APPLICATION OF LACTIC ACID BACTERIA AS POTENTIAL BIOPRESERVATIVE AGENTS OF MINIMALLY PROCESSED REFIRIGERATED FRUITS

By

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1994

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
May, 2002

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POTENTIAL BIOPRESERVATIVE AGENTS

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REFIRIGERATED

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Thesis Approved :

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ACKNOWLEDGEMENTS

I would like to extend my sincere appreciation to Dr. Stanley Gilliland for serving as my graduate advisor. It had been a privilege to work and learn from him. I wish to thank him for his patience, encouragement, knowledge, and constructive guidance. I wish to thank Dr. Christina Dewitt, and Dr. William McGlynn for serving on graduate committee and also for their valuable assistance and guidance.

I am very grateful to my loving husband and my best friend Upul, who has been a pillar of strength to me at all times especially during my graduate course work. And especially my wonderful parents Ranjit and Malani and rest of my loving family Sanjaya and Hasitha, for all their love, encouragement, and support and especially for their faith in me.

I would like to thank my dear friends Trenna, Jennifer, Eric, J.R, Keith and Sandra for their valuable friendship, encouragement and help. And I also would like to thank all the students working in the lab for their help with my research.

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CHAPTER I

INTRODUCTION

Minimally processed fresh cut fruits and vegetables are an important and rapidly expanding class of food products that primarily cater to the modern consumer needs. The ideology of "Minimally processed" denotes the concept of preparing and handling value added fresh produce that are high in quality while at the same time maintaining their fresh nature and providing convenience to the user.

Like other food products minimally processed fresh produce also has the tendency to act as a vehicle for food born pathogens. Contrary to the notion that fresh produce is one of the safest food products, contaminated produce can and does cause foodborne illnesses. The potential for microbial contamination of fruits is relatively high due to the wide variety of conditions to which the produce is subjected in the food production chain from production to consumption.

Fresh cut fruits also are highly perishable due to their lack of a skin or protective layer. This provides an ideal growth environment for spoilage flora as well, especially due to the cut surfaces that release cellular materials, which provide nutrients for microorganisms. Maintaining shelf-stability of fresh cut fruits over an extended period of time is a challenge that has to be met effectively by the industry. Implementing quality assurance programs that include strong SOPs, GMPs and HACCP principles is an effective way of assuring quality and safety of the product to the consumer.

However a considerable number of outbreaks of food borne illnesses attributed to fresh produce has shed light on the need for a more effective method to combat undesirable microorganisms on fresh produce. Use of biocontrol agents to enhance the keeping quality and safety of fresh produce is a phenomenon that is fast gaining popularity in the minimal processing industry. Present day consumer's preference for nonchemical preservatives in food products is another justification in considering biopreservatives as potential agents to extend shelf life of fresh cut fruits and vegetables. Lactic acid bacteria are the most preferred and suitable group of organisms to be considered as potential biopreservatives of fresh produce due to their past use in the manufacture of cultured and fermented products, which indicates they are safe to consume.

CHAPTER II

REVIEW OF THE LITERATURE

Preservation of Food by Lactic Acid Bacteria

One of the oldest technologies employed to preserve food is fermentation. Implementation of these phenomena goes back to biblical times (Gilliland, 1985) where food products underwent certain alterations resulting in desirable outcome in the sensory attributes of these products. Subjecting the food to specific conditions brought out these changes of the food product, that were more appealing to the pallet. Over the centuries fermentation has evolved and been refined. As the technology advanced it was understood that fermentation, which resulted in preservation, was due to the growth and activity of microorganisms and also that fermentation might enhance the nutritional quality of foods. Lactic acid bacteria are the major microorganisms that bring out these desired changes in food products by fermentation. It is understood that potential health or nutritional benefits (Gilliland, 1990) may be exerted due to the growth and activity of certain lactic acid bacteria in the fermented food.

Lactic acid bacteria belong to a group of microorganisms, which are Gram positive nonspore forming, nonmotile rods or cocci. *Lactobacillus*, *Streptococcus, Pediococcus,* and *Leuconostoc* are the genera included in this group of microorganisms. All the members of these genera are fastidious organisms, requiring

not only carbohydrates as energy sources but also preformed amino acids, nucleotides and vitamins. They do not contain cytochromes and thus obtain energy through fermentation rather than via oxidative phosphorylation. They are able to produce lactic acid from carbohydrates and unable to reduce nitrate. Lactic acid bacteria prefer low oxidation-reduction potential for growth and thus are facultative to microareophilic.

Fermentation carried out by lactic acid bacteria is a metabolic process, which results in the production of energy as a result of fermenting carbohydrates and other related compounds. The organic compounds, which are formed from the degradation of carbohydrates during the fermentation process, act as final electron acceptors. Oxygen can act as the electron acceptor (Gilliland & Speck, 1969) during oxidation and reduction reactions under aerobic conditions, which in turn leads to the formation and accumulation of H_2O_2. Since these microorganisms do not possess the mechanism to breakdown H_2O_2, it can accumulate in the growth media.

Two types of fermentation, homo and hetero, may be observed in lactic acid bacteria. In homofermentation, lactic acid is the primary end product. The lactic acid produced plays a significant role in the production of cultured food products by lowering the pH, which in turns favors the production of desired flavor, color and consistency and also suppresses undesirable microorganisms (Berwal and Dinchev, 1993). These homolactic bacteria metabolize sugars via the glycolisis pathway. Whereas in heterofermentation acetic acid, ethanol and CO_2 are formed in addition to lactic acid. Heterolactic bacteria belonging to this group ferment sugars by either pentose or hexose monophosphate pathways. Some heterolactics can ferment citrate

to produce diacetyl, which imparts a unique and characteristic aroma to some cultured products.

Most lactic acid bacteria are mesophiles having an optimum growth temperature of about 37^0C, some exhibit optimum growth at 45^0C while others can grow as low as 5^0C . These microorganisms usually have an optimal pH range of 5.5-6.2. Lactic acid bacteria are found in dairy products, meat and fish products, fruit and fruit juices, and pickled vegetables.

Natural fermentation of food products which is brought about by the indigenous or resident microflora is a time consuming process that sometimes results in undesirable changes or end products that can lead to spoilage (Desai and Sheth, 1997). As opposed to natural fermentation, controlled fermentation is executed by applying pure cultures of lactic acid bacteria and subjecting the food product to specific and controlled growth environment to obtain desired changes or end products. With the advance in food technology and increased demand and awareness by the consumers for better quality and safety products, controlled fermentation has become the norm in the food industry today.

Fermentation processes to produce cultured food products may encompass a diversity of functions, which lead to improved digestibility, enhanced food edibility, intensified sensory qualities, increased shelf life, and safe food products. There has been significant evidence to indicate that lactic fermentation inhibits growth and survival of pathogenic bacteria, as well as spoilage microflora (Adams and Nicolaides, 1997). Controlled lactic fermentation using one or more specific strains of one or more species of lactic acid bacteria ensures maximum growth and activity of

the starter culture in the inoculated product and formation of the end product with the desired flavor and aroma within the shortest time possible. Desai and Sheth (1997) reported that natural fermentation of vegetables led to the production of an undesirable yeast film on the vegetables. This was not observed when specific lactic starters were used for the fermentation. This indicates that inoculation of the vegetables with selected lactic acid bacteria could prevent the development of spoilage flora, presumably due to acid production. Therefore it can be concluded that controlled fermentation has the potential to prevent losses in seasonal vegetables as a result of poor cold storage facilities and at the same time adding variety and palatability to the diet.

Selected species of starter cultures produce end products like lactic acid (Lucke, 1997), bacteriocins (Tagg et al, 1976), and hydrogen peroxide. All the above mentioned components have the ability to exert antagonistic action toward pathogenic and spoilage microflora (Lucke, 1997). Application of starter cultures to foods for controlled fermentation ensures the potential dominance of desired microflora over the resident flora (Bacus and Brown, 1981). Daeschel (1989) concluded, based on his research, that lactic acid bacteria inhibit the growth and prevalence of undesirable microflora by converting fermentable sugars to lactic acid. Ceylan and Fung (2000) reported after conducting a study on fermentation of Turkish dry sausage, that low-temperature fermentation (25^0C) using strains of *Lactobacillus sake* and high-temperature fermentation using *Pediococcus acidilactici* (40^0C) were effective in destroying *Yersinia enterocolitica* . They concluded that lactic acid produced by the above mentioned two organisms was the primary component responsible for this

antagonistic reaction. Based on epidemiological studies it was concluded that *Y.enterocolitica,* which is an environmental organism, has the potential to be transmitted via foods of animal origin, such as milk, beef, lamb, pork, chicken and products that have come to contact with contaminated water (Zink et al, 1982). Tacket et al (1984 and 1985) reported that *Y.enterocolitica*, due to its ability to grow and multiply at refrigeration temperatures, could occur in foods produced or stored at these temperatures.

A number of food borne pathogens have the capability of surviving the fermentation process. Enteropathogens, such as enterohaemorrhagic *Escherichia coli* show some patterns of acid resistance and could survive the fermentation process. It was stated by Glass et al (1992) that *E.coli* O157:H7 does not grow at a pH below 4.0. Based on earlier research, yogurt and fermented meat have been identified as potential vehicles of the above food borne pathogen. *Escherichia coli* O157:H7 is a Gram negative facultative anaerobic foodborne pathogen that has created quite a safety concern in the dairy and food industry (Buchanan and Doyle, 1997). Doyle (1991) concluded that foods of animal origin were the primary sources of *E.coli* O157:H7 infection. Due to their apparent resistance to low pH, it was found that *E.coli* O157:H7 could survive and even exhibit growth to a certain extent during cottage cheese and cheddar cheese manufacturing process (Arocha et al,1992; Reitsma and Henning, 1996). Fortunately it also was noted by same scientists that the number of this pathogen decreased during the ripening process.

It has been reported that *E.coli* O157:H7 survived in unpasteurized apple cider at pH 3.6 to 4.0 for as long as 31 days at 4^0C (Zhao et al, 1993). Based on results

obtained by Chou et al (2000) *E.coli O*157:H7 could grow during the fermentation of skim milk in the presence of *Lactobacillus casei* or *Lactobacillus bulgaricus*. The growth pattern and survival of this pathogen during lactic fermentation was directly correlated to strain variation of the pathogen and the type of starter culture used for the fermentation process. Chou and Tsai (1992) reported that the addition of sugar enhanced the survival rate of the pathogen in the cultured milk drink. These researchers indicated that addition of sugar also extended the survival of *Listeria monocytogenes* in the fermented milk drink during refrigerated storage.

Due to the capability of some strains of pathogens to survive and grow during lactic fermentation, it is quite imperative to implement and maintain adequate standards of hygiene during the fermentation process.

Biopreservative Actions of Lactic Acid Bacteria

Production of Antimicrobial Compounds

Lactic acid bacteria play an important role not only in the fermentation process but also as active mediators in the preservation of food products. This group of microorganisms exerts these benefits either as natural microflora or as a starter culture added under controlled parameters. Lactic acid bacteria can augment hygiene quality and safety attributes of food products by inhibiting the natural competing flora, including spoilage and pathogenic bacteria. The primary agent responsible in

mediating such an antagonistic effect is lactic acid (Daeschel, 1989), which has the capacity to lower the pH in the surrounding media and create a hostile environment for the undesirable microflora. This acidic media allows the lactic acid bacteria to compete and establish dominance.

However lactic acid bacteria also produce other effective compounds in addition to lactic acid that have the potential to cause inhibition to other microflora. These antagonistic factors can be fundamentally grouped into metabolic end products such as organic acids and H_2O_2 or bactericidal / bacteriostatic proteins (Klaehammer, 1993; Stiles and Hastings, 1991; Nettles and Barefoot, 1993). The latter proteinaceous compounds could be either antibiotic substances such as acidolin (Hamdan and Mikolajcik, 1974) or bacteriocins (Barefoot and Klaenhammer, 1983; Ferreira and Gilliland, 1988).

Based on a study done by Gilliland and Speck (1975) to examine inhibition of psychrotrophic microflora by lactic acid bacteria during refrigerated storage of food it was concluded that H_2O_2 formed by lactobacilli was the main causative agent mediating inhibition at (5-7^{0}C). Hydrogen peroxide produced by lactobacilli in significant amounts has proven to be antagonistic towards spoilage flora, more specifically Gram negative psychrotrophs as well as some food-borne pathogens (Dahiya and Speck, 1968; Gilliland and Speck, 1975). Dahiya and Speck (1968) were able to show that *Latobacillus lactis* had the ability to produce hydrogen peroxide in sufficient amounts to cause an effective inhibition of growth of *Staphylococcus aureus* at 35^{0}C. These researchers also showed that the maximum amount of H_2O_2 was produced and accumulated in cell suspensions of lactobacilli at

5^0C even though *L.lactis* do not grow at refrigeration temperatures. The effect of H_2O_2 produced by lactobacilli on psychrotrophes was not studied during that period of time. But Gilliland and Speck (1975) were able to impart more knowledge on this topic in a later study. Bottazi 1972 stated that *L.lactis* produced the most H_2O_2 of all the other lactic acid bacteria evaluated.

Brashears et al 1998 reported that a selected strain of *L. lactis*, inhibited *E.coli* O157: H7 on raw chicken meat during refrigerated storage. These scientists also illustrated that the lactobacilli were able to exert antagonistic action toward coliforms and psychrotrophic spoilage flora. Several species of *Salmonella*, associated with food products including meats, fruits, and vegetables, were inhibited by *L.lactis* during incubation at 37^0C (Brashears and Durre, 1999). The causative agent responsible for inhibition in this study was identified as lactic acid and not H_2O_2. Although there is a sufficient production of H_2O_2 by *L.lactis* during refrigerated storage to give rise to a broad spectrum of inhibition of pathogens, the amount produced at growth temperature of *L.lactis* was not sufficient to destroy pathogens.

