INFLUENCE OF *LACTOBACILLUS DELBRUECKII* SSP. *LACTIS* ON *ESCHERICHIA COLI* O157:H7, *SALMONELLA CHOLERASUIS* AND *LISTERIA MONOCYTOGENES* ON MINIMALLY PROCESSED VEGETABLES

By

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Bachelor of Science

Oklahoma State University

Stillwater, Oklahoma

1998

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
December, 2001

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ACKNOWLEDGEMENTS

I would like to sincerely thank Dr. Stanley Gilliland for serving as my graduate advisor. Dr. Gilliland was patient, encouraging and has been a tremendous mentor. I also express thanks to Dr. Peter Muriana and Dr. William McGlynn for serving on my graduate committee and their contributions to my education. I would also like to thank Ms. Siobhan Reilly who introduced me to the world of food microbiology. Siobhan was always there providing support and new challenges during the time I worked in the microbiology lab.

I would also like to thank my wife, Courtney Harp, who has always been my encouragement, support, and best friend. I would also like to thank my parents, Robert and Jamae Harp, for their continued support and belief in me through out my entire life. I would like to thank my brothers, Ryan and Jared, and the rest of my family whom I have been blessed with to have a part of my life.

To Kevin "JR" McComas, Michelle Senne, Himali Senaratne, and all the girls who worked in the laboratory, thank you for your support, help and your valued friendship.

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CHAPTER I

INTRODUCTION

Ready-to-eat foods have become very popular with modern consumers. Of these foods, pre-packaged salads have experienced a tremendous growth in sales. Today's consumers demand food that is convenient, appetizing, nutritious and safe to eat. These demands provide a challenge for the produce industry. Producers must provide a product, which is fresh, nutritious, convenient and safe to eat. This demand has brought the age of commercially available minimally processed fruits and vegetables.

Packaging produce and keeping it fresh and safe over extended periods of time is a challenge. There is a microecology associated with each packaged vegetable that eventually causes spoilage of the product. To extend the life of the produce, the industry uses several techniques that slows the aging of the produce and inhibits the growth of microorganisms in this microecology. However the approved techniques available at the present time have limited effectiveness.

The use of a living organism or biopreservative is a potential alternative means of extending the shelf life and increasing the safety of minimally processed vegetables. Many consumers prefer food that is wholesome and does not contain harsh chemicals, which may be perceived as harmful to eat. By using selected lactic acid bacteria to preserve the freshness or safety of food, many chemical preservatives may be eliminated or reduced.

Lactobacillus delbrueckii ssp. *lactis* is a lactic acid bacterium, which has a demonstrated ability to inhibit both spoilage and pathogenic bacteria at refrigeration temperatures through production of hydrogen peroxide. The objective of this study was to use *L. delbrueckii* ssp. *lactis* as a biopreservative in minimally processed vegetables to exert antagonistic action toward undesirable microorganisms during storage.

CHAPTER II

REVIEW OF THE LITERATURE

Fresh cut vegetables have become increasingly popular with consumers during the last decade. Bagged salads offer the consumer a quality product at an affordable price along with the major advantage of convenience. Many of these fresh cut salads are composed of lettuce, cabbage, and carrots. These vegetables undergo a physiological process during storage, altering them from a living organism to decaying matter. Although the vegetables have been cut, washed and bagged, cell respiration continues indicating living tissue; however; the vegetables are not the only living organisms inside the bag. There is a microecology of organisms existing on the vegetables. This microecology not only affects the quality of the product but also can create an unsafe product for consumers. The microorganisms associated with minimally processed fruits and vegetables also can affect the overall quality and shelf life of these products.

FRESH CUT VEGETABLES

Many consumers today prefer the use of minimally processed packaged vegetables for salads, however there are risks associated with these products. They consist mainly of washed, peeled, sliced or shredded, packed raw vegetables stored below $10^{O}C$, and sold within 7 to 14 days. (Nguyen-The and Prunier, 1989; Garcia-gimeno and Zurera-Cosano, 1997). The nature of vegetables suggests that there are risks of pathogen contamination due to the environment where the vegetables are grown and harvested.

Furthermore, these risks are heightened when vegetables are processed and cut, which allows the harboring of additional microorganisms (Abdul-Raouf *et al*, 1993; Brackett, 1987; Brackett, 1992; Garg *et al*, 1990; Lund, 1981; Madden, 1992). Types of food, temperature, humidity, use of modified atmosphere or low dose irradiation can affect the microecology of minimally processed fruits and vegetables. The altering of this microecology can influence the safety and quality of fruits and vegetables (Brackett, 1987; Hotchkiss and Banco, 1992; Marchetti *et al*, 1992). While frozen processed vegetables are subjected to a critical control point during processing such as freezing, which eliminates growth of many ubiquitous microorganisms, fresh cut products do not have a critical control point in processing (Garg *et al*, 1990).

Minimally processed vegetables must be stored for a brief period, yet maintain their fresh characteristics. By reducing the rate of ripening, or delaying the onset of ripening, and preventing decay or other disorders, produce can be stored successfully and arrive to the consumer at an acceptable level of quality (Irving, 1984). This storage is achieved by altering the environment through various means such as lowering the temperature, application of chemicals, changing the composition of the atmosphere, or a combination of these treatments (Hotchkiss and Banco, 1992; Weichmann, 1987; Wills *et al* 1998). Such treatments can help maintain the desirable organoleptic qualities of the prepared salad.

Associated Undesirable Organisms

Many human pathogens are associated with vegetables used in ready-to-eat salads. These pathogens include *Listeria monocytogenes, Clostridium botulinum, Shigella* sp., *Salmonella* sp., *Escherichia coli* O157:H7, *Aeromonas hydrophila, Bacillus, cereus, Plesiomonas shigelloides, Yersinia entercolitica,* and others. (Bracket 1992; Brocklehurst *et al*, 1987; Sizmur and Walker, 1988; Tauxe *et al*, 1997). The nature and occurrence of these pathogens create a high potential for contamination on vegetables (Madden, 1992). Plants and vegetables are subject to their environment. Soil, waste, and animals may all contribute to the contamination of food plants and vegetables.

Escherichia coli O157:H7 is a pathogen that can be associated with minimally processed vegetables. It was first recognized as a food-borne pathogen in 1982 (Riley *et al*, 1983). It has been responsible for a number of illnesses including hemorrhagic colitis, hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) (Doyle, 1987; Doyle, 1991). Although *E. coli* O157:H7 has been isolated from fresh produce (Tauxe *et al*, 1997; Gonzalez *et al*, 1987), Doyle (1991) reported that most outbreaks associated with *E. coli* O157:H7 have been attributed to animal origin. Thus, these microorganisms can often gain entry onto vegetable plants when animal waste, waste -water, or contaminated irrigation water is used during production (Cieslak *et al*, 1993). Unfortunately, the environment where the vegetables are grown, is not the only potential source for contamination.

In addition to fecal material and contaminated water, soil and food handlers can also be sources for contamination (Hao and Brackett, 1993). If the product can stay free from contamination through growth, harvesting, and handling, it still can become indirectly contaminated through cross contamination. Cross contamination could be attributed to the processing equipment, which can transfer organisms from surfaces of the vegetable to the inside surfaces (Garg *et al*, 1990). As the name "minimally processed" implies, the vegetables undergo only a washing and a slicing process prior to packaging. Slicing or cutting causes a physical stress on the produce, which reduces the life and enhances microbial growth (Barry-Ryan and O'Beirne, 1998). The cutting and tearing of vegetable tissue results in the release of nutrients and water by the plant cells, which can support microbial growth. Seo (1999) confirmed this by reporting the accumulation of *E. coli* O157:H7 at the cut surfaces of lettuce. This study confirmed the survival and adherence of this pathogen to packaged vegetables. Not only can *E. coli* O157:H7 survive in fresh-cut vegetables (Abdul-Raouf *et al*, 1993; Richert *et al*, 2000), but also may have the ability to grow on them (Hao and Brackett, 1993; Richert *et al*, 200). These researchers indicated that *E. coli* O157:H7 was capable of surviving and in some instances growing under conditions where there is a modified atmosphere and the storage temperature is 10 ^{O}C or colder. The fact that *E.coli* accumulates at the cut edges of the vegetables and can grow under the right conditions presents a significant food safety hazard to the consumer. Abdul-Raouf *et al* (1993) reported that an initial decline in pH in salad vegetables was correlated with initial increases in the populations of *E. coli* O157:H7. However, a decline in the population of *E. coli* O157:H7 was observed when the pH dropped below 5.0. The decrease in pH was attributed to the fermentative

capability of the organism and other accumulated acids, which become toxic to the organism.

Listeria monocytogenes has been linked to food-borne illness outbreaks associated with vegetables for years (Schlech *et al*, 1983; Sizmur and Walker, 1988). Schlech reported that cabbage fertilized with sheep manure was responsible for an outbreak of listeriosis. Similar to *Escherichia coli* O157:H7, or other microorganisms, this pathogen can be introduced to vegetables through a variety of means. While the reduced oxygen conditions in a packaged salad inhibit growth of aerobic bacteria, they can be ideal for the growth of *L. monocytogenes,* which is facultative. Kakiomenou *et al* (1998) confirmed this by reporting the survival of *L. monocytogenes* on vegetables in a modified atmosphere. Even when the vegetables are stored at refrigeration temperatures, *L. monocytogenes* is a psychrotroph and has the ability to grow. This is especially true if other intrinsic microflora are removed through chemical cleaning, which results in better growth for *L. monocytogenes* (Carlin *et al*, 1996). Omary *et* al (1993) confirmed this by reporting the growth of *Listeria* in packaged cabbage. They concluded that prolonged storage at cold temperatures may have encouraged/enhanced the growth of *L. monocytogenes* and reduced normal cabbage microflora.

Due to the agronomic system of growing vegetables, *Salmonella* sp. can easily gain entry into vegetables. In 1995 a major food-borne illness outbreak resulted from alfalfa sprouts contaminated with *Salmonella* (Tauxe *et al* 1997). They reported the isolation of *Salmonella* from various vegetables such as endive, lettuce, salad greens, bean sprouts, and eggplants, as well as fruit products, such as orange juice. *Salmonella* is a very resilient organism. It has a tremendous ability to survive in harsh environments.

Even with the use of a modified atmosphere on packaged salad vegetables, Kakiomenou *et al* (1998) reported the survival of *Salmonella* on shredded carrots and lettuce after 15 days of storage at refrigeration temperatures. This is not surprising since it is facultative and not a strict aerobe.

