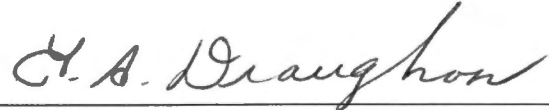


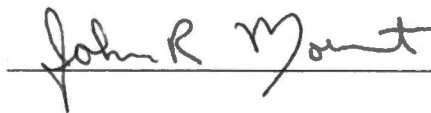
To the Graduate Council:

I am submitting herewith a thesis written by Ronald D. Smiley entitled "Growth and Survival of *Listeria monocytogenes* on Minimally Processed, Osmotically Dehydrated Broccoli With or Without Antimicrobial Treatment". 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.



Dr. F. A. Draughon, Major Professor

We have read this thesis
and recommend its acceptance:



Accepted for the Council:



Associate Vice Chancellor
and Dean of the Graduate School

**Growth and Survival of *Listeria monocytogenes*
on Minimally Processed , Osmotically Dehydrated
Broccoli With or Without Antimicrobial Treatment**

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Ronald D. Smiley

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Dedication

This thesis is dedicated to my mom for everlasting support and love which she has always given freely throughout my college career.

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Abstract

Growth and survival of *Listeria monocytogenes* on minimally processed broccoli, with and without osmotic dehydration and chemical pretreatment, stored under modified atmospheric conditions was evaluated. Inoculated fresh broccoli florets were osmotically dehydrated by immersion in a saturated NaCl solution then chemically treated by immersion in a sanitizer solution before packaging. Fresh broccoli florets were packaged in gas permeable film, with and without atmospheric evacuation, and stored at either 4 or 10°C for 15 days. *L. monocytogenes* increased 2 logs on fresh broccoli florets stored at 10°C but increased only slightly less than 1 log during storage at 4°C. When inoculated onto osmotically dehydrated broccoli, *L. monocytogenes* increased 5 logs at 10°C and 2 logs at 4°C. *L. monocytogenes* was more resistant to antimicrobial treatment when broccoli was stored at 10° than at 4°C. Metabisulfite was better at controlling growth of *L. monocytogenes* than was hypochlorite when broccoli was stored at 10°C. The effects of antimicrobial treatment were similar for both osmotically dehydrated and non-osmotically dehydrated broccoli. Osmotic dehydration neither increased or decreased the resistance of *L. monocytogenes* to antimicrobial treatment.

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Chapter I

Introduction

Minimally processed vegetables (MPV) are vegetables that have undergone some preservation method just short of complete preservation. The goal of MPV is to provide convenience to the consumer while maintaining the fresh characteristics of the original commodity (King and Bolin, 1989). Many of the preservation methods that maintain the freshness of a commodity are not severe enough to destroy or inhibit the growth of pathogenic microorganisms. Three of the most popular preservation methods for MPV includes: modified atmosphere packaging, refrigeration and chemical treatment (Shewfelt, 1986). These methods used alone may not be sufficient to control the growth of psychrotrophic, pathogenic microorganisms. Some organisms, including *Listeria monocytogenes*, have been shown to grow following exposure to these types of preservation methods (Beuchat and Brackett, 1990; Palumbo, 1986; Steingruegge et al., 1988).

Modified atmosphere packaging (MAP) is a technique in which the air in the package is altered to achieve some desired combination of gases, usually carbon dioxide (CO₂), oxygen (O₂) and/ or nitrogen (N₂). MAP has been used to increase the shelf life of MPV by reducing respiration and slowing microbial growth (Buick and Damoglou, 1989; Zagory and Kadar, 1988). Since vegetables continue to respire after harvest, the modified atmosphere may change within the package during storage. One popular method of MAP

has been to lower the concentration of O₂ in the package. However, it has been noted that a continually respiring vegetable may soon deplete the package of its available O₂ leading to anaerobic conditions and possibly germination of spores of anaerobic microorganisms. A new approach to MAP has been the use of semi-permeable plastic films for packaging of fresh vegetables. The film permeability maintains a steady state between the O₂ consumed (CO₂ released) and the O₂ which enters the package. Although this prevents an anaerobic environment from forming, it may not be sufficient to inhibit pathogenic microorganisms. The microbiological implication of MAP is that shelf life of MPV may be increased, from a spoilage and quality standpoint, for a period long enough to allow pathogenic psychrotrophic organisms to reach unsafe levels. From a safety issue, this situation is important since MPV are typically consumed uncooked.

Osmotic dehydration is a method of concentrating solutes in fruits and vegetables, typically prior to the application of some other type of preservation. Osmotic dehydration is accomplished by immersing the product in an aqueous solution of osmotic agents (e.g., NaCl, sucrose, ethanol) (Biswal and Bozorgmehr, 1990). During osmotic dehydration, vegetables lose water as they intake solutes. The resulting loss of water may impact the respiration rate of vegetables by slowing enzymatic activity. Changes in respiration will affect the atmosphere within the package. These atmospheric changes may have an impact on both spoilage and pathogenic microorganisms. Little research is available on the effects of osmotic dehydration on microorganisms, either spoilage or pathogenic. Torreggiani (1993) reported the need for investigation of microbial safety in osmotically dehydrated fruits and vegetables.

The purpose of this research was to determine the effects of a combination of osmotic dehydration, modified atmosphere and antimicrobial on the growth and survival of *Listeria monocytogenes* on broccoli stored at 4 and 10° C.

Chapter II

Review of the Literature

A. Minimally Processed Vegetables

Minimally processed vegetables (MPV) are usually considered within the food industry as vegetables that have undergone one or more of the following processes: washing, peeling, trimming, coring and slicing. MPV are generally harvested, shipped, processed, and then preserved in some additional manner (Huxsoll and Bolin, 1989). Shewfelt (1986) states that one of the contentions of MPV is to extend shelf life of the commodity while still maintaining its freshness and providing convenience to the consumer.

Preservation of MPV may include any one or combination of the following methods: packaging, refrigeration, chemical, ionizing radiation and reducing water activity (Aw) (Huxsoll and Bolin, 1989). Microbiological safety, package integrity, packaging materials, methods and packaging component migration are considerations for packaging MPV (Ronk et al., 1989). Refrigerated storage of MPV may be utilized provided that the commodity is not susceptible to chilling injury (Huxsoll and Bolin, 1989). Although chemical treatment may be used to preserve a vegetable commodity, the chemical may have a detrimental effect on the quality of the commodity (flavor, color, texture) (Huxsoll and Bolin, 1989). Consumer acceptability to irradiated foods is growing (Resurreccion et

al., 1995). However, the number of irradiation facilities still remains low. Reduction of Aw has been shown to be an effective preservation method for MPV (Lazer, 1966), although, the appealing aspect of MPV being “ready to eat” is lost since the product usually must be rehydrated before being consumed.

The microbiological flora of minimally processed vegetables has been extensively reviewed (Brackett, 1987; King and Bolin, 1989; Koek et al., 1983; Lund, 1992; Nguyen-the and Carlin, 1994). Nguyen-the and Carlin (1994) report that between 80 and 90% of all mesophilic organisms recovered from fresh vegetables were Gram negative rods including, but not limited to, species of *Pseudomonas*, *Enterobacter*, and *Erwinia*. Lund (1992) reported that some of the properties that influence the growth of bacteria on MPV include pH, organic acid content, available nutrients and presence of naturally occurring antimicrobials.

Preservation methods are necessary to control microorganisms and increase shelf life. Rolle and Chism (1987) reviewed the physiological consequences of minimally processing vegetables. They reported that MPV have substantial tissue injury including cell rupture due to cutting. Bolin et al. (1977) reported that the presence of cellular fluids from physically damaged lettuce increased microbial growth on lettuce when stored at refrigeration temperatures. In their research, Bolin et al. (1977) examined several different shredding methods to determine what affect physical damage had on shelf life of fresh lettuce. This research supports similar observations by Priepke et al. (1976), who indicated that spoilage (as indicated by aerobic plate counts) of shredded vegetables in a prepared salad mix increased at a faster rate than that of intact vegetables. The authors

reported that microorganisms on the intact vegetables exhibited a two day lag phase before increasing in numbers, whereas, microorganisms on the cut vegetables did not. Microbial counts on cut vegetables were reported to have increased 1000-fold within eight days, whereas the counts on uncut vegetables took ten days to increase the same amount. For all of the cut vegetables, the aerobic plate count reached levels greater than 10^8 CFU/g in ten days. For the uncut vegetables it was reported that only lettuce supported aerobic counts as high as 10^8 CFU/g in ten days, (Priepke et al., 1976).

In addition to spoilage organisms, MPV may harbor organisms that cause serious illness. Several species of pathogenic bacteria, which may cause foodborne illness, have been isolated from MPV. Brocklehurst et al. (1987) reported that up to 76% of retail packets of salad tested contained *Yersinia enterocolitica*, but only one isolate was a virulent strain. Other bacteria which have been isolated from MPV include *Aeromonas hydrophila*, *L. monocytogenes*, and *Escherichia coli* (Archer and Kvenberg, 1988; Beckers et al., 1988; Mossel, 1978).

B. Refrigerated Storage of Vegetables

It is generally considered that storage temperature has the greatest influence on microbial growth (Brackett, 1987; Palumbo, 1986; Shewfelt, 1986). Several reports indicate that reduced temperatures help to control microbiological growth on many vegetables (Bolin et al., 1977; Priepke, 1976; Steinbruegge et al., 1988). Several psychrotrophic bacteria (capable of growth at temperatures below 5°C) have been implicated as causative agents of foodborne illness and include *Clostridium botulinum*, *Y.*

entercolitica, *E. coli*, *A. hydrophila* and *L. monocytogenes* (Palumbo, 1986). One psychrotroph, *L. monocytogenes*, was shown to increase several log cycles on shredded lettuce held under refrigeration (Steinbruegge et al., 1988).

Refrigeration is used to maintain safety and quality of a commodity. Bolin et al. (1977) reported that shredded lettuce retained its quality as much as 2.5 times longer when stored at 2°C as compared with storage at 10°C. In their study, fresh lettuce was shredded and stored. The lettuce was then visually scored by two judges. Bolin et al. (1977) concluded that storage stability of shredded lettuce could be maximized by storage at a temperature just above freezing. Cold storage reduces enzyme activity, thus slowing respiration. Pripke et al. (1976) reported that respiration of many of the vegetables in a prepackaged salad halted after a few days when held at 4°C. By slowing respiration, shelf life, as based on stage of maturity, is increased (Shewfelt, 1986). Kader et al. (1989) reported that respiration for a variety of commodities (green beans, broccoli, cabbage, pepper) was reduced when the product was held at colder temperatures.

C. Sanitizers and Antimicrobials

A wide range of chemical compounds are approved and are currently being used in the food industry to control microorganisms. Descriptions of these chemicals and their applications are extensively covered in the literature. The approved use of chemicals in foods for the control of microorganisms is described in Title 21 of the Code of Federal Regulations. Many chemicals are not approved for application to fresh vegetables. Those that are may, in some instances, be approved at such levels that inhibition of foodborne

disease causing organisms does not occur.

Chlorine (hypochlorite) is commonly used as a disinfectant on whole vegetables (Nguyen-the and Carlin, 1994). Chlorine may act as a nonspecific oxidizing agent inside bacterial cells, oxidizing proteins and other metabolically important molecules (Davidson and Branen, 1983). Once oxidized, these molecules no longer perform their specific functions, thereby resulting in cell death.

Brackett (1987) reported that chlorine concentrations of less than 50 ppm had no antimicrobial effect on *L. monocytogenes* when tested in a cell suspension. Also, brussel sprouts that had been dipped in a 200 ppm solution of chlorine exhibited a 2 log reduction in numbers of recovered cells, only a 10-fold difference than that observed when the product was dipped in water alone (Brackett, 1987). Similar results were obtained by Beuchat and Brackett (1990) where it was determined that a chlorine treatment (200 ppm) did not influence the growth of *L. monocytogenes* on shredded lettuce, and that any significant difference between chlorinated and non-chlorinated treatments disappeared after a few days. Sallam and Donnelly (1992) reported the efficiency of an organo-chlorine compound, in reducing populations of *Listeria*, as greater than 99.999%, but noted that, given sufficient time to recover, many *Listeria* were able to repair themselves and become viable.

Sulfites are commonly used antimicrobial agents. Sulfites are available in several salt forms and are generally recognized as safe (21 CFR 182). The use of sulfites on fresh fruits and vegetables is restricted, but sulfites may be applied to dehydrated fruits and vegetables. The ban of sulfite use on fresh fruits and vegetables, that are consumed raw, is

due to allergic responses that some individuals have shown. Davidson and Branen (1983) reviewed the antimicrobial effects of various forms of sulfites. In solution, sulfites may assume various forms, including sulfurous acid (H_2SO_3), bisulfite (HSO_3^-), sulfite (SO_3^{2-}) or sulphur dioxide (SO_2). Sulfurous acid inhibits yeasts, molds and bacteria with bacteria generally being the least sensitive (Jay, 1992). Sulphur dioxide inhibits microbial growth by interacting with cellular proteins and enzymes (Jay, 1992).

D. Modified Atmosphere Packaging

Modified atmosphere packaging (MAP) of vegetables is a technique in which the gaseous environment surrounding the commodity is altered. Traditionally, this has been accomplished by reducing the amount of O_2 and increasing the amount of CO_2 in the package (Kader et al., 1989; Zagory and Kader, 1989). Research has shown that MAP can reduce the respiration rate of vegetables, increasing shelf life, and affect the microbial population of the commodity (Kader et al., 1989; Solomous, 1994; Zagory and Kader, 1989). With the advent of semipermeable plastic films, modified atmospheres no longer have to be created by gas flushing. The passive entry and exit of atmospheric gases and product respiration create the modified atmosphere. This type of MAP is generally referred to as commodity generated or passive MAP (Zagory and Kader, 1989).

The effect of MAP on the respiration rate of vegetables has been reviewed extensively and has been mathematically modeled (Lee et al., 1991; Platenius, 1946; Solomos, 1994). In a review of the physical principles of MAP, Solomos (1994) presented several theories for the effect of low O_2 and high CO_2 on reducing product

respiration. The most commonly held theory states that there are regulatory enzymes that exert feedback inhibition on glycolysis, under low concentrations of O_2 , thereby lowering respiration rate. Regardless of the mechanism involved, research supports the conclusion that MAP slows respiration rates in many vegetables. Kader et al. (1989) reported the respiration rates for a variety of vegetables (green beans, broccoli, cabbage, pepper) when stored under air and modified atmosphere. In most cases, the respiration rate decreased regardless of the temperature at which the commodity was stored. Leshuk and Saltveit (1990) reported that the level of O_2 beneficial for extending shelf life of broccoli is between 1 and 3%, but that detrimental effects may occur at levels around 0.5%. The authors also reported that the beneficial level of CO_2 is between 5 and 15% and that detrimental effects may occur at levels around 15%. It was further reported that atmospheric injury to broccoli may result in off flavors, off aromas and discoloration (Leshuk and Saltveit, 1990). Perishability of harvested vegetables is proportional to the rate of respiration (Kader, 1992). Research by Bash et al. (1995) showed that there is a negative correlation between respiration rate and shelf life of fresh asparagus spears.

Early research showed that some modified atmospheres, especially increased levels of CO_2 and decreased levels of O_2 , may slow microbial growth (Baker et al., 1986; Eklund and Jarmund, 1983; Hintlian and Hotchkiss, 1986). The microbial aspects of MAP have recently been reviewed (Farber, 1991; Hintlian and Hotchkiss, 1986b). Two potential safety problems with MAP have been cited. The first is the potential growth of clostridia in anaerobic or near anaerobic environments. Hintlian and Hotchkiss (1986) point out that research has shown that increased levels of CO_2 may stimulate the germination of

clostridial spores especially on a product which has been temperature abused. The second potential safety problem with MAP is that spoilage organisms, which may signify an unsafe product, may be inhibited while psychrotrophic pathogens grow to unsafe levels. These two problems have been addressed in recent research (Barriga et al., 1991; Post et al., 1985).

Conclusions as to the effects of MAP on spoilage organisms varies depending on gas composition, commodity and storage temperature (Barriga et al., 1991; Buick and Damoglou, 1989; Hao and Brackett, 1993). Buick and Damoglou (1989) report that vegetable salad had a predominant spoilage micro flora consisting of the following; *Enterobacter*, *Pseudomonas*, *Lactobacillus*, *Bacillus* and yeasts. These authors noted that the predominant spoilage organisms determining the shelf life of vegetable salad, held under atmospheres other than air, were yeasts and that the shelf life of the product could be extended by as much as 14 days when held at 4° C. Barriga et al. (1991) determined that the visual quality and shelf life of shredded lettuce can be extended by altering the storage atmosphere, but that it did not affect the microbial counts obtained. Hao and Brackett (1993) determined that MAP did not suppress the growth of spoilage organisms on bell peppers and concluded that extension of shelf life was a physiological consequence and not a microbial one.

In the circumstance that signs of spoilage are delayed by MAP, pathogenic organisms may reach unsafe levels. The behavior of several pathogenic organisms when studied under modified atmospheres is well documented (Farber, 1991). Grau and Vanderlinde (1992) detected *Listeria* on 93 of 175 samples of vacuum packaged

processed meats. Berrang et al. (1989) reported that *L. monocytogenes* increased in numbers when stored under an atmosphere different than air. Manu-Tawiah et al. (1993) reported that *Y. enterocolitica* grew under modified atmosphere at the same rate as other psychrotrophic spoilage organisms possibly compromising the safety of meat products. In the same study, it was also noted that vacuum packaging was no more effective than gas flushing for controlling growth of *Y. enterocolitica*.

E. Osmotic Dehydration of Vegetables

Biswal and Bozorgmehr (1988) define osmotic dehydration as a process in which part of the natural water content of cellular materials such as fruits and vegetables is removed by immersing the materials in concentrated aqueous solutions of soluble solids. Osmotic dehydration is typically applied prior to some other form of preservation. Benefits of osmotic dehydration are reviewed by Torreggiani (1993) and Biswal et al. (1991). Some of the benefits include: reduced weight of the product, improved organoleptic properties, reduced refrigeration load, savings in packaging, and savings in distribution. Hartel (1967) reported that as much as 32% of the original weight of potatoes is lost when osmotically dehydrated in salt solution for up to 1 hour. Osmotic dehydration is achieved due to the countercurrent flow of water out of the product and solute into the product. The amount of solute uptake is largely dependent on the membrane structure of the commodity and usually varies between different commodities (Biswal and Bozorgmehr, 1988).

Biswal and Bozorgmehr (1988) described the application of osmotic dehydration

of vegetables using NaCl (table salt). Speck et al., (1977) demonstrated that vegetables osmotically dehydrated with salt are organoleptically acceptable. This was later supported by Biswal et al. (1991) who reported that osmotically dehydrated green beans were still acceptable for consumption. In this particular work, osmotically dehydrated green beans were frozen, later thawed and then cooked in boiling water. The salt level in the beans prior to cooking was reported to be near 5%, and dropped to 1% after cooking. This level was reported to be acceptable by an untrained sensory panel.

Osmotic dehydration is expected to reduce respiration rates for vegetables . It stands to reason that, coupled with MAP, osmotic dehydration should reduce respiration even further. Currently, no information is available on the effects, of coupling osmotic dehydration with MAP, on the respiration rate of vegetables. Ortega (1993) found that osmotically dehydrated apples had a lower respiration rate than did non-dehydrated apples when stored under modified atmosphere. Additionally, Torreggiani (1993) reported that no microbial analysis had been performed on osmotically dehydrated vegetables and that this type of testing is necessary before any industrial development is performed.

F. Preservation of Broccoli

Broccoli may achieve respiration rates of 240 mL/kg/h at 20°C, 91 mL/kg/h at 10°C and 39 mL/kg/h at 0°C (Day, 1990). Controlling respiration rates is important in extending shelf life of fresh vegetables. It has been shown that there is an inverse relationship between respiration rate and shelf life (Brash et al., 1995; Kader, 1992). MAP of broccoli has been evaluated with variable results (Ballantyne et al., 1988; Barth et

al., 1993; Bastrash, et al., 1993; Berrang et al., 1990; Wu and Salunkhe, 1976) (Table 1).

Ballatyne et al. (1988) reported that modified atmosphere packaging of broccoli in oxygen permeable films (3000 to 32,500 mL/m²/day/atm), did not improve quality retention of broccoli florets when compared to florets stored in air. Barrang et al. (1990) reported that broccoli stored in an atmosphere of 11% O₂/ 10% CO₂/ 79% N₂ did not differ in color retention, firmness and microbial activity from broccoli stored in air. Other researchers (Barth et al., 1993; Bastrash et al., 1993; Wu and Salunkhe, 1976) reported positive results from packaging broccoli in modified atmosphere. Barth et al. (1993) reported that moisture content, chlorophyll content and color retention were higher in broccoli packaged under modified atmosphere (8% CO₂/ 10% O₂) than when stored in air. Wu and Salunkhe (1976) demonstrated that vacuum packaging broccoli extended the shelf life for 11 days at 4°C and 6 days at 15°C as a result of delayed senescence. Bastrash et al. (1993) reported that broccoli florets stored in a atmosphere consisting of 6% CO₂/ 2% O₂ (balance with N₂) resulted in extended shelf life due to delayed yellowing, prolonged chlorophyll retention, reduced mold development and increased moisture retention.

G. Post-Harvest Physiological Effects of Vegetables

Plant commodities differ from other types of food products in that they continue to respire after harvest. Respiration is generally considered to be the breakdown of carbon compounds and the formation of carbon skeletons necessary for maintenance and synthetic reactions after harvest (Kays, 1991). Products of respiration are CO₂, H₂O and energy in the form of ATP (Bettelhem and March, 1990). During respiration, plant cells utilize

Table 1. Review of the Effects of Modified Atmosphere Packaging on the Shelf Life of Broccoli.

Author(s)	Atmosphere	Shelf Life	Microbial Growth
Barth et al., 1993	9%/ 3% (CO ₂ / O ₂)	increased moisture retention	no difference in APC between MAP and air
Ballantyne et al., 1988	3%/ 3% (CO ₂ / O ₂)	improved retention of quality	NR ^a
Lee et al., 1991	various	reduced respiration as O ₂ levels decreased	NR
Wu and Salunkhe, 1976	vacuum	extended shelf life decreased yellowing	NR
Berrang et al., 1990	10%/ 11% (CO ₂ / O ₂)	no effect on firmness, no effect on color	lower microbial counts than when packaged in air
Bastrash et al., 1993	6%/ 2% (CO ₂ / O ₂)	extended shelf life	NR
Barth et al., 1993b	8%/ 10% (CO ₂ /O ₂)	Better color retention, better moisture retention	NR

a= Not Reported

existing stores of sugars and oxygen from the surrounding environment (Kays, 1991). During respiration sugar breakdown occurs along four major pathways: glycolysis, Krebs cycle, electron transport and the pentose phosphate pathway (Bettelhem and March, 1990). Respiration has a direct effect on product quality after harvest. Intermediate compounds in the respiratory pathway are used to build amino acids, nucleotides, pigments, lipids and flavor compounds (Kays, 1991).