Vescovo et al (1996) proposed the idea of utilizing antimicrobial producing lactic acid bacteria as potential biopreservatives of ready to eat vegetables. They suggested that certain strains of lactic acid bacteria isolated from commercial salads demonstrated a negative impact on the growth and activity of *Aeromonas hydrophila*, *Listeria monocytogenes, S.typhimurium* and *S. aureus* in salads and in juice prepared from vegetable salads by producing antimicrobial substances. Shahani et al (1976) obtained a lactic acid free preparation called acidophilin, produced by *L.acidiphilus* spp, which had an inhibitory effect on Gram positive organisms.

Application of bacteriocins as a potential biopreservative agent in meat, dairy, and vegetables products has been investigated and documented by many researchers. Berry et al (1991) and Foegeding et al (1992) in two separate challenge tests conducted to study the effect of cells of *P.acidilacti* on *L.monocytogenes* in American style fermented sausages, construed based on the results that the antilisterial activity was due to a bacteriocin produced by this strain of lactic acid bacteria. Taking into consideration the current interest of consumers for commercially processed products that do not contain any nonfood preservatives, bacteriocin producing lactic acid bacteria or their purified bacteriocins could be employed to further upgrade hygiene and quality of food products. Yang and Ray (1994) implied that bacteriocin production by lactic acid bacteria was governed and greatly augmented by parameters including the type of strains used, composition of growth media, final pH and incubation time at optimum growth temperature.

Nisin produced by *Lactococcus lactis* ssp *lactis* is one of the most studied and characterized bacteriocin which is commercially available and permitted in various countries to be utilized as a food preservative (Hurst, 1981). The additional benefit in utilizing cells of *Pediococcus acidilactici* in the fermentation of sausage was demonstrated by its ability to produce a bacteriocin, capable of exerting inhibitory activity against a range of Gram positive bacteria including *L. monocytogenes*. Chou and Beuchat (1994) suggested the utilization of bacteriocin produced by *P. acidilactici* to control the growth of *L. monocytogenes* during the production of Kimchi, a spiced fermented cabbage.

Gourama (1996) concluded that lactic acid bacteria display a broad spectrum of inhibition not only towards bacteria but also have the potential to produce antifungal and antimycotoxigenic compounds. The inhibitory action in this study was not correlated with the production of lactic acid or H_2O_2 but was attributed to the presence of bacteriocins. The genus *Penicillium* plays an important role as a food contaminant especially at lower temperatures, lower pH and also poses a health hazard due to the production of mycotoxins (Cole andCox, 1981).

Production of Organic Acids

Acids have played a major role as one of the contributory factors involved in preservation of foods (Knochel and Gould, 1995). Some foods are acidic by nature while others attain acidity either by direct addition of acids to the food, or due to the production of acids as metabolites during the growth and activity of lactic acid bacteria. Acids like benzoic and sorbic, are intentionally added to foods as preservatives, which are very effective in inhibiting growth of microflora especially fungi (Dziezak, 1986). On the other hand acids like lactic, acetic, fumaric and propionic are specifically added in order to prevent or delay the onset of growth of pathogenic or spoilage bacteria (Dziezak, 1986; Greer and Dilts, 1995) in order to improve safety and shelf life of foods. Lactic acid bacteria can produce a variety of metabolites including lactic and acetic acids and thus have the ability to reduce the pH in the surrounding growth media. Lactic acid bacteria in general have the potential

of exhibiting resistance to acidic conditions, whereas it is inhibitory to other competing bacteria including most pathogens (DeVuyst and Vandamme, 1994).

General recognition and acceptance of organic acids as low toxic components to human beings, have led to the innovation of wide spectrum of applications in the food industry, to reduce microbial growth on meat carcasses (Smulders et al, 1986) for instance. It is well documented that different organic acids vary considerably in their inhibitory effects. The inhibitory effect of organic acids is mainly due to their undissociated nature (Lund et al, 1987 ; Adams and Hall, 1988). Emanation of the antimicrobial activity of these undissociated acids is further enhanced by their ability to permeate cellular membranes of bacteria (Adams and Hall, 1988). Once inside the cell, the acid begins to dissociate and release protons and conjugated bases. This activity is greatly influenced by the high pH in the cytoplasm. It was found that the release of these protons can lead to the disruption of proton motive force which in turn can disable energy-yielding and transport process (ICMSF, 1980). Therefore it is safe to conclude that antimicrobial effect of an organic acid depend on it's pK_a value and the pH of the external medium.

Of the organic acids, acetic acid is the strongest inhibitor, displaying a broad range of inhibitory activity towards yeasts, molds and bacteria (Blom and Mortvedt, 1991). Acetic acid therefore has the potential to extend shelf life of food products when present as a food component or as an end product of fermentation by inhibiting psychrotrophic bacteria. Rubin (1978) reported that inhibition of *Salmonella typhimurium* was observed in the presence of acetic and lactic acids, indicating an apparent synergistic activity. Adams et al (1988) conducted a study on the inhibition

of *S. enteritidis* and *E.coli* by lactic and acetic acids, individually and in combined mixtures. Results from their study confirmed that the undissociated acid is the active inhibitory agent. These scientists were also able to confirm the synergistic activity of the two acids. The lower pH environment created by the lactic acid, augmented potentiation of acetic acid as an inhibitory substance.

Hydrogen Peroxide (H_2O_2) Production

A number of lactic acid bacteria have the capacity to produce hydrogen peroxide as a metabolic by-product. It is documented that certain strains of streptococci and lactobacilli produce detectable quantities of H_2O_2 (Dahiya and Speck, 1968; Farrell, 1935). It was concluded that in some cultures hydrogen peroxide would be accumulated to levels that could mediate autoinhibitory action or inhibitory effects on other bacteria (Anders et al, 1970; Gilliland and Speck, 1974, 1975). Hydrogen peroxide has been categorized as an inhibitory agent synthesized by lactobacilli in sufficient amounts to create an adverse effect on the growth and development of some food borne pathogens as well as on Gram negative psychrotrophic bacteria (Gilliland and Speck 1969, 1974, 1975, 1977). Inhibition of pathogens at refrigeration temperatures by H_2O_2 producing bacteria (Martin and Gilliland, 1980 ; Gilliland and Ewell, 1983) can be utilized as an effective tool in food preservation.

Gilliland (1980) proposed, based on conclusive results that of all the lactobacilli, that *Lactobacillus delbrueckii* subsps. *lactis* tends to produce the highest amount of hydrogen peroxide at refrigeration temperatures without growing or changing the pH of the medium. Brashears et al (1998) reported similar supporting evidence to this effect by concluding that *Lactobacillus delbrueckii* subsps.lactis I, among other strains of *L.delbrueckii* subsps. *lactis* tested produced sufficient hydrogen peroxide to inactivate cells of *Escherichia coli* O157:H7 on refrigerated raw chicken meat. Villegas and Gilliland (1988) concluded that in addition to NADH oxidase, which oxidizes NADH forming hydrogen peroxide, *L. delbrueckii* ssp. *lactis* also contains lactate oxidase, which forms hydrogen peroxide by oxidizing lactate, also was active at refrigeration temperature. These two enzymes were construed to be responsible for accumulation of hydrogen peroxide by *L.delbrueckii* ssp. *lactis* at refrigeration temperatures.

Yap and Gilliland (1999) conducted a study to isolate new strains of *L.delbrueckii* subsp. *lactis* from raw milk that could synthesize higher concentrations of H_2O_2 at refrigeration temperature (5^0C) than *L.delbrueckii* ssp. *lactis* 1 used by Brashears et al (1998). The study produced an isolate from raw milk (RM 2-5) able to produce more H_2O_2 than *L.delbrueckii* ssp. *lactis* I. It thus should be more effective in causing inhibitory actions as a biopreservative of refrigerated foods.

Species of lactobacilli isolated from oysters produced sufficient amounts of H_2O_2 to inactivate *Pseudomonas*, *Bacillus*, and *Proteus* species (Price and Lee, 1970). Hydrogen peroxide synthesized by *Lactobacillus lactis* inhibited the growth of *Staphylococcus aureus*, at 5^0C (Dahiya and Speck, 1968). Based on similar studies

conducted to determine the inhibitory effect of H_2O_2, it was found out that growth of *L.monocytogenes* (Tharrington and Sorrells, 1992) and *Salmonella* sp. (Watson and Schubert, 1969) also were inhibited.

Lactoperoxidase System

The bactericidal property of bovine milk having the ability to inhibit the growth of certain bacteria has been studied and documented for a considerable period of time (Hansen, 1924). The factor responsible for initiating inhibition was elucidated and coined as lactoperoxidase system. The lactoperoxidase-thiocyanate system (LP system) is a naturally occurring antimicrobial defense mechanism in raw milk, which has proven to be bacteriostatic or bactericidal to a variety of Gram positive and Gram negative microorganisms (Pruitt and Reiter, 1985). It also was proven to be lethal to Gram negative organisms such as *Pseudomonas* species and *E.coli* species (Bjorck et al, 1975). The LP system is only activated in the presence of lactoperoxidase, thiocyanate and hydrogen peroxide. The enzyme lactoperoxidase catalyzes the oxidation of thiocyanate by hydrogen peroxide (Reiter, 1985) to form a variety of oxidized agents, the major inhibitory intermediary product being hypothiocyanite (Aune and Thomas, 1977).

Out of the three required components to activate the LP system, lactoperoxidase and thiocyanate occur naturally in milk in adequate amounts to catalyze the reaction (Bjorck et al, 1975). Hydrogen peroxide can be added exogenously, generated enzymatically or produced by lactobacilli (Bjorck, 1978 ;

Martin and Gilliland, 1980). Bjorck et al (1975) reported that the effect of the LP system is greatly influenced by low temperatures. The LP system has the ability to emit a much stronger antibacterial effect than H_2O_2 alone (Thomas et al, 1993). The augmented antimicrobial effect of the LP system could be credited to it's inherent ability to react more actively with components of microbial cells (Thomas et al, 1993). Some studies also indicate that microorganisms might not exhibit specific defense mechanisms against the LP system whereas most bacterial cells synthesize enzymes to detoxify H_2O_2.

The LP system has been reported to be antibacterial to *S. typhimurium* (Wolson and Summer, 1994), *L.monocytogenes* (Zapico et al, 1993), species of *Pseudomonas* and *Escherichia coli* (Bjorck et al, 1975). Even thiocyanate compounds alone have been studied for their antibacterial effect. Lin et al (2000) reported the bactericidal effect of two types of isothiocyanate compounds (allyl and methyl forms) on *Salmonella montevideo*, *L. monocyotogenes*, and *Escherichia coli* inoculated onto iceberg lettuce. Isothiocyanates are a group of compounds responsible for the pungent flavor of vegetables like cabbage and horseradish. These compounds were determined to be the key ingredients propagating antimicrobial activity in Japanese green mustard (Delaquis and Mazz, 1995).

Minimally Processed Fresh Cut Fruits

Quality Maintenance of Fresh-cut Fruits

Minimally processed fresh cut fruits are an important and rapidly developing class of food products that are fast gaining popularity with the modern society and catering to the needs of an ultra busy consumer. " Minimally processed " horticultural products are prepared and handled to maintain their fresh nature while providing convenience, nutrition and variety to the user. These food products are precut so that additional preparation is not required prior to consumption. These fresh fruit and vegetable products are especially useful for restaurants, dining commons, fast food outlets and retail markets. The projected shelf life for fresh cut produce ranges from seven to twenty days if held under appropriate temperature and conditions (Watada and Ling, 1999).

Fresh cut fruits and vegetables are highly sensitive and perishable due to their exposed internal tissues and the absence of a protective outer layer (Watada, 1997). An increased metabolic rate is normally observed in the tissues of these fresh cut products due to processing. As a result of the physical damage the tissues are subjected to stress, which in turn will affect the shelf life and quality of the product. Consumer satisfaction, which is generally related to product quality is the ultimate challenge and the desired goal in a marketing strategy of a commercial product. Quality can be defined in the most basic terms as the degree of excellence. Food quality embraces both sensory attributes that are easily perceived by the senses of the

user and hidden attributes such as safety and nutrition, which can only be determined objectively (Shewfelt, 1999). A product with reasonable shelf stability and quality is a goal only attainable by adhering to certain parameters in every aspect of production, processing, distribution and consumption.

The appearance of fresh fruits and vegetables is the primary criterion used by consumers in making purchasing decisions (Kays, 1990). Product appearance is determined by size, shape, form, color, condition and absence of defects. These characteristics play a significant role in influencing consumer appeal. Maturity is an important quality attribute of fresh cut fruit because immature fruit lacks good sensory quality and over-mature fruit has limited shelf life (Kim et al , 1993). Varieties do differ in quality characteristics and therefore varieties need to be selected for optimum quality of fresh-cut products. Microbial population of fresh cut fruits has an effect on quality and shelf stability. Proper sanitation at every step is essential in keeping the microbial load to a minimum. Native microorganisms should not be destroyed completely, due to their potential ability to control the growth of any contaminating pathogens (Bennik et al, 1996).

Chemical and physical disorders caused by processing also contribute to diminishing quality of fresh produce. The most common processing induced disorder is discoloration. Gunes and Lee (1997) indicated that applying an antibrowning agent in conjunction with modified atmosphere could effectively negotiate this hurdle. Reducing mechanical damage and utilizing sharp cutting equipment during cutting improves the quality of the end product.

Temperature is the most influential factor in maintaining quality of fresh-cut products. Fresh cut fruits should be held at a lower temperature than that recommended for intact commodities. The ideal handling, holding and transient temperature for these commodities is near 0^0C (Watada et al, 1996). Increase in the temperature results in elevating the rate of respiration, which in turn hastens the onset of senescence by catalyzing physical and biochemical reactions at a faster phase than normal. Decreasing the temperature of fresh-cut fruits has a two-fold effect on storage. Low temperatures decrease the metabolic rate by decreasing the respiration rate of the fresh produce and thus delaying the on set of senescence. Low temperatures also have the capability of either slowing down or stopping the growth of residing microflora and thus helping to prolong the shelf stability of the product. However low temperature storage utilized as means of preservation of fresh-cut fruits does not stop the growth of psychrotrophic organisms (Brackett, 1987). The weight and water loss due to augmented metabolic rate also has a consequence on the potential quality of the fresh produce (Watada et al, 1996). Atmosphere within the packaged container of fresh fruits is modified due to respiration of the cut commodities and this environment has been proven to be beneficial in maintaining quality of the fresh cut-product (Gorny, 1997).