Although not all bacteria present on the vegetables are pathogenic, many cause spoilage of the product, which can reduce the quality and shelf life. After 10 days of storage, packaged salads tend to brown or discolor around the leaf margins. Nguyen-The and Prunier (1989) reported that most spoilage and decay of leafy vegetables is caused by the species of *Pseudomonas*. *Pseudomonas marginalis* and *P. cichorii* were the two predominant organisms, which caused the spoilage of most ready-to–eat vegetables. These Gram-negative bacteria have pectinolytic activity and the ability to produce acids from sucrose from the vegetables (Nguyen-The and Prunier, 1989). Both of these characteristics of *Pseudomonas* degrade the cell walls of the vegetables and cause decay and premature rotting. Others also have concluded that *Pseudomonas*, were the predominant microflora on most fresh cut vegetables (Garg *et al*, 1990; Lund, 1981). After harvest, the cellular physiology of vegetables drastically changes. Cellular membrane damage of the plant cells occurs from the accumulation of natural plant chemicals such as hydrogen peroxide produced in defense of unwanted organisms, namely *Pseudomonas* (Bestwick *et al*, 1997). This hypersensitive reaction causes rapid and localized death of infected cells. This response is similar to apoptosis in animal cells, the process of programmed cell death (Bestwick *et al*, 1998). Other enzymes and chemicals are released in the event of cell damage or bacterial attachment. Peroxidases and other super oxide radicals are produced both intra and extracellularly (Bestwick *et al*,

1998). To retard these damaging metabolic activities, produce is kept at refrigeration temperatures after harvest; however, many *Pseudomonas* sp. grow well in conditions that are cold and aerobic. If all available oxygen were removed from a package of fresh cut vegetables, an anaerobic condition would be created inside the bag, which is undesirable for the storage of the produce. Most vegetables require a minimum oxygen concentration of 2 to 3 percent (Irving, 1984). Reducing the available oxygen during storage can help control this organism; however, its psychrotrophic nature enables it to grow well in refrigerated fresh cut vegetables (Bestwick *et al*, 1997).

Temperature and Humidity

Since the age of refrigeration, food has kept fresh longer than when it was previously stored at ambient temperatures. Decreasing the temperature of fresh cut vegetables has a two-fold effect on storage. First, the low temperature slows metabolic activity in the vegetables, and secondly it slows the growth of many bacteria intrinsic to the produce. Lowering the temperature of the produce to a temperature between 5^{O} C and 15 ^{O}C slows the rate of deterioration three different ways. First, the low temperatures slow the respiration of the produce. Second, it reduces the production of ethylene, and third, it reduces the response to ethylene (Wills, 1998). Ethylene is a gas produced by vegetables and other environmental sources that promotes ripening and decay (Bohling and Hansen 1983; Wills 1998). This lower temperature range also retards the growth of many bacteria (Lund, 1981). These lower temperatures, however, do select for psychrotrophic bacteria (Brackett, 1987). Many of these psychrotrophs have been

indicated as the primary cause for deterioration or spoilage of the product (Nguyen-The and Prunier, 1989).

Another technique used to extend shelf life of vegetables is to control the relative humidity of the storage conditions. Relative humidity is described as the ratio of water vapor pressure to the saturation water vapor pressure at that temperature, expressed as a percent (Weichmann, 1987). Increasing the relative humidity of the vegetable environment to 95% reduces moisture loss from the produce (Cantwell, 1992). Low relative humidity causes the produce to physiologically change. The plant stomata close, which reduces transpiration. The loss of transpiration prevents the plant from incorporating moisture from the atmosphere for respiration. The plant is forced to use its own cellular fluids and water creating an overall water loss in the produce. This water loss is proportional to weight loss and loss of organoleptic qualities. Keeping water and other nutrients in the vegetables increases the life of the produce. Not only does this prevent the loss of product weight by not dehydrating it, but promotes the vegetative cells to retain their original and desirable characteristics.

Chemical Application

Extensive research has been done on the application of chemical rinses to vegetables in order to remove or destroy bacteria that are associated with the raw vegetables (Adams and Hall 1988; Garcia-Gimeno and Zurera-Cosano, 1997; Seo, 1999). Hypochlorite rinses appear to be the most widely used in the produce industry. Adams *et al* (1989) reported the effects of various rinse treatments on prepared salads.

Hypochlorite solutions with varying acidities were used to wash chopped lettuce. This caused a significant decrease in overall numbers of microorganisms, although the survival of some organisms indicated the inability of the hypochlorite solution to completely cover the surface of the lettuce. Pockets or hydrophobic areas limited the effectiveness of the solutions to cover the entire surface of the lettuce, thus reducing their effectiveness.

Despite the fact that these chemical treatments are very effective in killing microorganisms, they still do not completely eliminate harmful bacteria from the produce. Even if a chlorine rinse can completely cover the surface of a vegetable, *E. coli,* still can be found active in the stomata and cut edges (Seo, 1999). Although these washes are recommended for post harvest application to produce, they do not remove all intrinsic microorganisms (Garg, 1990). The small numbers of organisms left on the produce after washing can grow and multiply, which spoils the produce. Nicholl and Prendergast (1998) reported that although initial populations of natural micro flora were reduced due to a hypochlorite dip, there was no significant difference between the dipped treatment and the control after four days of storage at refrigeration temperature. In fact increasing the levels of free chlorine did not improve the antimicrobial effect. Zhang and Farber (1996) investigated the effects of various other disinfectants against *L. monocytogenes* on lettuce and found that numbers were reduced only by approximately one log cycle by disinfectants such as chlorine, chlorine dioxide, *Salmide*®- a sodium chlorite-based oxy-halogen compound, trisodium phosphate, and other organic acids. These disinfectants were applied prior to packaging. Even though they reduced the numbers they allowed survival of *Listeria* and perhaps other pathogens. Omary *et al* (1993) found an initial

decline in numbers of *L. monocytogenes*, inoculated on freshly shredded cabbage treated with citric acid and sodium erythorbate. However results showed an increase in levels of *L. monocytogenes* above initial levels after 21 days of storage. Reducing the initial population of microorganisms on produce must be accomplished to gain extended life of the product. A treatment that could continue to inhibit unwanted microbial growth after a primary application would extend shelf life plus help ensure the safety of the product.

Modified Atmosphere Packaging

The proper altering of the atmosphere in a prepared bagged salad can extend the shelf life of the product. The use of controlled atmosphere or modified atmosphere slows respiration and other metabolic activities in the vegetables (Exama *et al,* 1993; Weichmann, 1987; Wills *et al* 1998). After harvest, fresh vegetables go through senescence or ripening. The vegetables still respire and when packaged, modify the atmosphere inside the package (Finn and Upton, 1997). Modified atmosphere (MA) packaging of produce is the process in which the storage environment of the bag is altered to have higher levels of carbon dioxide and lower levels of oxygen than normal outside air. By limiting the amount of oxygen required for transpiration, MA slows the metabolism, delays senescence, and extends the life of the produce. Modified atmosphere packaging (MAP) uses special film barriers that allow the formation or retention of a modified atmosphere to extend the shelf life of fresh cut vegetables. Ballantyne *et al* (1988) reported that packages of shredded lettuce with modified

atmosphere doubled the shelf life compared to control packs. Acceptable sensory and visual qualities of produce are extended due to MA. This storage, however, is also known to increase the chances of pathogenic bacteria or toxins reaching the consumer (Abdul-Raouf *et al,* 1993; Finn and Upton, 1997; Hao and Brackett, 1993; Hao *et al*, 1998; Hotchkiss and Banco, 1992; Madden, 1992). While a low oxygen atmosphere slows growth of microorganisms, it does not stop growth. Many reports indicate the growth of *Pseudomonas* sp. and other spoilage organisms causing the eventual spoilage of produce packaged in a modified atmosphere (Brocklehurst *et al*, 1987; Hao and Brackett, 1993).

BIOPRESERVATIVES

Inhibition of undesirable microorganisms by lactobacilli

The use of lactic acid bacteria to control undesirable microorganisms in refrigerated foods has been proposed by several researchers (Gilliland and Speck, 1975; Watson and Schubert, 1969, Garver and Muriana, 1993, Brashears *et al*, 1998). Lactic acid bacteria have been used for years in the fermentation of vegetables for preservation (Desai and Sheth, 1997). This fermentation through acidification changes the original characteristics of the food. These foods are generally considered safe for consumption. Bacteria used for culturing these foods not only produce acid that inhibits pathogens, but also are known to produce other antimicrobial compounds (Franz *et al*, 1997; Gourama, 1997). Lactic acid, acetic acid and bacteriocins produced by many of these cultures and can contribute to the control of undesirable organisms. The bacteriocin nisin, produced by *Lactococcus* sp., can inhibit the growth of many Gram-positive organisms such as *L. monocytogenes,* and *Clostridium perfringens*, but is not effective in inhibiting many Gram-negative bacteria (Franz *et al*, 1997). Adams and Hall (1988) reported the inhibition of *S. enteritidis* and *E. coli* in a low pH environment created by lactic and acetic acids. This environment would most likely be found in a fermented product such as sauerkraut, cucumber pickles and olives but not in a fresh cut vegetable package. This large decrease in pH harms the living plant cells and would eventually lead to the death of vegetable cells (Siriphanich and Kader, 1986).

The use of cells of lactic acid bacteria with minimally processed foods is a new idea that is gaining interest. Using lactic acid bacteria to control undesirable organisms without changing the organoleptic qualities of the vegetables would prove to be very beneficial. This potential method could control unwanted microorganisms on a product, which is very perishable.

Lactobacillus delbrueckii* ssp. *lactis

Lactobacillus delbrueckii ssp. lactis are Gram-positive, rod shaped bacteria, which are nonspore forming, nonmotile, and catalase negative (Kandler and Weiss, 1986). They are homofermentative and grow well between 40-52^{O}C however, do not grow at or below 15^{O}C. Although *L. delbrueckii* ssp. *lactis* does not grow at refrigeration temperature, it still has the ability to produce high levels of hydrogen peroxide (H_2O_2) (Gilliland and Speck, 1975; Villegas and Gilliland, 1998). This ability of this organism has a potential benefit to the food industry as a biopreservative (Watson and Schubert, 1969; Gilliland and Speck, 1975; Garver and Muriana, 1993; Brashears *et al*, 1998, Brashears and Durre, 1999).

Hydrogen Peroxide (H_2O_2) and the Lactoperoxidase System

The dairy industry has used hydrogen peroxide as an agent, to kill undesirable organisms found in milk (Dahiya and Speck, 1968; Gilliland, 1969; Price and Lee, 1970; Tharrington and Sorrells, 1992). Lactic acid bacteria such as lactococci and lactobacilli, can utilize lactate to generate hydrogen peroxide (Kandler, 1983; Villegas and Gilliland, 1998). Of the lactic acid bacteria, *L. delbrueckii* ssp. *lactis* (originally known as *Lactobacillus lactis*) has been reported to produce the most H_2O_2 (Premi and Bottazzi, 1972). Numerous studies have documented the antimicrobial effect of H_2O_2 produced by lactobacilli against undesirable organisms. *Lactobacillus* species isolated from oysters produced sufficient amounts of H_2O_2 to inhibit the growth of *Pseudomonas, Bacillus,* and *Proteus* species (Price and Lee, 1970). Dahiya and Speck (1968) found that H_2O_2 produced by lactobacilli, namely *Lactobacillus lactis*, inhibited the growth of *Staphylococcus aureus,* at $5^{O}C$ (Dahiya and Speck, 1968). Other investigators have reported the ability of lactobacilli to produce enough H_2O_2 to inhibit the growth of *L. monocytogenes* (Tharrington and Sorrells, 1992), *Salmonella* sp. (Watson and Schubert, 1969; Brashears and Durre, 1999), *Escherichia coli* O157:H7 (Brashears *et al*, 1998,1999), and psychrotrophic spoilage bacteria (Gilliland and Speck, 1975; Martin and Gilliland, 1980; Gilliland and Ewell, 1983). These studies have promoted further research to test the antimicrobial effects of lactobacilli on other non-dairy refrigerated foods. Select strains of *L. delbrueckii* ssp. *lactis* were found to be antagonistic to *Escherichia coli* O157:H7 on refrigerated raw chicken meat (Brashears *et al*, 1998).