Glycolysis can occur in the absence of oxygen (Alberts et al., 1994). The other three pathways, however, require oxygen to be present in order for them to operate (Alberts et al., 1994). Plant tissues are not capable of anaerobic respiration (Kays, 1991). In the absence of O_2 , pyruvate (the end product of glycolysis) is converted to acetaldehyde and may be converted further to ethanol (Kays, 1991). This process is generally referred to as fermentation. The conversion of pyruvate to acetaldehyde does not produce energy, but rather, requires energy and is thus detrimental to plant tissue (Kays, 1991).

One measure of shelf life of a vegetable commodity is how long that commodity is capable of maintaining its existing condition after harvest (Kays, 1991). This depends on how much sugar the plant has stored in reserve form. Loss of energy reserves leads to tissue starvation and accelerated senescence. Leaves, flowers, and other structures that do not serve as carbohydrate storage sites are especially susceptible to accelerated senescence.

Respiration rate affects the removal of O_2 from the surrounding environment while at the same time the O_2 concentration affects the respiration rate of the commodity.

Reduction in O_2 in the surrounding environment therefore will slow the respiration rate in the commodity. By reducing the respiration rate, the rate at which energy stores are utilized is slowed, resulting in an increased shelf life. Although the exact mechanism is unclear, low O_2 concentrations seem to affect several enzymes in the Krebs cycle (Kays, 1991). Respiration rates can be slowed by several means, including dehydration, refrigeration and MAP. Dehydration serves to slow respiration by reducing the moisture present inside the plant cells which is critical for enzymatic activity. Refrigeration may also slow respiration by slowing the enzymatic reactions of the respiratory chain. MAP also is used to regulate enzyme activity of the respiratory chain. It is generally believed that high levels of CO_2 stimulate some sort of feedback inhibition, slowing reactions of the Krebs cycle. High levels of CO_2 may also form carbonic acid inside the cells leading to a decrease in pH, thus altering enzymatic activity (Solomos, 1994).

While refrigeration, dehydration and modified atmospheres may be used to slow respiration in minimally processed vegetables, wounding by slicing and cutting increases respiratory activity. Secondary products of the respiratory chain are needed to initiate the healing process in the cut tissue. The increased need for these secondary products causes an increase in respiration in order to meet the demand. Other responses to wounding of plant tissue may include increased DNA and RNA synthesis, induction of new enzymes and membrane degradation (Solomos, 1994).

H. *Listeria monocytogenes*

Listeria monocytogenes is a Gram positive psychrotrophic rod (Jay, 1992). *L. monocytogenes* is a facultative anaerobe capable of growth with or without oxygen but is not capable of forming spores (Bille and Doyle, 1992). *L. monocytogenes* is motile due to the presence of peritrichous flagella (Bille and Doyle, 1992). The organism grows well over a wide temperature range from 1.1 to 42° C. and over a wide pH range from 5.5 to 9.5 (Jay, 1992). The *Listeria* are known to tolerate salt contents of up to 10% (Jay, 1992). *Listeria spp.* are catalase positive, oxidase negative, ferment glucose, methyl red positive, Vogues-Proskauer positive, do not produce indole, do not produce H₂S, and are not capable of hydrolyzing urea (Hitchins, 1992). *L. monocytogenes* may be found in a wide range of habitats including soil, water, plants and meat. *L. monocytogenes* has been isolated from a variety of raw and processed foods such as meat, vegetables and seafood (Bille and Doyle, 1992).

Foodborne listeriosis is a major concern to the food industry due to the seriousness of the disease. *L. monocytogenes* may affect the central nervous system causing meningitis or encephalitis (Bille and Doyle, 1992). *Listeria* poses a more particular health threat to certain segments of the population including elderly, immune compromised and pregnant women (Bille and Doyle, 1992).

L. monocytogenes has been shown to survive a number of techniques used to inhibit pathogenic bacteria in the food industry (Brackett, 1987; Manu-Tawiah, 1993; Miller, 1992). Brackett (1987) demonstrated that dipping brussel sprouts in a solution containing 200 ppm sodium hypochlorite yielded only a 2 log reduction in numbers which

was only 10 times less than dipping sprouts in water alone. El-Kest and Marth (1988) reported that *L. monocytogenes* survived in a 1 ppm sodium hypochlorite solution after exposure for up to 1 hour. Miller (1992) showed that *L. monocytogenes* could grow in media with A_w , lowered by the addition of NaCl, as low as 0.90. The effects of several other preservation methods on survival of *L. monocytogenes* are summarized in Table 2.

I. Safety Considerations

The combination of osmotic dehydration, MAP and refrigeration coupled with applications of sanitizers presents a vast, relatively unexplored dynamic system in which little is known about the behavior of pathogenic microorganisms. The effects of MAP on spoilage and pathogenic microorganisms and respiration of vegetables has been documented (Wiley, 1994). It has been shown that reduced O_2 concentrations (Solomos, 1994) and refrigeration (Wiley, 1994), typically decrease respiration rates. It is known that wounding increases respiration rate (Kays, 1992). The effects of a combination of these parameters on safety, however, has not yet been investigated.

Table 2. Effect of Preservation Methods on the Growth and Survival of *Listeria monocytogenes*.

Author	Preservation Method	Commodity	Storage Temperature	Effect on <i>Listeria</i>
Miller, 1992	Aw 0.90	Bacteriological media	30° C.	D-value 123 h
Miller, 1992	Aw 0.80	Bacteriological media	30° C.	D-value 3 h
Manu-Tawiah et al., 1993	modified atmosphere	pork chops	4° C.	similar growth between air and vacuum
Brackett, 1987	chlorine	brussel sprouts	30° C.	2 log reduction
El-Kest and Marth, 1988	pH and chlorine	bacteriological media	25° C.	higher pH higher survivors
Sorrells et al., 1989	acidulants	bacteriological media	10° C.	growth at pH4.4 with malic acid
Sallam and Donnelly, 1992	sanitizing compounds	bacteriological media	-	<i>Listeria</i> recovered after 72 h enrichment

Chapter III

Materials and Methods

A. Broccoli Samples

Broccoli samples were obtained at a local produce distributor in Knoxville, Tennessee. Upon arrival, broccoli was rinsed in tap water to remove dirt and other debris. The florets were then separated from the stalks and stored in a 30 gallon covered rubber container at 4°C until packaging the following day. All broccoli was packaged within 24 hours of arrival.

B. Osmotic Dehydration

An osmotic solution consisting of NaCl and water was prepared by dissolving 26g of NaCl per liter of water (final volume 12 L). The osmotic solution was prepared in a 40 L rubber container, which had been sanitized by washing with a 400 ppm hypochlorite solution and rinsing to remove residual chlorine. Osmotically dehydrated broccoli florets were placed inside a sterile, nylon mesh bag and submerged in the osmotic solution.

In order to determine the length of time necessary to achieve a given NaCl concentration inside the florets, a standard curve was prepared by submerging florets for varying time periods in the osmotic solution at 25°C. Salt content was determined using silver nitrate titration according to AOAC procedures 50.025, 50.027 and 50.029 (AOAC, 1990). Prior to analysis, 5 g of broccoli was immersed in liquid nitrogen, ground into a

powder, mixed with 25 ml of distilled/ deionized water and analyzed for salt content as described above. The data obtained were analyzed using the Proc Reg procedure of SAS (1989) (SAS; Cary, NC).

C. Inoculum

Three strains of *Listeria monocytogenes* (Scott A, ATCC 7644, ATCC 15313) were activated and grown in 100 mL of sterile Tryptic Soy Broth (Difco; Detroit, MI), and then centrifuged in a Biofuge 17R refrigerated centrifuge (Baxter Scientific; McGaw Park, IL) at 10,000 x g at 4°C to obtain a cell pellet. Cell pellets from the three strains of *L. monocytogenes* were resuspended in sterile 0.1% peptone water and then pooled. The pooled cell suspension was then diluted to yield approximately 10⁵ CFU/ mL and was used to inoculate the broccoli samples. Samples (in a nylon mesh bag) were inoculated by immersion in 10 L of buffer containing the inoculum. Samples were immersed for 50 minutes and allowed to drip dry for 30 minutes.

D. Antimicrobial Agents

The effect of a 200 ppm hypochlorite or 100 ppm sodium metabisulfite dip in conjunction with osmotic dehydration was evaluated. The 200 ppm hypochlorite solution was prepared in a 30 L container by mixing 38.1 mL of a 5.25% sodium hypochlorite solution with 10 L of deionized water. Broccoli florets were sanitized by immersing the broccoli in the hypochlorite solution. A 100 ppm solution of sodium metabisulfite was prepared by dissolving 0.1 gram of anhydrous sodium metabisulfite in 1L of deionized

water. Broccoli florets were immersed in the solution for approximately 5 seconds, and allowed to drip dry.

E. Sample Packaging

After inoculation, samples were packaged separately in Cryovac PD941 (W. R. Grace & Co; Simpsonville, SC) semi-permeable packaging film (40mm x 40mm). The film was designed primarily for the packaging of high respiration vegetables such as broccoli. The film has an O₂ transmission rate of 16,544 cm³/M²/24 h, a CO₂ transmission rate of 22,000-26,000 cm³/M²/24 h and a water vapor transmission rate of 5 g/100 in²/ 24 h. The packages were sealed and flushed with air or vacuum packaged using a Multivac heat-seal packaging machine. The packages were then stored at 4 or 10° C. A septum (Modern Control Inc.; Minneapolis, MN) was applied to each non-inoculated package so that gas analysis could be performed without disturbing the integrity of the package.

F. Headspace Gas Analysis

In order to determine the effects of osmotic dehydration and MAP on the respiration rate of minimally processed broccoli, gas analysis was performed on the non-inoculated dehydrated and non-dehydrated samples. Gas analysis was performed, at the time of packaging and again at each sampling period, using a Hewlett Packard 5890 A gas chromatograph (Hewlett Packard; Wilmington, DE) equipped with an Alltech CTR column (Alltech; Deerfield, IL) with a detector temperature of 30°C and a column temperature of 25°C. The gas chromatograph was fitted with a thermal conductivity

detector. A flow rate of 60 mL/min, with helium as the carrier gas, was used to perform gas analysis. Headspace gas was obtained by inserting a 10 cm³ syringe with a 27 gauge needle into a preapplied septum. The gas sample was injected into the gas chromatograph which contained a splitter so that 1 μ L of the sample was analyzed. The quantity of each component was determined from the sample retention time and peak area as compared with a standard curve obtained using a gas of known composition.

G. Other Chemical Analysis

Water activity (A_w) of the broccoli was measured prior to and after osmotic dehydration using an AquaLab CX2 Water Activity Measurement System (Decagon Devices; Pullman, WA). A_w was measured by immersing the broccoli florets in liquid nitrogen until frozen and then grinding the florets into a powder. The powdered form was then analyzed following manufacturers instructions.

Titrateable acidity (AOAC, 1990) was performed on each of the uninoculated samples at the end of each sampling period. Titrateable acidity was determined by immersing broccoli floret in liquid nitrogen until frozen and grinding the florets into a powder. The powdered form (5 g) was added to 25 ml of deionized water in an Erlenmeyer flask with 2 g NaCl. The flask was heated to precipitate color pigments and the solution was filtered through cheesecloth. Two drops of phenolphthalein indicator was added to the filtered solution followed by titration with 0.1 N NaOH. Titrateable acidity was expressed as percent malic acid.

H. Microbiological Analysis

Microbial counts, on broccoli, were determined initially and at three day intervals for up to 15 days. Microbial analysis was performed to enumerate aerobic microorganisms, coliform bacteria, lactic acid bacteria, psychrotrophic bacteria, combined yeast and molds, and *L. monocytogenes* (Table 3). All media was purchased from Difco (Detroit, MI), Oxoid (Columbia, MD) or BBL (Cockeysville, MD) and prepared according to manufacturers instructions unless otherwise stated.

A 25 g sample of broccoli was blended (2 minutes) with 225 ml of sterile 0.1% peptone water, in sterile Seward Medical Stomacher Closure Bags (Tekmar; Cincinnati, OH) using a Seward Model 400 Stomacher Lab Blender (Tekmar; Cincinnati, OH). Dilutions were either pour or surface plated onto the appropriate medium as indicated in Table 3.

A direct plate count of *L. monocytogenes* was determined by surface plating 0.1 ml of the diluted samples onto PALCAM agar (Oxoid; Columbia, MD). Colonies of *L. monocytogenes* were enumerated after incubation at 32°C for 48 hours.

Confirmation of *L. monocytogenes* was performed on all samples following the FDA procedure (Hitchins, 1992) for isolation and confirmation with omission of the 45° transillumination procedure since PALCAM is a selective and differential media and gives presumptive identification based on the dark appearance of the colonies. PALCAM agar was substituted for Modified McBride and LPM agars. In addition to direct plating, a 25 g sample of broccoli was removed at the time of sampling and enriched for 48 hours in

Table 3. Microbiological evaluation performed on minimally processed broccoli stored at 4° and 10° C. for 15 days.

Microbial Analysis	Media	Incubation Period	Incubation Temperature
Aerobic Plate Count	Trypticase Soy Agar	48 hours	32° C.
Psychrotrophic Plate Count	Trypticase Soy Agar	5 days	4° C.
Coliform Count	Violet Red Bile Agar	25 hours	32° C.
Yeast and Mold Count	Rose Bengal Agar ^a	3 days	28° C.
Lactic Acid Bacteria Count	MRS ^b	24 hours	32° C.

a= supplemented with chloramphenicol (Oxoid; Columbia, MD)

b= deMann, Rogosa, Sharpe agar with pH decreased to 5.9 using 1 N hydrochloric acid

University of Vermont (UVM) enrichment broth (BBL Microbiology Systems; Cockeysville, MD) to detect for the presence of *L. monocytogenes* in both inoculated and uninoculated samples. The following confirmation tests, taken from the FDA procedure for confirmation of *L. monocytogenes*, was used: β -hemolysis on 5% sheep blood agar, catalase activity, motility on SIM agar (BBL Microbiology Systems; Cockeysville, MD), carbohydrate (rhamnose, xylose and mannitol) utilization and oxidase reaction.

I. Experimental Design

A fractional factorial design, with a factorial treatment arrangement, was used to evaluate the effects of osmotic dehydration, inoculation, vacuum packaging, antimicrobial agent and temperature on the growth of indigenous bacteria and the growth and survival of inoculated *L. monocytogenes* on fresh broccoli. Upon arrival, the broccoli was separated into groups according to the treatment combination that would be applied. Treatment combinations were randomly assigned to each group of broccoli. Due to time and equipment constraints, all samples which required osmotic dehydration were treated together, all samples requiring inoculation were treated together, and samples receiving the same antimicrobial were treated together. Inoculated and uninoculated samples were not treated together to avoid cross contamination. Additionally there were no concerns that dehydrating and inoculating samples at the same time would affect the outcome of the research. Each sample was then separately packaged in air or vacuum and stored at 4 or 10°C. Sampling period were altered with 4°C storage period being evaluated first followed by 10°C storage to help control any seasonal differences between replications of

the experiment.

Due to the tremendous amount of time required to plate each sample, plating all 48 possible treatment combinations was impractical. A fractional factorial was designed using E4 (Evolutionary Software Inc, 1990). Nine treatment combinations were used during each sampling period in the study (Table 4). Each sampling period lasted approximately one month. Storage temperatures were alternated each sampling period with 4° C being evaluated first followed by 10° C storage.

J. Statistical Analysis

Statistical analysis was performed using Proc Mixed procedure in SAS (1994) (SAS; / Cary, NC). Main effects and lower order interactions were analyzed. Higher order interactions were not analyzed since they may be inaccurate due to low numbers of observations created by using a fractional factorial and higher order interactions are difficult to interpret. Mean separation of statistically significant ($P < 0.05$) effects was performed using the least significant difference option in the Proc Mixed procedure.

Table 4. Experimental observations created for a fractional factorial to analyze the effects of temperature, antimicrobial dip, osmotic dehydration and atmosphere on the growth and survival of microorganisms on fresh broccoli.

Observation	Month	Temperature	Antimicrobial	Inoculation	Osmotic Dehydration	Atmosphere
1	1	4	none	yes	yes	air
2	1	4	chlorine	no	yes	air
3	1	4	sulfite	yes	yes	vac
4	1	4	sulfite	yes	yes	vac
5	1	4	chlorine	no	no	vac
6	1	4	chlorine	no	no	air
7	1	4	none	yes	yes	air
8	1	4	none	no	no	air
9	1	4	none	no	no	vac
10	1	10	sulfite	no	no	air
11	1	10	chlorine	no	no	air
12	1	10	none	yes	yes	air
13	1	10	chlorine	yes	yes	vac
14	1	10	chlorine	no	no	vac
15	1	10	sulfite	no	no	vac
16	1	10	sulfite	yes	yes	air
17	1	10	none	yes	yes	vac
18	2	10	sulfite	no	no	vac
19	2	10	none	yes	no	vac
20	2	10	none	yes	no	vac
21	2	10	chlorine	no	yes	air
22	2	10	sulfite	no	yes	air

Observation	Month	Temperature	Antimicrobial	Inoculation	Osmotic Dehydration	Atmosphere
23	2	10	none	no	yes	air
24	2	10	chlorine	no	yes	vac
25	2	10	sulfite	yes	no	vac
26	2	10	chlorine	no	yes	vac
27	3	4	chlorine	yes	yes	air
28	3	4	sulfite	yes	no	air
29	3	4	chlorine	yes	no	vac
30	3	4	chlorine	yes	no	air
31	3	10	sulfite	yes	no	vac
32	3	10	none	yes	no	air
33	3	10	chlorine	yes	no	air
34	3	4	none	yes	no	vac
35	3	4	chlorine	yes	no	air
36	3	4	chlorine	no	yes	air
37	3	4	sulfite	yes	no	air
38	3	4	none	no	yes	air
39	3	4	sulfite	no	yes	air
40	3	4	chlorine	no	yes	air
41	3	4	none	no	yes	vac
42	3	4	sulfite	no	yes	vac

Chapter IV.

Results and Discussion

Growth of Spoilage Bacteria on Minimally Processed Broccoli

4° C Storage

Microbial growth was evaluated on minimally processed broccoli stored at 4°C under air and vacuum packaging (Figures 1 and 2). Aerobic bacteria were initially present on fresh raw broccoli at levels of approximately Log_{10} 6.0 CFU/ g and increased to levels slightly above Log_{10} 7.0 CFU/ g after 15 days storage at 4° C (Figure 1). High initial psychrotrophic bacterial counts indicated that fresh raw broccoli is heavily populated with Gram negative psychrotrophic spoilage bacteria (Figure 1). These results are similar to current predictions reported in the literature for the predominant species of micro flora commonly found on fresh vegetables. Brackett (1994) indicated that Gram negative bacteria, including *Pseudomonas spp*, *Erwinia spp*, and *Enterobacter spp*, are most often isolated from raw vegetables. Yeast and mold contamination was initially lower than bacterial contamination but increased from Log_{10} 4.8 CFU/ g to Log_{10} 7.0 CFU/ g by the end of the storage period (Figure 1). These counts were primarily yeasts, rather than molds. These results support the observation reported by Brackett (1987) in which he reported that levels of bacterial and fungal contamination are generally about the same in

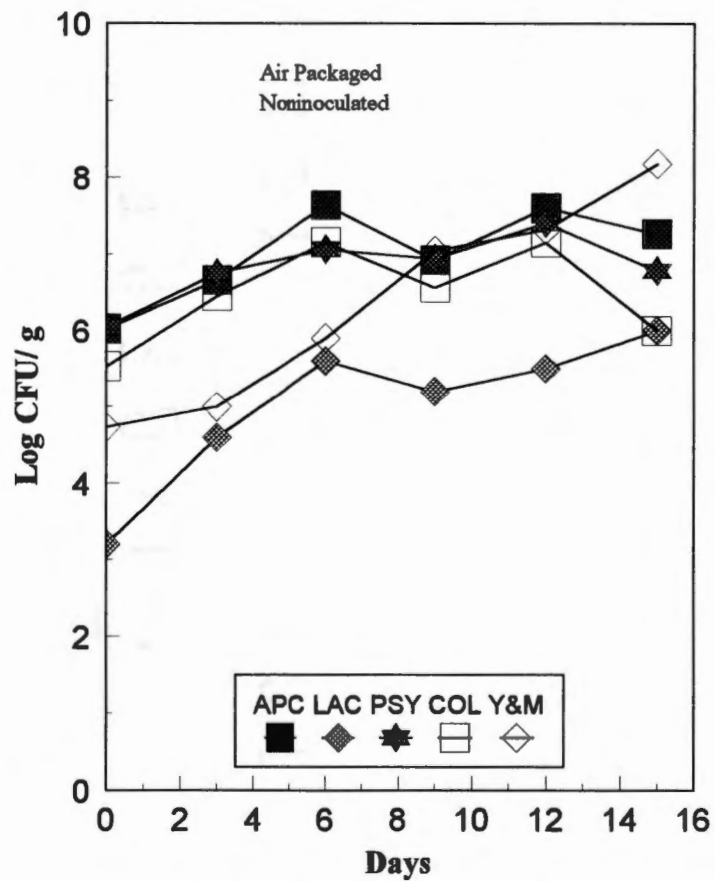


Figure 1: Microbial growth on minimally processed broccoli stored at 4°C under air.

