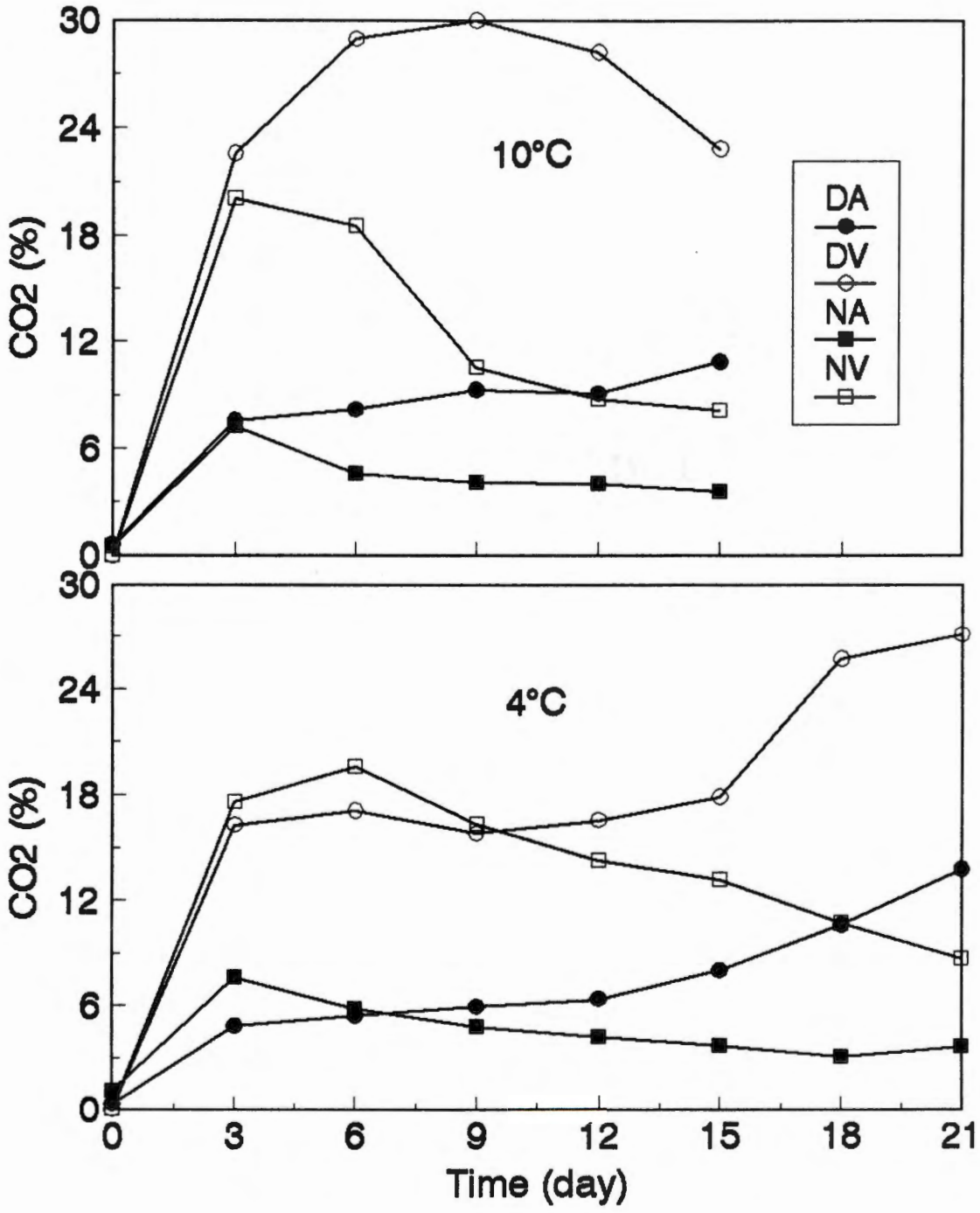

Fig. 12. CO_2 content of headspace in air or vacuum packaging of osmotically dehydrated or non-dehydrated carrot slices held at 4 or 10°C. [DA(V)=dehydrated under air(vacuum); NA(V)=non-dehydrated under air(vacuum).]