Food Safety Concerns

While consumer demand for fresh-cut fruits has accelerated tremendously during recent years, they also are a potential source of food borne illnesses (Brackett,

1999). Therefore it is imperative not only to minimize the risks of fresh produce being contaminated with pathogenic microorganisms but also to decrease the spoilage microorganisms in order to extend the shelf stability. It is reported that each year estimated 9000 deaths occur in the United States due to food borne illnesses (CAST, 1994). There have been several well-publicized food borne outbreaks relating to cantaloupe, tomatoes, lettuce, alfalfa sprouts, and both apple and orange juices. Both Gram positive and Gram negative bacteria are responsible for contaminating or residing in fresh-cut fruits. Generally the low pH and high sugar content of fresh-cut fruits provides an ideal growth media for the fungal population.

Virtually every type of fruit and vegetable is susceptible to the invasion of microflora. Fresh-cut produce is more susceptible to microbial contamination due to the absence of a protective outer skin and by increased surface area open to the environment. Many researches have speculated that every step from production to consumption posses a significant impact on the microbiological safety of foods (Beuchat et al, 1977; Brackett et al, 1993). In the case of fresh produce preharvest factors also influence the final quality and safety of the final product.

Watkins and Sleath (1981) demonstrated that *Salmonella* and *Listeria monocytogenes* have the ability to survive for months in sewage sludge applied to agricultural soils. Floodwater contaminated with microflora especially due to fecal matter can get deposited on flooded croplands and survive for a considerably long period of time (Beuchat and Ryu, 1997). Another factor contributing to the population of microflora is use of contaminated water for irrigation. Ackers et al (1977) concluded that agricultural workers were the most likely source of contaminants in an

outbreak of cholera associated with sliced melon. Therefore it is essential to raise the awareness of the producer of the significant role they play on the safety of the fresh produce.

Human contact, immersion in water, cutting or slicing, conditioning and packaging steps during processing all provide opportunities for the product not only to become contaminated with pathogenic bacteria but also enhance their growth (Brackett, 1993,and 1994). Even though the transportation industry has been ignored as a potential participant in contamination of fresh produce, it has been noted that the conditions to which these fresh produce are subjected during distribution have a profound potential effect on the microbial safety.

Modified atmosphere packaging (MAP) can prolong the shelf stability of fresh produce. Despite this fact there have been instances where MAP could have facilitated the growth and production of toxin of *Clostridium botulinum* (Conner et al, 1989) in case of temperature abuse.

Consumers are generally unaware that the conditions to which they subject the fresh produce can have a deleterious impact on the quality and safety of that product. One effective way of overcoming this lack of knowledge of users is by consumer education and awareness of the importance of temperature abuse of fresh produce. A systemic approach that encompasses all the various stages of production, processing, distribution, and consumption of fresh fruits and vegetables is required to maintain quality and safety of these products. Therefore a quality assurance program based on SOPs, GMPs, and HACCP system is most successful in keeping the food safe when it

is implemented throughout the entire food production chain, from farm to table (Childers, 1996).

Food Borne Pathogens on Fresh Produce

Salmonella is an important enteric pathogen that sometimes has been found to contaminate fruits. Morbidity And Mortality Weekly Report (1991) stated that *Salmonella* is the most frequently reported foodborne pathogen causing gastroenteritis in the United States. Usual sources of contamination are flooded soils, fertilizers, irrigation water, cross contamination, and infected handlers. *Salmonella* is a very resilient organism that can survive harsh environments. Salmonellosis has been linked to tomatoes, cantaloupe, honeydew melons, watermelons, apple juice and orange juice (Beuchat and Burnett, 2000). In 1977, there was an outbreak of salmonellosis in California affecting twenty-four residents (Abbott et al, 1990). The causative organism was identified, as *S.saphra* and cantaloupes imported from a specific region of Mexico were the responsible vehicle for the outbreak.

Salmonella miami and *S. bareilly* were reported to be responsible for two outbreaks relating to consumption of pre-cut watermelon (Wei et al, 1997). Epidemiological studies have found several other outbreaks relating to *Slamonella*. In Illinois (MMWR,1979), *S.oranienburg* caused an outbreak of salmonellosis and the incriminated food was watermelon. Contaminated tomatoes acted as a vehicle for *S.javiana* and *S. Montevideo* in outbreaks that occurred in 1990 and 1993 respectively. In 1991 there were two multistate outbreaks of salmonellosis

documented. *Salmonella chester* was found to affect 245 persons in 30 states, while the other causative agent was *S.poona*, which was responsible for 185 cases in 23 states and 56 cases in two Canadian provinces. Contaminated pre-cut cantaloupes served either in salad bars or fruit salads were the incriminated food in both of these outbreaks (CDCP, 1991).

Listeria monocytogenes is widespread in nature including agricultural habitat especially in soil and decaying vegetation (Brackett, 1988). Both domestic animals and humans can act as potential carriers for this organism (Lovett, 1988). The above characteristics facilitate the susceptibility of fresh produce to contamination by *L.monocytogenes*. This organism is a facultative anaerobic, low acid tolerant, and capable of survival and growth under low oxygen concentrations created within a modified atmosphere packaging (Francis and Beirne, 1997). Due to this capability and the added advantage of being psychrotophic, this organism is of concern in minimally processed food products that depend on refrigeration as a means of preservation. Data from literature indicate that *L.monocytogenes* has been isolated from minimally processed fruits that apparently were subjected to listericidal treatments thus emphasizing post process recontamination. Mejia (1997), based on a study conducted in Venezuela, reported the occurrence of *L.monocytogenes* in minimally processed fruit salad containing papaya, apple, watermelon, grape, guava, and pineapple.

Escherichia coli O157:H7 is another significant pathogen linked to gastroenteritis. Since its first recognition as a food pathogen (Riley et al, 1983) in 1982, this organism has been found to be the causative agent of bloody diarrhea, and

renal failure in humans (Doyle, 1991). Most of the outbreaks linked to this pathogen were due to foods of animal origin. However *E.coli O157:H7* has been isolated from fresh produce as well (Gonzalez et al, 1997; Tauxe et al, 1997). Therefore this organism can be a pathogen of concern in minimally processed fruit products. Borezyk et al (1987) reported that dairy cattle, especially young calves are the primary reservoir of *E.coli O157:H7.* Thus this pathogen can gain access to fresh commodities via fecal contaminated soil, fertilizers, and irrigation water during the production phase (Roberts, 1984). During various stages in processing, distribution and consumption of fresh produce, and especially during the assembly of ready-to-eat- meals containing cut fruits in food establishments this pathogen also may be transferred to other foods via cross contamination, and or infected food handlers.

Zhao et al (1993) demonstrated that unpasteurized apple juice held at $8^{O}C$ was able to support the growth of *E. coli* O157:H7 up to 31 days. Similar evidence was exhibited during outbreaks in the Western United States and Connecticut due to consumption of contaminated apple juice (CDC, 1997). Beuchat et al (1997) studied the survival and growth of *E.coli O157:H7* on cantaloupe and watermelon and concluded that even though this pathogen was able to survive low temperatures ($5^{o}C$) but could not grow at these temperatures. These findings are supported by outbreaks of illnesses caused by *E.coli O157:H7* linked to melons (Beuchat, 1996).

Aeromonas, a psychrotrophic and a facultative anaerobe is another pathogen of concern in fresh produce. Literature indicates that fruit salads are a potential source of *Aeromonas hydrophila.* A challenge study conducted by using *A.hydrophila* provided conclusive results, indicating that this pathogen can grow and survive under

refrigeration on fruit salad, even with a very low initial cell count at the point of storage (Revette, 1998). It was reported by Steinberg et al (2001) based on a study to evaluate recent trends in cholera caused by *vibrio chloreae* in the United States that two cases were acquired by sliced cantaloupe contaminated by an asymptomatic infected food handler.

Yeasts and molds are the main spoilage flora of minimally processed fruits due to their ability to withstand and even prefer low pH, high sugar and salt contents (Fleet et al, 1997). The specific type or fungus that will cause spoilage depends on the type of fruit. The prevalence of such spoilage flora on fresh-cut fruits diminishes keeping quality and consumer acceptance and renders these products undesirable for consumption due to growth and activity of the fungi. The discovery of mycotoxin production in foods by molds has highlighted their importance in food safety and quality. Mold growth can result in food spoilage, off-flavors, rotting, discoloration, and toxin production. Mycotoxins are secondary metabolites produced by certain molds, which can have pathogenic or allergenic properties (Tipples, 1995). Once mycotoxins are produced and present in the food, they can withstand processing and storage (Scott, 1991).

Spoilage Organisms on Fresh Produce

The majority of the bacteria responsible for spoilage of fresh produce during refrigeration storage are Gram negative psychrotrophic organisms. The ability of species of *Pseudomonas* to grow well at refrigeration temperatures makes them

dominant spoilage bacterial flora at low temperatures (Brackett, 1987). Literature indicates the growth of these organisms adversely affects fruit flavor, texture, nutrients, and overall quality. Since many *Pseudomonas* sp. are strictly aerobic, reducing the level of oxygen present within the packaged environment of fresh produce can help control their growth.

Utilization Of Chemicals as Sanitizers or Preservatives

The proper implementation of sanitizers to disinfect containers, equipment, and the surface of whole and cut produce is an effective way to compliment a strong sanitation program but at the same time these sanitizers do not have the capacity to completely eliminate pathogenic or spoilage microorganisms from contaminated fresh produce (Brackett, 1992). The food processing industry has utilized and preferred chlorine as a disinfectant for many years. There are three major groups of chlorine compounds (liquid chlorine, hypochlorites, and chlorine dioxide) all these compounds exhibit varying degrees of antibacterial activity (Barmore,1995). Of these, sodium hypochlorite is most extensively used in the fresh cut produce industry (Annon, 1996). The extent of bactericidal activity depends on the amount of free chlorine available in the water that comes to contact with microbial cells. Beuchat and Brackett (1990) observed that a chlorine wash has the capacity to reduce the microbial load present on fresh produce but not to destroy human pathogens like *Listeria monocytogenes*.

There are several documented challenge tests conducted to determine the efficacy of chlorine treatment. Nguyen and Carlin(1994), reported that the lack of effectiveness of chlorine wash of whole uncut produce could be due to the inaccessibility of the sanitizer to microbial cells residing in creases, crevices, pockets, and natural openings in the skin of the fresh commodities. The hydrophobic nature of waxy cuticles present in many fruits prevents and protects surface contaminants from been exposed to sanitizers. Cubed cantaloupe inoculated with *Salmonella serovars* were subjected to chlorine treatment (2000 ppm), which resulted in less than 1 $\log_{10}$ reduction of viable cells (Ryu et al, 1997). Researchers concluded that the organic matter in the juice of cantaloupe tissue that is released when cubed could be the responsible factor in buffering the toxic effect of chlorine. One potential way to minimize the microbial load on the surface of fruits and vegetables is by utilizing chlorine dioxide (Bernarde et al, 1965). It is a strong oxidizing agent 2.5 times stronger than chlorine. However information regarding use of this gas as a potential sanitizer for minimally processed refrigerated fruits and vegetables is lacking (Linton et al, 2000).

The lack of effectiveness of chlorine as a sanitizer for pathogen reduction and control and safety issues concerning chlorine by-products have led to the exploration of alternative disinfection methods. Gerald et al (1998) investigated the uses of hydrogen peroxide as a vapor and wash treatment for fresh-cut fruits and vegetables. They concluded that vapor treatment was effective in controlling postharvest decay in grapes and reducing the microbial load on whole cantaloupes, prunes, raisins, and walnuts. However, it was also found to induce browning in mushrooms and pigment

bleaching in strawberries. On the other hand hydrogen peroxide dip treatment (5% for 2 min) was able to extend the shelf life of fresh cut cantaloupe, honeydew melons, cucumber, zucchini, and green bell pepper. These studies indicate the potential use of hydrogen peroxide as an effective antimicrobial agent in conditioning fresh-cut fruits and vegetables during processing, but further research is required for successful practical application.

Modified Atmosphere Packaging

Prolonged shelf life of minimally processed fruits and vegetables can be attained by refrigerated storage in conjunction with modified atmosphere packaging (MAP). Increased temperatures lead to an increase in the respiration rate and catalyze several metabolic reactions that have a detrimental ripple effect on the quality of the fresh produce. The increased rate of metabolic reactions of the fresh produce can lead to an early onset of senescence and also render the product more susceptible to microbial break down. These changes affect the keeping quality of the produce by altering its organoleptic attributes (Sode et al, 1998). The above changes also will increase the susceptibility of the produce to a microbial spoilage. (Day, 1989).

Control of temperature is the essential key in regulating respiration to extend the shelf life of fruits and vegetables. The second most effective method to modulate respiration is MAP (Brecht, 1988). There are two modes of MAP (Zagory and Kader, 1988), passive and active. Passive MAP consists of placing the produce in a gas permeable package and sealing it, and allowing the atmosphere inside the package to

be modified by respiration of the produce. This will result in an atmospheric composition of increased CO_2 and decreased O_2 concentrations within the package different to that in the air (Stiles, 1991). Whereas active MAP involves placing the produce in a gas permeable package, evacuating the package atmosphere and replacing it by a specific gas mixture of O_2, and CO_2 with a balance of N_2 and sealing the package immediately. The desired gas mixture suitable for the modified atmosphere is based on what is recommended for the whole commodity (Salveit, 1997).

Modified atmosphere packaging has the ability to control some insects, retard growth of plant pathogens, and reduce sensitivity to ethylene as well (Hotchkiss et al, 1992). Researchers have indicated that the modified atmospheric composition (high CO_2 and low O_2) within the package creates an inhibitory effect towards the growth of a variety of microorganisms, like fungi (Reeleder et al, 1981; Daniels et al, 1985; Farber, 1991). Carbon dioxide appeared to be more inhibitory toward Gram negative organisms than Gram positive organisms.