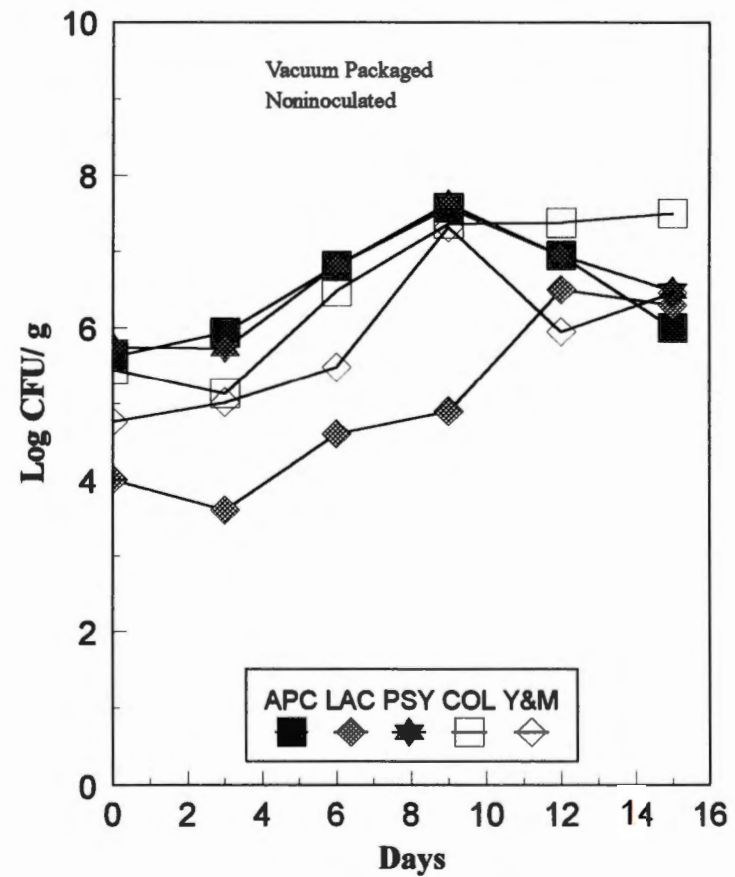


Figure 2: Microbial growth on minimally processed broccoli stored at 4°C under vacuum.

raw vegetables. Lactic acid (LA) bacteria and coliform bacteria increased similarly to aerobic and psychrotrophic organisms throughout the storage period (Figure 1). The highest increase in microbial populations (aerobic, psychrotrophic, LA, and coliform bacteria) was observed within the first three days of storage, after which, bacterial populations remained at a constant level (Figure 1). Yeasts and molds continued to increase in number up to day 15 (Figure 1). Storage at 4° C exhibited a slight inhibitory effect on yeasts and molds as indicated by the slow but continual increase in growth. Storage at 4° C had little effect on aerobic or psychrotrophic bacterial counts indicating the presence of large populations of bacteria capable of growth at 4° C.

Similar results were obtained for aerobic, psychrotrophic, LA, and coliform bacteria when minimally processed (MP) broccoli was vacuum package and stored at 4°C (Figure 2). Initial microbial populations were similar to those described above indicating the presence of Gram negative spoilage organisms. Maximum populations for both aerobic and psychrotrophic bacteria were similar to the air package (Figure 1). The microbial growth rate was slowed by vacuum packaging as indicated by the length of time necessary to achieve maximum populations (Figure 2). Maximum aerobic bacterial populations were achieved by day six for air packaged MP broccoli (Figure 1) but was extended to approximately day nine for vacuum packaged broccoli (Figure 2). Growth of yeasts and molds was slowed throughout the storage period when MP broccoli was vacuum packaged (Figure 2) as opposed to air packaged (Figure 1). The high count obtained on day nine may be the result of a weaker vacuum on that particular package. Coliform and LA bacteria exhibited similar growth patterns to aerobic and psychrotrophic

organisms. Coliforms remained consistently higher in the vacuum packaged broccoli (Figure 2) than in the air packaged broccoli (Figure 1). The decreased O₂ content of the vacuum packaged broccoli may have provided an environment which selected for facultative organisms, such as coliform bacteria, over other microorganisms which may have been present. No statistically significant differences ($P>0.05$) were observed between vacuum and air packaged broccoli for aerobic bacteria (Table A1), psychrotrophic bacteria (Table A2), LA bacteria (Table A3), coliform bacteria (Table A4) and yeasts and molds (Table A5) when stored at 4°C.

Figure 3 illustrates the behavior of indigenous microflora on air and vacuum packaged MP broccoli inoculated with Log₁₀ 5.0 CFU/ mL *L. monocytogenes* and stored at 4°C. Initial levels of coliform bacteria were lower on MP broccoli inoculated with *L. monocytogenes* (Figure 3) than on non-inoculated broccoli (Figures 1 and 2) although both aerobic and psychrotrophic organisms were present at similar levels to the non-inoculated broccoli (Figures 1, 2, and 3). Growth of coliform bacteria, however, was similar over the 15 day storage period for both the inoculated (Figure 3) and the non-inoculated (Figures 1 and 2). Similar results were observed with the growth of yeasts and molds (Figure 3).

Inoculation with *L. monocytogenes* caused changes in coliform bacterial counts, LA bacterial counts and yeasts and molds counts (Figure 3). Package atmosphere (air or vacuum) did not significantly ($P>0.05$) influence behavior of *L. monocytogenes* on MP broccoli stored at 4°C (Tables A1, A2, A3, A4 and A5).

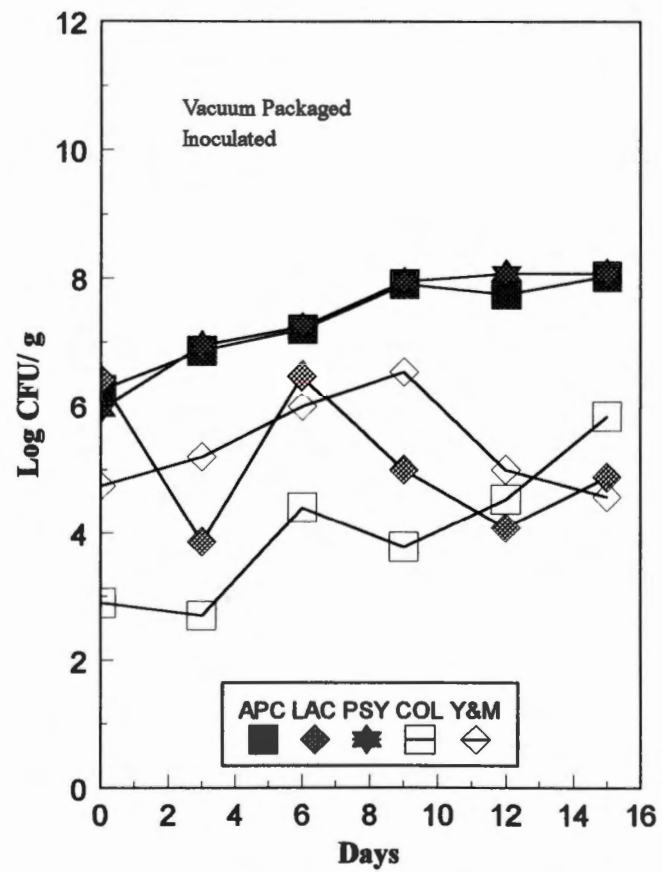


Figure 3: Microbial growth on minimally processed broccoli inoculated with Log 5.0 CFU/ mL *Listeria monocytogenes* and stored at 4°C. Atmospheres were not significantly different ($P>0.05$). Data for air packaged inoculated broccoli not show.

10° C Storage

Initial populations of microorganisms on MP broccoli stored at 10° C (Figure 4 and Figure 5) were similar to MP broccoli stored at 4° C (Figure 1 and Figure 2). Aerobic and psychrotrophic bacteria increased from Log₁₀ 7.0 CFU/ g to Log₁₀ 9.0 CFU/ g, by the sixth day of storage, on air packaged MP broccoli when stored at 10°C (Figure 4). Growth of coliform bacteria was statistically similar ($P>0.05$) (Table A4) at 10° C (Figure 4) as compared to 4° C storage (Figure 1). This would indicate that 10°C storage is equally effective as 4°C storage in controlling growth of coliform bacteria. LA bacteria, however, increased more rapidly and reached significantly higher ($P<0.05$) populations at 10°C than at 4°C (Table A3). Variability in the populations of yeasts and molds was observed at 10°C (Figure 4). The increased populations of aerobic bacteria may indicate that bacteria were increasing at a faster rate during storage at 10°C and were out-competing yeasts and molds. Inoculation with Log₁₀ 3.2 CFU/ mL *L. monocytogenes* (Figures 3-5) did not significantly influence growth of aerobic organisms (Table A1), psychrotrophic organisms (Table A2), or coliform bacteria (Table A4) on MP broccoli stored at 10°C. LA bacteria increased faster and with less variability on MP broccoli stored at 10°C (Figure 5) than during storage at 4°C (Figure 3). Growth of microorganisms on vacuum packaged inoculated MP broccoli, stored at 10°C, (Figure 5) did not significantly ($P>0.05$) differ from air packaged, inoculated MP broccoli stored at 10°C (Figure 4). Although there was variability between the growth of yeasts and molds between the air packaged (Figure 4) and the vacuum packaged (Figure 5) broccoli, these differences were determined to be not statistically significant ($P>0.05$) (Table A5).

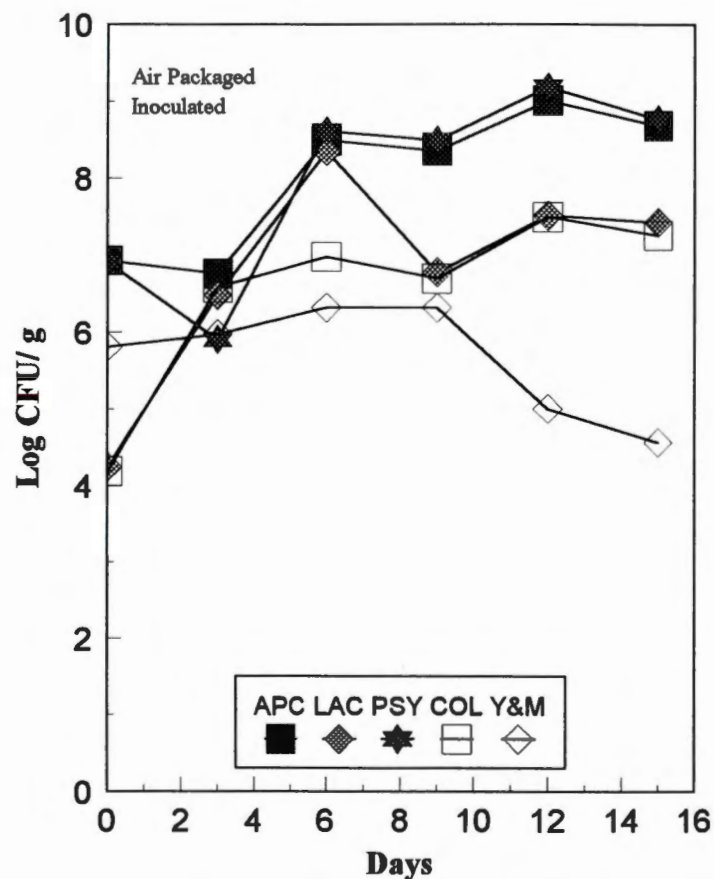


Figure 4: Microbial growth on minimally processed broccoli stored at 10°C. Aerobic and psychrotrophic organisms were not significantly different ($P>0.05$) between inoculated and noninoculated broccoli. Data for noninoculated broccoli not shown.

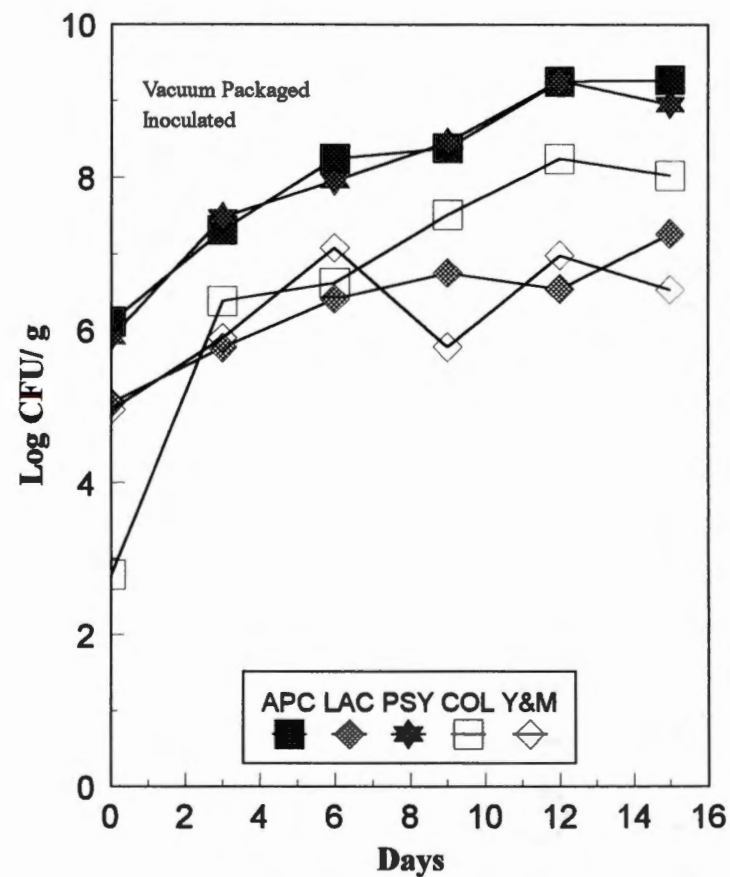


Figure 5: Microbial growth on minimally processed broccoli stored at 10°C under vacuum. Aerobic and psychrotrophic organisms were not significantly different ($P>0.05$) from noninoculated broccoli. Data for noninoculated broccoli not shown.

The initial levels of microorganisms determined in this experiment were similar to those reported by Nguyen-the and Carlin (1994) in which the authors reported the initial levels, on processed salads, for mesophilic organisms (10^5 CFU/ g), lactic acid bacteria (<10 CFU/ g), yeasts and molds (10^2), and coliform bacteria (10^2). The growth of aerobic microorganisms on MP broccoli was also found to be similar to that reported by Berrang et al., (1990) in which it was reported that aerobic microorganisms were present initially at levels of 10^4 - 10^5 CFU/ g on fresh broccoli. Berrang et al., (1990) also reported that aerobic organisms increased to levels greater than 10^7 after 21 days storage at 4°C which was similar to the final populations of aerobic organisms observed during this experiment.

L. monocytogenes

L. monocytogenes was recovered on inoculated MP broccoli at approximately Log_{10} 3.3 CFU/ g (Figure 6). The level of *L. monocytogenes* recovered was lower than the inoculum level (Log_{10} 5.0 CFU/ ml) used to distribute the organism onto the broccoli. This would be expected since only the surface of the broccoli florets came into contact with the inoculum solution and since the *L. monocytogenes* was enumerated on a per gram basis. *L. monocytogenes* multiplied much faster during storage at 10°C than at 4°C (Figure 6). Increases in numbers of *L. monocytogenes* were noted within the first three days of storage when MP broccoli was stored at 10°C (Figure 6). Populations of *L. monocytogenes* remained constant from day nine until the end of the storage period. Populations of *L. monocytogenes*, on MP broccoli stored at 4°C remained at approximately Log_{10} 3.0 CFU/ g from day zero to day 12 of storage (Figure 6). However, a sharp increase in numbers of *L. monocytogenes* were observed after twelve days of

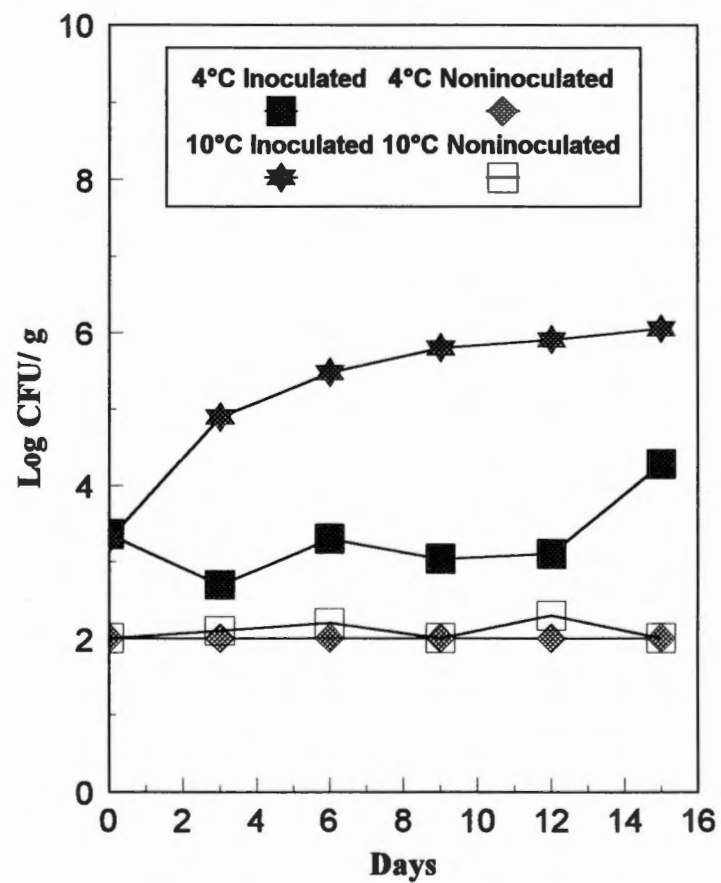


Figure 6: Growth of *Listeria monocytogenes* on inoculated/ noninoculated minimally processed broccoli stored at 4 and 10°C. Atmosphere was not significantly different ($P>0.05$)

storage. Storage of MP broccoli at 10°C was determined to significantly increase ($P<0.05$) the growth rate of *L. monocytogenes* over storage at 4°C (Table A6).

L. monocytogenes was only detected sporadically in noninoculated MP broccoli indicating that low levels of the organism were present or that it was absent on the broccoli. *L. monocytogenes* was not detected on any broccoli, after 48 hours enrichment in UVM broth, that was not detected by direct plate count on PALCAM.

The growth of *L. monocytogenes* on MP broccoli was similar to that reported by Berrang et al. (1989). Berrang et al. (1989) reported that *L. monocytogenes*, when inoculated onto fresh broccoli, increased from initial levels of 10^3 to levels slightly above 10^4 after 21 days storage at 4°C indicating that although a psychrotroph *L. monocytogenes* grows slow at colder temperatures. The sporadic detection of *L. monocytogenes* on non-inoculated broccoli may be expected since Nguyen-the and Carlin (1994) reported that the organism was detected on 13% of all of the salad vegetables that they tested.

Microbial Growth on Osmotically Dehydrated MP Broccoli

4°C Storage

Aerobic and psychrotrophic bacteria were initially present on osmotically dehydrated MP broccoli at levels of approximately Log_{10} 6.5 CFU/ g (Figures 7 and 8). Aerobic and psychrotrophic bacteria were observed to increase from their initial levels to populations greater than Log_{10} 9.0 CFU/ g by the end of the 15 days storage period at 4°C. The growth of aerobic and psychrotrophic organisms was statistically similar

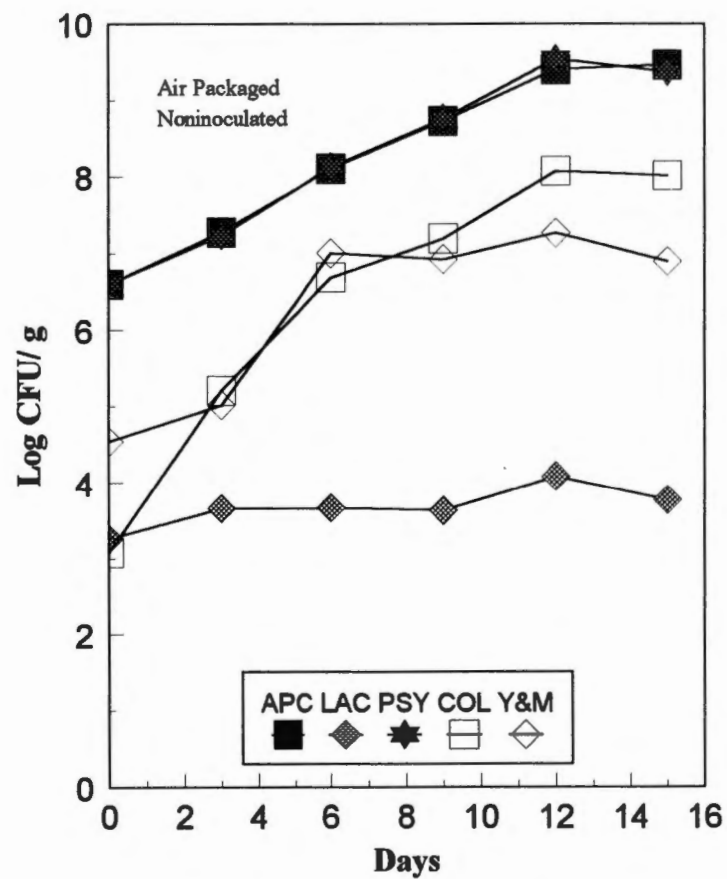


Figure 7: Microbial growth on osmotically dehydrated minimally processed broccoli stored at 4°C.

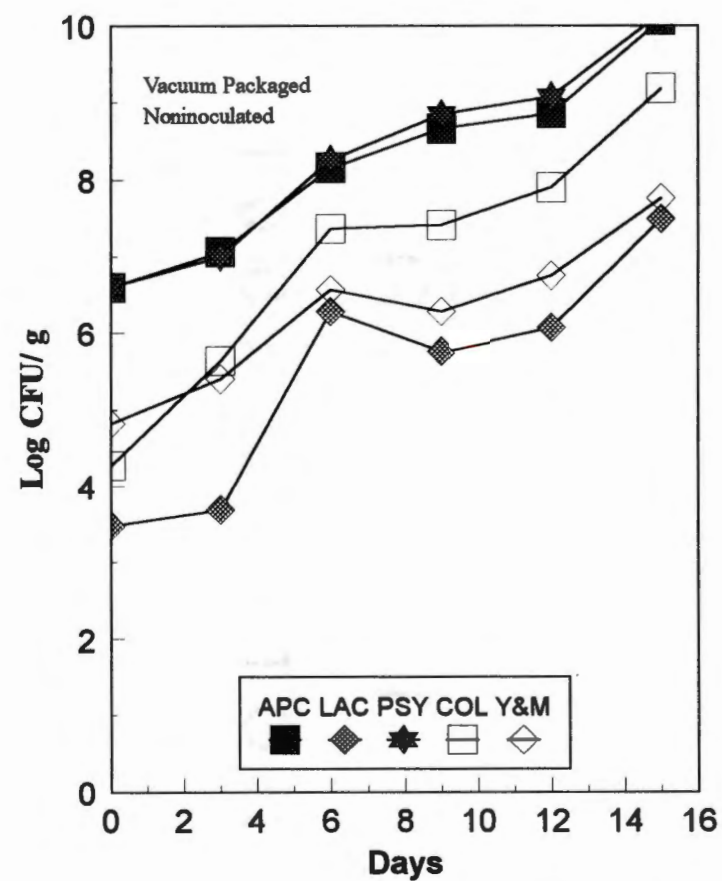


Figure 8: Microbial growth on osmotically dehydrated (2% NaCl), vacuum packaged, minimally processed broccoli stored at 4°C.

($P>0.05$) between both air packaged (Figure 7) and vacuum packaged (Figure 8) osmotically dehydrated MP broccoli. Osmotic dehydration was found to significantly increase ($P<0.05$) the growth of aerobic and psychrotrophic bacteria (Tables A1 and A2). Populations of the organisms were initially similar to nondehydrated MP broccoli (Figures 1 and 2) however, populations increased more steadily and reach higher levels on the osmotically dehydrated broccoli (Figures 7 and 8). Growth of aerobic and psychrotrophic organisms was statistically the same ($P>0.05$) on air package (Figure 7) and vacuum packaged (Figure 8) osmotically dehydrated MP broccoli (Tables A1 and A2).