respiration of plant tissues and microorganisms of packages. The air content of non-dehydrated carrots reached approximate equilibrium at 4-8% O_2 and 4-5 CO_2 after 6 days until the end of storage. In vacuum packages, CO_2 rapidly increased to approximately 17% over the first 3 days storage and then reduced from 19% to 8% by 21 days storage(Figure 11) due to the interaction between the gas permeability of packaging film and the respiration of plant tissues and organisms. A large amount of O_2 was rapidly exhausted by rapid respiration of carrots with a large amount of CO_2 produced. As the concentration of CO_2 increased, the reduction of O_2 and the increase rate of CO_2 were reduced (Figures 11 and 12). Finally, a new equilibrium was established between the respiration of carrot tissues and organisms and the permeability of gases through the packaging film. In effect, a modified atmosphere environment was present in these packs. The beneficial effects of high CO_2 in the MA storage of vegetables are generally thought to arise through a suppression of respiration (Kader, 1989). However, the report of Kubo et al. (1990) showed that high CO_2 affected the respiration rate little if at all in vegetables such as carrots that do not produce measurable levels of C_2H_4 because the high CO_2 mainly affects the action and/or synthesis of C_2H_4. The higher storage temperature (10°C) significantly increased the respiration rates of plant tissues and organisms within osmotically treated carrots ($P < 0.05$) and a higher concentration of CO_2 and a lower O_2 level were obtained at 10°C (Figures 11-12; Tables A30-A33). The mean CO_2 contents were 14.73% for the packages held at 10°C and 11.91% for the packages held at 4°C. The mean O_2 levels were 4.69% for the packages held at 10°C and 6.18% for the packages held at 4°C (Tables A31). Osmotic dehydration significantly increased th CO_2

content of packages ($P < 0.05$) (Tables A23-A24). The mean CO_2 levels of packages were 14.73% in dehydrated carrots and 7.43% in non-dehydrated carrots. The O_2 levels of atmospheres were 4.69% in dehydrated carrots and 5.33% in non-dehydrated carrots held at 10°C. The mean CO_2 levels of atmospheres were 11.91% in dehydrated carrots and 7.89% in non-dehydrated carrots (Table A27), but the O_2 levels of packages were not significantly affected by dehydration at 4°C ($P > 0.05$) (Table A23). The increase in respiration rate of carrots might be due to the physiological injury of carrot tissue by osmotic dehydration and the rapid growth of lactic acid bacteria and yeasts in the osmotically dehydrated carrot packages. The packaging conditions significantly contributed to the gas contents of packages ($P < 0.05$) (Tables A23-A24). The mean CO_2 levels were 16.14% and 14.78% in vacuum packages and 6.16% and 5.68% in air packages held at 10 and 4°C, respectively. The O_2 levels were 2.90% and 4.62% in vacuum packages, and 7.0% and 7.75% in air packages held at 10 and 4°C, respectively (Table A27). The highest CO_2 concentrations (27% and 30%) occurred in osmotically dehydrated carrots with vacuum packaging held at 4 and 10°C. This is the exact opposite of the results expected since osmotic dehydration generally reduces respiration of vegetables. The results indicated that the film system used might be inadequate to provide total gas exchange in the osmotically dehydrated samples at the higher storage temperature. However, the most reasonable explanation may be due to increased CO_2 from microbial action in osmotically dehydrated carrots. Figure 4 shows the increased growth of lactic acid bacteria in osmotically dehydrated carrots. Since a major metabolic product of heterofermentative lactics is CO_2 , they probably contributed significant

amounts of CO_2 to the overall atmosphere inside packages. The dehydrated carrot samples stored at 10°C were spoiled more quickly than the others. The researches (Carlin et al., 1990; Nguyen-the and Carlin, 1994) showed that many minimally processed vegetables, such as shredded carrots, were better maintained under moderately high CO_2 concentrations (5 to 20%) and moderately low O_2 concentrations (2 to 5%), and the right packaging conditions can be an efficient and simple way to delay microbial development and spoilage. Anaerobic conditions developed more rapidly as respiration rate increased and O_2 consumption was increased rapidly when temperature was increased from 4 to 10°C (Figures 11-12). Therefore, film permeability was not sufficient to balance the consumption of O_2. This risk was increased in cut and osmotically dehydrated carrots because cutting increased the respiration rate and salt dehydration caused the rapid growth and CO_2 production of lactic acid bacteria and yeasts.