Although hydrogen peroxide is effective in killing psychrotrophs and other undesirable organisms, the lactoperoxidase system may prove to be more lethal to psychrotrophs (Thomas *et al*, 1994). Hydrogen peroxide produced by lactic acid bacteria in milk can react with thiocyanate (SCN^-), in the presence the enzyme lactoperoxidase to form hypothiocyanite ($OSCN^-$), which is inhibitory to microorganisms (Bjorck *et al,* 1975, Thomas *et al,* 1994, Thomas *et al,* 1981). This lactoperoxidase system has been reported to be antibacterial to *S. typhimurium* (Wolfson and Sumner, 1994; Wolfson *et al,* 1994), *L. monocytogenes* (Zapico *et al,* 1993) and psychrotrophic, Gram-negative organisms (Bjorck, 1978; Uceda *et al,* 1994). Even the thiocyanate compound used in the lactoperoxidase system has itself been investigated for bactericidal properties. Lin *et al* (2000) reported bactericidal effects of two types of isothiocyanate compounds. Vapors from allyl and methyl forms of isothiocyanate were tested on iceberg lettuce inoculated with *Salmonella montevideo*, *L. monocytogenes,* and *E. coli* O157:H7. These compounds were sealed in each treatment bag. While the methyl form proved to be more antagonistic against *L. monocytogenes,* the allyl form had a higher bactericidal activity against *S. montevideo* and *Escherichia coli* O157:H7 (Lin *et al,* 2000).

Biopreservatives and Fresh Cut Vegetables

Vescovo *et al* (1996) investigated the use of lactobacilli to control undesirable microorganisms on ready-to-use vegetables. The investigators found that a strain of *Lactobacillus casei* isolated from vegetables proved to be effective in inhibiting *Aeromonas hydrophila, L. monocytogenes, Salmonella typhimurium,* and *S. aureus*. Later, Torriani *et al* (1997) reported that *L. casei* added to ready-to-use vegetables reduced total numbers of mesophilic bacteria and suppressed coliforms, enterococci, and *A. hydrophilia* populations during the storage of 6 days at 8^{O}C. These population reductions were attributed to lactic acid production and perhaps another active antimicrobial agents produced by *L. casei.*

The most effective way to control pathogens on fresh cut produce is to use a series of processes and techniques. The use of biopreservatives as a final hurdle prior to packaging could be an effective way to provide a safer product. Another study showed the effectiveness of using hurdle processing by combining modified atmosphere packaging, temperature, and lactobacilli to control *Aeromonas hydrophilia* (Vescovo *et al*, 1997). These studies relied on the ability of *L. casei* to grow at refrigeration temperatures and produce lactic acid or other antimicrobial agents. However, the growth of lactic acid bacteria on fresh cut vegetables can itself cause spoilage or visual discoloration of the produce.

The high levels of acid produced by lactic acid bacteria during growth could alter the qualities and fresh characteristics of produce. This fact could be another benefit of using lactic acid bacteria that do not grow at refrigeration temperatures as biocontrol

agents. Breidt and Fleming (1997) suggested the use of lactic acid bacteria as agents that will cause spoilage of the produce if temperature abuse occurs. Temperature abuse could result in the growth of harmful pathogens that may not cause spoilage resulting in a product, which appears to be edible, but in fact is very dangerous to eat. By adding lactic acid bacteria that would cause spoilage in produce that was subjected to temperature abuse would be very useful in alerting consumers of spoilage.

OBJECTIVE OF PRESENT STUDY

Lactobacillus delbrueckii ssp. *lactis* has the ability to produce sufficient quantities of H_2O_2 to be antagonistic towards many undesirable organisms. This organism can produce these quantities of H_2O_2 at refrigeration temperatures even though it does not grow. *Lactobacillus delbrueckii* ssp. *lactis* could be an ideal candidate as a biopreservative for minimally processed vegetable products. The addition of *L. delbrueckii* ssp. *lactis* to packaged minimally processed vegetables could enhance the shelf life and safety of the product without changing the organoleptic properties of the produce. Thus, the objective of this study was to determine if a selected strain of *L. delbrueckii* ssp. *lactis* could create an antagonistic action towards selected pathogens on fresh cut vegetables during storage at refrigeration temperature.

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CHAPTER III

EVALUATION OF A SELECT STRAIN OF *LACTOBACILLUS DELBRUECKII* SSP *LACTIS* AS A BIOLOGICAL CONTROL AGENT FOR PATHOGENS ON FRESH CUT VEGETABLES STORED AT 7 ^{O}C

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ABSTRACT

Raw vegetables inoculated with selected pathogenic bacteria were treated with a strain of *Lactobacillus delbrueckii* spp. *lactis*, which was selected for its ability to produce hydrogen peroxide at refrigerated temperatures. The vegetables included broccoli, cabbage, carrots and lettuce. Each vegetable was rinsed, chopped and stored under conditions similar to ready-to-eat vegetables sold at retail. Portions of each vegetable were separately inoculated with one of three pathogenic bacteria: *Escherichia coli* O157:H7, *Salmonella cholerasuis*, and *Listeria monocytogenes*. Prior to packaging, one portion of the each inoculated vegetable was treated with a cell suspension of the selected strain of *L. delbrueckii* ssp. *lactis*. The vegetables were stored at 7 ^{O}C for 6 days. The numbers of pathogens and lactobacilli on each sample were enumerated on days of 0, 3 and 6 of storage. Although populations of *L. delbrueckii* ssp. *lactis* remained at high levels during the storage, there was no noticeable antagonistic effect against the pathogens under conditions similar to conditions of these products at the retail level. Each pathogen survived on all vegetables throughout the storage. Further testing revealed that there was apparently sufficient catalase activity in the cut vegetables to destroy enough of the hydrogen peroxide so that antagonistic action toward the pathogens was prevented.

INTRODUCTION

Select strains of lactobacilli have the ability to produce sufficient amounts of hydrogen peroxide at refrigeration temperatures to inhibit various undesirable organisms such as *Escherichia coli* O157:H7 (Brashears *et al,* 1998). Watson and Schubert (1969) reported the inhibitory action of hydrogen peroxide against *Salmonella typhimurium.* Despite the presence of bacterial catalase, sufficient amounts of hydrogen peroxide inhibited *S. typhimurium.* Milk culture filtrates from cells of lactobacilli containing hydrogen peroxide were found to be inhibitory to *Listeria monocytogenes* (Tharrington and Sorrells, 1992). Although other compounds were present in the milk culture filtrate, hydrogen peroxide was a primary antimicrobial. Price and Lee (1970) isolated strains of *Lactobacillus* from oysters that produced hydrogen peroxide, which was found to be inhibitory to *Pseudomonas*, *Bacillus,* and *Proteus* species. These experiments were conducted in a 1% peptone broth at 30°C for 2-5 days. Although *L. delbrueckii* spp. *lactis* does not grow at refrigeration temperatures; it can produce sufficient amounts of hydrogen peroxide to inhibit the growth of organisms such as these at refrigeration temperatures (Gilliland and Speck, 1975). Brashears *et al* (1998) applied cells of *L. delbrueckii* ssp. *lactis* to refrigerated raw chicken previously inoculated with *E. coli* O157:H7. They observed that the lactobacilli produced sufficient amounts of hydrogen peroxide to cause decline in the numbers of *E. coli* O157:H7. Those studies indicated the potential for *L. delbrueckii* ssp. *lactis* to be used as a biopreservative in some other refrigerated foods. The addition of beneficial organisms that continually produce hydrogen peroxide without changing the organoleptic qualities of the food could enhance the safety and shelf life of fresh cut vegetables.

The objective of this study was to determine if a selected strain of *L. delbrueckii* ssp. *lactis* could produce an antagonistic action towards selected pathogens and spoilage organisms on fresh cut vegetables during storage at refrigeration temperature.

MATERIALS AND METHODS

Sources of Cultures

Lactobacillus delbrueckii ssp. *lactis* RM 2-5 used in this study was from the stock culture collection of the Food Microbiology Laboratory in the Oklahoma Food & Agriculture Products Research and Technology Center at Oklahoma State University. The culture was maintained by subculturing in MRS broth (Difco Laboratories, Detroit MI) using a 1 % inoculation and 18 hours incubated at 37^{O}C.

Pathogens for this study, *Escherichia coli* O157:H7 (ATCC 43894) *Salmonella cholerasuis* (ATCC 13706) and *Listeria monocytogenes* (27-2, V7-2, 383-2, & Scott-A), were also from the stock culture collection of the Food Microbiology Laboratory. All strains (27-2, V7-2, 383-2, & Scott A) of *L. monocytogenes* were used in a cocktail combination. These cultures were maintained by subculture in Tryptic Soy Broth (Difco, Detroit MI) using a 1% inocula and18 hours incubation at 37^{O}C.

All cultures were stored at refrigeration temperatures (2-5^{O}C) between subcultures. Each was subcultured three times immediately prior to each experimental use.

Enumeration of Bacteria

Lactobacilli were enumerated by the pour plate technique with an overlay or by spiral plate technology on *Lactobacillus* selection (LBS) agar. The LBS agar was prepared from individual ingredients according to the manufacturer's (BBL Microbiology Systems, Cockeysville, MD) formulation. Each sample was diluted according to the procedures in the Compendium of Methods for the Microbiological Examination of

Foods (Swanson et al, 1992). Decimal dilutions were prepared using 99-ml sterile dilution blanks containing 0.1% peptone and 0.001% antifoam in distilled water. The plates were then placed in plastic bags and flushed with carbon dioxide (30 secs), sealed and incubated at 37^{O}C for 48 hours. The colonies from the pour plates were counted with the aid of a Quebec Colony Counter.

A Whitley automatic spiral plater or (WASP)© was used to perform the spiral plating according to the manufacturer's directions (Don Whitley Scientific Limited, West Yorkshire, England). Pre-poured plates of the appropriate agar medium were used for enumeration of microorganisms. Ten to fifteen milliliters of an appropriate decimal dilution of each sample was aseptically placed into sampling cups for the Spiral-plater. The Spiral plater then automatically transferred 50 µl of the sample dilution onto the appropriate plate in a spiral fashion. Colonies on the plates were enumerated using Synbiosis ©Protocol Spiral plate counter and software (Synbiosis, 1998).