Coliform bacteria and LA bacteria also demonstrated significant increases ($P<0.05$) in numbers throughout the 15-day storage period at 4°C (Tables A3 and A4). Lactic acid bacteria increased slowly on osmotically dehydrated, air packaged broccoli stored at 4°C (Figure 7). However, when osmotically dehydrated broccoli was vacuum packaged and stored under similar conditions, populations increased from initial levels near Log_{10} 3.5 to levels slightly higher than Log_{10} 7.0 CFU/ g (Figure 8). Vacuum packaging was determined to significantly increase ($P<0.05$) the growth of LA bacteria on osmotically dehydrated MP broccoli held at 4°C (Table A3). Coliform bacteria increased from initial levels of Log_{10} 3.0 CFU/ g to levels slightly greater than Log_{10} 7.5 CFU/ g by the end of storage on air package broccoli (figure 7). A statistically similar ($P>0.05$) growth pattern was observed for coliform bacteria on vacuum packaged broccoli stored under the same conditions (Figure 8).

A significant ($P<0.05$) increase in numbers of yeasts and molds, during storage for 15 days, was observed on osmotically dehydrated MP broccoli stored at 4°C (Table A5).

The growth of yeasts and molds was determined not to be influenced by osmotic dehydration or package atmosphere (Table A5).

Inoculation with *L. monocytogenes* did not significantly ($P>0.05$) influence the growth of aerobic and psychrotrophic organisms on osmotically dehydrated MP broccoli held at 4°C (Tables A1 and A2). Aerobic and psychrotrophic organisms demonstrated similar growth patterns on inoculated broccoli (Figures 9 and 10) as they did on noninoculated broccoli under the same storage conditions (Figures 7 and 8). LA bacteria increased from initial levels of Log_{10} 5.0 CFU/ g to Log_{10} 6.0 CFU/ g by the sixth day of storage at 4°C (Figure 9). Coliform bacteria were initially detected at a level of Log_{10} 4.0 CFU/ g to final populations of Log_{10} 8.5 CFU/ g by the ninth day of storage at 4°C (Figure 9). The rate of growth for both LA bacteria and coliform bacteria were similar in both air packaged (Figure 9) and vacuum packaged (Figure 10) MP broccoli. The growth of both LA bacteria and coliform bacteria were not affected by the presence of *L. monocytogenes*.

10°C Storage

A significant ($P<0.05$) increase in populations of aerobic and psychrotrophic bacteria was observed on osmotically dehydrated MP broccoli stored at 10°C (Tables A1 and A2). Aerobic and psychrotrophic organisms increased from initial populations near Log_{10} 5.0 CFU/ g to levels approaching Log_{10} 9.0 CFU/ g by the sixth day of storage at 10°C (Figure 11). Populations of these organisms remained constant from the sixth day of storage until the end of 15 days storage period. Aerobic and psychrotrophic organisms increased at a significantly ($P<0.05$) faster rate when stored at 10°C (Figure 11) than when

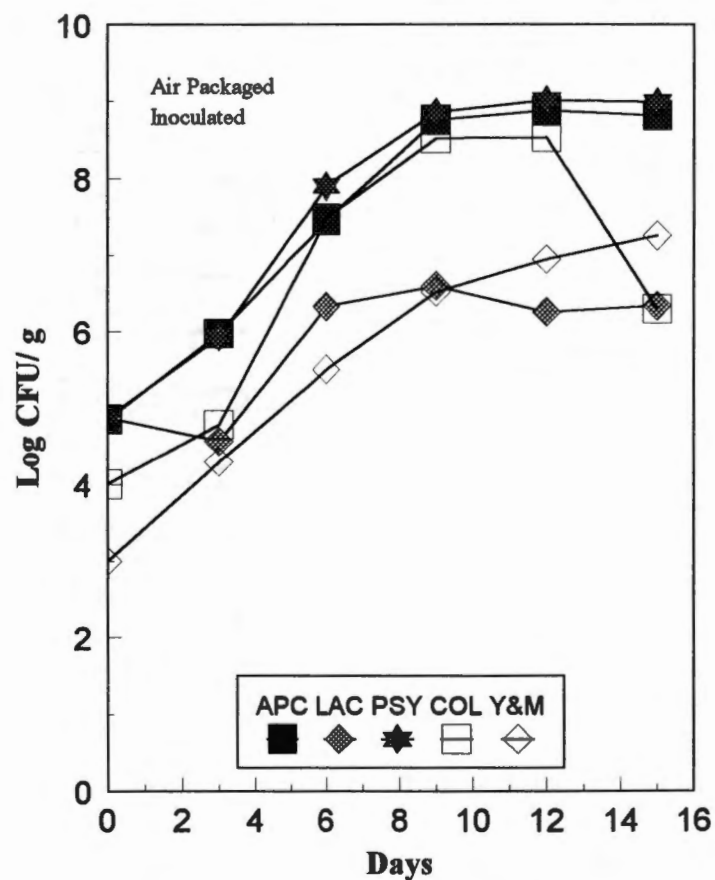


Figure 9: Microbial growth on osmotically dehydrated minimally processed broccoli, inoculated with Log 5.0 CFU/ mL *Listeria monocytogenes* and stored at 4°C under vacuum.

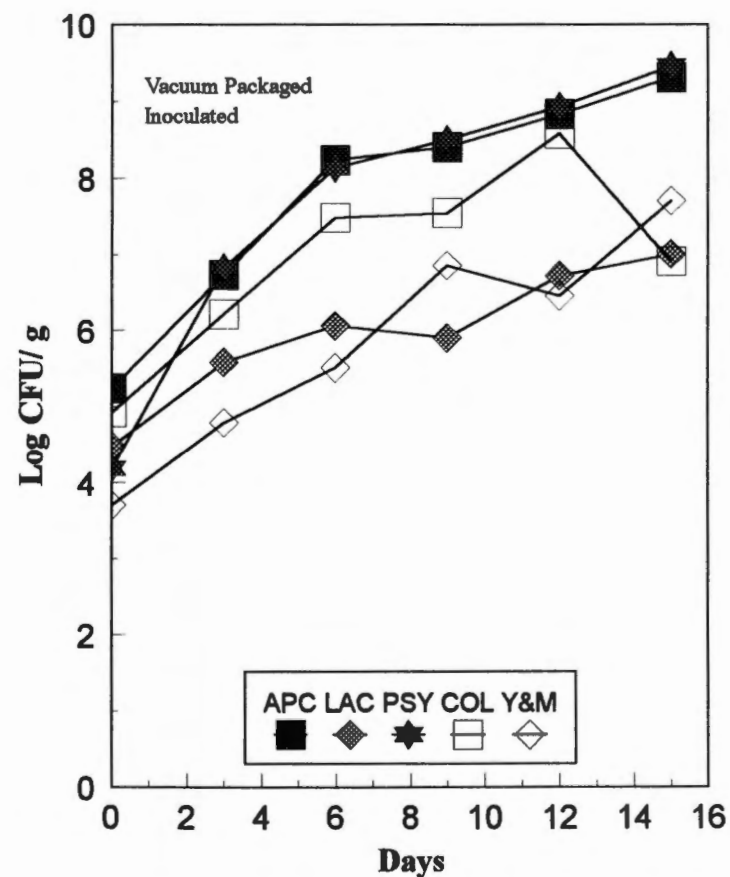


Figure 10: Microbial growth on osmotically dehydrated minimally processed broccoli, inoculated with Log 5.0 CFU/ ml *Listeria monocytogenes* and stored at 4°C and stored under vacuum.

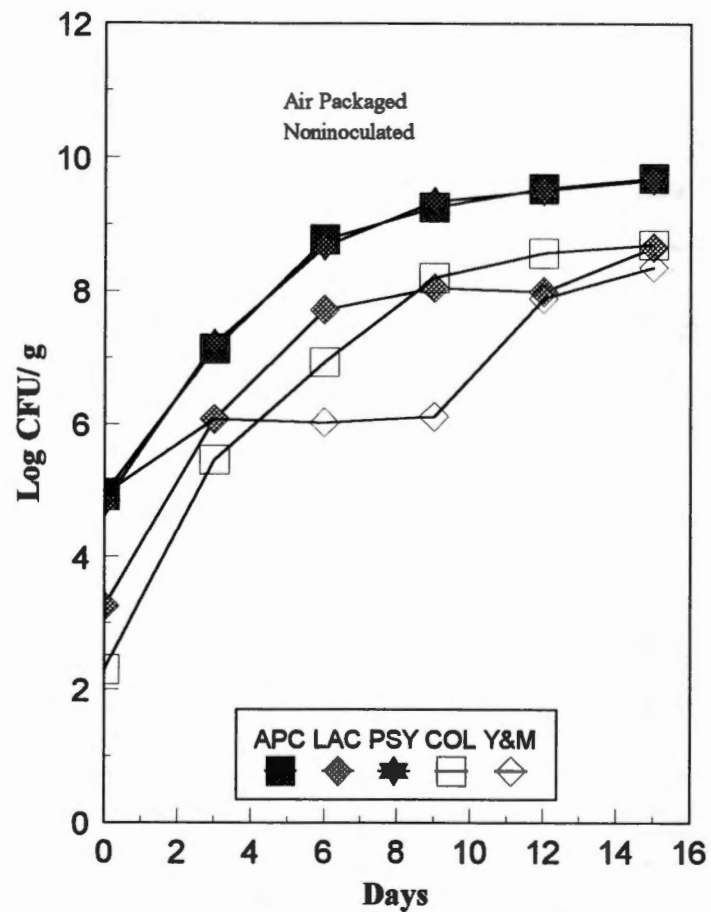


Figure 11: Microbial growth on osmotically dehydrated minimally processed broccoli stored at 10°C under air or vacuum. Atmospheres were significantly different ($P > 0.05$) Data for vacuum packaged, osmotically dehydrated broccoli not shown.

stored at 4°C (Figures 7 and 8) (Tables A1 and A2).

LA bacteria and coliform bacteria increased significantly ($P<0.05$) over the 15 days storage period on osmotically dehydrated MP broccoli stored at 10°C (Table A3).

Populations of LA bacteria increased from initial levels of approximately Log_{10} 3.0 CFU/ g to levels approaching Log_{10} 9.0 CFU/ g by day 15 (Figure 11). Coliform bacteria increased from initial levels of Log_{10} 2.2 CFU/ g to levels slightly above Log_{10} 8.0 CFU/ g by day 12 twelve of storage. Air packaged and vacuum packaged osmotically dehydrated MP broccoli was determined to be statistically the same ($P<0.05$) for both LA bacteria (Table A3) and coliform bacteria (Table A4).

Yeasts and molds were not influenced by osmotic dehydration (Table A5).

Populations of yeasts and molds increased from initial levels near Log_{10} 4.5 CFU/ g to levels slightly higher than Log_{10} 8.0 CFU/ g by the end of the storage period (Figure 11).

Inoculation of osmotically dehydrated MP broccoli with *L. monocytogenes* did not significantly ($P>0.05$) influence growth of aerobic and psychrotrophic organisms at 10°C (Tables A1 and A2). Aerobic and psychrotrophic organisms increased from initial levels of approximately Log_{10} 4.0 to Log_{10} 9.5 CFU/ g by the end of storage (Figure 12).

Statistically similar ($P>0.05$) results were observed for vacuum packaged, osmotically dehydrated MP broccoli stored at 10°C (Figure 13). Aerobic and psychrotrophic organisms on inoculated osmotically dehydrated broccoli demonstrated (Figures 12 and 13) similar growth rates as noninoculated osmotically dehydrated broccoli (Figure) when stored at 10°C. This indicates the presence of bacteria that are capable of competing with *L. monocytogenes* at refrigeration temperatures.

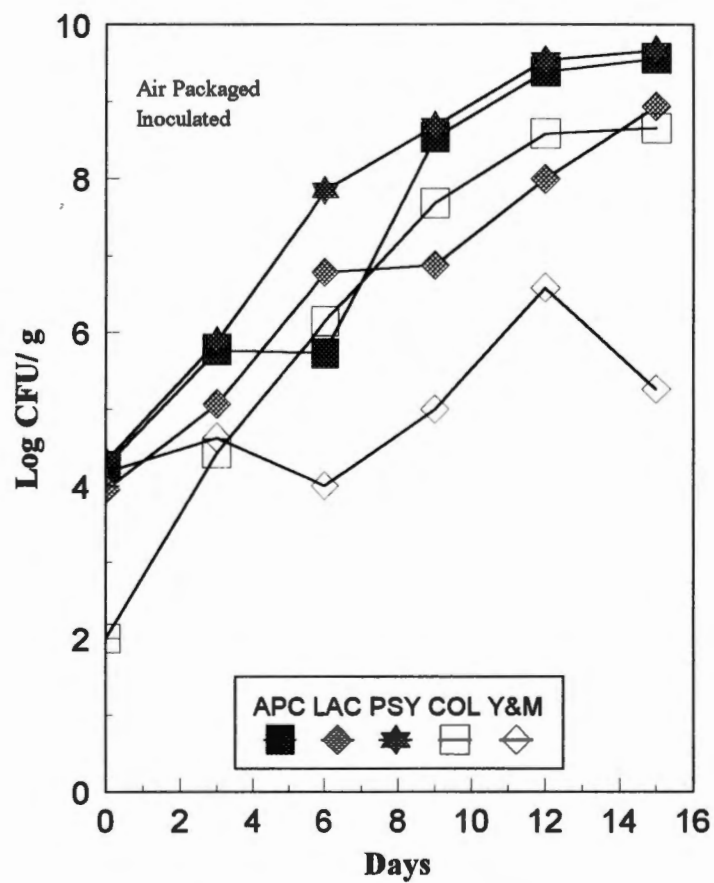


Figure 12: Microbial growth on osmotically dehydrated minimally processed broccoli, inoculated with Log 5.0 CFU/ ml *Listeria monocytogenes*, and stored at 10°C.

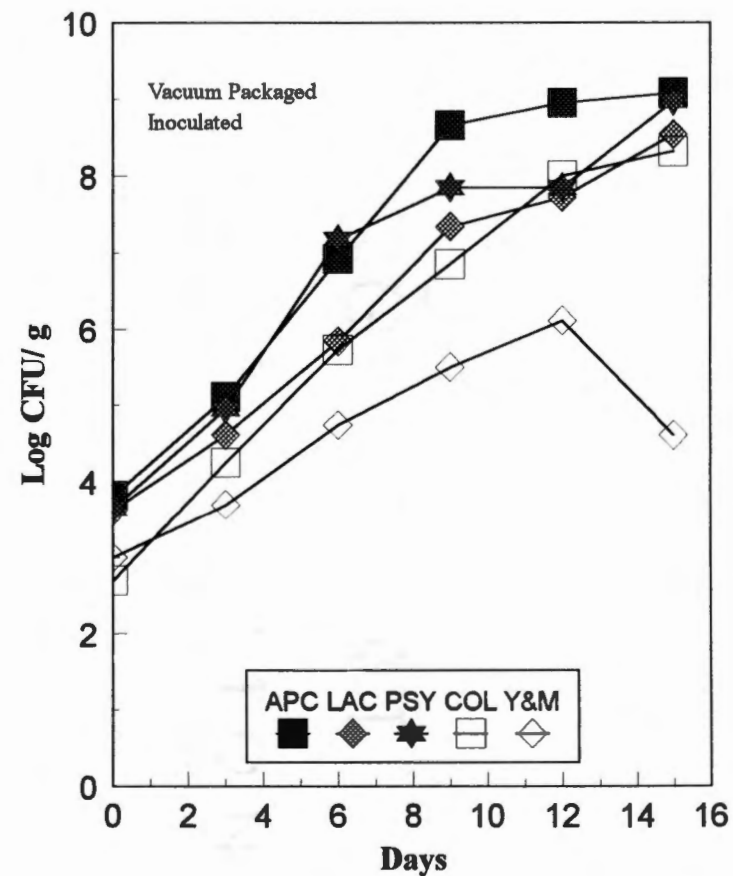


Figure 13: Microbial growth on osmotically dehydrated, vacuum packaged, minimally processed broccoli, inoculated with Log 5.0 CFU/ ml *Listeria monocytogenes* and stored at 10°C.

Coliform bacteria behaved similarly on inoculated osmotically dehydrated broccoli (Figure 12) as compared to noninoculated osmotically dehydrated broccoli (Figure 11). Coliforms increased from initial levels of approximately Log_{10} 2.0 CFU/ g to levels slightly greater than Log_{10} 8.0 CFU/ g by day 12 of storage. A significant ($P < 0.05$) difference was observed for the behavior of coliform bacteria on osmotically dehydrated and nondehydrated inoculated broccoli stored at 10°C (Table A4). Variability among the daily coliform counts was observed on nonosmotically dehydrated broccoli (Figure 4) indicating that inoculation with *L. monocytogenes* may have caused a competition between the two organisms. This variability was not noted on osmotically dehydrated inoculated broccoli (Figure 12) stored under similar conditions. Coliform bacteria were able to increase faster and achieve higher populations on the osmotically dehydrated broccoli.

LA bacteria fared slightly better on osmotically dehydrated broccoli (Figures 12 and 13) than on nondehydrated broccoli (Figures 4 and 5) when inoculated with *L. monocytogenes* and stored at 10°C. LA bacteria increased from initial levels of approximately Log_{10} 4.0 CFU/ g to levels above Log_{10} 8.0 CFU/ g by the end of storage (Figure 12). A similar response was also noted on vacuum packaged broccoli under the same conditions (Figure 13). LA bacteria demonstrated some difficulty competing with *L. monocytogenes* in nondehydrated broccoli (Figure 4) but grew well in the osmotically dehydrated broccoli (Figure 12) indicating that the increased salt content of the broccoli may have had a stimulatory effect on the LA bacteria. This would not seem to be surprising since a NaCl level just slightly higher than two percent is used to select for LA bacteria in some vegetable fermentations such as the production of sauerkraut (Jay, 1992).

Inoculation, with *L. monocytogenes*, of osmotically dehydrated broccoli was determined not to have a significant ($P>0.05$) effect on the growth of yeasts and molds when stored at 10°C (Table A5). Yeasts and molds increased from initial levels of Log_{10} 4.0 at the beginning of storage to levels slightly above Log_{10} 6.0 CFU/ g by the twelfth day of storage (Figure 12). Statistically similar ($P>0.05$) results were obtained on inoculated osmotically dehydrated broccoli which had been vacuum packaged (Figure 13).

L. monocytogenes

Populations of *L. monocytogenes* demonstrated a significant ($P<0.05$) increase over the 15 days storage period on osmotically dehydrated MP broccoli held at 4 and 10°C (Table A6). The initial population of *L. monocytogenes* increased from Log_{10} 3.2 CFU/ g to levels slightly higher than Log_{10} 8.0 CFU/ g by the fifteenth day of storage at 10°C (Figure 14). An approximate 2.0 Log increase in numbers was observed for *L. monocytogenes* at 4°C. *L. monocytogenes* increased at a faster rate and reach higher final populations when broccoli was stored at 10°C than when broccoli was stored at 4°C (Figure 14). Only isolated populations of *L. monocytogenes* were recovered in noninoculated broccoli (Figure 14).

L. monocytogenes increased at faster rates and reached higher final populations, regardless of storage temperature, on osmotically dehydrated broccoli (Figure 14) than on nondehydrated broccoli (Figure 6) even though similar initial populations were observed for both the dehydrated and nondehydrated. *L. monocytogenes* demonstrated a 2.0 log increase on osmotically dehydrated broccoli at 4°C (Figure 14) but only showed a 0.5 log increase on nondehydrated broccoli at the same storage temperature (Figure 6). *L.*

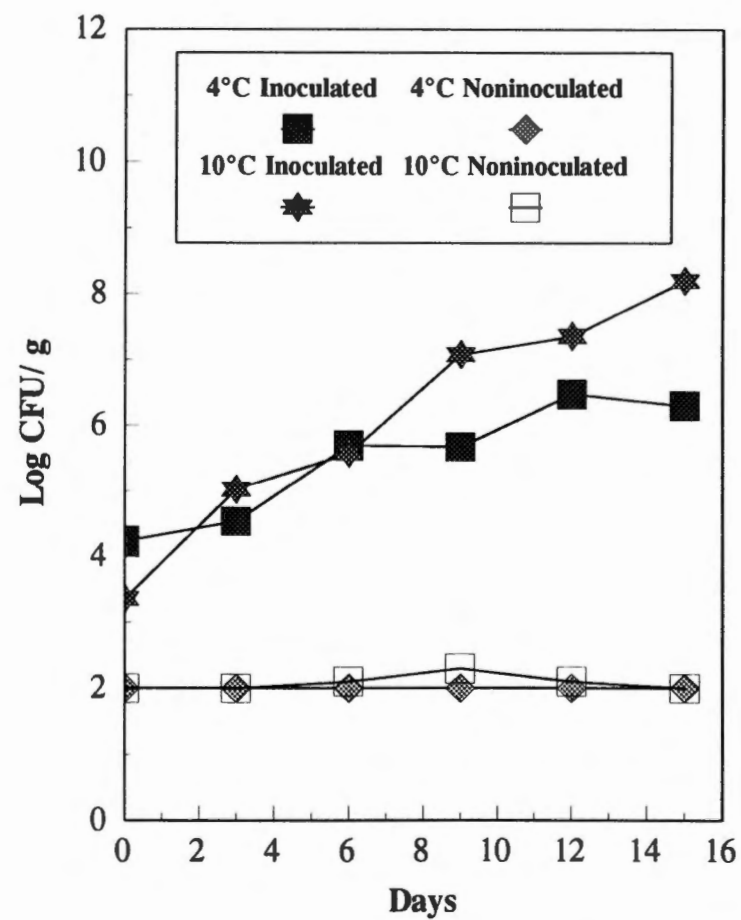


Figure 14: Growth of *Listeria monocytogenes* on osmotically dehydrated minimally processed broccoli. (Atmospheres were not significantly different $P > 0.05$.)

monocytogenes demonstrated an approximately 5.0 log increase in numbers on osmotically dehydrated broccoli at 10°C (Figure 14) but only increased by approximately 2.5 logs on nondehydrated broccoli at the same storage temperature (Figure 6). Osmotic dehydration (final level 2% NaCL) appeared to have a stimulatory effect on the growth of *L. monocytogenes* at either storage temperature and in fact was confirmed by the statistical analysis which demonstrated that osmotic dehydration was having a significant effect ($P<0.05$) (Table 6) on growth of *L. monocytogenes*.