Appearance of Carrots

The average initial salt content of osmotically treated carrot slices was 1.78%. The water activity of salt treated carrots was 0.981. The salt content and water activity of non-dehydrated carrots were evaluated as 0.00% and 0.999, respectively. As cell membranes of carrot tissue are not completely semi-permeable, and a large proportion of cells were damaged during cutting, a 1.78% salt uptake to the tissue and a 0.081 unit reduction of water activity of samples occured during 5 min soaking in 10% salt solution. Osmotic dehydration produced a softening of tissue and deep, uniform color of the carrot slices due to the removal of water from carrots. General organoleptic appearance of the

samples was acceptable. Observation showed that osmotically dehydrated carrots spoiled earlier than non-dehydrated samples. The osmotic dehydration greatly promoted the spoilage of carrots because salt treatment appeared to cause physiological damage of carrot tissues and increased the respiration rate and moisture level of the surfaces of carrots or stimulated growth of microorganisms, especially, lactic acid bacteria. During the storage peroid, the osmotically dehydrated carrots had a more uniform appearance and deeper orange color than did non-dehydrated carrot samples which had a bright color and crispy texture, but later showed signs of drying out with a development of white materials on the surface after 12 days storage. The osmotically dehydrated carrots under vacuum at 4°C appeared spoiled after 12 days storage with a wet surface, softening texture and dark-colored center. The dehydrated carrots under air held at 4°C looked better with a deep and uniform color, and moist surfaces, but had softer texture than that of non-dehydrated carrots. All samples with osmotic dehydration were spoiled with dark color, wet surfaces and off-odor after 15 days storage at 4°C. The carrots without dehydration did not appear spoiled, but the surface appeared dried out with a rough texture with white materials on the surfaces until 21 days storage at 4°C. The 10°C storage temperature accelerated the spoilage of carrot samples dehydrated. The samples under air or vacuum began to be spoiled after 9 days storage. The non-dehydrated carrots were not spoiled until 15 days storage, but the surfaces were dry, shrinked with white materials and uneven color after 12 days storage. The carrots with vacuum packaging had a better appearance than samples under air because the vacuum condition prevented moisture loss. Howard and Griffin (1993) reported that lignin formation occured and corresponded with development of

white discoloration in storage of carrot slices and sticks in packs. Two key enzymes in the lignin pathway, phenylalanine ammonia-lyase and peroxidase were stimulated by wounding and presumably were important in lignification. Salt dehydration might have affected the activities of the enzymes and prevented the formation of the white materials. Also, high relative humidity inside the packages of dehydrated carrots may create an environment that retarded moisure loss and tissue dehydration. However, osmotic dehydration of carrots stimulated growth of spoilage organisms and the respiration of plant tissues due to the physiological damage of the plant tissues and selectivity of NaCl for lactic acid bacteria, and accelarated the spoilage speed of the treated carrots.

Replication differences

There was a significant difference between replications of the psychrotrophic, pectinolytic, yeast, and *Y. enterocolitica* counts at 4°C (Tables A2-A9), aerobic, psychrotrophic, coliform, anaerobic, yeast, and *Y.enterocolitica* counts at 10°C (Tables A12-A20). Mean separations of the microbial counts according to replication are shown in Tables A10 and A22. A general pattern observed was that replication one was significantly different from two and three ($P < 0.05$). This might be attributed to sampling across a seasonal change by using a different group of carrots for each replication, and replication two and three were closer together in time than replication one.

Chapter V

CONCLUSIONS

In this study, osmotic dehydration and a storage temperature of 10°C accelerated the spoilage and shortened the shelf life of minimally processed carrot slices when packaged in modified atmospheres and held at 4 or 10°C. The packaging conditions were not an effective way to prevent growth of most organisms. A comparison between data for dehydrated and non-dehydrated samples showed that the dehydrated carrots were spoiled after 12 days stored at 4°C and after 9 days at 10°C, respectively, but the non-dehydrated carrots were not spoiled during the storage time at either temperature. The growth of psychrotrophic flora held at 4 or 10°C closely resembled that of mesophilic flora held at the same conditions because vegetables contained significant numbers of psychrotrophs that were comparable with mesophilic bacteria. Although growth of bacteria in dehydrated carrots was suppressed during first 9 days storage at 4°C, the final maximum numbers of mesophilic and psychrotrophic bacteria, coliforms, and *Yersinia enterocolitica* were higher than that of non-dehydrated samples. The growth of these bacteria was stimulated and the shelf life of products was shortened by the higher storage temperature from 4°C to 10°C, particularly, in dehydrated samples. The osmotic dehydration greatly stimulated growth of lactic acid bacteria held at 4 and 10°C because sodium chloride and reduced oxygen conditions provide favorable growth condition for lactic acid bacteria. The rapid growth and CO_2 production of lactic acid bacteria may play a key role in causing the faster spoilage of dehydrated carrots. Growth of mesophilic