Modified atmosphere packaging seemingly reduced the rate of deterioration of the produce but nonetheless provided ample time for human pathogens to grow and render the product unsafe to consume while still maintaining edibility (Brackett, 1990). Brackett (1992,1994), indicated that the MAP decreased the rate of deterioration but had no affect whatsoever on the growth of *Listeria monocytogenes*. or *Escherichia coli O157:H7*. The modified atmospheric composition within the package also may provide a suitable environment for *Clostridium botulinum* to grow and produce toxin, especially during temperature abuse if pH is suitable (Conner et al,

1989). Ideally MAP can be implemented as an effective tool in extending shelf life by delaying the onset of senescence by means of respiration modulation. Therefore it appears the extension of shelf life in MAP could be attributed to the physiological state of the minimally processed produce rather than inhibiting the growth of microorganisms.

Use Of Biopreservatives

The heightened preference of the modern consumer for commercially processed foods without chemical preservatives has given the incentive to search for alternative methods. Biopreservation is an example of an alternate method. Due to their potential nutritional benefits and extensive exploitation in the production of fermented and cultured food products, lactic acid bacteria (LAB) are the most important and appropriate group of organisms that are considered as biopreservatives (Daeschel, 1990; Luke, 1993). Lactic acid bacteria also are considered GRAS (Generally Recognized As Safe), to recognize the fact that there are no known negative health effects due to consumption of food products containing them (Ray et al, 1993).

It is a documented fact that LAB can occur as natural contaminants on minimally processed produce. These bacteria gain access via raw materials or are introduced during processing (Nguyen and Carlin, 1994). Some authors have in conjunction with refrigeration to control undesirable microorganisms, proposed the potential of applying selected LAB as biocontrol agents on minimally processed fruits

and vegetables (Gombas, 1989 ; Stiles, 1996). Selected LAB may produce bactericidal components like organic acids, bacteriocins, hydrogen peroxide, antibiotic like substances that may provide a broad spectrum of inhibition toward foodborne pathogens and spoilage flora on minimally processed produce. However, Gilliland and Speck(1975), observed an antagonistic reaction towards psychrotrophic bacteria in nonfermented food during refrigeration by nongrowing cells of selected lactobacilli. These authors were able to shed light on a new concept of using nongrowing cells as biopreservatives that resulted in no alteration of the intrinsic qualities of the food.

A recent study conducted by Gilliland and Yap (2000), led to the discovery of a new strain of *Lactobacillus delbrueckii* subsp.*lactis* (RM2-5), isolated from raw milk that has the ability to produce more hydrogen peroxide than strains previously tested. The ability of the above strain to produce sufficient hydrogen peroxide at refrigeration temperature without any growth suggests the potential application of the strain to extend shelf life of refrigerated food products. Since there is no growth by the strain, the organoleptic or aesthetic attributes of the food product would probably be altered very little or none at all.

The main criterion in implementing biocontrols in minimally processed produce is not fermentation but to accomplish dominance and control over the microenvironment of the produce by inhibition of pathogens and spoilage flora (Breidt and Fleming, 1997). Another factor that justifies the consideration of LAB as biopreservatives is their role as indicator organisms of temperature abuse or prolonged storage of fresh produce. During these conditions LAB will grow actively

and not only alter the food product but also inhibit the growth of pathogens (Brashears et al, 1999). Their active growth will render the product unfit to eat and serve as an effective tool in warning the consumer of possible contamination of the product.

Microbial control of pathogens like *L.monocyotgenes*, *Salmonella typhimurium*, *Stapylococcus aureus* , and *E.coli* O157:H7, on meat, cheese and fresh produce by selected strains of LAB has been proposed (Janisiewicz et al, 1999). Garver and Muriana (1993), isolated a bacteriocin producing strain of *Pediococcus acidilactici* from fresh fruits and suggested the practicality of using this strain as a biocontrol agent. Their data portrayed the possibility of production of bacteriocin in foods by specific strains of LAB used as biocontrols without the excessive growth of the producing organism.

Even though there is data supporting the use of purified bacteriocins as biopreservatives, there are some potential drawbacks to such applications (Bergh, 1993). Use of LAB cells that produce hydrogen peroxide could be more effective in controlling the growth of pathogens and spoilage flora than purified bacteriocins. Bacteriocins usually have the ability to inhibit only a narrow range of microorganisms, which is a major limiting factor to their use (Daeschel, 1992). Peroxide produced by cells of LAB on the other hand will continuously be replenished and its action has a broader spectrum of inhibition than bacteriocins. The implementation of biopreservatives in minimally processed food products has the potential to enhance the concept of hurdle technology (Leistner and Gorris, 1995), utilized in the processing industry to control pathogens.

OBJECTIVE OF STUDY

The production of bactericidal components by lactic acid bacteria was the fundamental justification in considering these organisms as biocontrol agents in minimally processed produce. However it is essential that the selected strain of lactic acid bacteria exhibiting inhibitory effect towards the target pathogen can survive refrigeration temperatures, be metabolically active during storage, and not alter the sensory attributes of the produce unless temperature abuse and prolong storage occurs. It is a well-documented fact that cells of *Lactobacillus delbrueckii* ssp. *lactis* can produce sufficient quantities of hydrogen peroxide to exert antagonistic effect against undesirable organisms without growing at refrigeration temperatures. *Pediococuus acidilactici* is another species of lactic acid bacteria believed to create an antagonistic action toward pathogens. The main contributing component responsible for the inhibition is thought to be a bacteriocin produced even during refrigerated storage.

The objective of the current study was to determine whether the two selected lactic acid bacteria, *Lactobacillus delbrueckii* ssp. *Lactis* RM2-5 and *Pediococcus acidilactici* D3 could create an antagonistic action toward pathogens and spoilage flora on fresh cut fruits during refrigerated storage to enhance the safety and shelf stability of the products.

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CHAPTER III

EVALUATION OF CELLS OF *LACTOBACILLUS DELBRUECKII* SPP. *LACTIS* RM2-5 AND *PEDIOCOCCUS ACIDILACTICI* D3 AS BIOPRESERVATIVES ON FRESH CUT CANTALOUPE AT 7^0C

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ABSTRACT

The objective of the current study was to evaluate the potential antibacterial effect of selected cultures of lactic acid bacteria toward *Salmonella choleraesuis* and spoilage organisms on fresh cut cantaloupe during storage at 7^0C. Cells of *S.choleraesuis* were directly applied onto the surface of the fresh cut cubes of cantaloupe placed in sterile petri plates. Some cubes were additionally inoculated with cells of *Lactobacillus delbrueckii* ssp. *lactis* RM 2-5 or *Pediococcus acidilactici* D3. The cubes were placed at 7^0C for a 10day storage period. Microbiological analyses of the samples on day 0, 5, and 10 revealed that the population of the lactobacilli and pediococci remained fairly constant at approximately 7 $\log_{10}$ CFU/g over the ten-day storage period. A small although statistically significant ($P < 0.05$) inactivation of *S.choleraesuis* was observed when cantaloupe cubes were additionally inoculated with either cells of *L.delbrueckii* spp. *lactis* suspended in 5.5mM sodium lactate or *P.acidilactici* suspended in 5.5mM lactic acid or 5.5mM glacial acetic acid solutions. No significant decline in numbers of *S.choleraesuis* was attained when the cells of *L.delbrueckii* spp.*lactis* were suspended in 5.5mM lactic acid or when *P.acidilactici* was suspended in sterile water. However, none of the treatments contributed to an effective ($P > 0.05$) inhibition of the spoilage flora on the melons. Further studies showed, that the amount of H_2O_2 produced by the lactobacilli was reduced in the presence of the melon.

INTRODUCTION

Fresh cut fruits are considered as perishable food products due to their lack of protective covering and exposed internal tissues. The increased growth area and availability of nutrients in the tissue fluid of the cut fruits provide an excellent environment for microbiological growth and activity. Breidt and Flemming (1997), proposed the concept of utilizing lactic acid bacteria as biocontrol agents to control food borne pathogens and spoilage flora in minimally processed refrigerated produce.

Certain strains of lactobacilli have been shown to exert antagonistic actions against undesirable microorganisms. *Lactobacillus* species isolated from oysters were found to be bactericidal to *Pseudomonas, Bacillus,* and *Proteus* species (Price and Lee, 1970). The responsible component was identified as hydrogen peroxide produced by the lactobacilli. Tharrington and Sorrells (1992) reported that inhibition of *Listeria monocytogenes* by lactobacilli was due to hydrogen peroxide. The ability of *Lactobacillus delbrueckii* ssp. *lactis* to produce sufficient quantities of hydrogen peroxide at refrigeration temperatures without active growth highlights the importance of utilizing these organisms as preservatives of refrigerated products (Gilliland and Speck, 1975; Villegas and Gilliland, 1998).

Lactobacillus delbrueckii ssp. *lactis* has been shown to produce more hydrogen peroxide than other species of lactobacilli (Premi and Bottazzi, 1972). This species can produce sufficient amounts of hydrogen peroxide to negate the growth and activity of *L.monocytogenes*, *Salmonell*a spp (Brashears and Durre, 1999), *Escherichia coli* O157:H7 (Brashears et al, 1999), and psychrotrophic spoilage

bacteria (Gilliland and Speck, 1975; Gilliland and Ewell, 1983) In 1999 Yap and Gilliland isolated a strain of *L.delbrueckii* ssp. *lactis* (RM 2-5) from raw milk which had the ability to produce more hydrogen peroxide than the other strains of this species tested and therefore could be more effective as a biopreservative.

Bacteriocins are proteinaceous in nature and are antibacterial compounds that may be produced by lactic acid bacteria (Daeschel, 1989). These bactereocins or the producing organisms can be used as bioperservatives to enhance the safety and shelf stability of many food products (Hurst, 1981). Many bacteriocins have been found not only active against other lactic acid bacteria but also against pathogens like *L.monocytogenes, Clostridium perfringens, Bacillus cereus,* and *Stapylococcus aureus* (Bhunia et al, 1988). Kim et al (1997) found that a bacteriocin produced by *Pediococcus acidilactici* had the ability to prevent the growth of *S.aureus, Salmonella* spp, *E.coli* O157:H7, *L.monocytogenes* in sausage fermentation. Pucci et al (1988) reported similar findings of antilisterial activity of bacteriocins in food systems. Gourama (1996) elucidated antifungal and antimycotoxigenic activities of bacteriocins by lactobacilli isolated from fresh produce. A bacteriocin produced by *P.acidilactici* was responsible in inhibiting the growth of *L.monocytogenes* in frankfurters and cooked ham at 5^0C (Brashears et al, 2001).

MATERIALS AND METHODS

Sources and Maintenance of Cultures

Lactobacillus delbrueckii ssp. *lactis* RM 2-5 utilized in the study was obtained from the stock culture collection of the Food Microbiology Laboratory in the Oklahoma Food & Agricultural Products Research and Technology Center at Oklahoma State University. *Pediococcus acidilactici* D3 was generously provided by Dr. Mindy Brashears from the Department of Animal Science & Food Technology at Texas Tech University. Both cultures were maintained by subculturing (1% inocula) in MRS broth (Difco Laboratories, Sparks, Maryland) and incubating for 18 hours at 37^0C. They were held at 5^0C between subculturing. Subculturing was done three times consecutively prior to each experimental use.

Escherichia coli O157:H7 (ATCC 43894) and *S.choleraesis* (ATCC 13706) were obtained from the American Type Culture Collection (Manassas, Virginia). These cultures were maintained by subculturing in Tryptic Soy Broth (Difco Laboratories) using 1% inocula and 18 hours of incubation at 37^0C. They were held at refrigeration temperature (4^0C) between subcultures. The cultures were subcultured three times consecutively prior to experimental use.

Microbial Analyses

Procedures from the Compendium of Methods for the Microbiological Examination of Foods (Swanson et al, 1992) were followed for sample dilution. Using sterile diluent composed of 0.1% peptone in distilled water, initial dilutions

were prepared by aseptically placing and weighing the sample in a stomacher bag and adding an equal amount of sterile diluent. Following pummeling for 60 seconds, further dilutions were prepared. The appropriate dilutions were plated by either spread plate or pour plate technique, depending upon the type of media used and microorganism to be enumerated. Cells of lactobacilli and pediococci were enumerated by pour plate technique on Lactobacillus Selection Agar (LBS; Difco Laboratories) with an overlay. (The media was prepared using individual ingredients and according to the manufacturer's formulation). The LBS plates were placed in a plastic bag, flushed with carbon dioxide for sixty seconds, sealed and incubated at 37^0C for 48 hours.

Enumeration of *S.choleraesuis* was accomplished by spread plating on Xylose Lysine Desoxycholate Agar (XLD; Difco Laboratories) and incubating at 37^0C for 24 hours. The background flora on the fruits was enumerated by pour plate using Plate Count Agar (PCA; Difco Laboratories) with an overlay and aerobic incubation for 7 days at 15^0C. Prior to plating, 0.5 % 2,3,5 triphenyl tetrazolium chloride (TTC) solution was added to the tempered molten PCA (1ml/100ml) prior to plating to enhance visibility of colonies. For experiments involving *P.acidilactici*, Crystal Violet Tetrazolium chloride Agar (CVT) which is selective for Gram negative organisms was used (Swanson et al 1992), since the pediococci formed colonies on PCA at 15^0C. The CVT agar was PCA to which 1ml of 0.1% of ethanolic solution of Crystal Violet and 1ml of 0.5% aqueous TTC was added. Plates were incubated aerobically at 15^0C for 7 days.

Preparation of Bacterial Cell Suspensions for Inoculation of Melons

Ten ml of TSB was inoculated with 0.1ml of a freshly prepared TSB culture of *S.choleraesuis* (ATCC 13706) and incubated at 37^0C for 18 hours. The cells were harvested by centrifugation in sterile centrifuge bottles, (7000 x g, 20min, at 2^0C) the supernatant was discarded and the cells were resuspended in 20 ml cold sterilized deionized water and held in ice water until use. The cell suspension was used within 15 minutes of preparation.