Antimicrobial Effect on Microbial Growth on MP Broccoli

4°C Storage

Aerobic and psychrotrophic organisms showed a significant ($P<0.05$) increase in numbers throughout the 15 days of storage on MP broccoli treated with a hypochlorite dip (Tables A1 and A2). Aerobic and psychrotrophic organisms increased from initial levels of approximately Log_{10} 3.5 CFU/ g to levels approaching Log_{10} 7.5 CFU/ g toward the end of the storage period at 4°C (Figure 15). Similar results were observed on vacuum packaged MP broccoli stored at 4°C (Figure 16). Aerobic and psychrotrophic organisms did not differ significantly ($P>0.05$) from MP broccoli not treated with antimicrobials when stored at either 4 or 10°C (Table A1 and A2). The initial levels of aerobic and psychrotrophic organism on hypochlorite treated MP broccoli (Figure 15) were observed to be less than initial levels of the same organisms on nontreated MP broccoli (Figure 1). Aerobic and psychrotrophic organisms did increase in numbers, on

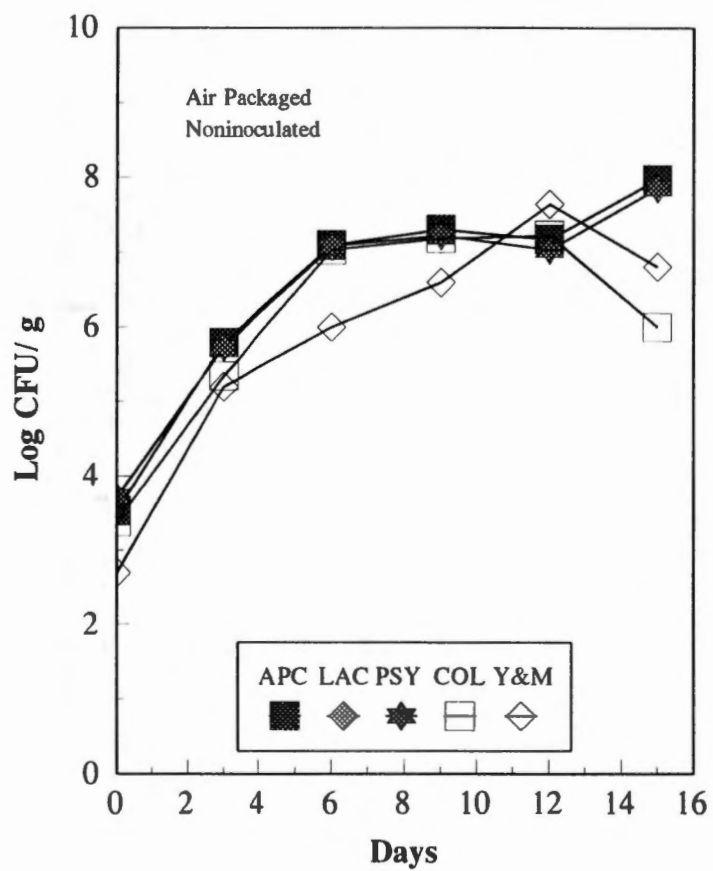


Figure 15: Microbial growth on hypochlorite treated, minimally processed broccoli stored at 4°C under air.

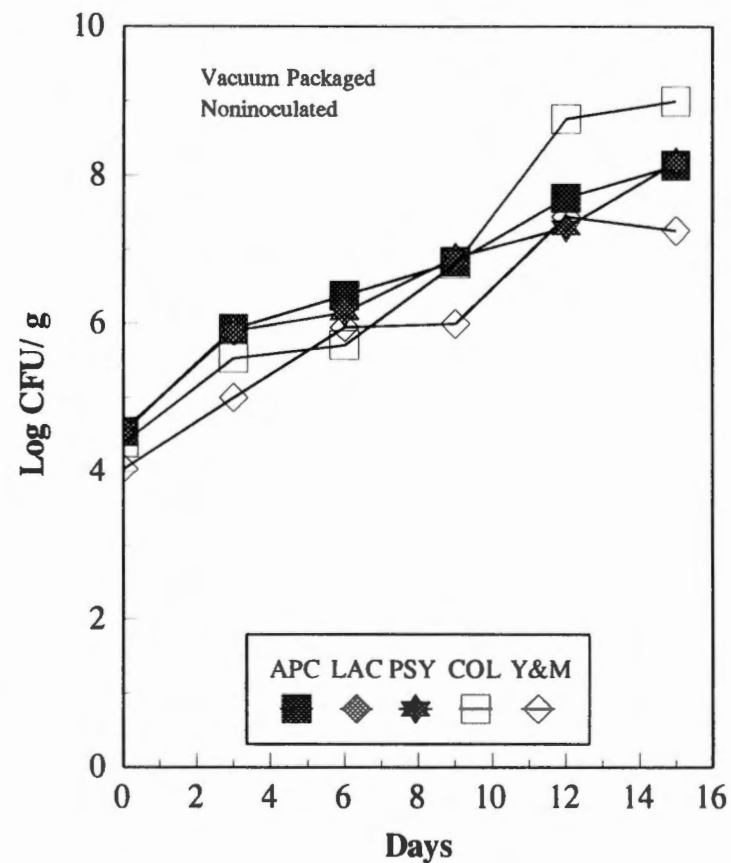


Figure 16: Microbial growth on hypochlorite treated minimally processed broccoli stored at 4°C under vacuum.

hypochlorite treated MP broccoli, throughout the storage period (Figure 15). This indicates that fresh MP broccoli contains a significant amount of hypochlorite (200 ppm) resistant organisms. Similar growth patterns were observed for both coliform bacteria and LA bacteria on hypochlorite treated MP broccoli at 4°C (Figures 15 and 16). No significant differences ($P>0.05$) were observed in LA bacteria counts between air or vacuum packaged MP broccoli stored at 4°C (Table A3 and A4). LA bacteria were able to increase from initial populations of Log_{10} 3.5 CFU/ g to levels slightly above Log_{10} 7.0 CFU/ g by day 9 of storage at 4°C (Figure 15). Coliform bacteria demonstrated a similar growth pattern as that of LA bacteria under the same conditions (Figure 15). Both coliform and LA bacteria reached peak populations by day 9 of storage and remained consistently level for the rest of the storage period (Figure 15).

Inoculation of MP broccoli with *L. monocytogenes* did not significantly ($P>0.05$) influence growth of aerobic and psychrotrophic organisms during storage at 4°C (Tables A1 and A2). Growth of aerobic and psychrotrophic organism on inoculated MP broccoli, treated with hypochlorite (Figures 17 and 18) demonstrated similar patterns to the same organisms on noninoculated MP broccoli at 4°C (Figures 15 and 16). No apparent differences were observed for aerobic and psychrotrophic bacterial counts on air packaged (Figure 17) or vacuum packaged (Figure 18) inoculated MP broccoli when stored at 4°C.

Aerobic and psychrotrophic organisms also demonstrated a significant increase ($P<0.05$) in numbers during storage at 4°C on metabisulfite treated MP broccoli (Tables A1 and A2). Aerobic and psychrotrophic organisms increased from an initial population of approximately Log_{10} 6.5 CFU/ g to levels nearing Log_{10} 8.5 CFU/ g by the ninth day of

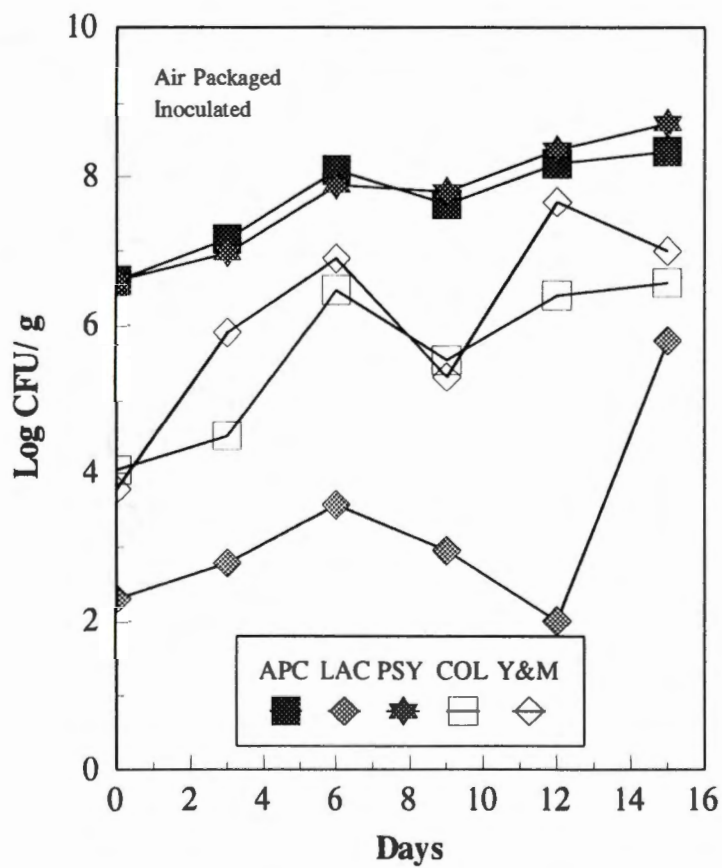


Figure 17: Microbial growth on hypochlorite treated minimally processed broccoli, inoculated with Log 5.0 CFU/ mL *Listeria monocytogenes*, and stored at 4°C under air.

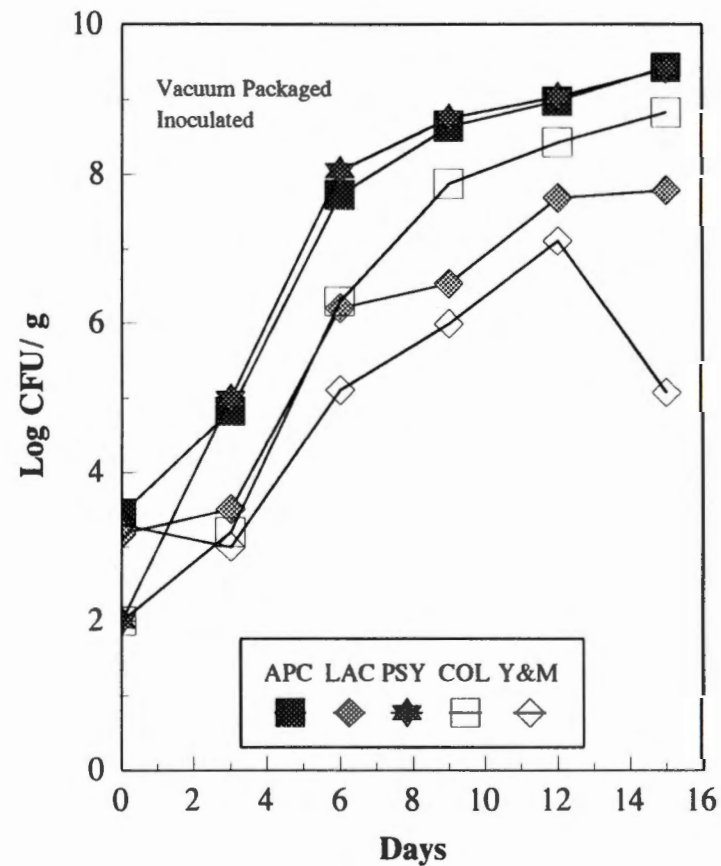


Figure 18: Microbial growth on hypochlorite treated, vacuum packaged minimally processed broccoli, inoculated with Log 5.0 CFU/ mL *Listeria monocytogenes*, and stored at 4°C.

storage at 4°C (Figure 19). Aerobic and psychrotrophic bacterial growth on air packaged metabisulfite treated MP broccoli stored at 4°C (Figure 19) was not significantly ($P>0.05$) influenced by inoculation with *L. monocytogenes* and was not significantly different from vacuum packaged broccoli under similar storage conditions (data not shown) (Table A1 and A2). Aerobic and psychrotrophic bacteria on metabisulfite treated MP broccoli (Figure 19) reached similar bacterial count levels as that of hypochlorite treated MP broccoli (Figure 17) and nontreated broccoli (Figure 1) at 4°C.

Metabisulfite treatment showed some bacteriostatic effect against LA bacteria on MP broccoli stored at 4°C (Figure 19). LA bacteria counts remained consistently low for the first 6 days of storage before increasing slightly (Figure 19). Coliform bacteria appeared not to be affected by metabisulfite treatment (Figure 19) as they maintained similar growth levels as nontreated broccoli during storage at 4°C (Figure 1).

10°C Storage

Aerobic and psychrotrophic organisms increased significantly ($P<0.05$) from initial levels of Log_{10} 5.7 CFU/ g to levels slightly above Log_{10} 9.0 CFU/ g on hypochlorite treated MP broccoli by the twelfth day of storage at 10°C (Figure 20). Growth of aerobic and psychrotrophic organisms was not significantly ($P>0.05$) influenced by air or vacuum packaging or by inoculation with *L. monocytogenes* (Tables A1 and A2). Aerobic and psychrotrophic organisms (Figure 20) were present, initially, at levels slightly greater than levels on hypochlorite treated MP broccoli stored at 4°C (Figures 15 and 16). This may indicate that one shipment of broccoli was more heavily contaminated than others and indicates the variability that may be observed between different cases of broccoli. The

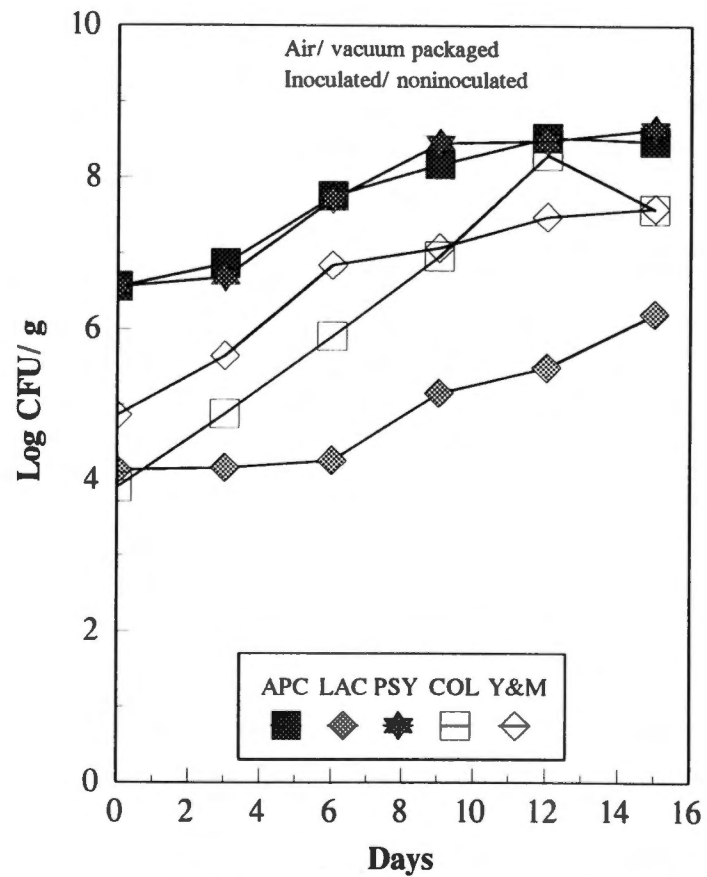


Figure 19: Microbial growth on metabisulfite treated minimally processed broccoli, inoculated with Log 5.0 CFU/ mL *Listeria monocytogenes* and stored at 4°C. Atmosphere and inoculation did not significantly ($P > 0.05$) influence growth of microorganisms. Data for vacuum packaged, noninoculated broccoli not shown.

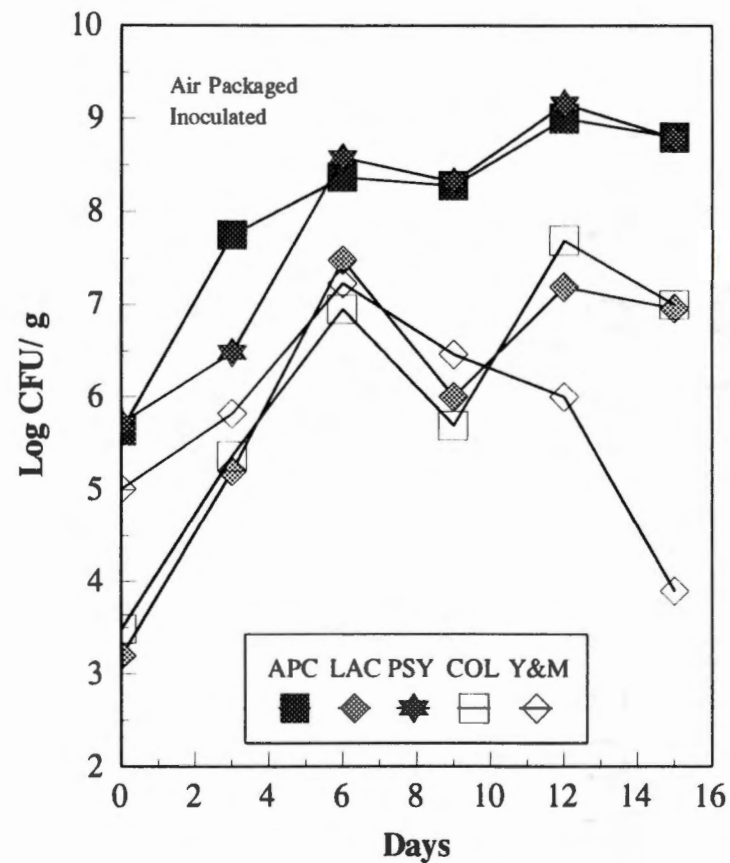


Figure 20: Microbial growth on hypochlorite treated minimally processed broccoli, inoculated with Log 5.0 CFU/ mL *Listeria monocytogenes* and stored at 10°C. Atmosphere and inoculation did not significantly ($P>0.05$) influence growth of microorganisms. Data for vacuum packaged, inoculated broccoli not shown.

increase in numbers of aerobic and psychrotrophic organisms at 10°C indicates the presence of numerous hypochlorite resistant bacteria which were present on fresh broccoli and indicates that the washing in a hypochlorite solution (200 ppm) may not be sufficient to remove most spoilage organisms.

Both LA bacteria and coliform bacteria experienced some difficulty competing with *L. monocytogenes* on hypochlorite treated MP broccoli, inoculated with *L. monocytogenes* and stored at 10°C (Figure 20). The variability in LA bacteria counts on the hypochlorite treated, inoculated broccoli at 10°C (Figure 20) is similar to the variability observed in hypochlorite treated and untreated, inoculated broccoli stored at 4°C (Figure 17) and 10°C (Figure 4) was similar. The consistency with which inoculation interferes with the growth of LA bacteria indicates that the LA bacteria struggle to compete with *L. monocytogenes* regardless of the treatment application that the broccoli received. Coliform bacteria demonstrated some variability between daily counts, however it was determined that statistically there was no significant difference ($P>0.05$) between inoculated and noninoculated broccoli at 10°C storage (Table A4).

Microbial growth was evaluated on minimally processed broccoli treated with metabisulfite (Figures 21 and 22) Aerobic and psychrotrophic organisms increased from initial levels of Log_{10} 4.0 CFU/ g to populations greater than Log_{10} 9.0 CFU/ g by the end of the 15 days storage period, on metabisulfite treated MP broccoli stored at 10°C (Figure 21). The significant ($P<0.05$) increase in microbial populations over time indicate the presence of metabisulfite resistant psychrotrophs on fresh broccoli (Table A2). Aerobic and psychrotrophic organisms increased at a faster rate and reached slightly higher final

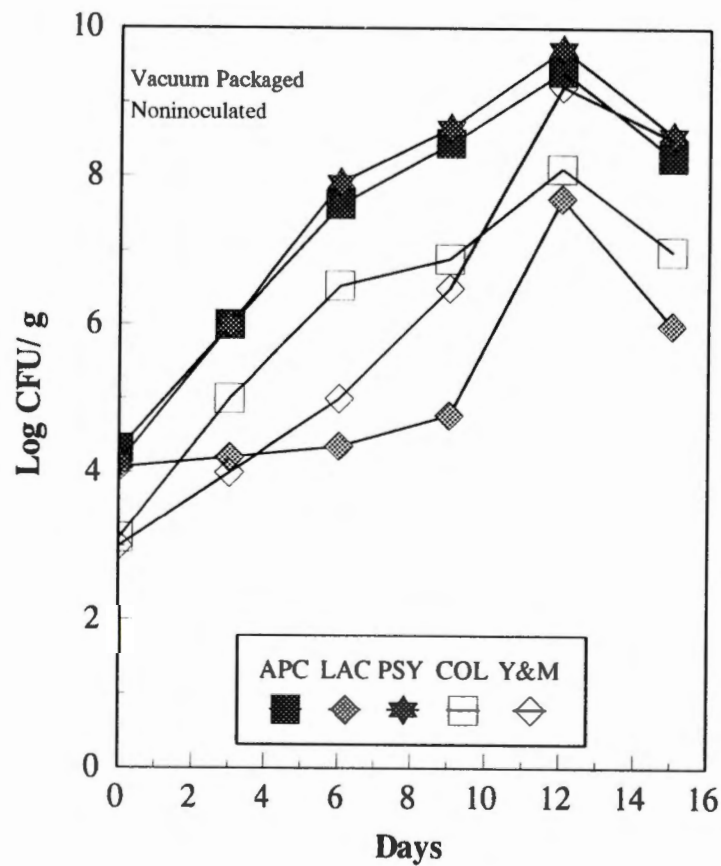


Figure 21: Microbial growth on metabisulfite treated minimally processed broccoli stored at 10°C. Atmosphere did not significantly influence microbial growth ($P > 0.05$). Data for air packaged broccoli not shown.

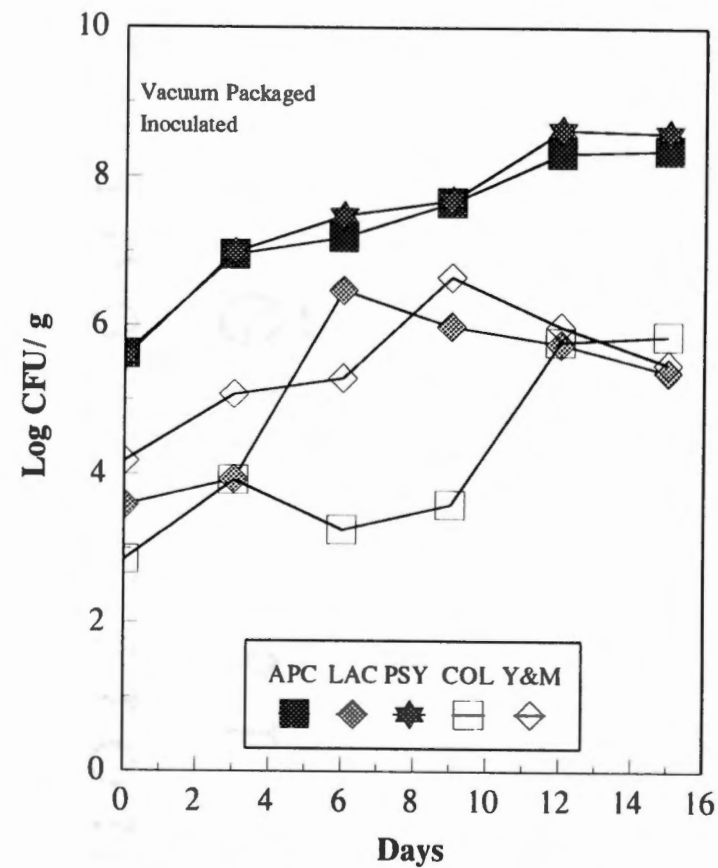


Figure 22: Microbial growth on metabisulfite treated minimally processed broccoli, inoculated with Log 5.0 CFU/ mL *Listeria monocytogenes*, and stored at 10°C. Atmosphere did not significantly ($P > 0.05$) influence microbial growth. Data for air packaged broccoli not shown.

populations during storage at 10°C (Figure 21) than during storage at 4°C (Figure 19).