Enumeration of *E. coli* O157:H7 was done by pour plate method with overlay using Violet Red Bile Agar (VRBA; Difco Laboratories, Detroit MI) and incubation at 35 ^{O}C for 18 to 24 hours. Enumeration of psychrotrophic bacteria was done using the spiral plating techniques on Plate Count Agar (PCA; Difco Laboratories, Detroit MI) plus 1% TTC solution and incubation at 7 ^{O}C for seven days. *Salmonella* was enumerated using Brilliant Green Agar (BGA; Difco Laboratories, Detroit MI) on the spiral plater and incubated at 37 ^{O}C for 24-48 hours. The cultures of *Listeria* used in this study were streptomycin resistant strains of *Listeria monocytogenes*. These strains were enumerated using the spiral-plater and plated on TSA with added streptomycin 0.1mg/ml (Sigma, St. Louis, MO) at 30 ^{O}C for 48 hours.

Preparation of bacterial cell suspensions for treatments

A frozen concentrated culture of *L. delbrueckii subs. lactis* RM 2-5 was used to treat the vegetables. Cells of the lactobacilli from 1000 ml of a MRS broth culture (1% inoculum and incubation for 18 hours at 37 ^{O}C) were harvested by centrifugation at 5000-x g for 20 minutes at 2^{O}C. The supernatant from each centrifuge bottle was discarded and the pellet resuspended in 100 mls of cold 10 % NFMS with the aid of 10-20 sterile glass beads (2mm diameter) per centrifuge bottle. The resuspended cells were combined into one container. The resulting concentrated culture was aseptically dispensed into 2-gram aliquots into sterile cryogenic vials and submerged in liquid nitrogen (-196 °C) until use. On the day of use, the required numbers of vials were thawed by immersion in 1 liter of tap water at room temperature for 10 minutes. Once the vials were thawed and the tops sanitized with 70 % ethanol, 5-grams of concentrated culture were added to 500 ml of sterile 5mM sodium lactate solution. This cell suspension constituted the *L. delbrueckii ssp. lactis* RM 2-5 dip solution for the treatments.

Each pathogen dip was created by culturing (1% inoculum) the desired pathogen in 100 ml TSB at 37 ^{O}C for 18 hours. The cells were harvested by centrifugation at 7000-x g for 20 minutes at 2 ^{O}C. The supernatant was discarded and the cells were washed twice in 10 ml volumes of cold phosphate buffer. The washed cells were resuspended in 10 ml of cold phosphate buffer and stored on ice until ready for use (within one hour). The required amount of the cell suspension was added to 1-L of sterile water to achieve the desired inoculum level for the vegetables.

Treatment of Vegetables

Vegetables used in this study were purchased from a local supermarket and held at refrigeration temperature, until use for experimentation (not more than 4 hours). The vegetables were aseptically cut on a sterile cutting board. The lettuce and cabbage were cut in large salad size pieces (approximately 10 cm x 10 cm). The broccoli was cut in a fashion similar to that of fresh broccoli found on commercially prepared snack or party platters. The carrots were shredded into salad size shreds using a sterile hand vegetable grader. To wash the cut vegetables a total of 375 grams of each was weighed and placed in 2-L of sterile water and agitated for 2-minutes. The water was then poured from the containers and the vegetables allowed to drain. Two hundred-fifty grams of the cut and washed vegetable were placed into the appropriate pathogen dip and agitated for 2-minutes. The pathogen dip was then poured off and the vegetables allowed to drain through sterile cheesecloth. Half (125 g) of the vegetable inoculated with the pathogen was placed in the cell suspension of *L. delbrueckii* ssp. *lactis* RM 2-5 dip solution (labeled RM 2-5 Treatment) and the remaining 125 grams were placed into 500 ml of 5mM sodium lactate solution which contained 5 grams of sterile 10% NFMS (labeled Pathogen Control). Both treatments were agitated for 2 minutes. The solutions were then poured off and the vegetables drained through sterile cheesecloth. The treated vegetables were aseptically divided into three poly-olefin special modified atmosphere packages (8" x 14" Cryovac PD961 multilayer poly-olefin) generously provided by Ms. Myra Hughes of Cryovac Sealed Air Corporation of Duncan, South Carolina. It is the same packaging material used for packaging fresh-cut vegetables in retail markets. Each bag was then

flushed approximately 30 seconds with a gas mixture containing 85% nitrogen, 10 % carbon dioxide, and 5% oxygen, and heat-sealed. The vegetables were stored at 7 ^{O}C.

The remaining 125 grams of vegetables from the initial wash in 2-L of sterile water, were then placed into 500 ml of sterile 5mM sodium lactate solution which contained 5 grams of 10% NFMS (Uninoculated Control Treatment). It was agitated for 2-minutes. The product was drained as was done for the other treatments and dispensed into three separate packages (Cryovac PD961) flushed, heat sealed, and stored as was done for the other treatments. One bag of each treatment was removed from storage on days 0, 3, and 6 for microbial analysis.

Hydrogen Peroxide Production

Lactobacillus delbrueckii ssp. *lactis* RM 2-5 was grown in 10 ml of MRS broth (1% inoculum for 18 hours at 37 ^{O}C). The cells were harvested by centrifugation at 12,000-x g at 4 ^{O}C for 10 minutes and washed twice with 9 ml volumes of cold sodium phosphate buffer (1M, pH 6.5) and resuspended in 9 ml of cold 1 M sodium phosphate buffer (pH 6.5) containing 5 mM of sodium lactate. The cell suspension was inoculated (0.5 ml) into each of two tubes containing 9.5 ml of the 5mM sodium lactate buffer. A portion of selected cut vegetable weighing approximately 0.1-gram also was added to one of the tubes. The tubes were incubated at 7 ^{O}C. After 1 hour and 24 hours of incubation, the cells were removed by centrifugation at 12,000-x g at 4 ^{O}C for 10 minutes and the supernatants were assayed for hydrogen peroxide according to the method of Gilliland (1969).

RESULTS

Effect on *Escherichia coli* O157:H7 on Fresh Produce

The plate counts on VRBA for all samples inoculated with *E. coli* O157:H7 on day 0 were at least 2 log cycles higher than on the uninoculated samples for each vegetable (Table 1). Thus we assume the VRBA counts to be a count for *E. coli* O157:H7. This enabled us to monitor the numbers of *E. coli* O157:H7 throughout the six days storage for the broccoli, cabbage and through day 3 for the carrots and lettuce. On day 6 the counts of VRBA for the uninoculated (control) carrots and lettuce were equal to or greater than the inoculated samples. Thus making it impossible to draw conclusions about the actual numbers of *E. coli* O157:H7 on these two products on day 6.

For each experiment involving a different vegetable, statistical analyses were done to determine if any interaction existed between the treatments and time. There was no significant interaction ($P > 0.05$) between time and the treatments for the vegetables except for broccoli (SAS ® Institute, Cary, NC). No significant differences ($P > 0.05$) were observed on any day between the counts on VRBA during storage at 7 °C for the samples inoculated with *E. coli* O157:H7 and those additionally inoculated with *L. delbrueckii* ssp. *lactis* (Table 1).

Populations of *E. coli* O157:H7 significantly decreased ($P < 0.05$) on the cabbage during the six-day storage for both treatments inoculated with *E. coli* O157:H7, however, there was a significant increase in numbers of coliforms in the uninoculated control on day 6. The initial population of *E. coli* in the inoculated samples was approximately 5.5 $\log_{10}$ CFU/g on day 0 and after six days of storage the final population of *E. coli* O157:H7 was approximately 4.5 $\log_{10}$ CFU/g, which was a significant decline ($P < 0.05$). The cells of

lactobacilli had no influence on the decline since both treated and untreated samples shared the decline in population levels.

Similar to the cabbage experiment, populations of *E. coli* O157:H7 on the inoculated carrots significantly declined ($P < 0.05$) during storage by day 3. There was no difference in the decline with or without added cells of lactobacilli. The uninoculated carrots had coliforms initially (3.16 $\log_{10}$ CFU/g), which was higher than initial coliform levels in the broccoli or cabbage and exhibited significant growth ($P < 0.05$) during the six days. There was no significant difference ($P > 0.05$) among the treatments on day six of storage indicating that the background coliforms had reached the levels in the samples that had been inoculated with *E. coli* O157:H7.

Counts on VRBA for the fresh cut lettuce, which had been inoculated with *E. coli* O157:H7 did not change significantly ($P > 0.05$) over time. The cells of *lactobacilli* had no significant effect ($P > 0.05$) in the counts obtained in VRBA during any of the days of storage. As with the carrots, coliform populations on the uninoculated sample were significantly lower ($P < 0.05$) than were those for the inoculated samples however, the numbers did significantly ($P < 0.05$) increase over time and eventually reached levels of the inoculated samples.

TABLE 1

INFLUENCE OF *LACTOBACILLUS DELBRUECKII* SSP. *LACTIS* RM 2-5 ON *ESCHERICHIA COLI* O157:H7 (ATCC 43894) INOCULATED ON FRESH PRODUCE STORED AT 7 ^{O}C FOR 6 DAYS.

		Counts on VRBA (log_{10} CFU/g) [1]		
Vegetable	**Inoculum**	**Day 0**	**Day 3**	**Day 6**
Broccoli	None (Control)[2]	0.00 ^{Aa}	0.00 ^{Aa}	2.22 ^{Aa}
	E.coli[3]	5.51 ^{Ba}	5.02 ^{Ba}	5.02 ^{Ba}
	E.coli + RM 2-5[4]	5.52 ^{Ba}	5.11 ^{Ba}	5.06 ^{Ba}
Cabbage	None (Control)[2]	0.00 ^{Aa}	0.00 ^{Aa}	3.19 ^{Ab}
	E.coli[3]	5.43 ^{Ba}	4.30 ^{Bb}	4.06 ^{Bb}
	E. coli + RM 2-5[4]	5.46 ^{Ba}	4.49 ^{Bb}	4.47 ^{Bb}
Carrots	None (Control)[2]	3.16 ^{Aa}	4.66 ^{Ab}	6.01 ^{Ac}
	E.coli[3]	6.30 ^{Ba}	5.89 ^{Ba}	5.78 ^{Ab}
	E. coli + RM 2-5[4]	6.60 ^{Ca}	5.92 ^{Bb}	5.89 ^{Ab}
Lettuce	None (Control)[2]	3.29 ^{Aa}	3.66 ^{Aa}	5.40 ^{Ab}
	E.coli[3]	5.43 ^{Ba}	5.36 ^{Ba}	5.40 ^{Aa}
	E. coli + RM 2-5[4]	5.44 ^{Ba}	5.51 ^{Ba}	5.40 ^{Aa}

[1]VRBA counts are expressed as log_{10} CFU/g; each value is the mean from three replicate trials. Broccoli SE = 0.31; Cabbage SE= 0.14; Carrots SE = 0.09; Lettuce SE = 0.13.

[2]Fresh-cut vegetables rinsed in sterile water served as a control; no *Lactobacillus* or *E. coli* added.