sporaforming anaerobic bacteria was suppressed when the samples were stored at 4°C regardless of sample treatment. The higher storage temperature and dehydration also caused a increase in the population of anaerobes, especially, in vacuum packaging which contained low O_2 and high CO_2 concentrations. Modified atmosphere packaging with the higher storage temperature (10°C) could increase the risk of botulinum poisoning in vegetables since anaerobic sporeforming bacteria grew well under these conditions. Therefore, a storage temperature of 4°C or below is strongly recommended for storing minimally processed carrots. Growth of pectinolytic organisms was inhibited by salt treatment when carrots were held at 4°C. The higher storage temperature also increased their growth; however, the role of pectinolytic organisms in spoilage of carrots was not clear. Yeasts may be the other important spoilage organisms in minimally processed carrots, possibly due to their sugar content and the high CO_2 concentration of headspace gases. The role of yeasts in spoilage of carrots under modified atmospheres should be further investigated. Molds were not an important spoilage organisms in carrots under modified atmospheres because high CO_2 concentration in packaging inhibited their growth. The rapid growth of *Yersinia enterocolitica* in carrots under modified atmospheres held at both temperatures was of great concern. The modified atmospheres, refrigeration temperature and salt dehydration were not an effective way to inhibit its growth due to its psychrotrophic and facultative capabilities. Minimally processed vegetables are ready-to-eat foods. The cut surfaces and high moisture level, with anaerobic condition, provided *Y. enterocolitica* with good growth conditions. An additional barrier(s) is necessary to prevent growth of *Y. enterocolitica* during storage of

the products. The increased temperature and osmotically dehydration caused a increased respiration rate of plant tissue and microbial metabolism resulting in high CO_2 concentrations in packaging. This caused a decrease in shelf life of products due to the physiological damage to plant tissue and rapid growth of lactic acid bacteria and yeasts. A better film system might to provide better gas exchange in the osmotically dehydrated carrot slices held at 10°C. However, the film used was one of the high oxygen permeable films currently available. Another alternative which could increase quality of the product is to incorporate a CO scavenger into the packaging system, thus reducing CO_2 in headspace gases. Although osmotic dehydration increased the spoilage of carrots, it provided a way to prevent moisture loss and discoloration of carrot surfaces during storage. This might be a new clue for improving appearence of carrots during cold storage.

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APPENDIX

Table A1. Anova table for aerobic plate count in carrot slices stored at 4°C

Source	DF	Mean square	Prob > F
Day	7	23.9683	0.0001
Atmosphere	1	0.2114	0.3342
Osmotic dehydration	1	5.6059	0.0001
Replication	2	0.6306	0.0684
Day*Atmosphere	7	0.2449	0.3769
Day*Osmotic	7	2.6718	0.0001
Atmosphere*Osmotic	1	0.0729	0.5694
Error	48	0.2222	

Table A2. Anova table for psychrotrophic count in carrot slices stored at 4°C

Source	DF	Mean square	Prob > F
Day	7	26.4554	0.0001
Atmosphere	1	0.0822	0.5576
Osmotic dehydration	1	7.4328	0.0001
Replication	2	1.6671	0.0020
Day*Atmosphere	7	0.2061	0.5335
Day*Osmotic	7	3.3018	0.0001
Atmosphere*Osmotic	1	0.1628	0.4100
Error	48	0.2357	

Table A3. Anova table for coliform count in carrot slices stored at 4°C

Source	DF	Mean square	Prob > F
Day	7	23.5746	0.0001
Atmosphere	1	0.0484	0.6763
Osmotic dehydration	1	2.2383	0.0063
Replication	2	0.8049	0.0627
Day*Atmosphere	7	0.3348	0.3101
Day*Osmotic	7	2.1301	0.0001
Atmosphere*Osmotic	1	0.0908	0.5677
Error	48	0.2742	

Table A4. Anova table for lactic acid bacteria in carrot slices stored at 4°C

Source	DF	Mean square	Prob > F
Day	7	22.5500	0.0001
Atmosphere	1	5.4150	0.0353
Osmotic dehydration	1	27.5885	0.0001
Replication	2	1.6488	0.2496
Day*Atmosphere	7	0.2367	0.9827
Day*Osmotic	7	6.4396	0.0001
Atmosphere*Osmotic	1	0.0712	0.8049
Error	48	1.1541	

Table A5. Anova table for anaerobic bacteria count in carrot slices stored at 4°C