Cell suspensions of *L.delbrueckii* spp. *lactis* RM2-5 or *P.acidilactici* D3 were prepared by inoculating 500ml of MRS broth with 5ml of a freshly prepared MRS broth culture of *L.delbrueckii* ssp. *lactis* RM2-5 or *P.acidilactici* D3 and incubating at 37^0C for 18 hours. The cells were harvested by centrifugation in sterile centrifuge bottles (7000 x g, 20min, at 2^0C) and resuspended in 20ml of either sterile deionized water, 5.55mM sodium lactate, 5.55mM lactic acid, or 5.5mM acetic acid depending upon the protocol of the experiment and held in ice water until use (not more than 15 minutes).

Preparation of Cantaloupe Cubes

The exterior of the cantaloupe was washed with water and sprayed with a 75% ethanol solution. Then the cantaloupe was peeled and cut into cubes (approximately 2.54 x 2.54 x 2.54cm) under aseptic conditions using sterile equipment. The melon cubes were then aseptically placed into sterile petri plates (150

x 25 mm). Four cubes were placed in each of 12 plates to correspond to 4 treatment conditions with 3 storage days (0,5, and10) at 7^0C storage. The 12 plates were labeled as A, B, C, and D to correspond with the treatments.

Treatments

For the three plates labeled A (control), 0.1ml of sterilized deionized water was aseptically placed onto the center of top of each cube of cantaloupe. For treatment B (*S.choleraesuis* alone), 0.1ml of aqueous suspension of cells of *S.choleraesuis* was inoculated onto the surface center of each cantaloupe cube for all the three plates. For treatment C (lactobacilli), 0.1ml cell suspension of *L.delbrueckii* ssp. *lactis* RM 2-5 was inoculated onto the surface center of each cantaloupe cube for the three plates. For treatment D (*S.choleraesuis* plus either lactobacilli), 0.1ml of cell suspension of *S.choleraesuis* was inoculated onto the surface center of each four cubes in all three plates followed by 0.1ml of cell suspension of *L.delbrueckii* ssp. *lactis*, plates were then placed at 7^0C and one plate from each treatment was retrieved on day, 0, 5, and 10 for microbiological analyses. Same treatment protocol was repeated utilizing cells of *P.acidilactici* D3.

Hydrogen Peroxide Production

Lactobacillus delbrueckii ssp. *lactis* RM 2-5 was grown twice in 10ml of MRS broth (1% inoculum) for 18 hours at 37^0C. After incubation the cells were harvested by centrifugation at 12,000x g at 4^0C for 10 minutes. The cells were then washed twice with 9ml of cold sodium phosphate buffer (1M, pH 6.5) and

resuspended in 9ml of cold 1M sodium phosphate buffer, containing 5 mM of sodium lactate. Five tenths of cell suspension was inoculated into each of twelve tubes containing 9.5ml of 5mM sodium lactate buffer. Fresh cut slices of watermelon weighing approximately 0.1g were added into four tubes and fresh cut slices of cantaloupe (approximately 0.1g) were added into four more tubes of the twelve tubes containing the cell suspension in sodium lactate buffer. The remaining four tubes contained only the cells of lactobacilli in sodium lactate buffer. The twelve tubes were incubated at 7^0C. After 1 hour and 24 hours of incubation the cells were removed by centrifugation at 12,000 x g at 4^0C for 10 minutes and hydrogen peroxide assay was performed on the supernatant. The assay was conducted using the peroxidase, O-dianisidine method described by Gilliland (1969). The protocol was repeated employing cells of pediococci as well.

Statistical Analyses

Statistical analyses for all experiments were performed as a split plot in a randomized block design. Treatments were main units and different storage time were subunits in 4 x 3 factorial arrangement of treatments. All the experiments were conducted at least three times. Least significant difference analyses were employed to compare means for significant differences at the 5% level of confidence. All data were analyzed with the SAS program PROC MIXED and LSMEANS (SAS, 1985)

RESULTS

Enumeration and Analyses of Data for *Salmonella choleraesuis* and Spoilage Flora on Fresh Cut Cantaloupe

For all experiments, conducted on fresh cut cantaloupe the counts detected on XLD agar for treatments B and D were approximately 5 log cycles higher than for A and C. Treatments B and D were inoculated with *Salmonella choleraesuis* whereas treatments A and C were not. Based on this observation it was assumed that the XLD counts provided counts for the *Salmonella choleraesuis* inoculated onto the samples. Statistical analyses were done for the counts on XLD agar for samples B and D to determine if the numbers of *Salmonella* differed significantly during storage at 5% level of confidence.

Enumeration of the background flora was achieved by plating either on Plate Count Agar (PCA) or Crystal Violet Agar (CVT) incubated at 15^0C for 7-10 days. This was to determine if significant influence was exerted on growth during storage on the background flora by the selected strain of lactobacilli or pediococci. The CVT agar was used, for those experiments involving *P.acidilactici*, since it formed colonies on PCA at 15^0C. Since *L.delbrueckii* ssp. *lactis* does not grow at 15^0C or lower PCA was suitable for monitoring background flora in experiments in which it was used. To determine if significant influence was exerted on flora, sample A treated only with water was considered as the control for sample C inoculated with cells of *L.delbrueckii* ssp. *lactis* or *P.acidilactici*. Therefore, for possible effect on background flora statistical comparisons were done between A and C treatments.

Effect of *Lactobacillus delbrueckii* ssp. *lactis* RM 2-5 Suspended in Sodium Lactate

The overall interaction between time and the treatments for this experiment was small enough to declare that the interaction was not significant ($P > 0.05$) but when comparisons were done within treatments significant differences were observed. With treatment D there was a significant decline ($P < 0.05$) in the numbers of *S.choleraesuis* between day 0 and day 10 (Table1). The starting population per gram of *S.choleraesuis* on sample D was approximately 6.1 $\log_{10}$ and the final population on the day 10 was in the region of 4.3 $\log_{10}$. There was a numerical, but non significant ($P > 0.05$), decline for the control (sample B). Even though the decline for sample D was significant, there were no significant differences ($P > 0.05$) detected on any day (day 0, day 5 or day 10) during the 10 day storage between samples B and D. The decrease in numbers of *Salmonella* on sample D is accredited to the antagonistic action of *L.delbrueckii* ssp. *lactis* RM 2-5 towards the pathogen. When evaluating the background microoraganisms monitored on PCA (Table 1) it was evident that the populations increased significantly ($P < 0.05$) for both sample A and sample C. There were no significant differences ($P > 0.05$) between samples A and C on any day during the storage period.

TABLE 1

EFFECT OF *LACTOBACILLUS DELBRUECKII* SSP. *LACTIS* RM 2-5 SUSPENDED IN SODIUM LACTATE ON *SALMONELLA CHOLERAESUIS* (ATCC 13706) AND SPOILAGE FLORA ON FRESH CUT CANTALOUPE AT 7^0 C.

Media	Treatment	Count/g [1]		
		Day 0	Day 5	Day 10
XLD [2]	B (*S.choleraesuis*)	5.60 ^{Aa}	5.50 ^{Aa}	4.85 ^{Aa}
	D (*S.choleraesuis* and lactobacilli)	6.08 ^{Aa}	5.20 ^{Aab}	4.28 ^{Ab}
PCA [3]	A (Control)	4.96 ^{Ac}	6.75 ^{Ab}	8.68 ^{Aa}
	C (Lactobacilli)	4.99 ^{Ac}	7.40 ^{Ab}	8.68 ^{Aa}

XLD counts for treatment A and C were not considered during statistical analyses.

[1] Microbial counts are expressed as log $_{10}$ CFU/g ; each value is the mean from three replicate trials.

[2] *Salmonella* counts detected on XLD are expressed as log $_{10}$ CFU/g ; each value is the mean from three replicate trials. SE = 0.3917

[3] Background flora counts detected on PCA incubated at 15^0C for 7 days are expressed as log $_{10}$ CFU/g ; each value is the mean from three replicate trials. SE = 0.3130

^{ABC} Means appearing in the same column followed by the same letter for each media used are not significantly different ($P > 0.05$).

^{abc} Means appearing in the same row followed by the same letter for each media used are not significantly different ($P > 0.05$).

Effect of *Lactobacillus delbrueckii* ssp. *lactis* RM 2-5 Suspended in Lactic Acid

There was no significant interaction observed between the time and treatments for fresh cut cantaloupe for this experiment. The XLD counts, which are an indicative of *S.choleraesuis*, exhibited declines although they did not decline significantly ($P > 0.05$) for any sample (Table 2). These observations imply that the cells of *L.delbrueckii* ssp. *lactis* apparently had no influence on the pathogen. Analyses of PCA for samples A and C counts (Table 2) showed the cells of lactobacilli to not suppress the growth of background microorganisms occurring on the cantaloupe.

TABLE 2

EFFECT OF *LACTOBACILLUS DELBRUECKII* SSP. *LACTIS* RM 2-5 SUSPENDED IN LACTIC ACID ON *SALMONELLA CHOLERAESUIS* (ATCC 13706) AND SPOILAGE FLORA ON FRESH CUT CANTALOUPE AT 7^0 C.

Media	Treatment	Count/g [1]		
		Day 0	Day 5	Day 10
XLD [2]	B (*S.choleraesuis*)	6.70 ^{Aa}	6.30 ^{Aab}	5.20 ^{Ab}
	D (*S.choleraesuis* and lactobacilli)	6.63 ^{Aa}	6.21 ^{Aa}	5.90 ^{Aa}
PCA [3]	A (Control)	4.77 ^{Aab}	3.21 ^{Ab}	6.58 ^{Aa}
	C (Lactobacilli)	4.08 ^{Aa}	5.13 ^{Aa}	5.55 ^{Aa}

XLD counts for treatment A and C were not considered during statistical analyses.

[1] Microbial counts are expressed as log $_{10}$ CFU/g ; each value is the mean from three replicate trials.

[2] *Salmonella* counts detected on XLD are expressed as log $_{10}$ CFU/g ; each value is the mean from three replicate trials. SE = 0.5297

[3] Background flora counts detected on PCA incubated at 15^0C for 7 days are expressed as log $_{10}$ CFU/g ; each value is the mean from three replicate trials. SE = 0.9649

^{AB} Means appearing in the same column followed by the same letter for each media used are not significantly different ($P > 0.05$).

^{ab} Means appearing in the same row followed by the same letter for each media used are not significantly different ($P > 0.05$).

Effect of *Pediococcus acidilactici* D3 Suspended in Sterile Water

An aqueous suspension of cells of *P.acidilactici* had no significant (P> 0.05) effect on *S.choleraesuis* (Table3). There were no significant differences (P > 0.05) observed on any of the days between the sample inoculated with *S.choleraesuis* (B) and the sample inoculated with *S.cholerasuis* and pediococci (D) .The sample inoculated with just *S.cholerasuis* declined over the ten day storage period, however this decline was not statistically significant (P > 0.05). Parallel observations were detected on sample D as well, which was inoculated with both *S.choleraesuis* and *P.acidilactici*.

The overall interaction between the time and treatments for background microbial counts (CVT at 15^0C) on fresh cut cantaloupe utilizing *P.acidilactici* suspended in water was found to be significant (P < 0.05). Significant growth (P <0.05) was observed during the ten day storage for both the samples, A and C (Table 3). However, no significant differences were observed between these two samples on any day indicating that *P.acidilactici* had no inhibitory effect on the naturally occurring background flora residing on the surface of the fresh cut melon.

TABLE 3

EFFECT OF *PEDIOCOCCUS ACIDILACTICI* D3 SUSPENDED IN WATER ON *SALMONELLA CHOLERAESUIS* (ATCC 13706) AND SPOILAGE FLORA ON FRESH CUT CANTALOUPE AT 7^0 C.

Media	Treatment	Count/g [1]		
		Day 0	Day 5	Day 10
XLD [2]	B (*S.choleraesuis*)	5.22 ^{Aa}	4.60 ^{Aa}	4.71 ^{Aa}
	D (*S.choleraesuis* and pediococci)	5.07 ^{Aa}	4.84 ^{Aa}	4.38 ^{Aa}
CVT [3]	A (Control)	1.28 ^{Ac}	6.16 ^{Ab}	8.60 ^{Aa}
	C (Pediococci)	1.57 ^{Ac}	5.53 ^{Ab}	8.44 ^{Aa}

XLD counts for treatment A and C were not considered during statistical analyses.

[1] Microbial counts are expressed as log $_{10}$ CFU/g ; each value is the mean from three replicate trials.

[2] *Salmonella* counts detected on XLD are expressed as log $_{10}$ CFU/g ; each value is the mean from three replicate trials. SE = 0.8488

[3] Gram negative counts detected on CVT incubated at 15^0C for 7 days are expressed as log $_{10}$ CFU/g ; each value is the mean from three replicate trials. SE = 0.8424

^{ABC} Means appearing in the same column followed by the same letter for each media used are not significantly different ($P > 0.05$).

^{abc} Means appearing in the same row followed by the same letter for each media used are not significantly different ($P > 0.05$).

Effect of *Pediococcus acidilactici* D3 Suspended in Lactic Acid.

Statistical analysis of the XLD data (Table 4) for the two relevant samples (B and D) revealed no interaction between treatments and time ($P > 0.05$). Conversely significant differences were detected between the samples during the 10-day period. Sample B (*S.choleraesuis* alone) did not display significant decline ($P > 0.05$) from day 0 to day 10.Whereas treatment D inoculated with both *P.acidilactici* and *S.choleraesuis* exhibited a significant decline ($P < 0.05$) between the initial and the final population from day 0 to day 10. This decline could be accredited to the ability of the pediococci cells to disperse inhibitory action towards the pathogen on the surface of the fresh cut melon cubes held at 7^0C for ten days. However no significant differences were observed when comparisons were made on any day (day 0,5,and 10) between sample B and D.

No significant interaction ($P>0.05$) between time and treatments for Gram negative spoilage counts detected on CVT incubated at 15^0C for 7 days were found. Individual comparisons were still made to detect any significant differences between sample A and sample C (Table 4). The population increased significantly ($P<0.05$) for both samples held at 7^0 C for ten days. There was a numerical but non significant ($P>0.05$) difference in the counts on day 5 and day 10 between sample A (treated only with water) and sample C (inoculated with the pediococci) held at 7^0C. Sample C had a numerically lower population than sample A. The results indicated that the cells of pediococci had no inhibitory effect on the growth of background flora on the surface of cut melons.