[3] Fresh-cut vegetables inoculated with *E. coli* O157:H7

[4]Fresh-cut vegetables inoculated with *E. coli* O157:H7 and treated with a cell suspension of *L. delbrueckii* ssp. *lactis* RM 2-5; the initial population of lactobacilli on each product was approximately $1x10^{8}$CFUg

[ABC] Means in the same column having the same letter in common for each vegetable are not significantly different ($P > 0.05$)

[abc] Means in the same row having the same letter in common for each vegetable are not significantly different ($P > 0.05$)

Effect on *Salmonella cholerasuis* on Fresh Produce

For each experiment involving *S. cholerasuis* and different vegetables, statistical analysis revealed that there was no significant ($P > 0.05$) time by treatment interaction except for the experiment involving broccoli. There were no significant differences ($P > 0.05$) between the counts for each vegetables inoculated with *Salmonella.* Background flora detected on BGA for the uninoculated vegetables all increased significantly ($P < 0.05$) during storage. These counts reached the same level on day six for all vegetables, which had been inoculated with the *Salmonella* except for the cabbage. The background flora, which formed colonies on BGA, was not identified. However, once this flora reached counts on BGA comparable to the counts obtained on the inoculated samples it was not possible using this medium to determine the fate of the *S. cholerasuis.* While there was a slight decline in counts on BGA after 3 days of storage on the broccoli, which had been inoculated, they had increased significantly ($P < 0.05$) after 6 days of storage (Table 2). This however does not indicate the growth of the *Salmonella cholerasuis* on the broccoli during refrigerated storage. Because the background flora on the broccoli had reached a level equal to the BGA counts on the inoculated samples by day 3, it is not possible to determine if the lactobacilli had any benefit.

The results for experiments involving *Salmonella* on carrots and lettuce were similar to those for the broccoli and cabbage. The lactobacilli again had no apparent affect on the *Salmonella.*

TABLE 2

INFLUENCE OF *LACTOBACILLUS DELBRUECKII* SSP. *LACTIS* RM 2-5 ON *SALMONELLA CHOLERASUIS* (ATCC 13706) INOCULATED ON FRESH PRODUCE STORED AT 7 ^{O}C FOR 6 DAYS.

		Counts of BGA (log_{10} CFU/g)[1]		
Vegetable	**Inoculum**	**Day 0**	**Day 3**	**Day 6**
Broccoli	None (Control)[2]	4.32 ^{Aa}	5.20 ^{Ab}	6.29 ^{Ac}
	Salmonella[3]	5.41 ^{Ba}	5.33 ^{Aa}	6.40 ^{Ab}
	Salmonella + RM 2-5[4]	5.53 ^{Ba}	5.32 ^{Aa}	6.40 ^{Ab}
Cabbage	None (Control)[2]	1.97 ^{Aa}	2.92 ^{Aa}	4.84 ^{Ab}
	Salmonella[3]	5.28 ^{Ba}	4.95 ^{Bb}	5.45 ^{Bc}
	Salmonella + RM 2-5[4]	5.48 ^{Ba}	4.95 ^{Bb}	5.46 ^{Bc}
Carrots	None (Control)[2]	5.09 ^{Aa}	5.24 ^{Aa}	7.34 ^{Ab}
	Salmonella[3]	6.73 ^{Ba}	6.74 ^{Ba}	7.24 ^{Ab}
	Salmonella + RM 2-5[4]	6.75 ^{Ba}	6.61 ^{Ba}	7.39 ^{Ab}
Lettuce	None (Control)[2]	2.87 ^{Aa}	5.44 ^{Ab}	5.02 ^{Ac}
	Salmonella[3]	5.80 ^{Ba}	5.44 ^{Ab}	5.32 ^{Bc}
	Salmonella + RM 2-5[4]	5.85 ^{Ba}	5.45 ^{Ab}	5.41 ^{Bc}

[1] BGA counts are expressed as log_{10} CFU/g; each value is the mean from three replicate trials. Broccoli SE = 0.14; Cabbage SE = 0.07; Carrots SE = 0.09; Lettuce = 0.05.

[2] Fresh-cut vegetables rinsed in sterile water served as a control; no *Lactobacillus* or *Salmonella* added.

[3] Fresh-cut vegetables inoculated with *S. cholerasuis.*

[4] Fresh-cut vegetables inoculated with *S. cholerasuis* and treated with a cell suspension of *L. delbrueckii* ssp. *lactis* RM 2-5; the initial population of lactobacilli on each product was approximately $1x10^{8}$CFUg

[ABC]Means in the same column having the same letter in common for each vegetable are not significantly different ($P > 0.05$).

[abc]Means in the same row having the same letter in common for each vegetable are not significantly different ($P > 0.05$).

Effect on *Listeria monocytogenes* on Fresh Produce

For each series of experiments involving a different vegetable inoculated with *L. monocytogenes*, statistical analysis indicated that there was no significant time by treatment interactions ($P > 0.05$). No colonies were detected on any of the vegetables on any storage day on TSA with added antibiotics for samples not inoculated with *L. monocytogenes*. Thus the counts in the inoculated samples were taken to be a true count for *L. monocytogenes* on each of the vegetables throughout the 6-day storage.

While no significant differences ($P > 0.05$) were observed between the samples inoculated with *L. monocytogenes* and those inoculated both with the pathogen and lactobacilli on any day of storage for the broccoli or carrots experiments, there were significant differences noted in the cabbage and lettuce experiments. On day three of storage in the cabbage experiment, there were a significantly fewer ($P < 0.05$) *L. monocytogenes* in the sample treated with *L. delbrueckii* spp. *lactis* than in the samples inoculated with the pathogen alone, however after six days of storage there was no significant difference ($P > 0.05$) between the two treatments.

There was no difference ($P > 0.05$) on days 0 and 3 between the samples inoculated with *Listeria* and the ones inoculated with both *Listeria* and the lactobacilli for the lettuce experiment. However, on day six significant fewer ($P < 0.05$) *Listeria* were recovered from the one containing the lactobacilli.

TABLE 3

INFLUENCE OF *LACTOBACILLUS DELBRUECKII* SSP. *LACTIS* RM 2-5 ON *LISTERIA MONOCYTOGENES* (27-2, V7-2, 383-2, & SCOTT A) INOCULATED ON FRESH PRODUCE STORED AT 7 ^{O}C FOR 6 DAYS.

		Counts of TSA (log_{10} CFU/g)[1]		
Vegetable	**Inoculum**	**Day 0**	**Day 3**	**Day 6**
Broccoli	None (Control)[2]	0.00 ^{Aa}	0.00 ^{Aa}	0.00 ^{Aa}
	Listeria[3]	4.23 ^{Ba}	4.19 ^{Ba}	4.40 ^{Bb}
	Listeria + RM 2-5[4]	4.16 ^{Ba}	4.22 ^{Ba}	4.44 ^{Bb}
Cabbage	None (Control)[2]	0.00 ^{Aa}	0.00 ^{Aa}	0.00 ^{Aa}
	Listeria[3]	4.51 ^{Ba}	4.93 ^{Bb}	4.63 ^{Bc}
	Listeria + RM 2-5[4]	4.43 ^{Ba}	4.28 ^{Ca}	4.58 ^{Bb}
Carrots	None (Control)[2]	0.00 ^{Aa}	0.00 ^{Aa}	0.00 ^{Aa}
	Listeria[3]	5.78 ^{Ba}	4.70 ^{Bb}	5.01 ^{Bb}
	Listeria + RM 2-5[4]	5.85 ^{Ba}	4.95 ^{Bb}	4.88 ^{Bb}
Lettuce	None (Control)[2]	0.00 ^{Aa}	0.00 ^{Aa}	0.00 ^{Aa}
	Listeria[3]	4.29 ^{Ba}	4.12 ^{Ba}	3.92 ^{Ba}
	Listeria + RM 2-5[4]	4.22 ^{Ba}	3.98 ^{Ba}	3.55 ^{Cb}

[1] TSA counts are expressed as log_{10} CFU/g; each value is the mean from three replicate trials. Broccoli SE = 0.04; Cabbage SE= 0.06; Carrots SE= 0.13; Lettuce SE= 0.09.

[2] Fresh-cut vegetables rinsed in sterile water served as a control; no *Lactobacillus* or *L. monocytogenes* cells were added.

[3] Fresh-cut vegetables inoculated with *L. monocytogenes*

[4] Fresh-cut vegetables inoculated with *L. monocytogenes* and treated with a cell suspension of *L. delbrueckii* ssp. *lactis* RM 2-5; the initial population of lactobacilli on each product was approximately $1x10^{8}$CFUg

[ABC] Means in the same column having the same letter in each column in common for each vegetable are not significantly different ($P > 0.05$).

[abc] Means in the same row having the same letter in each column in common for each vegetable are not significantly different ($P > 0.05$).

L. delbrueckii spp. *lactis* on Fresh Produce with Psychrotrophic organisms

Psychrotrophic organisms were enumerated on PCA incubated at 7 °C for 6- 10 days. The results revealed population levels of psychrotrophic organisms exceeding 6 $\log_{10}$ CFU/g on the vegetables after 6 days of storage. These population levels of bacteria indicate the prevalence and growth of spoilage organisms on the fresh-cut vegetables during storage. There was no significant difference ($P > 0.05$) observed among treatments containing *L. delbrueckii* ssp. *lactis* and treatments without the lactobacilli during storage (Appendix' A, B & C).

Influence of vegetables on hydrogen peroxide produced by *L. delbrueckii* spp. *lactis*

To ascertain whether or not the vegetables interfered with production of H_2O_2, by the lactobacilli, experiments were done to compare its production by *L. delbrueckii* ssp. *lactis* at 7°C in the presence and absence of each of the vegetables. There was significantly less ($P < 0.05$) hydrogen peroxide produced by *L. delbrueckii* ssp. *lactis,* in the presence of the fresh cut vegetables than without them (Table 4). Hydrogen peroxide produced by *L. delbrueckii* ssp. *lactis* RM 2-5 in presence and absence of the vegetables was measured after 1 hour and 24 hours of storage at 7 ^{O}C. While there was a significant increase ($P < 0.05$) in hydrogen peroxide levels after 24 hours in *L. delbrueckii* ssp. *lactis* in buffer alone, there was either no change or a decline in peroxide levels in the presence of vegetable. These results indicate that the fresh cut vegetables either inhibited

hydrogen peroxide production by *L. delbrueckii* ssp. *lactis* or destroyed it. After 24 hours of incubation, hydrogen peroxide levels in presence of all vegetable was significantly less ($P < 0.05$) than in the control containing only cells of *L. delbrueckii* spp. l*actis*.