Source	DF	Mean square	Prob > F
Day	7	3.2352	0.0001
Atmosphere	1	0.2717	0.3451
Osmotic dehydration	1	7.3103	0.0001
Replicatoion	2	0.2151	0.4002
Day*Atmosphere	7	0.1310	0.8660
Day*Osmotic	7	1.9840	0.0001
Atmosphere*Osmotic	1	0.0657	0.6406
Error	29	0.2949	

Table A6. Anova table for pectinolytic bacteria in carrot slices stored at 4°C

Source	DF	Mean square	Prob > F
Day	7	16.8955	0.0001
Atmosphere	1	0.4920	0.3197
Osmotic dehydration	1	53.4970	0.0001
Replication	2	3.3210	0.0017
Day*Atmosphere	7	0.4308	0.5288
Day*Osmotic	7	1.5296	0.0050
Atmosphere*Osmotic	1	0.0400	0.7761
Error	107	0.4923	

Table A7. Anova table for yeast count in carrot slices stored at 4°C

Source	DF	Mean square	Prob > F
Day	7	15.959	0.0001
Atmosphere	1	1.2195	0.2610
Osmotic dehydration	1	2.2022	0.1328
Replication	2	24.2926	0.0001
Day*Atmosphere	7	0.1226	0.9957
Day*Osmotic	7	5.6184	0.0001
Atmosphere*Osmotic	1	0.0216	0.8804
Error	51	0.9439	

Table A8. Anova table for mold count in carrot slices stored at 4°C

Source	DF	Mean square	Prob > F
Day	7	3.7339	0.0001
Atmosphere	1	0.0486	0.7545
Osmotic dehydration	1	0.1751	0.5532
Replication	2	0.3816	0.4656
Day*Atmosphere	7	0.4476	0.5058
Day*Osmotic	7	0.7988	0.1485
Atmosphere*Osmotic	1	0.0541	0.7413
Error	51	0.4915	

Table A9. Anova table for *Yersinia enterocolitica* count in carrot slices stored at 4°C

Sources	DF	Mean square	Prob > F
Day	7	18.0109	0.0001
Atmosphere	1	0.0022	0.9183
Osmotic dehydration	1	0.0134	0.8004
Replication	2	2.1779	0.0002
Day*Atmosphere	7	0.1849	0.5198
Day*Osmotic	7	2.7910	0.0001
Atmosphere*Osmotic	1	2.4424	0.0012
Error	48	0.2071	

Table A10. Mean seperations between replication for organism counts (log_{10} CFU/g) in carrot slices stored at 4°C

Organism	Rep. 1	Rep. 2	Rep. 3
Aerobic	6.1^{b}	6.5^{a}	6.5^{a}
Psy.	5.9^{b}	6.5^{a}	6.3^{a}
Coliform	5.7^{a}	5.6^{a}	5.5^{a}
Lactic	4.6^{a}	4.9^{a}	4.5^{a}
Anaerobic	-	2.6^{a}	2.5^{a}
Pectinoytic	4.2^{b}	4.8^{a}	4.9^{a}
Yeast	3.3^{c}	4.2^{b}	5.1^{a}
Mold	2.3^{a}	2.5^{a}	2.3^{a}
Yersinia	6.5^{a}	6.1^{b}	6.1^{b}

Within each row, means with the same letter are not significantly different ($P > 0.05$) using the Student-Newman-Keuls mean seperation test.

Table A11. Mean separations between atmospheres/osmotic dehydration for organism counts (log_{10} CFU/g) in carrot slices stored at 4°C

Organism	N	Atmosphere		Osmotic dehydration	
		air	vacuum	DE	ND
Aerobic	46	6.3[a]	6.4[a]	6.1[b]	6.6[a]
Psy.	46	6.2[a]	6.3[a]	6.0[b]	6.5[a]
Coliform	46	5.6[a]	5.6[a]	5.4[b]	5.7[a]
Lactic	46	4.4[b]	4.9[a]	5.2[a]	4.1[b]
Anaerobic	32	2.5[a]	2.6[a]	2.9[a]	2.2[b]
Pectin.	40	4.7[a]	4.8[a]	4.1[b]	5.3[a]
Yeast	44	4.3[a]	4.1[a]	4.0[a]	4.3[a]
Mold	44	2.3[a]	2.4[a]	2.3[a]	2.4[a]
Yersinia	46	6.2[a]	6.2[a]	6.2[a]	6.2[a]

Within each row of each treatment, means with the same letter are not significantly different ($P > 0.05$) using the Student-Newman-Keuls mean separation test. (DE=dehydrated; ND=non-dehydrated)