TABLE 4

EFFECT OF *PEDIOCOCCUS ACIDILACTICI* D3 SUSPENDED IN LACTIC ACID ON *SALMONELLA CHOLERAESUIS* (ATCC 13706) AND SPOILAGE FLORA ON FRESH CUT CANTALOUPE AT 7^0 C.

Media	Treatment	Count/g [1]		
		Day 0	Day 5	Day 10
XLD [2]	B (*S.choleraesuis*)	5.30 ^{Aa}	5.23 ^{Aa}	4.75 ^{Aa}
	D (*S.choleraesuis* and pediococci)	5.11 ^{Aa}	4.88 ^{Aab}	4.34 ^{Ab}
CVT [3]	A (Control)	2.74 ^{Ac}	7.30 ^{Ab}	9.31 ^{Aa}
	C (Pediococci)	2.65 ^{Ac}	6.95 ^{Ab}	8.88 ^{Aa}

XLD counts for treatment A and C were not considered during statistical analyses.

[1] Microbial counts are expressed as log $_{10}$ CFU/g ; each value is the mean from three replicate trials.

[2] *Salmonella* counts detected on XLD are expressed as log $_{10}$ CFU/g ; each value is the mean from three replicate trials. SE = 0.1982

[3] Gram negative counts detected on CVT incubated at 15^0C for 7 days are expressed as log $_{10}$ CFU/g ; each value is the mean from three replicate trials. SE = 0.2887

^{ABC} Means appearing in the same column followed by the same letter for each media used are not significantly different ($P > 0.05$).

^{abc} Means appearing in the same row followed by the same letter for each media used are not significantly different ($P > 0.05$).

Effect of Pediococcus *acidilactici* D3 Suspended in Acetic Acid

The interaction amid time and treatments on counts of *S.choleraesuis* (Table5) for the melons was not significant at 5% level ($P > 0.05$). Nonetheless comparisons made among the treatments did suggest certain significant differences among the pathogen counts. Both the samples, B (inoculated with *S.choleraesuis* only) and D (*S.choleraesuis* and *P.acidilactici*) exhibited significant declines ($P < 0.05$) in the population over the storage duration of 10 days at 7^0C. On day 0 and day 5 no significant differences were observed between B and D. Nevertheless statistically lower ($P < 0.05$) final population of *S.choleraesuis* was observed on day 10 for sample D comparing to sample B.

Contradictory results were obtained on CVT counts (Samples A and C) at 15^0C pertaining to the ability of the pediococci to inhibit the growth of Gram negative organisms on the surface of cut melons stored at 7^0C (Table 5). The interaction between treatment conditions and time was not significant ($P > 0.05$). The numbers increased significantly during the 10 day storage ($P<0.05$) for both the samples. No significant inhibitory effect ($P>0.05$) due to the pediococci was observed.

TABLE 5

EFFECT OF *PEDIOCOCCUS ACIDILACTICI* D3 SUSPENDED IN ACETIC ACID ON *SALMONELLA CHOLERAESUIS* (ATCC 13706) AND SPOILAGE FLORA ON FRESH CUT CANTALOUPE AT 7^0 C.

Media	Treatment	Count/g [1]		
		Day 0	Day 5	Day 10
XLD [2]	B (*S.choleraesuis*)	6.64 ^{Aa}	5.54 ^{Ab}	5.95 ^{Ab}
	D (*S.choleraesuis* and pediococci)	6.48 ^{Aa}	5.60 ^{Ab}	5.41 ^{Bb}
CVT [3]	A (Control)	2.32 ^{Ac}	5.68 ^{Ab}	7.40 ^{Aa}
	C (Pediococci)	2.14 ^{Ac}	6.42 ^{Bb}	7.40 ^{Aa}

XLD counts for treatment A and C were not considered during statistical analyses.

[1] Microbial counts are expressed as log $_{10}$ CFU/g ; each value is the mean from three replicate trials.

[2] *Salmonella* counts detected on XLD are expressed as log $_{10}$ CFU/g ; each value is the mean from three replicate trials. SE = 0.1492

[3] Gram negative counts detected on CVT incubated at 15^0C for 7 days are expressed as log $_{10}$ CFU/g ; each value is the mean from three replicate trials. SE = 0.1409

^{ABC} Means appearing in the same column followed by the same letter for each media used are not significantly different ($P > 0.05$).

^{abc} Means appearing in the same row followed by the same letter for each media used are not significantly different ($P > 0.05$).

Influence of Sliced Fruits on Hydrogen Peroxide Production

The objective of this experiment was to determine if the presence of cut fruits had the ability to influence the level of detectable hydrogen peroxide produced by the cells of *L.delbrueckii* ssp. *lactis* at 7^0C. The amount of hydrogen peroxide produced by *L.delbrueckii* ssp. *lactis* in the presence or absence of sliced fruits was monitored after 1 hour and 24 hours. The data revealed a significant interaction ($P < 0.05$) between time and treatments for the experiment. Significant differences were detected on the quantity of hydrogen peroxide among the treatment conditions (Table 6). The amount in the control sample (without fruit) increased significantly ($P<0.05$) during the 24 hr at 7^0C. However, no increase ($P>0.05$) was detected in the samples containing either cantaloupe or watermelon. The amount of hydrogen peroxide present after 24 hours in the control treatment was significantly higher ($P<0.05$) than in the other two samples. These results indicate the presence of sliced fruits had the ability to either interfere with the production of hydrogen peroxide by the cells of *L.delbrueckii* ssp. *lactis* RM 2-5 at 7^0C or to destroy it once it was produced.

When cells of *P.acidilactici* D3 were tested for hydrogen peroxide at 7^0C, negative results were obtained. Results indicated that there was no production of hydrogen peroxide at 7^0C by the cells of pediococci (data not shown). Thus no data is presented to show the influence of cut fruit on hydrogen peroxide production by this organism.

TABLE 6

HYDROGEN PEROXIDE PRODUCTION OF CELLS OF *LACTOBACILLUS DELBRUECKII* SSP. *LACTIS* RM 2-5 IN THE PRESENCE AND ABSENCE OF FRESH CUT FRUIT SLICES AT 7^0C IN BUFFER CONTAINING 5 Mm SODIUM LACTATE. [1]

Treatment[2]	H_2O_2 Produced (ug/ml)	
	0 Hour	24 Hours
Cantaloupe	0.30 ^{Aa}	0.07 ^{Ab}
Watermelon	0.22 ^{Aa}	0.10 ^{Aa}
Control	0.65 ^{Ba}	1.12 ^{Bb}

[1] 1mM sodium phosphate buffer (pH 6.5) containing 5 mM sodium lactate.

[2] Each treatment contains 9.5 ml of the buffer solution and 0.5 ml of the *L.lactis* RM 2-5 cell suspension and a fresh cut slice of either watermelon or cantaloupe; Control does not contain any fruit. SE = 0.0468

[AB] Value in same column followed by different letter differ significantly ($P < 0.05$).

[ab] Value in same row followed by different letter differ significantly ($P < 0.05$).

DISCUSSION

The increasing demand for fresh and convenient food products by the modern consumers has led the fresh cut produce industry to a new era of convenience foods. At the same time it is imperative to possess a discernment regarding the factors that eventually affect the quality and safety of the product. Empirically it has been proven that microbiological flora on fresh produce can influence quality and safety (Brackett, 1992). Application of lactic acid bacteria as a source of fermentation preservative is not a novel idea but a concept that has been utilized for many years. Conversely use of lactic acid bacteria as a biological preservative in nonfermented refrigerated food products (Gilliland and Speck, 1975) and minimally processed fruits and vegetables (Breidt and Flemming, 1997) is a concept that has been proposed in recent times.

The potential beneficial application of *L.delbrueckii* ssp. *lactis* as a biopreservative was demonstrated by it's ability to produce significant amounts of hydrogen peroxide, the main inhibitory component, at refrigeration temperatures without exhibiting any growth at these low temperatures (Gilliland and Speck, 1975; Gilliland and Villegas, 1998). Brashears et al (1998) reported the ability of *L.delbrueckii* ssp. *lactis* to inactivate the cells of *E.coli* O157:H7 on raw chicken meat at refrigeration temperature. They concluded that the main factor responsible for inactivation of the pathogen was hydrogen peroxide. Yap and Gilliland (1999), isolated a new strain of *L.delbrueckii* ssp. *lactis* from raw milk that produced more hydrogen peroxide than did the strain of lactobacilli used by Brashears et al at (1998) at 5^0C. Based on this finding the current research was conducted using this new strain

of *L.delbrueckii* ssp. *lactis* RM 2-5 as one of the selective cultures of lactic acid bacteria.

Based on the analyzed data no significant ($P > 0.05$) reduction of the pathogen *S.choleraesuis* or background spoilage flora was attained on melon cubes at 7^0C when the cells of *L.delbrueckii* ssp. *lactis* RM 2-5 were suspended in 5.5mM lactic acid. The reduction of *S.choleraesuis* observed when melon was treated with *L.delbrueckii* spp. *lactis* RM 2-5 suspended in 5.5mM sodium lactate, even though significant, likely was not great enough to be of practical importance. Sodium lactate has been shown to enhance the production of hydrogen peroxide by *L. delbrueckii* ssp. *lactis* indicating the presence of the enzyme lactate oxidase in cells of the lactobacilli (Villegas and Gilliland, 1998). No significant ($P < 0.05$) inhibition of growth of naturally occurring background flora on the melons was caused by the lactobacilli. Further testing however confirmed that hydrogen peroxide was produced by the *L.delbrueckii* ssp. *lactis* RM 2-5 strain at 7^0C, however none was detected in the presence of the fresh cut melons. The lack of detectable levels of hydrogen peroxide was attributed to the presence of catalases and peroxidases found in fresh cut melons (Lamikanra and Watson, 2000). Enzymes that are normally synthesized and located in cellular compartments in intact fruits are liberated to the surrounding environment along with tissue fluids as a result of destruction to the cells by cutting the fruit. Therefore most likely the presence of catalase and peroxidase was an encumbrance to the antibacterial mode of hydrogen peroxide. Thus not allowing hydrogen peroxide to have an antagonistic effect on the unfavorable organisms as desired.

Goff et al (1996) reported complete inhibition of low levels of *L.monocytogenes* inoculated onto raw chicken meat mediated by a bacteriocin produced by a strain of *P.acidilactici* at 5^0C storage. Brashears and Amezquita (2001) test were able to attain significant inhibition of the pathogen *L. monocytogenes* in cooked ham and frankfurters at refrigeration (5^0C) temperatures by selective cultures of lactic acid bacteria isolated from ready-to-eat meat products. One of the selected strains of lactic acid bacteria was identified as *Pediococcus acidilactici* D3. The authors concluded empirically that the antilisterial activity demonstrated by this specific strain could be due to a bacteriocin synthesized at refrigeration temperatures by this strain. The most promising observation of practical importance was the nature of *P.acidilactici* to mediate inhibition of the targeted pathogen at refrigeration storage at the same time exhibiting a considerably low growth rate and also causing no deleterious impact on the organoleptic properties correlating to qualitative attributes of the meat product.

For this reason we obtained *P.acidilactici* D3, from Dr. Brashears to study its impact on undesirable organisms on fresh cut melons at 7^0C. A small, although significant, reduction of *S.choleraesuis* by *P.acidilactici* was observed. However the reduction was small enough that it likely, has no practical importance. No effect on Gram negative organisms on cut melons at 7^0C was observed. Thus the pediococci do not appear to offer reasonable means for controlling undesirable microorganisms on refrigerated fresh cut melons.

Based on the report by Amezquita and Brashears (2001), key compound likely to have caused inactivation of the cells of *S.choleraesuis* by the pediococci was a

bacteriocin. We did not find that this organism produced hydrogen peroxide at 7^0C. Proteolytic enzymes are synthesized and located in cellular compartments of fruits at different stages of growth and development (Varoquaux and Wiley, 1994). Production of the proteases in fruits is augmented specially during ripening stage to aid in certain metabolic reactions and to hydrolyze cellular components as an integral part of ripening. Niidome et al (1993), found a high level of protease activity in melon juice as well. He also observed, an increase in the level of preoteolytic enzymes synthesized and released to the surrounding media due to stress induced by fresh cut processing of the melons. Such proteases could have reduced the effectiveness of any bacteriocin produced by *P.acidilactici*. Additionally such bacteriocins would not be expected to be very effective against Gram negative bacteria such as *Salmonella*.

Results obtained in the current study suggested minimal potential for beneficial effect of using biocontrol cultures of lactobacilli to prevent or inactivate the growth of unfavorable organisms of fresh cut melons during refrigeration storage. The observed differences are not of sufficient magnitude to justify the practicality of implementing the concept of utilizing such cultures as biopreservatives of fresh cut produce.

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APPENDIX A

INTERACTION OF LACTOBACILLUS DELBRUECKII SSP. LACTIS RM2-5 WITH *ESCHERICHIA COLI* O157:H7 ON FRESH CUT STRWABERRIES

Dip Experiments

"Dip" experiments were conducted in the preliminary stage of the study to determine the potential inhibitory effect of cells of *L.delbrueckii* spp. *lactis* RM 2-5 on pathogens such as *E.coli* O157:H7, *S.choleraesuis* and spoilage flora on fresh cut produce during refrigeration storage. The primary phase of the study was to test the potential antibacterial action of the lactobacilli against undesirable organisms on whole strawberries and fresh cut cubes of watermelon. The intact strawberries or cubes of melon were dipped into a cell suspension of approximately 6 $\log_{10}$ CFU/ml of *E.coli* O157:H7 for 3 minutes and allowed to drain. The inoculated samples were divided into two groups. One group was then dipped in a sterile deionized water suspension and the other group was dipped into a cell suspension of *L.delbrueckii* spp. *lactis* RM 2-5 with an initial cell population of 9 log10 CFU/ml. After draining, the samples were stored at 7^0C.

Enumeration of microorganisms during storage did not reveal inhibition of the undesirable organisms by the lactobacilli. The strawberries were overgrown by molds after 5 days of storage to an extent that prevented continuation of the experiment to obtain valid data. The reason for growth of molds on the surface of the strawberries could be accredited to acidic nature of strawberries and subsequent environmental conditions evolved within the stored atmosphere during the storage creating favorable growth conditions for the molds. Data obtained for the above studies are included in appendixes A, B, and C.