TABLE 4

HYDROGEN PEROXIDE PRODUCTION BY CELLS OF *LACTOBACILLUS DELBRUECKII* SSP. *LACTIS* IN THE PRESENCE AND ABSENCE OF SELECTED VEGETABLES AT 7 ^{O}C IN BUFFER CONTAINING 5 mM SODIUM LACTATE[1]

	H_2O_2 Produced (ug/ml)	
Treatment[2]	**0 Hours**	**24 Hours**
Control	0.24 ^{Aa}	0.62 ^{Ab}
Broccoli	0.08 ^{Ca}	0.00 ^{Db}
Cabbage	0.05 ^{Ca}	0.23 ^{Bb}
Carrots	0.25 ^{Aa}	0.10 ^{Cb}
Lettuce	0.16 ^{Ba}	0.03 ^{CDb}

[1] 1M sodium phosphate buffer (pH6.5) containing 5 mM sodium lactate

[2] Each treatment contains 9.5 ml of buffer solution and 0.5 ml cell suspension of *L. lactis* RM 2-5 & 0.1 gram of indicated vegetable; the control was the same without any vegetable.

^{ABCD}Value in same column followed by different letter differ significantly ($P < 0.05$)

^{abcd}Value in same row followed by different letter differ significantly ($P < 0.05$)

DISCUSSION

Some lactobacilli produce sufficient amounts of hydrogen peroxide to inhibit many undesirable organisms. Although *L. delbrueckii* spp. *lactis* has been shown effective (due to production of hydrogen peroxide) in controlling *E. coli* O157:H7 on refrigerated raw chicken, a large number of cells were needed for this effect (Brashears *et al* 1998). Yap and Gilliland (2000), realized this issue and selected a strain of *L. delbrueckii* spp. *lactis*, which produced significantly more hydrogen peroxide than did the one in the study reported by Brashears *et al*, 1998. Selected lactobacilli, which produced higher amounts of hydrogen peroxide than other lactic acid bacteria, could prove to be more effective in controlling undesirable organisms with fewer cells of lactobacilli. The strain labeled "RM 2-5" from Yap and Gilliland (2000), was selected for use in the present study because it produced the most hydrogen peroxide of all tested cultures including the one used by Brashears *et al* (1998).

The storage conditions used in the present study were similar to those used for retail storage of fresh-cut vegetables. This included the use of a modified atmosphere in the package. This atmosphere contained a high concentration of nitrogen and carbon dioxide and a very low concentration of oxygen, which increases the shelf life of the produce (Kader, 1992). The fresh-cut produce was stored in special poly-olefin packaging used by the fresh-produce industry for retail sales (Cryovac PD 961). These bags were designed to allow the appropriate amount of oxygen to transfer through the film to maintain a micro-aerobic atmosphere. The vegetables also were maintained at 7

^{O}C (45 ^{O}F), which is similar to normal retail cooler conditions. Since these conditions simulated the conditions of retail packages of fresh-cut vegetables we were able to evaluate the effect of added cells of *L. delbrueckii* ssp. *lactis* on the produce under retail conditions. The cells of *L. delbrueckii* ssp. *lactis* were suspended in a 5 mM sodium lactate solution since the organism apparently contains lactate oxidase, which forms H_2O_2 when oxidizing lactate (Villegas and Gilliland, 1998). This solution supplied a substrate for the production of hydrogen peroxide by the lactobacilli without supplying nutrients for growth for pathogens used in the study.

Although the vegetables treated with *L. delbrueckii* ssp. *lactis* RM 2-5 contained approximately 7 $\log_{10}$ CFU/g of lactobacilli, they had no significant effect ($P > 0.05$) on the pathogens in most cases. Even though in one or two situations there were significantly lower numbers of pathogens (Listeria) in the presence of the added lactobacilli, the differences were not enough to be of practical importance. The lack of beneficial effect likely was due to catalase or peroxidase in the vegetables, which inactivated peroxide, produced by the lactobacilli.

Abdul-Raouf *et al*, 1993, showed the survival and growth of *Escherichia coli* O157:H7 on salad vegetables stored in a modified atmosphere under refrigeration temperatures during a 14-day shelf life. The cellular fluids from the sliced vegetables, which contained simple sugars and other nutrients, appeared to be the nutrient source for the *E. coli* O157:H7. This same cellular fluid would contain enzymes (Baardseth and Slinde, 1987) that could destroy at least some hydrogen peroxide produced by the lactobacilli, thus reducing or eliminating the potential for having an adverse effect on pathogenic or spoilage organisms on fresh cut vegetables.

Regardless of the mechanism, the results (Table 4) indicate that compounds produced by the vegetables either caused the destruction of hydrogen peroxide or inhibited its production by the lactobacilli.

Baardseth and Slinde (1987) reported the amounts of catalase and peroxidase present in various vegetables. This research showed carrots and cabbage both contained high levels of peroxidase and catalase as well as other enzymes. Both peroxidase and catalase compounds can neutralize hydrogen peroxide, thus eliminating the antagonistic effects upon pathogenic bacteria.

While *L. delbrueckii* spp. *lactis* did not control pathogenic bacteria on these vegetables, there could still be potential uses for this organism in other refrigerated food products. Since the effects of hydrogen peroxide are neutralized on the vegetables during storage, perhaps other lactic acid bacteria, which inhibit pathogenic bacteria through another means, might prove to be effective.

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APPENDIX A

LACTOBACILLUS DELBRUECKII SSP. *LACTIS* INTERACTION WITH *E.COLI* O157:H7, ON FRESH CUT BROCCOLI, CABBAGE, CARROTS AND LETTUCE.

TABLE 5

ENUMERATION DATA OF ESCHERICHIA COLI 0157:H7 AND PSYCHROTROPHIC ORGANISMS ON FRESH CUT BROCCOLI

VRBA				PCA			
	Day 0	Day 3	Day 6		Day 0	Day 3	Day 6
Replication 1				**Replication 1**			
Control	0.00	0.00	2.70	Control	5.20	5.54	6.38
E. coli	5.68	5.23	5.26	E. coli	5.57	5.60	6.87
RM 2-5	5.52	5.28	5.04	RM 2-5	4.86	5.68	6.59
Replication 2				**Replication 2**			
Control	0.00	0.00	0.00	Control	5.41	4.54	6.32
E. coli	5.28	4.95	4.81	E. coli	4.32	5.00	6.00
RM 2-5	5.43	4.94	4.99	RM 2-5	5.49	5.00	6.53
Replication 3				**Replication 3**			
Control	0.00	0.00	0.00	Control	5.68	5.23	7.32
E. coli	5.49	4.74	4.84	E. coli	5.43	5.23	6.41
RM 2-5	5.60	5.04	5.15	RM 2-5	5.28	5.40	6.72
Means				**Means**			
Control	0.00	0.00	0.90	Control	5.43	5.11	6.67
E. coli	5.48	4.97	4.97	E. coli	5.11	5.28	6.43
RM 2-5	5.52	5.09	5.06	RM 2-5	5.21	5.36	6.62

E. coli detected on VRBA are expressed as $\log_{10}$ CFU/g; psychrotrophic counts detected on PCA incubated at 7 °C are expressed as $\log_{10}$ CFU/g.

Control – fresh cut broccoli rinsed in water to serve as a control; no *L. delbrueckii* spp. *lactis* or *E. coli* cells were added.

E. coli – fresh cut broccoli inoculated with *E. coli* O157:H7

RM 2-5 – fresh cut broccoli inoculated with *E. coli* O157:H7 and treated with 2.5×10^7 CFU/ml of *L. lactis delbrueckii ssp. lactis* RM 2-5 on day 0 as detected on LBS agar.

TABLE 6

ENUMERATION DATA OF ESCHERICHIA COLI O157:H7 AND PSYCHROTROPHIC ORGANISMS ON FRESH CABBAGE

VRBA				PCA			
	Day 0	Day 3	Day 6		Day 0	Day 3	Day 6
Replication 1				**Replication 1**			
Control	0.00	0.00	3.40	Control	4.18	7.30	6.04
E. coli	5.32	4.32	3.57	E. coli	3.49	7.45	6.18
RM 2-5	5.43	4.64	4.70	RM 2-5	3.95	7.53	6.08
Replication 2				**Replication 2**			
Control	0.00	0.00	2.95	Control	4.26	7.18	6.04
E. coli	5.51	4.45	3.60	E. coli	4.04	7.84	6.28
RM 2-5	5.45	4.46	4.00	RM 2-5	3.26	7.70	6.04
Replication 3				**Replication 3**			
Control	0.00	0.00	3.08	Control	3.91	7.04	6.40
E. coli	5.43	4.04	4.43	E. coli	3.89	7.40	6.18
RM 2-5	5.49	4.28	4.46	RM 2-5	3.40	7.28	6.57
Means				**Means**			
Control	0.00	0.00	3.14	Control	4.11	7.17	6.16
E. coli	5.42	4.27	3.87	E. coli	3.81	7.56	6.21
RM 2-5	5.46	4.46	4.39	RM 2-5	3.54	7.50	6.23

E. coli detected on VRBA are expressed as $\log_{10}$ CFU/g; psychrotrophic counts detected on PCA incubated at 7 °C are expressed as $\log_{10}$ CFU/g.

Control – fresh cut cabbage rinsed in water to serve as a control; no *L. lactis delbrueckii ssp. lactis delbrueckii ssp. lactis* or *E. coli* cells were added.

E. coli – fresh cut cabbage inoculated with *E. coli* O157:H7

RM 2-5 – fresh cut cabbage inoculated with *E. coli* O157:H7 and treated with 2.8×10^7 CFU/ml of *L. lactis delbrueckii ssp. lactis delbrueckii ssp. lactis* RM 2-5 on day 0 as detected on LBS agar.

TABLE 7

ENUMERATION DATA OF ESCHERICHIA COLI 0157:H7 AND PSYCHROTROPHIC ORGANISMS ON SHREDDED CARROTS

VRBA				PCA			
	Day 0	Day 3	Day 6		Day 0	Day 3	Day 6
Replication 1				**Replication 1**			
Control	3.18	4.61	5.99	Control	5.26	5.92	6.98
L.lactis	3.32	4.76	6.00	L.lactis	5.49	6.84	7.04
E.coli	6.41	5.93	5.58	E.coli	5.26	7.04	7.04
RM 2-5	6.63	5.48	5.83	RM 2-5	5.11	7.08	7.18
Replication 2				**Replication 2**			
Control	3.23	4.71	6.04	Control	5.28	6.11	7.15
L.lactis	3.46	4.70	6.08	L.lactis	5.30	7.00	7.15
E.coli	6.18	5.76	5.83	E.coli	5.18	7.00	7.28
RM 2-5	6.59	5.79	5.77	RM 2-5	5.08	6.88	7.20
Replication 3				**Replication 3**			
Control	3.04	4.65	5.99	Control	5.15	6.08	6.99
L.lactis	3.54	4.76	6.08	L.lactis	5.23	6.98	7.04
E.coli	6.28	5.98	5.88	E.coli	5.26	7.08	7.15
RM 2-5	6.56	6.20	6.04	RM 2-5	5.36	6.88	7.30
Means				**Means**			
Control	3.15	4.66	6.01	Control	5.23	6.04	7.04
L. lactis	3.44	4.74	6.05	L.lactis	5.34	6.94	7.08
E. coli	6.29	5.89	5.76	E. coli	5.23	7.04	7.16
RM 2-5	6.59	5.82	5.88	RM 2-5	5.18	6.95	7.23

E. coli detected on VRBA are expressed as $\log_{10}$ CFU/g; psychrotrophic counts detected on PCA incubated at 7 °C are expressed as $\log_{10}$ CFU/g.