Coliform bacteria increased from Log_{10} 3.0 CFU/ g to Log_{10} 8.0 CFU/ g on metabisulfite treated MP broccoli during storage at 10°C (Figure 21). Growth of coliform bacteria was somewhat variable on MP broccoli inoculated with *L. monocytogenes* (Figure 22). The variability observed in coliform bacteria counts was not observed on inoculated MP broccoli, packaged under similar conditions, but stored at 4°C (Figure 19). The warmer storage temperature was responsible for increased growth of *L. monocytogenes* which may contributed to the increased variability in coliform bacteria counts at 10°C.

LA bacteria increased slowly, on metabisulfite treated MP broccoli, during the first nine days of storage at 10°C (Figure 21). LA bacteria were able to increase to levels greater than Log_{10} 6.0 CFU/ g by day 10 of storage. LA bacteria did not fare as well on inoculated MP broccoli treated with metabisulfite (Figure 22). LA bacteria on inoculated broccoli (Figure 22) did not reach comparable levels to LA bacteria on noninoculated broccoli (Figure 21). This demonstrates the antagonistic effect that *L. monocytogenes* exhibited against LA bacteria, especially at 10°C. The antagonistic effect of *L. monocytogenes* was intensified at 10°C possibly due to increased numbers of the organism.

L. monocytogenes

L. monocytogenes increased only slightly from Log_{10} 2.0 CFU/ g to Log_{10} 3.0 CFU/ g on hypochlorite treated MP broccoli stored at 4°C (Figure 23). The combined effects of low temperature storage (4°C) and surface application of a 200 ppm hypochlorite (Figure 23) was similar to low temperature storage alone (Figure 6) in

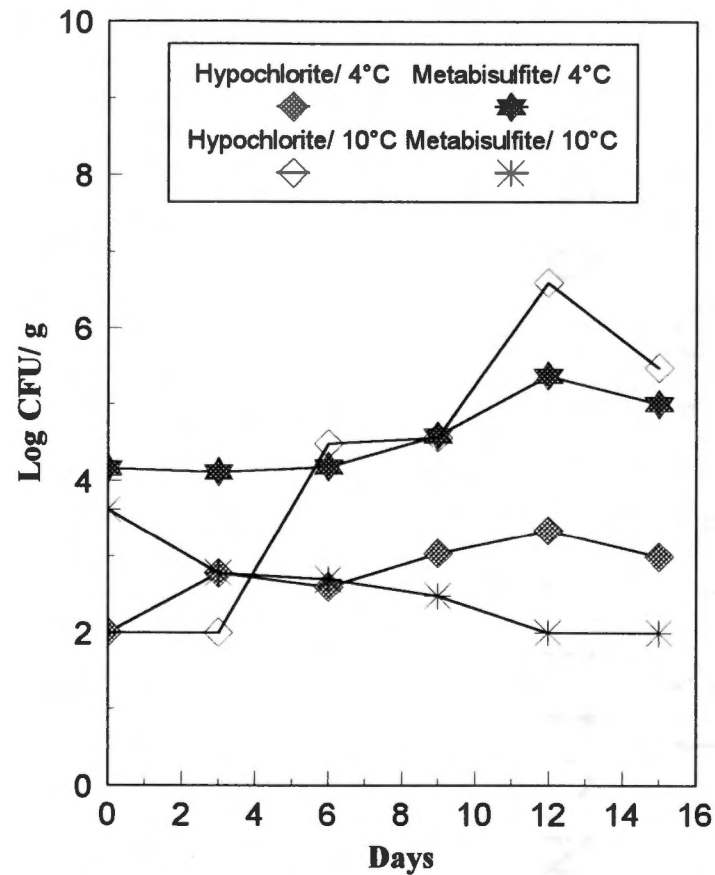


Figure 23: Growth of *Listeria monocytogenes* on minimally processed broccoli treated with either hypochlorite solution (200 ppm) or metabisulfite solution (100 ppm) and stored at 4 or 10°C. Atmosphere did not significantly ($P>0.05$) influence growth.

influencing growth of *L. monocytogenes* indicating that hypochlorite did not reduce numbers of the organism. Numbers of *L. monocytogenes* increased on hypochlorite treated MP broccoli stored at 10°C (Figure 23). Growth of *L. monocytogenes* at 10°C on hypochlorite treated broccoli (figure 23) was similar to growth of that organism on MP broccoli stored at 10°C with no antimicrobial treatment (Figure 6). Again this supports the observations at 4°C, that surface application of a 200 ppm hypochlorite solution was not effective at controlling growth of *L. monocytogenes* during refrigerated storage of MP broccoli. These observations support previously reported results for the effectiveness of hypochlorite treatment in reducing growth of *L. monocytogenes* on fresh vegetables (Brackett, 1987).

L. monocytogenes did not fare well when MP broccoli was treated with a surface application of a 100 ppm solution of metabisulfite (Figure 23). Populations of *L. monocytogenes* decreased during the 15 days storage period at 10°C and maintained similar numbers during storage at 4°C (Figure 23). Growth of the organism on metabisulfite treated MP broccoli (Figure 23) was lower than on hypochlorite treated MP broccoli (Figure 23) and on nontreated minimally processed broccoli (Figure 6). This indicates that the uses of a surface application of metabisulfite would be sufficient in controlling *L. monocytogenes* on MP broccoli. Growth of the organism at either temperature was not significantly influenced by package atmosphere (Table A6).

Antimicrobial Effect on Microbial Growth on Osmotically Dehydrated MP Broccoli

4°C Storage

Aerobic and psychrotrophic microorganisms increased, on osmotically dehydrated MP broccoli treated with hypochlorite, from initial populations of approximately Log_{10} 6.0 CFU/ g to levels slightly greater than Log_{10} 9.0 CFU/ g by the end of the storage period at 4°C (Figure 24). The initial counts indicate that the incoming broccoli was heavily contaminated with aerobic bacteria which may indicate that broccoli was mildly temperature abused during storage and shipment or may just be indicative of a product which is heavily contaminated with soilborne microorganisms from the field. The initial counts also indicate that the broccoli was heavily contaminated with aerobic and psychrotrophic bacteria which were resistant to hypochlorite at the levels applied. Storage atmosphere did not significantly ($P>0.05$) affect the growth of aerobic and psychrotrophic organisms on osmotically dehydrated MP broccoli treated with hypochlorite (Table A1 and A2).

Growth of LA bacteria was variable on osmotically dehydrated broccoli treated with hypochlorite (Figure 24). Populations of LA bacteria were significantly ($P<0.05$) (Table A3) lower on the osmotically dehydrated broccoli treated with hypochlorite (Figure 24) than on osmotically dehydrated broccoli which was not treated with an antimicrobial (Figure 8). LA bacteria began to increase slightly toward the end of the storage period

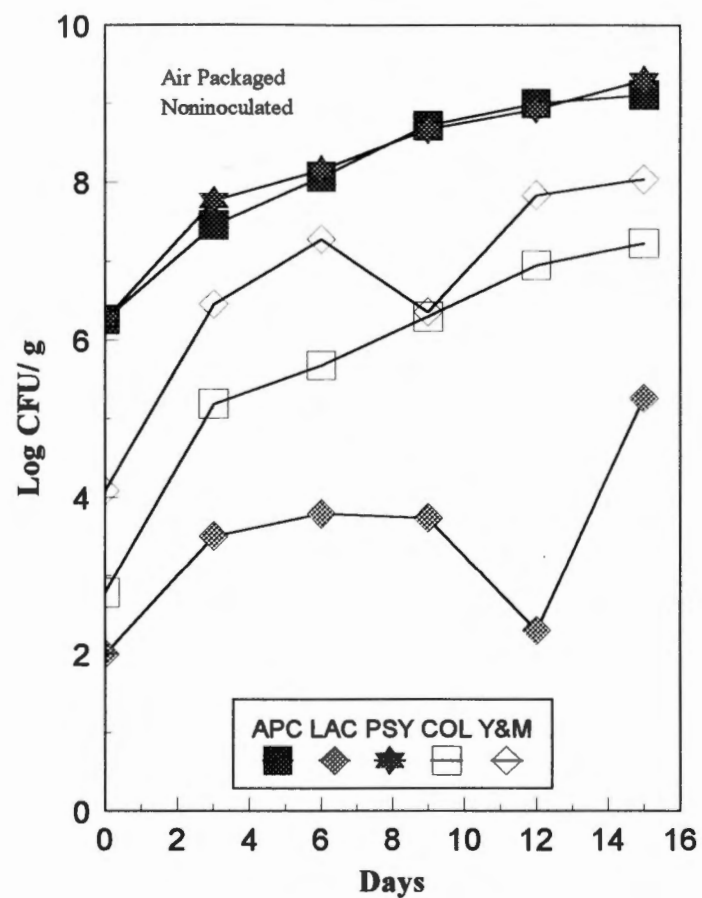


Figure 24: Microbial growth on hypochlorite treated, osmotically dehydrated air packaged broccoli and stored at 4°C. Atmospheres were not significantly ($P>0.05$) different. Data for vacuum packaged, hypochlorite treated, osmotically dehydrated broccoli not shown.

(Figure 24) which may have been attributed to the deteriorating concentrations of hypochlorite over time.

Coliform bacteria and yeasts and molds increased in numbers on the hypochlorite treated osmotically dehydrated MP broccoli (Figure 24). Similar growth patterns were observed on osmotically dehydrated MP broccoli with no antimicrobial treatment (Figure 8) indicating that the level of hypochlorite applied was not sufficient in retarding growth of these organisms.

Inoculation with *L. monocytogenes* did not significantly ($P>0.05$) influence the growth of aerobic and psychrotrophic organisms (Figures 25 and 26) on hypochlorite treated, osmotically dehydrated MP broccoli during storage at 4°C (Table A1 and A2). Some variability was observed in growth of coliform bacteria, LA bacteria and yeasts and molds when MP broccoli was inoculated with a cell suspension, of *L. monocytogenes* of approximately Log_{10} 5.0 CFU/ g and air packaged (Figure 25). The increase in microbial populations was more stable, however, when inoculated MP broccoli was vacuum packaged and stored under similar conditions (Figure 26). This indicated that package atmosphere had some influence on microbial growth on hypochlorite treated, osmotically dehydrated MP broccoli stored at 4°C.

Aerobic and psychrotrophic organisms increased, on metabisulfite treated, osmotically dehydrated MP broccoli, from initial populations of approximately Log_{10} 6.3 CFU/ g to populations greater than Log_{10} 9.0 CFU/ g toward the end of the storage period at 4°C (Figures 27 and 28). Growth of aerobic and psychrotrophic was similar between air packaged (Figure 27) and vacuum packaged (Figure 28) samples. The large increase in

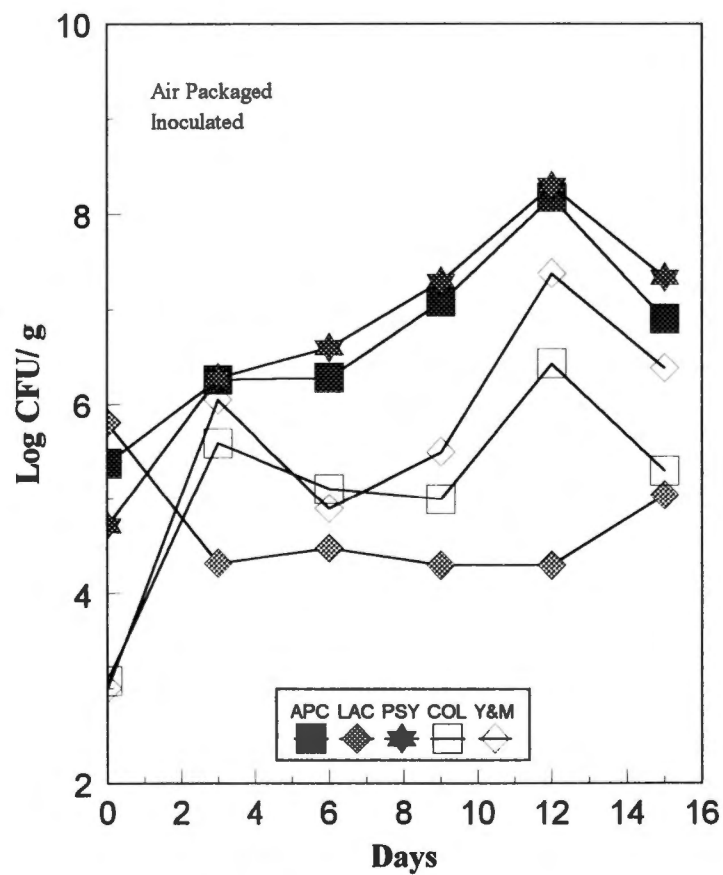


Figure 25: Microbial growth on hypochlorite treated, minimally processed broccoli, inoculated with Log 5.0 CFU/ mL *Listeria monocytogenes* and stored at 4°C.

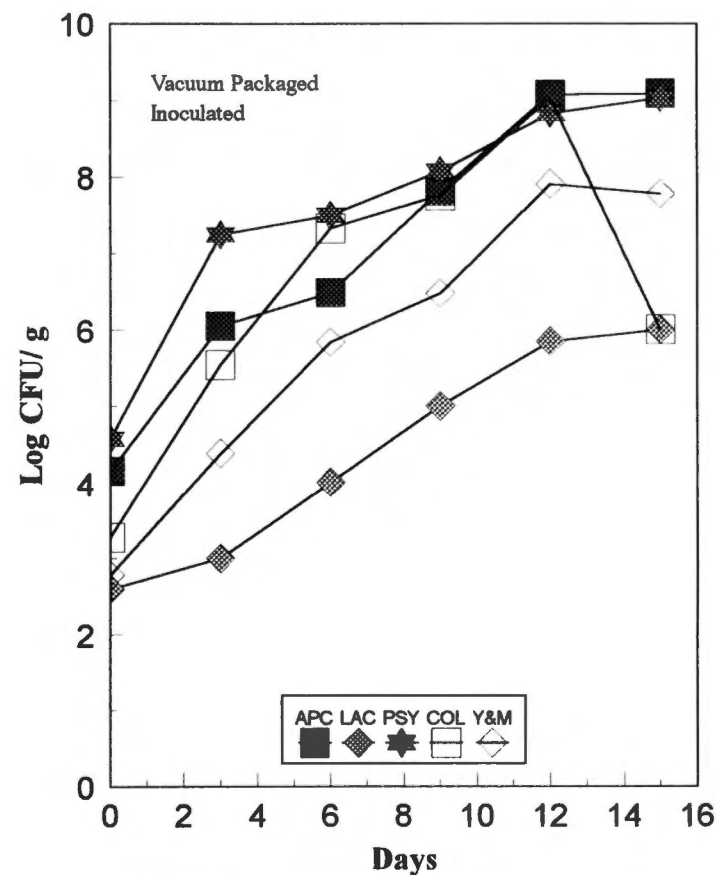


Figure 26: Microbial growth on hypochlorite treated, osmotically dehydrated, vacuum packaged minimally processed broccoli, inoculated with Log 5.0 CFU/ mL *Listeria monocytogenes* and stored at 4°C.

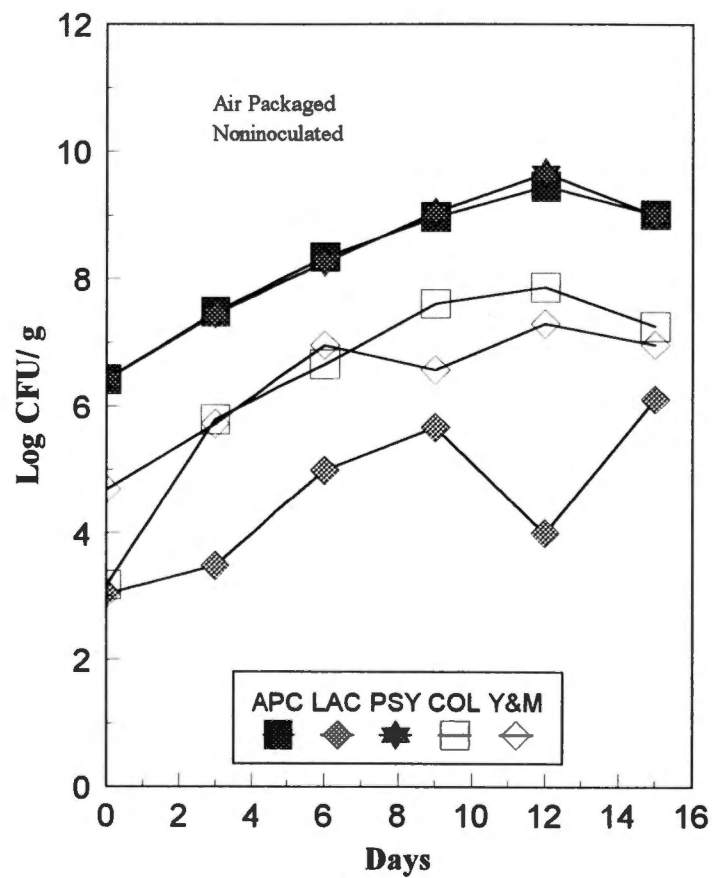


Figure 27: Microbial growth on metabisulfite treated, osmotically dehydrated, air packaged minimally processed broccoli stored at 4°C.

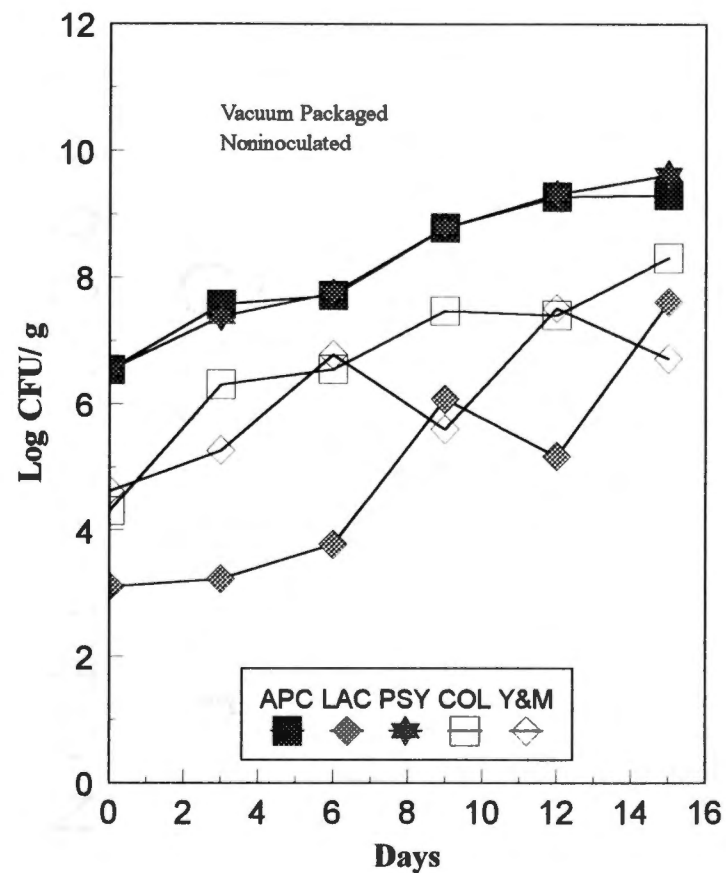


Figure 28: Microbial growth on metabisulfite treated, osmotically dehydrated, vacuum packaged, minimally processed broccoli stored at 4°C.

populations suggests that the broccoli was heavily contaminated with a variety of organisms resistant to the effects of metabisulfite. Growth on metabisulfite treated osmotically dehydrated MP broccoli was similar to nondehydrated broccoli under similar conditions (Figure 19) except that final microbial populations were approximately 1 log higher in the osmotically dehydrated samples indicating that the osmotic dehydration may have been slightly stimulatory to some aerobic and psychrotrophic organisms. Growth of aerobic and psychrotrophic organisms was slightly lower on metabisulfite treated osmotically dehydrated MP broccoli (Figure 27) than on nontreated osmotically dehydrated MP broccoli (Figure 8).

Growth of LA bacteria was somewhat variable on metabisulfite treated osmotically dehydrated MP broccoli (Figures 27 and 28). LA bacteria populations were slightly higher on vacuum packaged broccoli samples (Figure 28) than on air packaged samples (Figure 27). Growth of LA bacteria was slightly retarded during the first six days of storage but began to increase in numbers as the storage time progressed (Figure 28) indicating that the effects of the metabisulfite began to diminish after about six days.

Coliform bacteria and yeast and mold populations increased, on osmotically dehydrated MP broccoli treated with metabisulfite, throughout the 15 days storage period at 4°C (Figures 27 and 28). Growth was similar to that observed on nondehydrated MP broccoli treated with metabisulfite (Figure 19) indicating that osmotic dehydration had little effect on recovery of these organisms after exposure to metabisulfite.

Growth of microorganisms on metabisulfite treated, osmotically dehydrated MP broccoli stored at 4°C was not influenced by inoculation with *L. monocytogenes* (Log₁₀

5.0 CFU/ mL) (Figures 29 and 30). Aerobic and psychrotrophic organisms increased from initial levels of approximately Log_{10} 4-5 CFU/ g to levels greater than Log_{10} 9.0 CFU/ g by the end of the storage period. Growth was observed to be similar between the air packaged samples (Figure 29) and the vacuum packaged samples (Figure 30).

10°C Storage

Aerobic and psychrotrophic organisms increased, on hypochlorite treated, osmotically dehydrated MP broccoli, from initial counts of approximately Log_{10} 5.0 CFU/ g to populations greater than Log_{10} 9.0 CFU/ g by the end of the 15 days storage period at 10°C (Figures 31 and 32). Growth of aerobic and psychrotrophic organisms was statistically similar ($P>0.05$) (Tables A1 and A2) for both air packaged (Figure 31) and vacuum packaged (Figure 32) MP broccoli. Populations for aerobic and psychrotrophic organisms stored at 10°C peaked at similar levels (Figure 31) as those observed on broccoli stored at 4°C under similar conditions (Figure 24). Aerobic and psychrotrophic organism populations on hypochlorite treated osmotically dehydrated broccoli stored at 10°C (Figure 31) were similar to populations of the organisms on nondehydrated hypochlorite treated broccoli stored at 10°C (Figure 20) indicating that osmotic dehydration was not having a substantial influence on growth of these organisms at 10°C.