TABLE 7

RAW DATA FOR INFLUENCE OF *LACTOBACILLUS DELBRUECKII* SSP. *LACTIS* RM2-5 ON *ESHERICHIA COLI* O157:H7 AND SPOILAGE FLORA ON FRESH CUT STRAWBERRIES.

VRBA					**PDA**			
	Day 0	Day 5	Day10			Day 0	Day 5	Day10
Replication 1					Replication 1			
Control	1.54	3.34	-		Control	3.47	4.86	-
E.coli	5.6	4.04	-		E.coli	3.54	5.08	-
RM 2-5	5.64	4.25	-		RM 2-5	3.60	4.74	-
Replication 2					Replication 2			
Control	1.00	2.25	-		Control	4.27	5.95	-
E.coli	5.38	3.43	-		E.coli	4.30	5.82	-
RM 2-5	5.80	3.50	-		RM 2-5	4.41	6.27	-
Replication 3					Replication 3			
Control	2.84	1.00	-		Control	4.40	5.20	-
E.coli	5.80	5.25	-		E.coli	4.56	5 84	-
RM 2-5	5.84	4.00	-		RM 2-5	4.30	5.76	-
Means and SD					Means and SD			
Control	1.8(±0.94)	2.2(±1.17)	-		Control	4.0(±0.50)	5.3(±0.55)	-
E.coli	5.6(±0.21)	4.2(±0.93)	-		E.coli	4.1(±0.53)	5.6(±0.43)	-
RM 2-5	5.7(±0.10)	3.9(±0.38)	-		RM 2-5	4.1(±0.43)	5.6(±0.77)	-

E.coli : detected on VRBA are expressed as $\log_{10}$ CFU/g ; Yeasts and molds counts detected on PDA incubated at room temperature are expressed as $\log_{10}$ CFU/g.

Control : fresh cut strawberries rinsed in water and drained to serve as a control ; no *L.delbrueckii* spp. *lactis* or *E.coli* O157:H7 cells were added.

E.coli : fresh cut strawberries dipped in a suspension of *E.coli* O157:H7 cells and drained.

RM 2-5: fresh cut strawberries dipped in a *E.coli* O157:H7 cell suspension and treated with 7.7 x 10^7 CFU/ml of *L.delbrueckii* spp. *lactis* RM 2-5 in sodium lactate on day 0 as detected on LBS agar.

APPENDIX B

INTERACTION OF *LACTOBACILLUS DELBRUECKII* SSP. *LACTIS* RM2-5 WITH *ESCHERICHIA COLI* O157:H7 ON FRESH CUT WATERMELON

TABLE 8

RAW DATA FOR INFLUENCE OF *LACTOBACILLUS DELBRUECKII* SSP. *LACTIS* RM2-5 ON *ESCHERICHIA COLI* O157:H7 AND SPOILAGE FLORA ON FRESH CUT WATERMELON.

	VRBA				**PDA**		
	Day 0	Day 5	Day 10		Day 0	Day 5	Day10
Replication 1				Replication 1			
A	1.00	1.00	1.00	A	2.00	6.96	4.00
B	6.40	7.54	6.11	B	2.00	8.14	4.00
C	6.40	6.62	6.53	C	2.00	7.34	4.00
Replication 2				Replication 2			
A	2.80	1.00	1.00	A	2.00	1.00	1.00
B	6.90	6.65	5.75	B	2.00	1.00	1.00
C	7.04	6.43	6.36	C	2.00	1.00	1.81
Replication 3				Replication 3			
A	1.00	1.00	1.00	A	1.00	1.00	1.00
B	6.87	5.34	4.40	B	1.00	1.30	1.00
C	6.82	6.23	5.90	C	1.00	1.00	1.00
Means and SD				Means and SD			
A	1.6(±1.03)	1.0(±0.00)	1.0(±0.00)	A	1.6 (±0.57)	3.0(±3.40)	2.0(±1.73)
B	6.7(±0.28)	6.5(±1.10)	5.4(±0.90)	B	1.6(±0.57)	3.5(±4.03)	2.0 (±1.73)
C	6.7(±0.32)	6.4(±0.20)	6.3(±0.32)	C	1.6(±0.57)	3.1(±3.66)	2.3(±1.55)

E.coli : detected on VRBA are expressed as log $_{10}$ CFU/g ; yeasts and molds counts detected on PDA incubated at room temperature are expressed as log $_{10}$ CFU/g.

A : fresh cut watermelon cubes immersed in water and drained to serve as a control ; no *L.delbrueckii* spp. *lactis* or *E.coli* O157:H7 cells were added.

B : fresh cut watermelon cubes dipped in a suspension of *E.coli* O157:H7 cells and drained.

C : fresh cut watermelon cubes dipped in a *E.coli* O157:H7 cell suspension and treated with 3.6 x 10^8 CFU/ml of *L.delbrueckii* spp. *lactis* RM 2-5 in sodium lactate on day 0 as detected on LBS agar.

APPENDIX C

INTERACTION OF *LACTOBACILLUS DELBRUECKII* SSP. *LACTIS* RM2-5 WITH *SALMONELLA CHOLERAESUIS* ON FRESH CUT WATERMELON

TABLE 9

RAW DATA FOR INFLUENCE OF *LACTOBACILLUS DELBRUECKII* SSP. *LACTIS* RM2-5 ON *SALMONELLA CHOLERAESUIS* AND SPOILAGE ORGANISMS ON FRESH CUT WATERMELON.

XLD				**PCA**			
	Day 0	Day 5	Day 10		Day 0	Day 5	Day10
Replication 1				Replication 1			
A	1.00	1.00	1.00	A	1.00	1.00	1.00
C	1.00	1.00	1.00	C	1.00	1.00	1.00
B	6.34	6.14	6.14	B	6.60	6.55	6.47
D	6.41	6.11	5.94	D	6.70	6.34	6.11
Replication 2				Replication 2			
A	1.00	1.00	1.00	A	1.00	3.27	3.75
C	1.00	1.00	1.00	C	1.00	1.30	3.38
B	5.43	5.50	5.25	B	5.93	6.98	4.66
D	5.47	5.11	4.92	D	5.81	5.73	5.30
Replication 3				Replication 3			
A	1.00	1.00	1.00	A	2.63	5.62	3.50
C	1.00	1.00	1.00	C	3.30	5.64	3.62
B	6.77	6.14	5.47	B	6.04	6.68	4.68
D	6.47	5.84	4.04	D	5.90	5.62	5.25
Means and SD				Means and SD			
A	1.0(±0.00)	1.0(±0.00)	1.0(±0.00)	A	1.5(±0.94)	3.3(±2.31)	2.75(±1.52)
C	1.0(±0.00)	1.0(±0.00)	1.0(±0.00)	C	1.7(±1.32)	2.6(±2.59)	2.66(±1.44)
B	6.2(±0.68)	5.9(±0.37)	5.6(±0.46)	B	6.2(±0.36)	6.7(±0.22)	5.27(±1.03)
D	6.1(±0.56)	5.6(±0.36)	4.9(±0.95)	D	6.1(±0.49)	5.9(±0.38)	5.55(±0.48)

S.choleraesuis detected on XLD are expressed as $\log_{10}$ CFU/g ; spoilage cell counts detected on PCA incubated at 15^0C are expressed as $\log_{10}$ CFU/g.

A : fresh cut watermelon cubes rinsed in water and drained to serve as a control

C : fresh cut watermelon cubes dipped in a suspension of *L.delbrueckii spp. lactis*

B : watermelon cubes dipped in a *S.choleraesuis* cell suspension.

D : watermelon cubes dipped in a *E.coli O157*:H7 suspension and treated with 8.4×10^7 CFU/ml of *L.delbrueckii* spp. *lactis* in sodium lactate on day 0 as detected on LBS agar .

APPENDIX D

INTERACTION OF *LACTOBACILLUS DELBRUECKII* SSP. *LACTIS RM2-5* WITH *SALMONELLA CHOLERAESUIS* ON FRESH CUT CANTALOUPE

TABLE 10

RAW DATA FOR INFLUENCE OF *LACTOBACILLUS DELBRUECKII* SSP. *LACTIS* RM2-5 ON *SALMONELLA CHOLERAESUIS* AND SPOILAGE ORGANISMS ON FRESH CUT CANTALOUPE.

	XLD				PCA		
	Day 0	Day 5	Day 10		Day 0	Day 5	Day10
Replication 1				Replication 1			
A	1.00	1.00	1.00	A	4.56	7.47	8.40
C	1.00	1.00	1.00	C	4.81	7.40	8.40
B	5.70	5.71	5.74	B	4.71	8.11	9.04
D	6.75	5.27	5.61	D	6.50	8.40	9.11
Replication 2				Replication 2			
A	1.00	1.00	1.00	A	4.86	6.40	9.25
C	1.00	1.00	1.00	C	4.82	8.40	9.25
B	5.40	5.40	4.54	B	5.04	9.11	9.40
D	5.65	4.97	3.00	D	5.04	8.98	9.20
Replication 3				Replication 3			
A	1.00	1.00	1.00	A	5.47	6.40	8.40
C	1.00	1.00	1.00	C	5.34	6.40	8.40
B	5.70	5.40	4.27	B	5.46	8.40	9.40
D	5.85	5.34	4.23	D	4.92	8.40	9.40
Means and SD				Means and SD			
A	1.0(±0.00)	1.0(±0.00)	1.0(±0.00)	A	4.9(±0.46)	6.7(±0.62)	8.6(±0.49)
C	1.0(±0.00)	1.0(±0.00)	1.0(±0.00)	C	4.9(±0.30)	7.4(±1.00)	8.7(±0.49)
B	5.6(±0.17)	5.5(±0.18)	4.8(±0.78)	B	5.1(±0.37)	8.5(±0.51)	9.3(±0.20)
D	6.1(±0.58)	5.2(±0.19)	4.3(±1.30)	D	5.5(±0.88)	8.6(±0.33)	9.2(±0.15)

S choleraesuis detected on XLD are expressed as $\log_{10}$ CFU/g ; spoilage counts detected on PCA incubated at 15°C are expressed as $\log_{10}$ CFU/g.

A : cantaloupe cubes inoculated only with water to serve as a control.

C : cantaloupe cubes surface inoculated with *L.delbrueckii spp. lactis* in sodium lactate.

B : cantaloupe cubes surface inoculated with *S.choleraesuis*.

D : cantaloupe cubes surface inoculated with *S.choleraesuis* and then 4.3 x 10^6 CFU/ml of *L.delbrueckii* spp. *lactis* in sodium lactate on day 0 as detected on LBS agar.

TABLE 11

RAW DATA FOR INFLUENCE OF *LACTOBACILUS DELBRUECKII* SSP. *LACTIS* RM2-5 ON YEAST AND MOLD COUNT ON FRESH CUT CANTALOUPE.

PDA			
	Day 0	Day 5	Day 10
Replication	1		
A	4.11	3.54	3.00
C	3.30	3.00	2.00
B	4.11	1.00	4.00
D	6.40	1.00	3.00
Replication	2		
A	4.61	6.40	6.40
C	3.87	6.40	8.40
B	9.75	6.40	8.40
D	5.93	6.40	8.87
Replication	3		
A	2.11	2.00	5.51
C	1.74	1.60	6.40
B	5.00	2.47	5.68
D	1.30	1.30	4.87
Means and SD			
	3.6(±1.32)	3.9(±2.23)	4.9(±1.76)
	2.9(±1.10)	3.6(±2.46)	5.6(±3.27)
	6.3(±3.03)	3.3(±2.80)	6.0(±2.22)
	4.5(±2.81)	2.9(±3.03)	5.5(±3.00)

Yeasts and molds counts detected on PDA incubated at room temperature are expressed as $\log_{10}$ CFU/g.

A : cantaloupe cubes inoculated only with water to serve as a control.

C : cantaloupe cubes surface inoculated with *L.delbrueckii spp. lactis* in sodium lactate

B : cantaloupe cubes surface inoculated with *S.choleraesuis*.

D : cantaloupe cubes surface inoculated with *S.choleraesuis* and then 4.3×10^6 CFU/ml of *L.delbrueckii* spp. *lactis* in sodium lactate on day 0 as detected on LBS agar.

TABLE 12

RAW DATA FOR INFLUENCE OF *LACTOBACILLUS DELBRUECKII* SSP. *LACTIS* RM2-5 ON *SALMONELLA CHOLERAESUIS* AND SPOILAGE ORGANISMS ON FRESH CUT CANTALOUPE.

XLD				**PCA**			
	Day 0	Day 5	Day 10		Day 0	Day 5	Day10
Replication 1				Replication 1			
A	1.00	1.00	1.00	A	6.14	7.64	8.40
C	1.00	1.00	1.00	C	6.40	6.88	6.04
B	6.23	5.38	5.04	B	5.61	6.94	7.32
D	6.11	5.34	4.60	D	5.41	7.11	5.95
Replication 2				Replication 2			
A	1.00	1.00	1.00	A	1.77	1.00	4.54
C	1.00	1.00	1.00	C	2.90	4.47	4.96
B	6.90	6.88	3.81	B	6.98	6.97	7.51
D	6.88	6.74	6.56	D	6.88	6.56	6.38
Replication 3				Replication 3			
A	1.00	1.00	1.00	A	6.40	1.00	6.80
C	1.00	1.00	1.00	C	2.95	4.04	5.65
B	6.95	6.63	6.77	B	7.07	6.73	7.17
D	6.91	6.55	6.51	D	6.94	6.71	6.47
Means and SD				Means and SD			
A	1.0(±0.00)	1.0(±0.00)	1.0(±0.00)	A	4.7(±2.60)	3.2(±3.83)	6.6(±1.93)
C	1.0(±0.00)	1.0(±0.00)	1.0(±0.00)	C	4.1(±2.00)	5.1(±1.53)	5.5(±0.55)
B	6.7(±0.40)	6.3(±0.80)	5.2(±1.48)	B	6.5(±0.81)	6.8(±0.13)	7.3(±0.17)
D	6.6(±0.45)	6.2(±0.76)	5.9(±1.11)	D	6.4(±0.86)	6.8(±0.28)	6.3(±0.27)

S.choleraesuis detected on XLD are expressed as $\log_{10}$ CFU/g ; spoilage counts detected on PCA incubated at 15^{0}C are expressed as $\log_{10}$ CFU/g.