Control - shredded carrots rinsed in water to serve as a control; no *L. lactis delbrueckii ssp. lactis* or *E. coli* cells were added.

L. lactis - shredded carrots rinsed in water and treated with *L. lactis delbrueckii ssp. lactis* RM 2-5

E. coli - shredded carrots inoculated with *E. coli* O157:H7

RM 2-5 - shredded carrots inoculated with *E. coli* O157:H7 and treated with 3.7×10^7 CFU/ml of *L. lactis delbrueckii ssp. lactis* RM 2-5 on day 0 as detected on LBS agar.

TABLE 8

ENUMERATION DATA OF ESCHERICHIA COLI O157:H7 AND PSYCHROTROPHIC ORGANISMS ON FRESH CUT LETTUCE

VRBA				PCA			
	Day 0	Day 3	Day 6		Day 0	Day 3	Day 6
Replication 1				**Replication 1**			
Control	3.69	3.49	5.40	Control	3.94	6.61	7.38
E.coli	5.46	5.20	5.40	E.coli	3.92	6.46	7.38
RM 2-5	5.48	5.51	5.40	RM 2-5	3.88	6.49	7.36
Replication 2				**Replication 2**			
Control	2.54	3.62	5.40	Control	4.38	6.20	7.41
E.coli	5.46	5.58	5.40	E.coli	5.76	7.11	7.38
RM 2-5	5.41	5.56	5.40	RM 2-5	4.72	6.95	7.40
Replication 3				**Replication 3**			
Control	2.77	3.81	5.40	Control	4.34	6.32	7.20
E.coli	5.34	5.18	5.40	E.coli	3.92	6.49	7.08
RM 2-5	5.43	5.45	5.40	RM 2-5	3.65	7.32	7.08
Means				**Means**			
Control	3.00	3.64	5.40	Control	4.22	6.38	7.33
E. coli	5.42	5.32	5.40	E. coli	4.54	6.69	7.28
RM 2-5	5.44	5.50	5.40	RM 2-5	4.08	6.92	7.28

E. coli detected on VRBA are expressed as $\log_{10}$ CFU/g; psychrotrophic counts detected on PCA incubated at 7 °C are expressed as $\log_{10}$ CFU/g.

Control – fresh cut lettuce rinsed in water to serve as a control; no *L. lactis delbrueckii ssp. lactis* or *E. coli* cells were added.

E. coli – fresh cut lettuce inoculated with *E. coli* O157:H7

RM 2-5 – fresh cut lettuce inoculated with *E. coli* O157:H7 and treated with 2.7×10^7 CFU/g of *L. lactis delbrueckii ssp. lactis* RM 2-5 on day 0 as detected on LBS agar.

APPENDIX B

LACTOBACILLUS DELBRUECKII SSP. *LACTIS* INTERACTION WITH *SALMONELLA CHOLERASUIS*, ON FRESH CUT BROCCOLI, CABBAGE, CARROTS AND LETTUCE.

TABLE 9

ENUMERATION DATA OF SALMONELLA CHOLERASUIS AND PSYCHROTROPHIC ORGANISMS ON FRESH CUT BROCCOLI

BGA				PCA			
	Day 0	Day 3	Day 6		Day 0	Day 3	Day 6
Replication 1				**Replication 1**			
Control	4.41	5.41	6.26	Control	4.76	7.04	6.81
Salmonella	5.36	5.30	6.38	Salmonella	5.62	6.40	7.26
RM 2-5	5.58	5.38	6.52	RM 2-5	5.76	6.43	6.72
Replicaton 2				**Replicaton 2**			
Control	4.36	5.30	6.34	Control	4.95	6.99	6.86
Salmonella	5.54	5.23	6.43	Salmonella	5.65	6.88	7.26
RM 2-5	5.57	5.26	6.32	RM 2-5	5.40	7.04	6.88
Replication 3				**Replication 3**			
Control	4.11	4.18	6.28	Control	5.04	6.61	6.72
Salmonella	5.28	5.43	6.40	Salmonella	5.62	7.08	7.11
RM 2-5	5.41	5.30	6.32	RM 2-5	5.26	7.93	7.15
Means				**Means**			
Control	4.30	4.96	6.29	Control	4.92	6.88	6.79
E. coli	5.39	5.32	6.40	E. coli	5.63	6.78	7.21
RM 2-5	5.52	5.31	6.39	RM 2-5	5.47	7.13	6.92

Salmonella detected on BGA are expressed as $\log_{10}$ CFU/g; psychrotrophic counts detected on PCA incubated at 7 °C are expressed as $\log_{10}$ CFU/g.

Control – fresh cut broccoli rinsed in water to serve as a control; no *L. lactis delbrueckii ssp. lactis* or *Salmonella* cells were added.

Salmonella – fresh cut broccoli inoculated with *Salmonella cholerasuis*

RM 2-5 – fresh cut broccoli inoculated with *S. cholerasuis* and treated with 3.3×10^7 CFU/ml of *L. lactis delbrueckii ssp. lactis* RM 2-5 on day 0 as detected on LBS agar.

TABLE 10

ENUMERATION DATA OF SALMONELLA CHOLERASUIS AND PSYCHROTROPHIC ORGANISMS ON FRESH CUT CABBAGE

BGA				PCA			
	Day 0	Day 3	Day 6		Day 0	Day 3	Day 6
Replication 1				**Replication 1**			
Control	0.00	0.00	4.91	Control	3.87	4.65	5.54
Salmonella	5.30	5.08	5.38	Salmonella	3.89	4.80	4.95
RM 2-5	5.45	4.86	5.46	RM 2-5	3.56	4.96	5.61
Replication 2				**Replication 2**			
Control	0.00	0.00	4.95	Control	3.78	4.34	5.57
Salmonella	5.23	4.97	5.38	Salmonella	3.95	4.32	5.34
RM 2-5	5.51	5.00	5.34	RM 2-5	4.40	4.79	5.28
Replication 3				**Replication 3**			
Control	0.00	0.00	4.53	Control	5.26	4.30	5.00
Salmonella	5.30	4.71	5.57	Salmonella	5.41	4.20	5.18
RM 2-5	5.48	4.99	5.56	RM 2-5	5.32	4.81	5.28
Means				**Means**			
Control	0.00	0.00	4.80	Control	4.30	4.43	5.37
E. coli	5.28	4.92	5.44	E. coli	4.42	4.44	5.16
RM 2-5	5.48	4.95	5.45	RM 2-5	4.43	4.85	5.39

Salmonella detected on BGA are expressed as $\log_{10}$ CFU/g; psychrotrophic counts detected on PCA incubated at 7 °C are expressed as $\log_{10}$ CFU/g.

Control – fresh cut cabbage rinsed in water to serve as a control; no *L. lactis delbrueckii ssp. lactis* or *Salmonella* cells were added.

Salmonella – fresh cut cabbage inoculated with *Salmonella cholerasuis*

RM 2-5 – fresh cut cabbage inoculated with *S. cholerasuis* and treated with 2.2×10^7 CFU/ml of *L. lactis delbrueckii ssp. lactis* RM 2-5 on day 0 as detected on LBS agar.

TABLE 11

ENUMERATION DATA OF SALMONELLA CHOLERASUIS AND PSYCHROTROPHIC ORGANISMS ON SHREDDED CARROTS

BGA				PCA			
	Day 0	Day 3	Day 6		Day 0	Day 3	Day 6
Replication 1				**Replication 1**			
Control	5.28	5.11	7.38	Control	5.72	7.26	7.38
Salmonella	6.84	6.78	7.20	Salmonella	5.69	7.08	7.15
RM 2-5	6.76	6.70	7.57	RM 2-5	5.36	7.26	7.08
Replication 2				**Replication 2**			
Control	5.08	5.11	7.11	Control	5.78	7.11	6.95
Salmonella	6.72	6.75	7.36	Salmonella	5.61	7.08	7.43
RM 2-5	6.71	6.61	7.40	RM 2-5	5.48	7.15	7.04
Replication 3				**Replication 3**			
Control	4.76	5.41	7.46	Control	5.77	7.15	7.23
Salmonella	6.60	6.69	7.11	Salmonella	5.72	7.00	7.45
RM 2-5	6.79	6.49	7.08	RM 2-5	5.52	7.20	7.34
Means				**Means**			
Control	5.04	5.21	7.32	Control	5.76	7.17	7.19
E. coli	6.72	6.74	7.23	E. coli	5.67	7.05	7.34
RM 2-5	6.75	6.60	7.35	RM 2-5	5.45	7.20	7.15

Salmonella detected on BGA are expressed as $\log_{10}$ CFU/g; psychrotrophic counts detected on PCA incubated at 7 °C are expressed as $\log_{10}$ CFU/g.

Control – shredded carrots rinsed in water to serve as a control; no *L. lactis delbrueckii ssp. lactis* or *Salmonella* cells were added.

Salmonella – shredded carrots inoculated with *Salmonella cholerasuis*

RM 2-5 – shredded carrots inoculated with *S. cholerasuis* and treated with 3.7×10^7 CFU/ml of *L. lactis delbrueckii ssp. lactis* RM 2-5 on day 0 as detected on LBS agar.

TABLE 12

ENUMERATION DATA OF SALMONELLA CHOLERASUIS AND PSYCHROTROPHIC ORGANISMS ON FRESH CUT LETTUCE

BGA				PCA			
	Day 0	Day 3	Day 6		Day 0	Day 3	Day 6
Replication 1				**Replication 1**			
Control	0.00	5.38	4.95	Control	4.70	5.59	4.96
Salmonella	5.85	5.45	5.30	Salmonella	3.73	4.97	5.18
RM 2-5	5.81	5.57	5.45	RM 2-5	3.84	5.83	5.15
Replication 2				**Replication 2**			
Control	0.00	5.40	4.97	Control	2.70	5.20	5.08
Salmonella	5.81	5.45	5.32	Salmonella	3.69	5.30	5.08
RM 2-5	5.88	5.20	5.49	RM 2-5	4.51	4.49	5.04
Replication 3				**Replication 3**			
Control	0.00	5.52	5.11	Control	4.04	5.49	5.04
Salmonella	5.72	5.43	5.34	Salmonella	3.28	5.20	5.20
RM 2-5	5.87	5.51	5.28	RM 2-5	4.18	5.41	5.23
Means				**Means**			
Control	0.00	5.43	5.01	Control	3.81	5.43	5.03
E. coli	5.79	5.44	5.32	E. coli	3.57	5.16	5.15
RM 2-5	5.85	5.43	5.41	RM 2-5	4.17	5.25	5.14

Salmonella detected on BGA are expressed as $\log_{10}$ CFU/g; psychrotrophic counts detected on PCA incubated at 7 °C are expressed as $\log_{10}$ CFU/g.

Control – fresh cut lettuce rinsed in water to serve as a control; no *L. lactis delbrueckii ssp. lactis* or *Salmonella* cells were added.