Table A12. Anova table for aerobic plate count in carrot slices stored at 10°C

Source	DF	Mean square	Prob > F
Day	5	32.2588	0.0001
Atmosphere	1	0.4387	0.1199
Osmotic dehydration	1	3.3282	0.0001
Replication	2	8.0603	0.0001
Day*Atmosphere	5	0.6403	0.0082
Day*Osmotic	5	2.3124	0.0001
Atmosphere*Osmotic	1	0.0021	0.9149
Error	37	0.1731	

Table A13. Anova table for Psychrotrophic count in carrot slices stored at 10°C

Source	DF	Mean square	Prob > F
Day	5	36.1554	0.0001
Atmosphere	1	0.0059	0.8796
Osmotic dehydration	1	2.6719	0.0024
Replication	2	10.4128	0.0001
Day*Atmosphere	5	0.6154	0.0492
Day*Osmotic	5	3.2424	0.0001
Atmosphere*Osmotic	1	0.2145	0.3623
Error	37	0.2521	

Table A14. Anova table for coliform count in carrot slices stored at 10°C

Source	DF	Mean square	Prob > F
Day	5	37.5488	0.0001
Atmosphere	1	0.0517	0.6853
Osmotic dehydration	1	11.2733	0.0001
Replication	2	10.7294	0.0001
Day*Atmosphere	5	1.0248	0.0145
Day*Osmotic	5	2.7688	0.0001
Atmosphere*Osmotic	1	4.0946	0.0008
Error	37	0.3101	

Table A15. Anova table for lactic acid bacteria count in carrot slices stored at 10°C

Source	DF	Mean square	Prob > F
Day	5	29.1219	0.0001
Atmosphere	1	0.8602	0.3116
Osmotic dehydration	1	79.8637	0.0001
Replication	2	1.5334	0.1675
Day*Atmosphere	5	2.4261	0.0237
Day*Osmotic	5	8.2921	0.0001
Atmosphere*Osmotc	1	0.0847	0.7493
Error	37	0.8175	

Table A16. Anova table for anaerobic counts in carrot slices stored at 10°C

Source	DF	Mean square	Prob > F
Day	5	21.4247	0.0001
Atmosphere	1	2.5619	0.0434
Osmotic dehydration	1	3.8605	0.0145
Replication	2	7.7951	0.0001
Day*Atmosphere	5	7.7951	0.0001
Day*Osmotic	5	1.6943	0.0264
Atmosphere*Osmotic	1	1.8416	0.0845
Error	37	0.5869	

Table A17. Anova table for pectinolytic counts in carrot slices stored at 10°C

Source	DF	Mean square	Prob > F
Day	5	48.8408	0.0001
Atmosphere	1	0.8719	0.2670
Osmotic dehydration	1	2.2925	0.0733
Replication	2	1.9832	0.0636
Day*Atmosphere	5	0.7772	0.3597
Day*Osmotic	5	0.4972	0.6168
Atmosphere*Osmotic	1	1.7194	0.1202
Error	97	0.6996	

Table A18. Anova table for yeast counts in carrot slices stored at 10°C

Source	DF	Mean square	Prob > F
Day	5	13.0045	0.0001
Atmosphere	1	0.2424	0.3096
Osmotic dehydration	1	22.7362	0.0001
Replication	2	6.3718	0.0001
Day*Atmosphere	5	0.3918	0.1567
Day*Osmotic	5	3.6305	0.0001
Atmosphere*Osmotic	1	2.3794	0.0027
Error	34	0.2279	

Table A19. Anova table for mold counts in carrot slices stored at 10 °C

Source	DF	Mean square	Prob >F
Day	5	3.0596	0.1191
Atmosphere	1	2.3549	0.2344
Osmotic dehydration	1	0.0069	0.9481
Replication	2	1.0414	0.5296
Day*Atmosphere	5	1.1779	0.6042
Day*Osmotic	5	2.4693	0.2043
Atmosphere*Osmotic	1	0.0445	0.8688
Error	35	1.6088	

Table A20. Anova table for *Yersinia enterocolitica* counts in carrot slices stored at 10°C

Source	DF	Mean square	Prob > F
Day	5	37.2558	0.0001
Atmosphere	1	0.5563	0.0936
Osmotic dehydration	1	7.8847	0.0001
Replication	2	3.5206	0.0001
Day*Atmosphere	5	0.1548	0.5394
Day*Osmotic	5	1.9221	0.0001
Atmosphere*Osmotic	1	2.6959	0.0006
Error	35	0.1872	

Table A21. Mean separations between replications for organism counts (log_{10} CFU/g) in carrot slices stored at 10°C