LA bacteria, coliform bacteria and yeasts and molds also demonstrated increases in populations on hypochlorite treated, osmotically dehydrated broccoli during storage (Figures 31 and 32). LA bacteria were able to surmount to effects of the hypochlorite during storage at 10°C (Figure 31) but were hindered by hypochlorite during storage at 4°C (Figure 24). Coliform bacteria were able to reach populations greater than Log_{10} 7.0

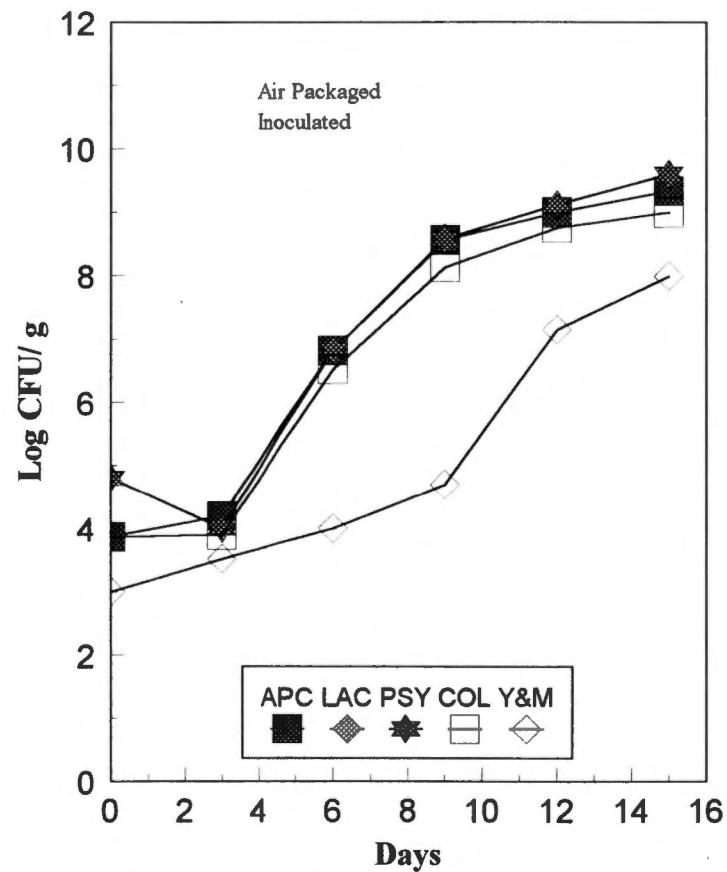


Figure 29: Microbial growth on metabisulfite treated, osmotically dehydrated, minimally processed broccoli, inoculated with Log 5.0 CFU/ mL *Listeria monocytogenes* and stored at 4°C.

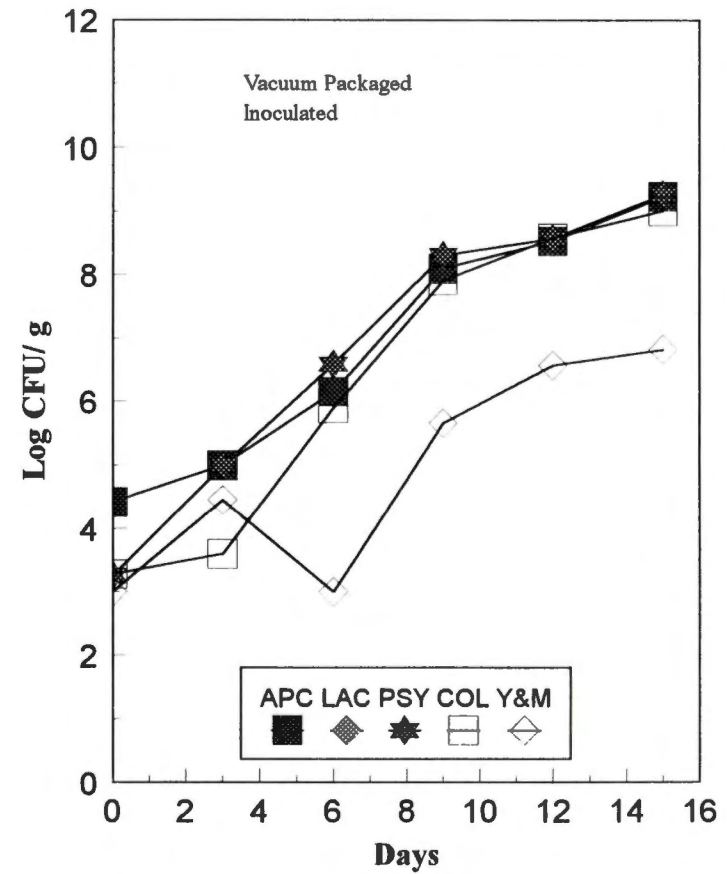


Figure 30: Microbial growth on vacuum packaged, metabisulfite treated, osmotically dehydrated, minimally processed broccoli, inoculated with Log 5.0 CFU/ mL *Listeria monocytogenes* and stored at 4°C.

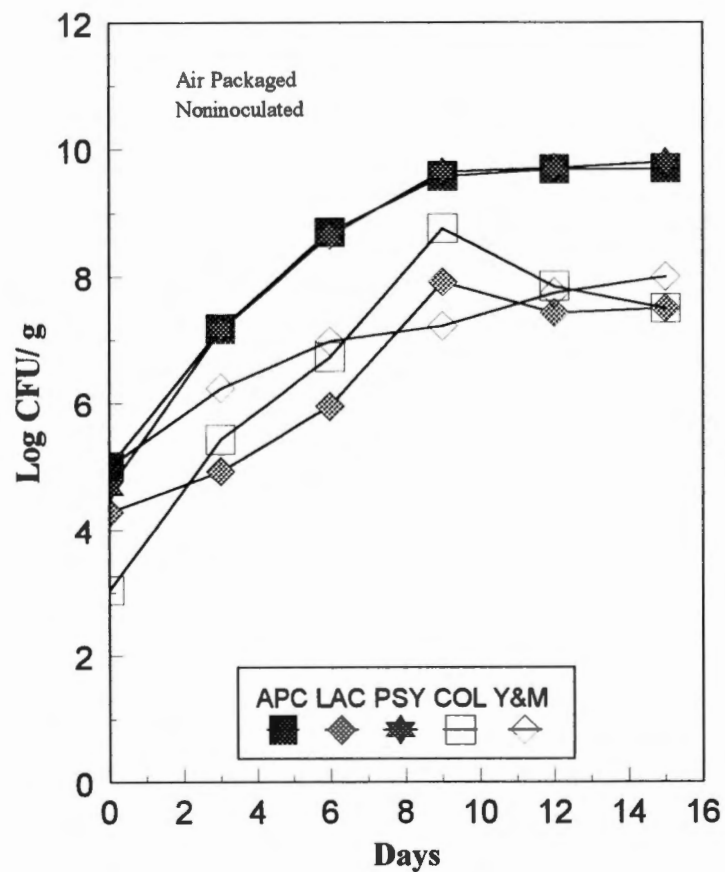


Figure 31: Microbial growth on hypochlorite treated, osmotically dehydrated, minimally processed air packaged broccoli stored at 10°C.

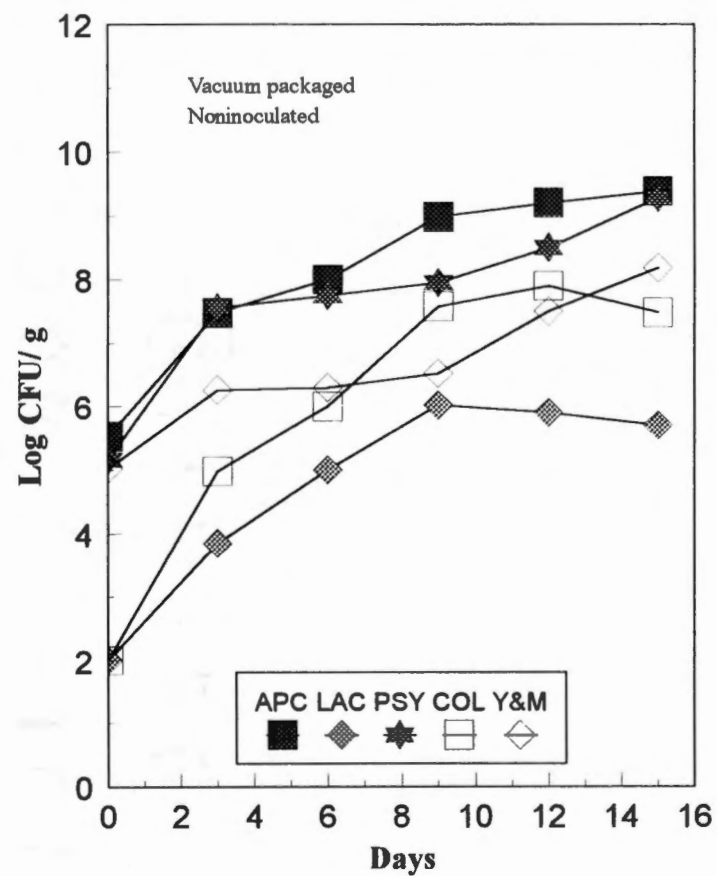


Figure 32: Microbial growth on vacuum packaged, hypochlorite treated, osmotically dehydrated, minimally processed broccoli stored at 10°C

CFU/ g at both 4 and 10°C storage although the growth rate was faster at 10°C (Figure 31) than at 4°C (Figure 24). Little variation was observed in microbial populations between air packaged osmotically dehydrated broccoli (Figure 31) and vacuum packaged osmotically dehydrated broccoli (Figure 32) treated with hypochlorite.

Microbial populations on hypochlorite treated, osmotically dehydrated MP broccoli were not influenced by inoculation with *L. monocytogenes* (Figure 33). It was observed that *L. monocytogenes* affected the growth of LA bacteria, coliforms and yeasts and molds on hypochlorite treated, osmotically dehydrated broccoli during storage at 4°C (Figure 25). The antagonistic effects of the *L. monocytogenes* were lessened by the warmer storage temperature.

Aerobic and psychrotrophic organisms increased on metabisulfite treated, osmotically dehydrated MP broccoli from initial levels of approximately Log_{10} 5.7 CFU/ g to populations greater than Log_{10} 9.0 CFU/ g by the end of storage at 10°C (Figure 34). Some variability was observed in LA bacteria counts (Figure 34) indicating that the metabisulfite was having an antagonistic effect on them. Coliform bacteria and yeasts and molds increased, on the metabisulfite treated broccoli (Figure 34) at rates similar to osmotically dehydrated broccoli with no antimicrobial treatment (Figure 11). Aerobic and psychrotrophic organisms increased at slower rates on vacuum packaged, metabisulfite treated, osmotically dehydrated MP broccoli (Figure 35) than on air packaged broccoli under similar conditions (Figure 34). This may indicate that the combination of vacuum packaging and metabisulfite treatment may have had a slight antagonistic effect on aerobic and psychrotrophic organisms although they still increased several logs over the 15 days

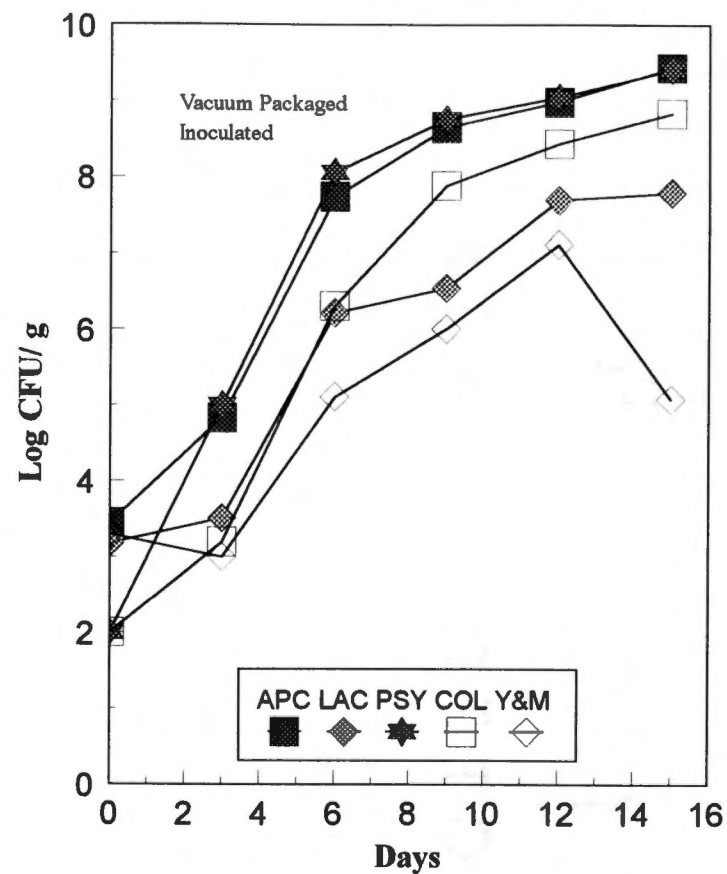


Figure 33: Microbial growth on hypochlorite treated, osmotically dehydrated, minimally processed broccoli, inoculated with Log 5.0 CFU/ mL *Listeria monocytogenes* and stored at 10°C after air and vacuum packaging. Atmospheres were not significantly ($P>0.05$) different. Data for air packaged, hypochlorite treated, osmotically dehydrated broccoli not shown.

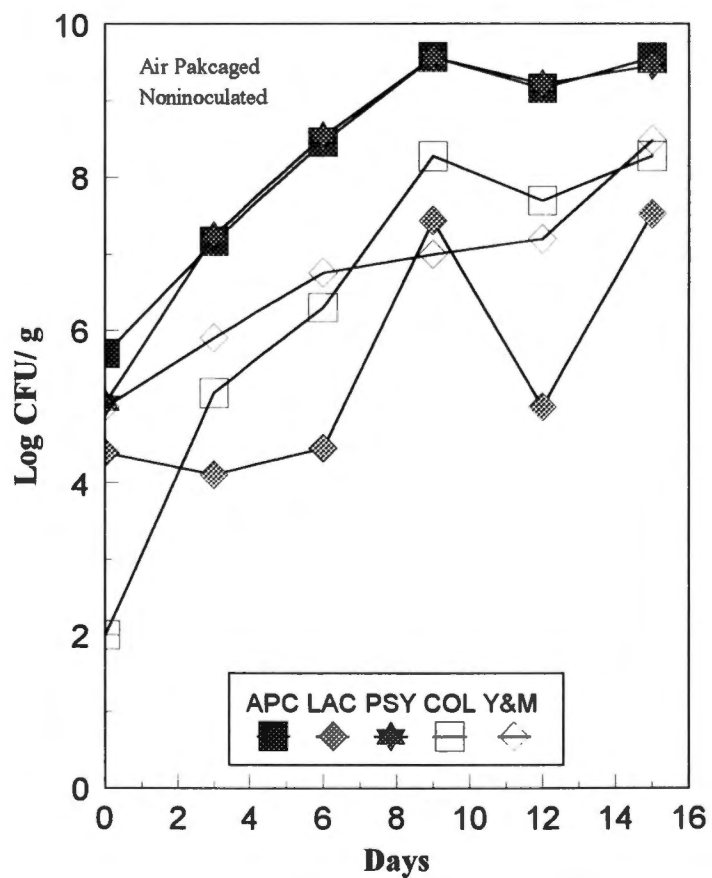


Figure 34: Microbial growth on metabisulfite treated, osmotically dehydrated, minimally processed broccoli stored at 10°C.

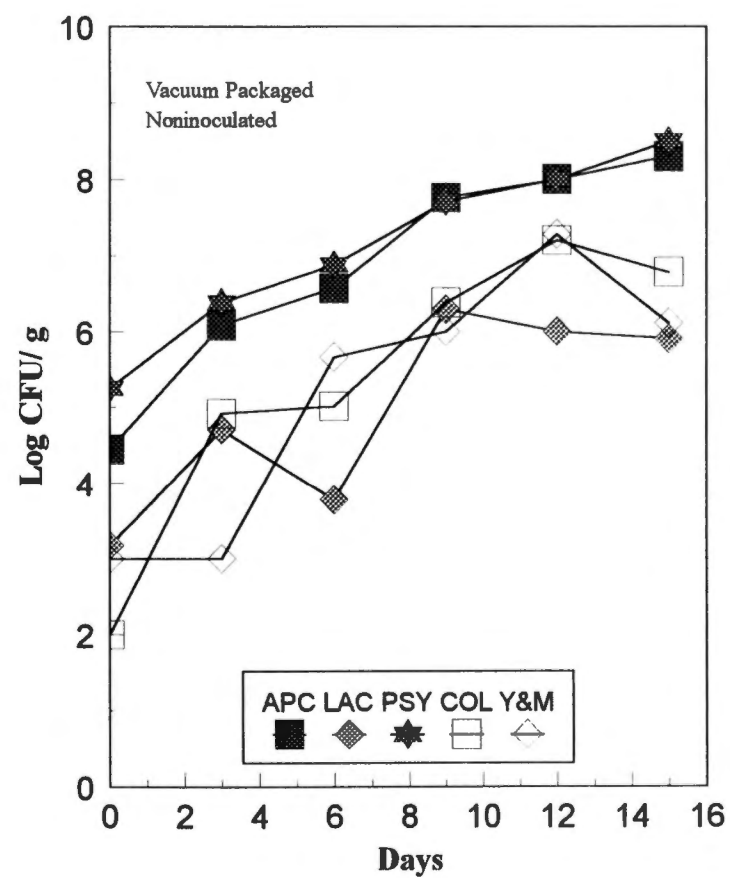


Figure 35: Microbial growth on vacuum packaged metabisulfite treated, osmotically dehydrated, minimally processed broccoli stored at 10°C.

storage period at 10°C. LA bacteria increased at a more rapid rate and with less variability on vacuum packaged, metabisulfite treated, osmotically dehydrated MP broccoli (Figure 35) than on air packaged broccoli receiving the same treatment (Figure 34). Coliforms and yeasts and molds behaved similarly on the vacuum package samples (Figure 35) as on the air packaged samples (Figure 34).

Growth of aerobic and psychrotrophic organisms were not significantly different ($P>0.05$) on metabisulfite treated, osmotically dehydrated MP broccoli stored at 10°C which was inoculated with *L. monocytogenes* (Log_{10} 5.0 CFU/ mL) (Figure 36) than on noninoculated broccoli (Figure 34). Some variability was observed in coliform bacteria and LA bacteria counts (figure 36) indicating that *L. monocytogenes* may have been having an antagonistic effect on these organisms.

L. monocytogenes

L. monocytogenes increased from an initial level of approximately Log_{10} 2.0 CFU/ g to levels greater than Log_{10} 8.0 CFU/ g on hypochlorite treated, osmotically dehydrated MP broccoli stored at 10°C (Figure 37). *L. monocytogenes* was slightly inhibited by hypochlorite treatment during storage at 4°C (Figure 37) indicating than the organism was having difficulty overcoming the combination of stresses of the low temperature and the hypochlorite. *L. monocytogenes*, at 4°C, began to overcome the stress of the hypochlorite and began to increase in numbers around the ninth day of storage (Figure 37). At either temperature, *L. monocytogenes* increased faster and reach higher final populations on the osmotically dehydrated broccoli (Figure 37) than on the nondehydrated broccoli (Figure 23). This may indicate the presence of a stimulatory effect due to the NaCl content in the

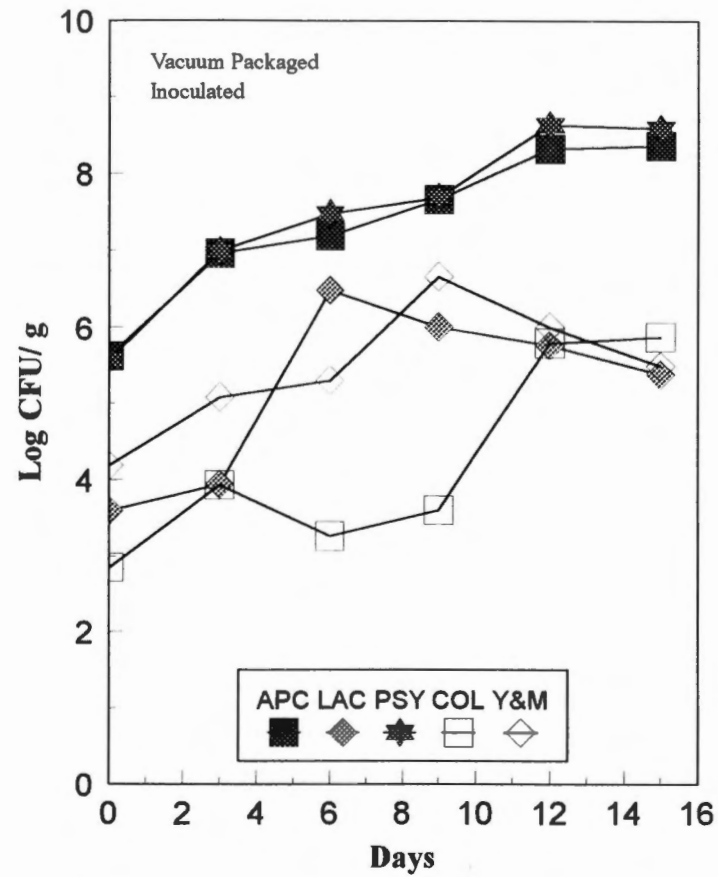


Figure 36: Microbial growth on metabisulfite treated, osmotically dehydrated, minimally processed broccoli, inoculated with Log 5.0 CFU/ mL *Listeria monocytogenes* and stored at 10°C. Atmosphere did not significantly ($P>0.05$) influence microbial growth. Data for air packaged, metabisulfite treated, osmotically dehydrated broccoli not shown.