Salmonella – fresh cut lettuce inoculated with *Salmonella cholerasuis*

RM 2-5 – fresh cut lettuce inoculated with *S. cholerasuis* and treated with 3.0×10^7 CFU/ml of *L. lactis delbrueckii ssp. lactis* RM 2-5 on day 0 as detected on LBS agar.

APPENDIX C

LACTOBACILLUS DELBRUECKII SSP. *LACTIS* INTERACTION WITH *LISTERIA MONOCYTOGENES*, ON FRESH CUT BROCCOLI, CABBAGE, CARROTS AND LETTUCE.

TABLE 13

ENUMERATION DATA OF LISTERIA MONOCYTOGENES AND PSYCHROTROPHIC ORGANISON FRESH CUT BROCCOLI

TSA				PCA			
	Day 0	Day 3	Day 6		Day 0	Day 3	Day 6
Replication 1				**Replication 1**			
Control	0.00	0.00	0.00	Control	5.54	6.45	6.58
Listeria	4.20	4.26	4.32	Listeria	5.60	6.46	6.43
RM 2-5	4.23	4.28	4.46	RM 2-5	5.68	6.73	6.26
Replication 2				**Replication 2**			
Control	0.00	0.00	0.00	Control	4.54	7.08	6.58
Listeria	4.20	4.28	4.41	Listeria	5.04	6.76	6.54
RM 2-5	4.18	4.28	4.43	RM 2-5	5.00	6.51	6.38
Replication 3				**Replication 3**			
Control	0.00	0.00	0.00	Control	5.23	6.41	6.48
Listeria	4.28	4.04	4.48	Listeria	5.23	6.38	6.54
RM 2-5	4.08	4.11	4.43	RM 2-5	5.40	6.23	6.92
Means				**Means**			
Control	0.00	0.00	0.00	Control	5.11	6.65	6.55
E. coli	4.23	4.19	4.40	E. coli	5.29	6.54	6.51
RM 2-5	4.16	4.22	4.44	RM 2-5	5.36	6.49	6.52

Listeria detected on TSA are expressed as $\log_{10}$ CFU/g; psychrotrophic counts detected on PCA incubated at 7 °C are expressed as $\log_{10}$ CFU/g.

Control – fresh cut broccoli rinsed in water to serve as a control; no *L. lactis delbrueckii ssp. lactis* or *Listeria* cells were added.

Listeria – fresh cut broccoli inoculated with *Listeria monocytogenes*

RM 2-5 – fresh cut broccoli inoculated with *L. monocytogenes* and treated with 2.7×10^7 CFU/ml of *L. lactis delbrueckii ssp. lactis* RM 2-5 on day 0 as detected on LBS agar.

TABLE 14

ENUMERATION DATA OF LISTERIA MONOCYTOGENES AND PSYCHROTROPHIC ORGANISMS ON FRESH CUT CABBAGE

TSA				PCA			
	Day 0	Day 3	Day 6		Day 0	Day 3	Day 6
Replication 1				**Replication 1**			
Control	0.00	0.00	0.00	Control	2.34	5.56	6.34
Listeria	4.45	4.82	4.74	Listeria	0.00	6.76	6.32
RM 2-5	4.38	4.38	4.54	RM 2-5	2.30	6.34	6.38
Replication 2				**Replication 2**			
Control	0.00	0.00	0.00	Control	3.56	4.85	6.80
Listeria	4.62	4.97	4.63	Listeria	4.88	6.59	6.40
RM 2-5	4.45	4.34	4.36	RM 2-5	0.00	6.80	6.49
Replication 3				**Replication 3**			
Control	0.00	0.00	0.00	Control	2.78	5.11	6.58
Listeria	4.46	5.00	4.53	Listeria	0.00	6.90	6.30
RM 2-5	4.45	4.11	4.84	RM 2-5	3.00	6.58	6.41
Means				**Means**			
Control	0.00	0.00	0.00	Control	2.89	5.17	6.57
E. coli	4.51	4.93	4.64	E. coli	1.63	6.75	6.34
RM 2-5	4.42	4.28	4.58	RM 2-5	1.77	6.57	6.43

Listeria detected on TSA are expressed as $\log_{10}$ CFU/g; psychrotrophic counts detected on PCA incubated at 7 °C are expressed as $\log_{10}$ CFU/g.

Control – fresh cut cabbage rinsed in water to serve as a control; no *L. lactis delbrueckii ssp. lactis* or *Listeria* cells were added.

Listeria – fresh cut cabbage inoculated with *Listeria monocytogenes*

RM 2-5 – fresh cut cabbage inoculated with *L. monocytogenes* and treated with 1.8×10^7 CFU/ml of *L. lactis delbrueckii ssp. lactis* RM 2-5 on day 0 as detected on LBS agar.

TABLE 15

ENUMERATION DATA OF LISTERIA MONOCYTOGENES AND PSYCHROTROPHIC ORGANISMS ON SHREDDED CARROTS

TSA				PCA			
	Day 0	Day 3	Day 6		Day 0	Day 3	Day 6
Replication 1				**Replication 1**			
Control	0.00	0.00	0.00	Control	5.38	7.51	7.32
Listeria	5.90	4.64	5.08	Listeria	5.30	7.38	7.23
RM 2-5	5.91	5.65	4.99	RM 2-5	5.32	7.20	7.28
Replication 2				**Replication 2**			
Control	0.00	0.00	0.00	Control	5.38	7.32	7.52
Listeria	5.64	4.89	5.08	Listeria	5.45	7.20	7.28
RM 2-5	5.78	4.68	4.76	RM 2-5	5.30	7.40	7.54
Replication 3				**Replication 3**			
Control	0.00	0.00	0.00	Control	5.41	7.20	7.57
Listeria	5.81	4.58	4.87	Listeria	5.40	7.36	7.18
RM 2-5	5.86	4.53	4.91	RM 2-5	5.15	7.46	7.40
Means				**Means**			
Control	0.00	0.00	0.00	Control	5.39	7.34	7.47
E. coli	5.78	4.71	5.01	E. coli	5.38	7.32	7.23
RM 2-5	5.85	4.96	4.89	RM 2-5	5.26	7.35	7.41

Listeria detected on TSA are expressed as $\log_{10}$ CFU/g; psychrotrophic counts detected on PCA incubated at 7 °C are expressed as $\log_{10}$ CFU/g.

Control - shredded carrots rinsed in water to serve as a control; no *L. lactis delbrueckii ssp. lactis* or *Listeria* cells were added.

Listeria - shredded carrots inoculated with *Listeria monocytogenes*

RM 2-5 - shredded carrots inoculated with *L. monocytogenes* and treated with 4.1×10^7 CFU/ml of *L. lactis delbrueckii ssp. lactis* RM 2-5 on day 0 as detected on LBS agar.

TABLE 16

ENUMERATION DATA OF LISTERIA MONOCYTOGENES AND PSYCHROTROPHIC ORGANISMS ON FRESH CUT LETTUCE

TSA				PCA			
	Day 0	Day 3	Day 6		Day 0	Day 3	Day 6
Replication 1				**Replication 1**			
Control	0.00	0.00	0.00	Control	3.81	6.00	6.58
Listeria	4.20	4.15	4.08	Listeria	3.90	6.11	6.48
RM 2-5	4.11	3.75	3.73	RM 2-5	4.04	5.76	7.38
Replication 2				**Replication 2**			
Control	0.00	0.00	0.00	Control	3.88	5.78	6.15
Listeria	4.40	4.00	3.65	Listeria	4.08	6.66	6.49
RM 2-5	4.36	4.11	3.67	RM 2-5	4.49	5.83	6.52
Replication 3				**Replication 3**			
Control	0.00	0.00	0.00	Control	3.64	6.43	6.18
Listeria	4.26	4.20	4.04	Listeria	3.54	5.84	6.49
RM 2-5	4.18	4.08	3.26	RM 2-5	4.36	5.85	6.43
Means				**Means**			
Control	0.00	0.00	0.00	Control	3.77	6.07	6.30
E. coli	4.29	4.12	3.92	E. coli	3.84	6.21	6.49
RM 2-5	4.22	3.98	3.55	RM 2-5	4.30	5.81	6.78

Listeria detected on TSA are expressed as $\log_{10}$ CFU/g; psychrotrophic counts detected on PCA incubated at 7 °C are expressed as $\log_{10}$ CFU/g.

Control – fresh cut lettuce rinsed in water to serve as a control; no *L. lactis delbrueckii ssp. lactis* or *Listeria* cells were added.

Listeria – fresh cut lettuce inoculated with *Listeria monocytogenes*

RM 2-5 – fresh cut lettuce inoculated with *L. monocytogenes* and treated with 3.3×10^7 CFU/ml of *L. lactis delbrueckii ssp. lactis* RM 2-5 on day 0 as detected on LBS agar.

APPENDIX D

HYDROGEN PEROXIDE PRODUCTION OF *LACTOBACILLUS DELBRUECKII* SSP. *LACTIS* AMOUNG FRESH CUT BROCCOLI, CABBAGE, CARROTS AND LETTUCE.

TABLE 17

HYDROGEN PEROXIDE PRODUCTION OF CELLS OF

LACTOBACILLUS DELBRUEKII SSP. *LACTIS* AT 7 °C WITH

ADDED FRESH CUT VEGETABLES

Treatment		H_2O_2 Produced (ug/ml)	
		1 Hour	24 Hours
Control	Replication 1	0.21	0.62
	Replication 2	0.28	0.65
	Replication 3	0.22	0.60
Broccoli	Replication 1	0.10	-0.03
	Replication 2	0.08	-0.02
	Replication 3	0.05	-0.03
Cabbage	Replication 1	-0.03	0.17
	Replication 2	0.09	0.29
	Replication 3	0.10	0.22
Carrots	Replication 1	0.23	0.04
	Replication 2	0.29	0.13
	Replication 3	0.25	0.13
Lettuce	Replication 1	0.20	0.01
	Replication 2	0.12	0.05
	Replication 3	0.16	0.04

L. lactis RM 2-5- control, cells of *L. lactis delbrueckii ssp. lactis* RM 2-5 in buffer alone

Carrots- cells of *L. lactis delbrueckii ssp. lactis* RM 2-5 with added 0.1 gram of shredded carrot

Broccoli- cells of *L. lactis delbrueckii ssp. lactis* RM 2-5 with added 0.1 gram of fresh cut broccoli

Lettuce- cells of *L. lactis delbrueckii ssp. lactis* RM 2-5 with added 0.1 gram of fresh cut lettuce

Cabbage- cells of *L. lactis delbrueckii ssp. lactis* RM 2-5 with added 0.1 gram of fresh cut cabbage

VITA

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Candidate for the Degree of

Masters of Science

Thesis: INFLUENCE OF *LACTOBACILLUS DELBRUECKII* SSP. *LACTIS* ON *ESCHERICHIA COLI* O157:H7, *SALMONELLA CHOLERASUIS* AND *LISTERIA MONOCYTOGENES* ON MINIMALLY PROCESSED VEGETABLES

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