A : cantaloupe cubes inoculated only with water to serve as a control.

C : cantaloupe cubes surface inoculated with *L.delbrueckii spp. lactis* in lactic acid.

B : cantaloupe cubes surface inoculated with *S.choleraesuis*.

D : cantaloupe cubes surface inoculated with *S choleraesuis* and 1.7 x 10^7 CFU/ml of *L.delbrueckii* spp. *lactis* in lactic acid on day 0 as detected on LBS agar respectively.

TABLE 13

RAW DATA FOR INFLUENCE OF *LACTOBACILLUS DELBRUECKII* SSP. *LACTIS* RM2-5 ON YEAST AND MOLD COUNT ON FRESH CUT CANTALOUPE.

PDA			
	Day 0	Day 5	Day 10
Replication	1		
A	1.47	1.65	4.50
C	1.00	1.54	3.85
B	1.00	1.00	6.40
D	1.00	1.65	3.17
Replication	2		
A	1.00	1.00	1.00
C	1.00	1.00	1.00
B	1.00	1.00	7.40
D	1.00	1.00	1.00
Replication	3		
A	1.00	1.00	1.00
C	1.00	1.00	1.00
B	1.00	1.00	1.00
D	1.00	1.00	1.00
Means and SD			
A	1.2(±0.27)	1.2(±0.37)	2.2(±2.02)
C	1.0(±0.00)	1.2(±0.31)	1.9(±1.64)
B	1.0(±0.00)	1.0(±0.00)	5.0(±3.44)
D	1.0(±0.00)	1.2(±0.37)	1.7(±1.25)

Yeasts and molds counts detected on PDA incubated at room temperature are expressed as $\log_{10}$ CFU/g.

A : cantaloupe cubes inoculated only with water to serve as a control.

C : cantaloupe cubes surface inoculated with *L.delbrueckii spp. lactis* in lactic acid.

B : cantaloupe cubes surface inoculated with *S.choleraesuis*.

D : cantaloupe cubes surface inoculated with *S.choleraesuis* and 1.7×10^7 CFU/ml of *L.delbrueckii* spp. *lactis* in lactic acid on day 0 as detected on LBS agar respectively.

APPENDIX E

INTERACTION OF *PEDIOCOCCUS ACIDILACTICI* D3 WITH *SALMONELLA CHOLERAESUIS* ON FRESH CUT CANTALOUPE

TABLE 14

RAW DATA FOR INFLUENCE OF *PEDIOCOCCUS ACIDILACTICI* D3 ON *SALMONELLA CHOLERAESUIS* AND SPOILAGE ORGANISMS ON FRESH CUT CANTALOUPE.

XLD					**CVT**			
	Day 0	Day 5	Day 10			Day 0	Day 5	Day10
Replication 1					Replication 1			
A	1.00	1.00	1.00		A	1.00	6.23	8.65
C	1.00	1.00	1.00		C	1.00	4.74	8.51
B	4.68	3.76	3.80		B	3.72	5.70	9.30
D	4.64	3.64	3.51		D	3.78	4.25	3.00
Replication 2					Replication 2			
A	1.00	1.00	1.00		A	1.84	6.09	8.40
C	1.00	1.00	1.00		C	2.71	7.40	8.27
B	6.44	6.36	6.62		B	6.00	7.95	9.07
D	5.97	7.27	5.92		D	5.55	8.14	9.11
Replication 3					Replication 3			
A	1.00	1.00	1.00		A	1.00	6.25	8.77
C	1.00	1.00	1.00		C	1.00	4.47	8.56
B	4.54	3.67	3.73		B	3.95	5.80	9.30
D	4.60	3.63	3.72		D	3.85	4.30	3.00
Means and SD					Means and SD			
A	1.0(±0.00)	1.0(±0.00)	1.0(±0.00)		A	1.3(±0.48)	6.2(±0.14)	8.6(±0.19)
C	1.0(±0.00)	1.0(±0.00)	1.0(±0.00)		C	1.6(±0.99)	5.5(±1.62)	8.4(±0.15)
B	5.2(±1.06)	4.6(±1.53)	4.7(±1.65)		B	4.5(±1.25)	6.5(±1.27)	9.2(±0.13)
D	5.0(±0.78)	4.8(±2.10)	4.4(±1.33)		D	4.4(±1.00)	5.5(±2.23)	5.0(±3.53)

S.choleraesuis detected on XLD are expressed as $\log_{10}$ CFU/g ;Gram negative counts detected on CVT incubated at 15^{0}C are expressed as $\log_{10}$ CFU/g.

A : cantaloupe cubes inoculated only with water to serve as a control.

C : cantaloupe cubes surface inoculated with *P.acidilactici* D3 .

B : cantaloupe cubes surface inoculated with *S.choleraesuis*.

D : cantaloupe cubes surface inoculated with *S.choleraesuis* and 2.4 x 10^8 CFU/ml of *P.acidilactici* D3 on day 0 as detected on LBS agar respectively.

TABLE 15

RAW DATA FOR INFLUENCE OF *PEDIOCOCCUS ACIDILACTICI* D3 ON *SALMONELLA CHOLERAESUIS* AND SPOILAGE ORGANISMS ON FRESH CUT CANTALOUPE.

XLD				CVT			
	Day 0	Day 5	Day 10		Day 0	Day 5	Day10
Replication 1				Replication 1			
A	1.00	1.00	1.00	A	1.90	6.14	7.40
C	1.00	1.00	1.00	C	2.11	6.43	7.40
B	6.27	5.57	5.79	B	6.17	5.92	8.40
D	6.17	5.62	5.32	D	6.00	6.00	8.40
Replication 2				Replication 2			
A	1.00	1.00	1.00	A	2.34	4.95	7.40
C	1.00	1.00	1.00	C	2.14	6.44	7.40
B	6.81	5.57	5.61	B	6.17	5.90	8.40
D	6.74	5.60	5.47	D	6.62	5.86	8.40
Replication 3				Replication 3			
A	1.00	1.00	1.00	A	2.73	5.96	7.40
C	1.00	1.00	1.00	C	2.17	6.41	7.40
B	6.84	5.50	6.47	B	6.27	5.84	8.40
D	6.54	5.57	5.44	D	6.20	6.17	8.40
Means and SD				Means and SD			
A	1.0(±0.00)	1.0(±0.00)	1.0(±0.00)	A	2.3(±0.42)	5.7(±0.64)	7.4(±0.00)
C	1.0(±0.00)	1.0(±0.00)	1.0(±0.00)	C	2.1(±0.03)	6.4(±0.02)	7.4(±0.00)
B	6.6(±0.32)	5.5(±0.04)	5.9(±0.45)	B	6.2(±0.06)	5.9(±0.04)	8.4(±0.00)
D	6.5(±0.29)	5.6(±0.02)	5.4(±0.08)	D	6.3(±0.32)	6.0(±0.15)	8.4(±0.00)

S.choleraesuis detected on XLD are expressed as $\log_{10}$ CFU/g ; Gram negative counts detected on PCA incubated at 15^{0}C are expressed as $\log_{10}$ CFU/g.

A : cantaloupe cubes inoculated only with water to serve as a control.

C : cantaloupe cubes surface inoculated with *P.acidilactici* D3 in acetic acid.

B : cantaloupe cubes surface inoculated with *S.choleraesuis*.

D : cantaloupe cubes surface inoculated with *S.choleraesuis* and 3.0 x 10^7 CFU/ml of *P.acidilactici* D3 in acetic acid on day 0 as detected on LBS agar respectively.

TABLE 16

RAW DATA FOR INFLUENCE OF *PEDIOCOCCUS ACIDILATICI* D3 ON *SALMONELLA CHOLERASUIS* AND SPOILAGE ORGANISMS ON FRESH CUT CANTALOUPE.

XLD				**CVT**			
	Day 0	Day 5	Day 10		Day 0	Day 5	Day10
Replication 1				Replication 1			
A	1.00	1.00	1.00	A	3.04	7.40	9.30
C	1.00	1.00	1.00	C	3.04	7.40	9.20
B	5.23	5.56	4.67	B	4.95	8.40	9.32
D	4.97	4.84	4.67	D	4.53	8.40	9.17
Replication 2				Replication 2			
A	1.00	1.00	1.00	A	3.14	7.40	9.23
C	1.00	1.00	1.00	C	2.98	7.40	9.23
B	5.34	5.44	4.40	B	4.98	8.40	9.23
D	4.97	5.27	3.96	D	4.56	8.40	9.17
Replication 3				Replication 3			
A	1.00	1.00	1.00	A	2.04	7.07	9.40
C	1.00	1.00	1.00	C	1.95	6.07	8.23
B	5.30	4.70	5.20	B	5.00	7.40	9.40
D	5.40	4.55	4.40	D	5.20	6.85	8.98
Means and SD				Means and SD			
A	1.0(±0.00)	1.0(±0.00)	1.0(±0.00)	A	2.7(±0.61)	7.3(±0.19)	9.3(±0.08)
C	1.0(±0.00)	1.0(±0.00)	1.0(±0.00)	C	2.6(±0.61)	6.9(±0.77)	8.8(±0.57)
B	5.3(±0.06)	5.2(±0.47)	4.8(±0.41)	B	4.9(±0.03)	8.1(±0.58)	9.3(±0.08)
D	5.1(±0.25)	4.8(±0.36)	4.4(±0.36)	D	4.8(±0.38)	7.9(±0.89)	9.1(±0.11)

S.choleraesuis detected on XLD are expressed as log $_{10}$ CFU/g ; psychrotrophic counts detected on PCA incubated at 15^{0}C are expressed as log $_{10}$ CFU/g.

A : cantaloupe cubes inoculated only with water to serve as a control.

C : cantaloupe cubes surface inoculated with *P.acidilactici* D3 in lactic acid.

B : cantaloupe cubes surface inoculated with *S.choleraesuis*.

D : cantaloupe cubes surface inoculated with *S.choleraesuis* and 1.0 x 10^8 CFU/ml of *P acidilactici* D3 in lactic acid on day 0 as detected on LBS agar respectively.

APPENDIX F

HYDROGEN PEROXIDE PRODUCTION OF *LACTOBACILLUS DELBRUECKII* SSP. *LACTIS* RM2-5 AT 7^0C WITH ADDED FESH CUT WATERMELON AND CANTALOUPE

TABLE 17

RAW DATA FOR HYDROGEN PEROXIDE PRODUCTION OF CELLS OF *LACTOBACILLUS DELBRUECKII* SSP. *LACTIS* RM2-5 AT 7°C WITH FRESH CUT FRUITS.

Treatment		**H_2O_2 Produced (ug/ml)**	
		1 Hour	24 Hours
Control	**Replication 1**	0.68	1.20
	Replication 2	0.72	1.12
	Replication 3	0.56	1.05
Watermelon	**Replication 1**	0.24	0.12
	Replication 2	0.18	0.16
	Replication 3	0.25	0.01
Cantaloupe	**Replication 1**	0.42	0.06
	Replication 2	0.15	0.08
	Replication 3	0.34	0.08
Means and SD			
	Control	0.65 (±0.08)	1.12 (±0.07)
	Watermelon	0.22 (±0.03)	0.10 (±0.07)
	Cantaloupe	0.30 (±0.13)	0.07 (±0.01)

Control – Cells of *L.delbrueckii* ssp. *lactis* RM 2-5 in buffer and lactate alone.

Watermelon – Cells of L. delbrueckii ssp. *lactis* RM 2-5 with a slice of fresh cut watermelon.

Cantaloupe – Cells of *L. delbrueckii* ssp. *lactis* RM 2-5 with a slice of fresh cut cantaloupe.

APPENDIX G

HYDROGEN PEROXIDE PRODUCTION OF *PEDIOCOCCUS ACIDILACTICI* D3 AT 7^0C WITH ADDED FRESH CUT WATERMELON AND CANTALOUPE

TABLE 18

RAW DATA FOR HYDROGEN PEROXIDE PRODUCTION OF CELLS OF *PEDICOCCUS ACIDILACTICI* D3 AT 7^0C WITH FRESH CUT FRUITS.

Treatment		H_2O_2 Produced (ug/ml)	
		1 Hour	24 Hours
Control	**Replication 1**	0.18	0.16
	Replication 2	0.19	0.14
Watermelon	**Replication 1**	0.13	0.05
	Replication 2	0.14	0.06
Cantaloupe	**Replication 1**	0.16	0.07
	Replication 2	0.15	0.08
Means and SD			
	Control	0.18 (± 0.01)	0.15 (± 0.02)
	Watermelon	0.13 (± 0.01)	0.05 (± 0.01)
	Cantaloupe	0.15 (± 0.01)	0.07 (± 0.01)

Control – Cells of *Pediococcus acidilactici* in buffer and lactate alone.

Watermelon – Cells of *Pediococcus acidilactici* with a slice of fresh cut watermelon.

Cantaloupe – Cells of *Pediococcus acidilactici* with a slice of fresh cut cantaloupe.

VITA

Gayathri Himali Senarante

Candidate for the Degree of

Master of Science

Thesis: APPLICATION OF LACTIC ACID BACTERIA AS POTENTIAL BIOPRESERVATIVE AGENTS OF MINIMALLY PROCESSED REFRIGERATED FRUITS

Major Field: Food Science

Biographical:

Education: Graduated from Bishop's College, Colombo, Sri-Lanka in December 1990. Received Bachelor of Science degree in Microbiology from University of Mysore, Mysore, India in May 1994.Completed the requirements for the degree of Master of Science at Oklahoma State University in May 2002.

Experience: Employed as a Food Service (Senior Supervisor) by Aramark Catering Service, Stillwater, Oklahoma; employed by Oklahoma State University, Oklahoma Food and Agriculture Products Research and Technology Center as a student research assistant; employed by Oklahoma State University Department of Animal Science as a graduate student.

Academic Honors: Member of PHI KAPPA PHI Honor Society.