Organism	Rep. 1	Rep. 2	Rep. 3
Aerobic	6.4[b]	7.5[a]	7.2[a]
Psy.	6.0[b]	7.3[a]	7.0[a]
Coiform	5.6[b]	6.9[a]	6.7[a]
Lactic	4.2[a]	4.7[a]	4.6[a]
Anaerobic	2.3[b]	3.3[a]	3.3[a]
Pectin.	4.4[b]	4.8[a]	4.6[ab]
Yeast	3.2[c]	4.3[a]	3.6[b]
Mold	2.1[a]	2.5[a]	2.3[a]
Yersinia	6.3[c]	7.2[a]	6.7[b]

Within each row, means with the same letter are not significantly different (P > 0.05) using the Student-Newman-Keuls mean separation test.

Table A22. Mean separations between atmospheres/dehydration for organism counts (log10 CFU/g) in carrot slices stored at 10°C

Organism	N	Atmosphere		Osmotic dehydration	
		air	vacuum	DE	ND
Aerobic	36	7.1[a]	6.9[a]	7.2[a]	6.8[b]
Psy.	36	6.8[a]	6.8[a]	7.0[a]	6.6[b]
Coliform	36	6.4[a]	6.4[a]	6.8[a]	6.0[b]
Lactic	36	4.4[a]	4.6[a]	5.6[a]	3.5[b]
Anaerobic	36	2.8[b]	3.2[a]	3.2[a]	2.8[b]
Pectin.	35	4.7[a]	4.6[a]	4.5[b]	4.8[a]
Yeast	34	3.6[a]	3.8[a]	4.3[a]	3.1[b]
Mold	34	2.5[a]	2.1[a]	2.3[a]	2.3[a]
Yersinia	35	6.6[a]	6.8[a]	7.0[a]	6.4[b]

within each row of atmosphere or dehydration treatment, the same letter are not significantly different (P > 0.05) using the Student-Newman-Keuls mean separation test. (DE=dehydrated; ND=non-dehydrated).

Table A23. Anova table for headspace gas analysis of carrot slices stored at 4°C

Gas	Source	DF	Mean square	Prob > F
CO_2	Day	7	389.5264	0.0001
	Atmosphere	1	3713.8864	0.0001
	Osmotic	1	553.8817	0.0001
	Replication	2	112.9706	0.0001
	Day*Atmosphere	7	104.0522	0.0001
	Day*Osmotic	7	180.9372	0.0001
	Atmosphere*Osmotic	1	10.7140	0.2794
	Error	118	9.071	
O_2	Day	7	101.4640	0.0001
	Atmosphere	1	453.4817	0.0001
	Osmotic	1	0.0609	0.9179
	Replication	2	41.6154	0.0010
	Day*Atmosphere	7	333.5523	0.0001
	Day*Osmotic	7	24.8791	0.0002
	Atmosphere*Osmotic	1	32.1500	0.0192
	Error	118	5.7082	

Table A24. Anova table for headspace gas analysis of carrot slices stored at 10°C

Gas	Source	DF	Mean square	Prob > F
CO_2	Day	5	727.3608	0.0001
	Atmosphere	1	3608.7857	0.0001
	Osmotic	1	1888.8818	0.0001
	Replication	2	8.8652	0.3883
	Day*Atmosphere	5	0.0484	0.9426
	Day*Osmotic	5	221.8596	0.0001
	Atmosphere*Osmotic	1	332.0502	0.0001
	Error	97	9.2800	
O2	Day	5	159.2285	0.0001
	Atmosphere	1	625.8822	0.0001
	Osmotic	1	15.1315	0.0001
	Replication	2	2.4086	0.0657
	Day*Atmosphere	5	379.7973	0.0001
	Day*Osmotic	5	20.1593	0.0001
	Atmosphere*Osmotic	1	3.9740	0.0341
	Error	97	0.8599	

Table A25. Mean separations of the replications of gas (%) analysis of carrot slices stored at 10°C

Gas	Rep. 1	Rep. 2	Rep. 3
CO_2	10.86[a]	10.68[a]	11.68[a]
O_2	5.22[a]	4.90[a]	4.92[a]

Within each row, means with the same letter are not significantly different (P > 0.05) using the Student-Newman-Keuls mean separation test.

Table A26. Mean separations of the replications of gas (%) analysis of carrot slices stored at 4°C

Gas	Rep. 1	Rep. 2	Rep. 3
CO_2	8.31[b]	10.28[a]	11.13[a]
O_2	7.32[a]	5.52[b]	6.10[a]

Within each row, means with the same letter are not significantly different (P > 0.05) using the Student-Newman-Keuls mean separation test.