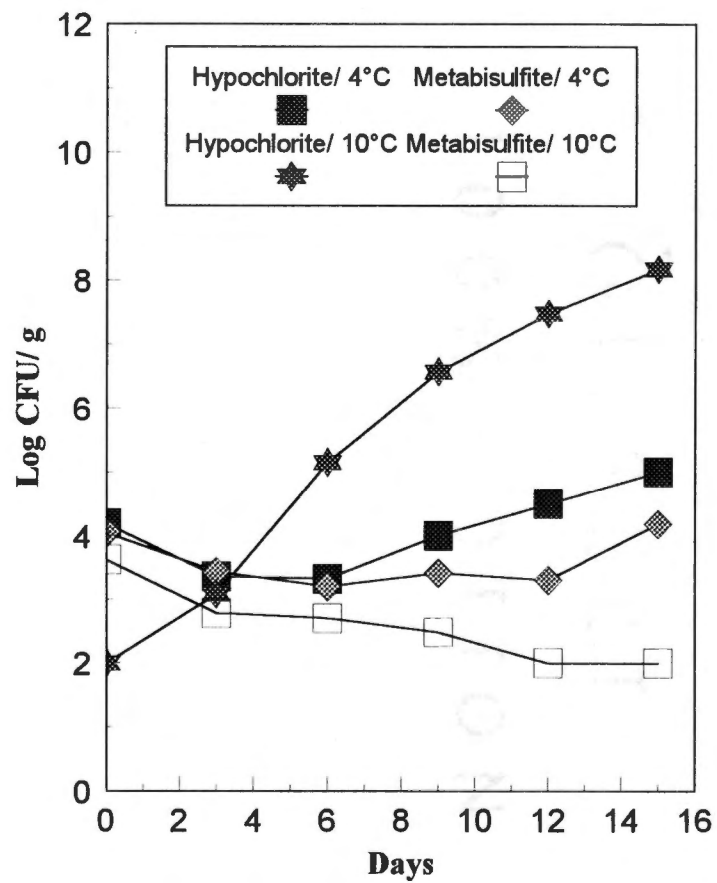


Figure 37: Growth of *Listeria monocytogenes* on osmotically dehydrated, minimally processed broccoli treated with antimicrobials. (Atmosphere did not significantly $P>0.05$ influence growth at 4 or 10°C.)

broccoli or the possibility of a preservative effect of the NaCl (2%) on *L. monocytogenes* cells, since osmotic dehydration was performed after inoculation with the organism.

L. monocytogenes did not fare well when osmotically dehydrated broccoli was treated with metabisulfite (100 ppm) (Figure 37). Little or no growth was observed for the organism at either storage temperature. Package atmosphere was not significant ($p>0.05$) in influencing the growth of the organism at either storage temperature (Table A6). Similar results were obtained for *L. monocytogenes* on nondehydrated broccoli receiving the same antimicrobial treatments (Figure 23).

Package Gas Composition for MP Broccoli Stored at 4 and 10° C.

CO₂ Concentrations

Initial levels of CO₂ were only slightly detectable for MP broccoli (air or vacuum packaged) at either 4 or 10°C (Figure 38). Initial levels of CO₂ were also similar for osmotically dehydrated (2%) MP broccoli (air or vacuum packaged) at either 4 or 10°C. The amount of CO₂ detected at the first sampling period was equivalent to the amount of CO₂ typically reported as present under normal atmospheric conditions. The amount of CO₂ increased in all packages of MP broccoli regardless of packaging method (air or vacuum) or storage temperature (4 or 10°C) during the 15 days storage (Figure 38). A similar increase in CO₂ was also noted for osmotically dehydrated MP broccoli packaged under similar conditions (Figure 39). The level of CO₂ was higher (for MP broccoli) at 10°C than at 4°C for vacuum packaged samples (Figure 38). The slightly warmer storage

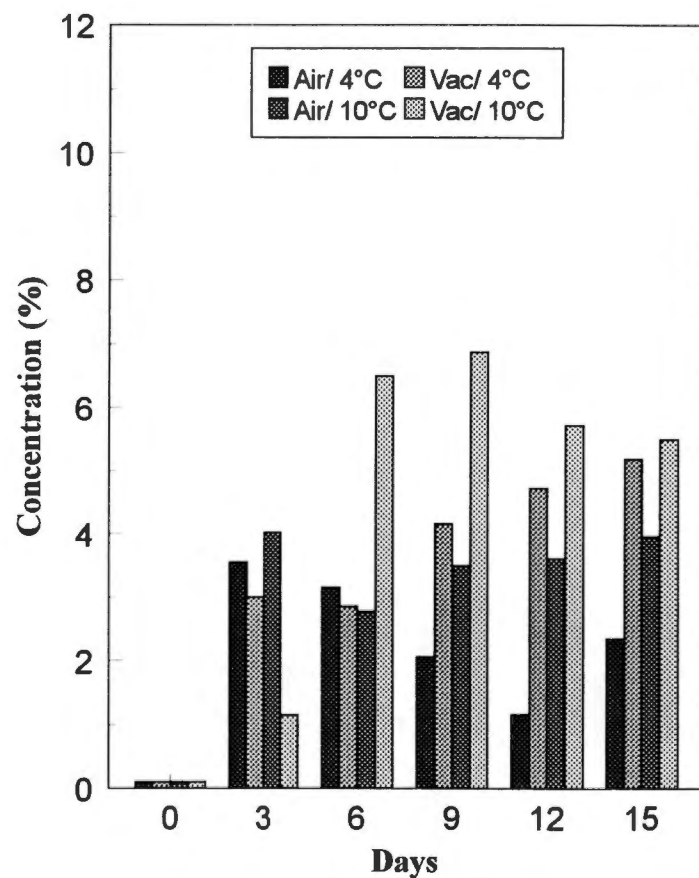


Figure 38: Carbon dioxide concentration in nonosmotically dehydrated, minimally processed broccoli during storage for 15 days

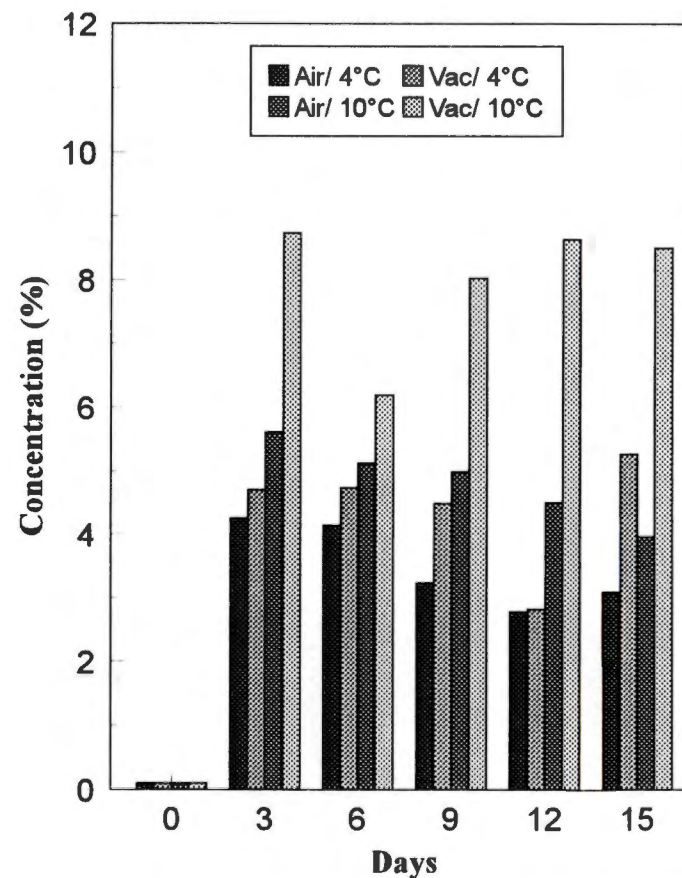


Figure 39: Carbon dioxide concentration in osmotically dehydrated, minimally processed broccoli during storage for 15 days.

temperature may have been responsible for a higher respiration rate in the broccoli which led to a depletion of any residual O₂ in the package and subsequent release of CO₂. The combination of vacuum packaging and storage at 10°C may have selected for the growth of LA bacteria which produce CO₂ as a result of their metabolic pathway. A third factor which needs to be considered and may be partially responsible for the increased CO₂ at 10°C for vacuum packaged MP broccoli is that the permeability rates of the packaging film are influenced by temperature. In general the concentration of CO₂ was greater when air packaged MP broccoli was stored at 10°C as opposed to 4°C (Figure 38). The warmer temperature was most likely responsible for increased respiration rates and subsequent release of CO₂ under those particular conditions.

Similar trends in CO₂ concentrations were observed for osmotically dehydrated MP broccoli (air and vacuum packaged) stored at 4 and 10°C (Figure 39). Concentrations of CO₂ increased throughout the 15 days of storage for all combinations of packaging (air and vacuum) and storage temperatures (4 and 10°C) (Figure 39). For all treatments, the concentration of CO₂ was greater in the osmotically dehydrated MP broccoli (Figure 39) than in the nondehydrated MP broccoli (Figure 38). Levels of CO₂ were higher in vacuum packaged osmotically dehydrated samples than in air packaged samples (Figure 39). For either the air or vacuum packaged osmotically dehydrated broccoli, the level of CO₂ was typically higher at the 10°C storage temperature (Figure 39). The salt content (2%) of the osmotically dehydrated broccoli may have been responsible for the increase in populations of LA organisms which release CO₂ as a product of their normal metabolism. Salt levels of approximately 2% have long been known for selecting for and stimulating growth of

LA bacteria such as in the lactic acid vegetable fermentation of saur kraut (Jay. 1992).

O₂ Concentrations

Initial levels of O₂ in packages of MP broccoli were approximately 22% (Figure 40), similar to reported values of O₂ under normal atmospheric conditions.

Concentrations of O₂ typically decreased during the 15 days storage (Figure 40). Air packaged broccoli at 4°C typically had higher concentration of O₂ than did air packaged broccoli stored at 10°C (Figure 40). This may reflect that the O₂ permeability of the packaging film was influenced by storage temperature. Typically the concentration of O₂ was higher in the air packaged broccoli than in the vacuum packaged broccoli (Figure 40). O₂ concentrations for vacuum packaged broccoli at 10°C were typically lower than for that of vacuum packaged broccoli stored at 4°C (Figure 40). The warmer storage temperature was responsible for increasing the growth of aerobic organisms which may have led to the increased rate of depletion of residual O₂.

The concentration of O₂ in packages of osmotically dehydrated broccoli decreased throughout the 15 days storage at both 4 and 10°C (Figure 41). Levels of O₂ were typically lower for packages of osmotically dehydrated broccoli (Figure 41) than for packages of nondehydrated broccoli (Figure 40). The increased NaCl level in the broccoli may have been responsible for increased populations of LA bacteria which may have contributed to the rapid decrease in O₂. In most instances lower concentrations of O₂ were observed in air packaged broccoli than in vacuum packaged broccoli (Figure 41). The osmotic dehydration may have had a stimulatory effect on certain aerobic microorganisms, increasing their numbers. The increases aerobic populations may have

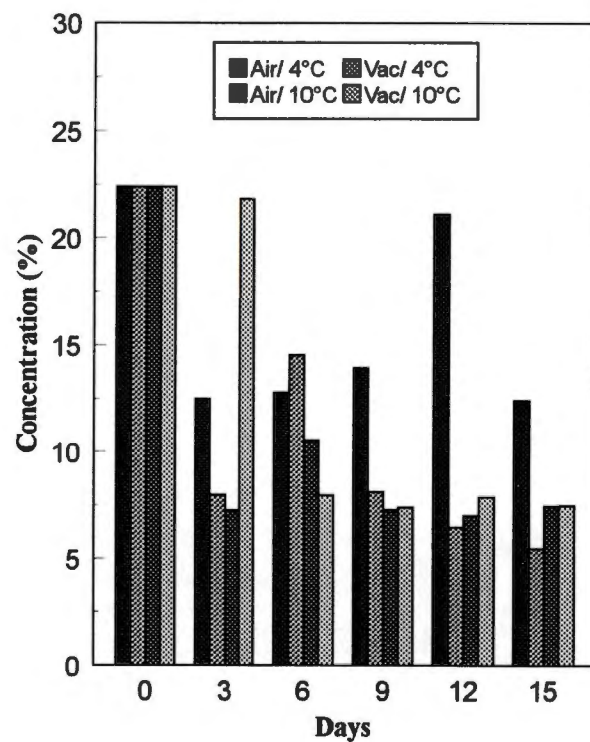


Figure 40: Oxygen concentration in nonosmotically dehydrated, minimally processed broccoli during storage for 15 days.

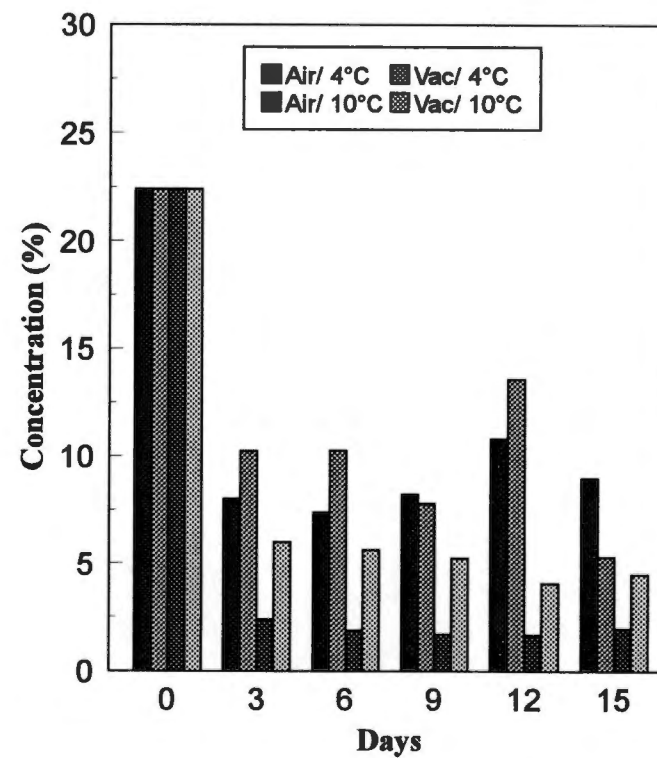


Figure 41: Oxygen concentration in osmotically dehydrated, minimally processed broccoli during storage for 15 days.

been responsible in depleting the O₂ at a faster rate than that which occurred in vacuum package broccoli.

Osmotic Dehydration of MP Broccoli

The data obtained from immersion of MP broccoli florets in a saturated NaCl solution were analyzed using the Proc Reg procedure (SAS; Cary, NC). Linear regression analysis was used to fit a line to the data in order to have a mathematical model which could be used to estimate the amount of immersion time necessary to achieve an internal salt concentration of 2%. The following equation was obtained by parameter estimates for the slope of the line and the Y intercept: $Y=0.04X + 0.20$. Using this equation the amount of immersion time to achieve a 2% level of salt was determined to be approximately 55 minutes.

Conclusions

Microbial analysis was performed to determine if *L. monocytogenes* could survive and grow on osmotically dehydrated, chemically pre-treated, MAP broccoli stored at 4 and 10°C. *L. monocytogenes*, when inoculated prior to chemical pre-treatment, was capable of surviving and growing on fresh broccoli when stored at 4 and 10°C. *L. monocytogenes* grew faster and reached higher levels when stored at 10°C than at 4°C regardless of chemical treatment (none, hypochlorite, metabisulfite). *L. monocytogenes*

was more sensitive to treatment with metabisulfite than to treatment with hypochlorite. Both hypochlorite and metabisulfite treatment reduced growth rates of *L. monocytogenes* over that observed on non-treated samples. Metabisulfite treatment was capable of inhibiting growth of *L. monocytogenes* at both 4° and 10°C whereas hypochlorite treatment was only successful at inhibiting growth at 4°C. Osmotic dehydration was shown to increase the resistance of *L. monocytogenes* to hypochlorite treatment when broccoli was stored at 4°C. Osmotic dehydration was shown not to influence the behavior of *L. monocytogenes* receiving metabisulfite treatment at either temperature or receiving hypochlorite treatment and storage at 10°C.

The results from this work indicates *L. monocytogenes* may be a potential problem to the fresh produce industry if contamination occurs. *L. monocytogenes*, when inoculated onto broccoli prior to processing, was shown to survive chemical treatment with hypochlorite and metabisulfite. Washing produce in water containing hypochlorite has historically been incorporated into fresh vegetable processing. This research indicates that this procedure alone will not be sufficient to control *L. monocytogenes*. Subsequent methods of preservation need to be incorporated into minimally processed broccoli to maintain the safety of the product since *L. monocytogenes* grows rapidly when temperature rises above 4°C.

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Appendices

Table A1. ANOVA for aerobic plate count of minimally processed broccoli held under refrigerated conditions.

Source	DF	Type III SS	F Value	Pr > F
Model	22	420.23	34.16	0.0001
Month	1	1.01	1.80	0.1809
Temp	1	3.41	6.09	0.0143
Anti	2	0.96	0.86	0.4238
Anti*Temp	2	1.09	0.97	0.3790
Inoc	1	0.02	0.04	0.8440
Inoc*Temp	1	0.06	0.11	0.7414
Anti*Inoc	2	0.52	0.46	0.6303
Osmo	1	7.96	14.24	0.0002
Osmot*Temp	1	1.03	1.83	0.1770
Anti*Osmo	2	1.84	1.64	0.1954
Inoc*Osmo	1	1.86	3.33	0.0693
Atmos	1	1.90	3.40	0.0666
Atmos*Temp	1	0.06	0.10	0.7538
Anti*Atmos	2	0.45	0.41	0.6669
Inoc*Atmos	1	0.25	0.45	0.5013
Osmo*Atmos	1	0.00	0.00	0.9439
Day	1	344.46	615.95	0.0001
Error	225	125.83		

Table A2. ANOVA for psychrotrophic plate count of minimally processed broccoli held under refrigerated conditions.

Source	DF	Type III SS	F Value	Pr > F
Model	22	460.26	30.85	0.0001
Month	1	0.43	0.63	0.4270
Temp	1	3.15	4.64	0.0322
Anti	2	2.97	2.19	0.1147
Anti*Temp	2	1.24	0.91	0.4024
Inoc	1	0.08	0.11	0.7391
Inoc*Temp	1	0.04	0.06	0.8062
Anti*Inoc	2	0.81	0.59	0.5530
Osmo	1	7.12	10.50	0.0014
Osmot*Temp	1	1.66	2.45	0.1187
Anti*Osmo	2	1.45	1.07	0.3447
Inoc*Osmo	1	2.48	3.66	0.0570
Atmos	1	1.95	2.87	0.0915
Atmos*Temp	1	0.61	0.90	0.3429
Anti*Atmos	2	0.55	0.41	0.6660
Inoc*Atmos	1	0.69	1.02	0.3143
Osmo*Atmos	1	0.03	0.05	0.8275
Day	1	387.63	571.69	0.0001
Error	224	151.88		

Table A3. ANOVA for lactic acid bacteria plate count of minimally processed broccoli held under refrigerated conditions.

Source	DF	Type III SS	F Value	Pr > F
Model	22	406.97	16.66	0.0001
Month	1	3.76	3.39	0.0671
Temp	1	27.21	24.51	0.0001
Anti	2	20.26	9.12	0.0002
Anti*Temp	2	6.35	2.86	0.0597
Inoc	1	5.43	4.89	0.0282
Inoc*Temp	1	0.50	0.45	0.5020
Anti*Inoc	2	0.00	0.00	0.9966
Osmo	1	8.54	7.69	0.0061
Osmot*Temp	1	0.00	0.00	0.9492
Anti*Osmo	2	0.10	0.05	0.9550
Inoc*Osmo	1	4.83	4.35	0.0384
Atmos	1	0.00	0.00	0.9837
Atmos*Temp	1	6.04	5.44	0.0207
Anti*Atmos	2	0.13	0.06	0.9445
Inoc*Atmos	1	3.30	2.97	0.0862
Osmo*Atmos	1	0.48	0.44	0.5098
Day	1	173.07	155.89	0.0001
Error	194	215.38		

Table A4. ANOVA for coliform bacteria plate count of minimally processed broccoli held under refrigerated conditions.

Source	DF	Type III SS	F Value	Pr > F
Model	22	592.09	20.89	0.0001
Month	1	0.02	0.02	0.8994
Temp	1	0.77	0.60	0.4402
Anti	2	10.81	4.19	0.0163
Anti*Temp	2	0.81	0.31	0.7301
Inoc	1	9.61	7.46	0.0068
Inoc*Temp	1	7.99	6.20	0.0135
Anti*Inoc	2	2.07	0.81	0.4483
Osmo	1	15.65	12.15	0.0006
Osmot*Temp	1	1.75	1.36	0.2450
Anti*Osmo	2	2.20	0.85	0.4268
Inoc*Osmo	1	0.13	0.10	0.7528
Atmos	1	0.07	0.05	0.8156
Atmos*Temp	1	0.68	0.53	0.4695
Anti*Atmos	2	4.71	1.83	0.1630
Inoc*Atmos	1	4.67	3.62	0.0582
Osmo*Atmos	1	0.75	0.58	0.4467
Day	1	491.73	381.69	0.0001
Error	223	287.30		

Table A5. ANOVA for combined yeast and mold plate count of minimally processed broccoli held under refrigerated conditions.

Source	DF	Type III SS	F Value	Pr > F
Model	22	246.59	11.33	0.0001
Month	1	0.07	0.07	0.7923
Temp	1	0.33	0.33	0.5666
Anti	2	0.43	0.22	0.8058
Anti*Temp	2	0.82	0.41	0.6621
Inoc	1	16.14	16.32	0.0001
Inoc*Temp	1	1.12	1.14	0.2875
Anti*Inoc	2	0.66	0.33	0.7157
Osmo	1	0.09	0.09	0.7663
Osmot*Temp	1	0.10	0.10	0.7561
Anti*Osmo	2	3.93	1.99	0.1394
Inoc*Osmo	1	1.90	1.92	0.1671
Atmos	1	0.09	0.09	0.7618
Atmos*Temp	1	1.86	1.88	0.1713
Anti*Atmos	2	0.83	0.42	0.6588
Inoc*Atmos	1	0.88	0.89	0.3459
Osmo*Atmos	1	0.62	0.63	0.4286
Day	1	185.25	187.32	0.0001
Error	208	205.70		

Table A6. ANOVA for *Listeria* plate count of minimally processed broccoli held under refrigerated conditions.

Source	DF	Type III SS	F Value	Pr > F
Model	22	611.03	27.22	0.0001
Month	1	1.93	1.89	0.1708
Temp	1	35.65	34.94	0.0001
Anti	2	22.82	11.18	0.0001
Anti*Temp	2	4.17	2.05	0.1317
Inoc	1	267.75	262.38	0.0001
Inoc*Temp	1	23.88	23.40	0.0001
Anti*Inoc	2	31.36	15.37	0.0001
Osmo	1	7.82	7.67	0.0061
Osmo*Temp	1	0.04	0.04	0.8395
Anti*Osmo	2	1.30	0.63	0.5309
Inoc*Osmo	1	4.67	4.57	0.0336
Atmos	1	0.50	0.49	0.4856
Atmos*Temp	1	0.10	0.10	0.7549
Anti*Atmos	2	7.47	3.66	0.0273
Inoc*Atmos	1	0.28	0.28	0.5996
Osmo*Atmos	1	1.20	1.17	0.2802
Day	1	32.76	32.10	0.0001
Error	225	229.60		

Vitae

Ronald Derike Smiley was born June 02, 1970 in Nashville, Tennessee to Ronnie F. Smiley and Beverly J. Simpson. He graduated from Father Ryan High School and May 1988. In January 1989 he enrolled at Tennessee Technological University in Cookeville, Tennessee and in May 1993 was awarded the Bachelor of Science degree in Agriculture with a concentration in Animal Science. In the fall of 1993, he began working toward a Master of Science degree in Food Science and Technology at the University of Tennessee in Knoxville. He was awarded the M.S. degree in December 1996.

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