Table A27. Mean separations between atmosphere/dehydration for gas (%) analysis of carrot slices

		Atmosphere		Dehydration	
Temp.	Gas	Air	Vacuum	Dehydrated	Nondehydrated
4°C	CO_2	5.68[b]	14.78[a]	11.91[a]	7.89[b]
	O_2	7.75[a]	4.62[b]	6.18[a]	6.38[a]
10°C	CO_2	6.16[b]	16.14[a]	14.73[a]	7.43[b]
	O_2	7.07[a]	2.90[b]	4.70[b]	5.33[a]

Within each row of each treatment, means with the same letter are not significantly different ($P > 0.05$) using the Student-Newman-Keuls mean separation test.

Table A28. Anova table for *Yersinia enterocolitica* in carrot slices held at different temperatures

Treatment	Source	DF	Mean square	Prob > F
Dehydrated carrots	Temperature	1	12.3591	0.0001
	Day	7	30.9511	0.0001
	Atmosphere	1	1.4622	0.0024
	Replication	2	0.7958	0.0064
	Temp*Day	5	4.6791	0.0001
	Temp*Atmosphere	1	0.0629	0.5043
	Day*Atmosphere	7	0.2405	0.1283
	Error	40	0.1386	
Non-dehydrated carrots	Temperature	1	0.7659	0.0897
	Day	7	14.1794	0.0001
	Atmosphere	1	3.8896	0.0004
	Replication	2	0.3784	0.2365
	Temp*Day	5	0.5616	0.0719
	Temp*Atmosphere	1	0.3003	0.2825
	Day*Atmosphere	7	0.2879	0.3597
	Error	38	0.2526	

Table A29. Mean separations between temperatures for *Yersinia enterocolitica* counts in dehydrated or non-dehydrated carrots

Treatment	4°C	10°C
Dehydrated	6.2b	7.0a
Non-dehydrated	6.2a	6.4a

Within each row, means with the same letter are not significantly different (P > 0.05) using the Student-Newman-Kuels mean separation test.

Table A30. Anova table for Gases contents (%) of packaging with osmotically dehydrated carrots held at different temperatures

Gas	Source	DF	Mean square	Prob > F
CO2	Temperature	1	315.8534	0.0001
	Day	7	819.2610	0.0001
	Atmosphere	1	5055.9931	0.0001
	Replication	2	192.7744	0.0001
	Temp*Day	5	56.1515	0.0043
	Temp*Atmosphere	1	116.8797	0.0069
	Day*Atmosphere	7	171.0393	0.0001
	Error	119	15.456	
O2	Temperature	1	87.4766	0.0001
	Day	7	148.0576	0.0001
	Atmosphere	1	352.8646	0.0001
	Replication	2	57.1216	0.0001
	Temp*Day	5	10.5066	0.0647
	Temp*Atmosphere	1	26.6342	0.0214
	Error	119	4.8976	

Table A31. Mean separations for gases (%) of packaging with osmotically dehydrated carrots held at different temperatures

Gas	4°C	10°C
CO_2	11.91^b	14.73^a
O_2	6.18^a	4.70^b

Within each row, means with the same letter are not significantly different (P > 0.05) using the Student-Newman-Keuls mean separation test.

Table A32. Anova table for gases (%) of packaging with non-osmotically dehydrated carrots held at different temperaures

Gas	Source	DF	Mean square	Prob > F
CO2	Temperature	1	7.9267	0.1667
	Day	7	329.5043	0.0001
	Atmosphere	1	2334.4506	0.0001
	Replication	2	3.0044	0.4821
	Temp*Day	5	20.5341	0.0003
	Temp*Atmosphere	1	19.1583	0.0326
	Day*Atmosphere	7	104.1314	0.0001
O2	Temperature	1	42.5120	0.0001
	Day	7	92.9801	0.0001
	Atmosphere	1	745.9239	0.0001
	Replication	2	0.9118	0.7186
	Temp*Day	5	9.9240	0.0046
	Temp*Atmosphere	1	0.4765	0.6781
	Day*Atmosphere	7	287.8367	0.0001

Table A33. Mean separations for gases (%) of packaging with non-osmotically dehydrated carrots held at different temperatures

Gas	4°C	10°C
CO_2	7.89[a]	7.43[a]
O_2	6.38[a]	5.33[b]

Within each row, means with the same letter are not significantly different (P > 0.05) using the Student-Newman-Keuls mean separation test.

VITA

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