Survival of *Listeria monocytogenes* Scott A to pediocin under various conditions in pork

by

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GENERAL INTRODUCTION

Listeria monocytogenes is an important foodborne pathogen that has been involved in several outbreaks within the past decade. The organism is salt tolerant, and it can also survive a wide pH range.

Listeria monocytogenes is widely distributed in nature, and it has been isolated from a variety of foods, such as vegetables, dairy products, and meats. The incidence of listeriae in fresh and ready-to-eat meat products has been reported to range from 8 to 92% in the United States and Europe (Johnson et al., 1990). During a preliminary survey in the U.S., it was found that about 70% of ground beef, 43% of pork sausages, and 48% of poultry were contaminated with *L. monocytogenes* (Brackett, 1988). Due to its psychrotrophic nature, the pathogen can readily grow and multiply in fresh meat and meat products when stored under refrigeration conditions.

There are several reports that indicate *L. monocytogenes* can undergo a phenonmenon commonly known as "heat-shock" after an exposure to elevated sublethal temperatures. As a result, the organism becomes thermotolerant and can survive higher temperature treatments. Processing and mishandling of meat either in the meat industry, restaurants, or in homes can produce thermotolerant bacteria. Presence of such heat-resistant *Listeria* in meat products can be a health risk to consumers.

Recently, the use of "natural biopreservatives" such as pediocin has been recommended as a possible method to control the growth of *L. monocytogenes* in meat. In addition, vacuum and modified atmosphere packaging are commonly employed in the meat industry to inhibit microorganisms and

increase shelf life of meat products. However, due to microaerophilic nature of *Listeria*, the organism can survive vacuum and gas mixture atmospheres. Therefore, an alternative method to inhibit the growth of this pathogen during storage is to use a combination of several methods.

The objectives of this study were to determine the effect of heat shock, pediocin (either added directly or produced by addition of *Pediococcus acidilactici*), and packaging atmosphere on survival of *L. monocytogenes* in fresh pork. A final objective was to determine the effect of pediocin on meat color, texture, and odor in order to ascertain the acceptability of pork meat treated with this biopreservative by sensory evaluation.

LITERATURE REVIEW

Listeria monocytogenes

A. History

The first occurrence of listeriosis probably dates back to as early as 1891 in tissue sections from patients in Germany (Gray and Killinger, 1966). It was about two decades after that, in 1911, that the bacterium was isolated from rabbits in Sweden (Gray and Killinger, 1966; Wehr, 1987). Murray et al. (1926) named the organism *Bacterium monocytogenes* due to its involvement in a typical monocytosis that had occurred in infected laboratory rabbits and guinea pigs in 1924. It was not until 1927 that Pirie also isolated the same bacterium from gerbil, *Tatera lobengulae,* in south Africa, and named it *Listerella hepatolytica* in honor of Lord Lister. The bacterium was eventually renamed *Listeria monocytogenes* by Pirie in 1940 (Welshimer, 1981).

Listeria monocytogenes was first described in a human patient by Nyfeldt (Seeliger, 1961). In 1936, Burns described *L. monocytogenes* as the causative agent for perinatal infection in humans (McLauchlin, 1987). It is known that the bacterium is also responsible for abortion and septicemia in animals. Potel in 1953 reported the first incidence of foodborne listeriosis in humans that involved direct relation to animals. In this case, consumption of raw milk from a cow with a listerial mastitis resulted in stillborn twins (Hird, 1987). Being a prevalent pathogen in the environment, *L. monocytogenes* has also been isolated from meat. The result of a survey that was done by Farber et al. (1989) showed that this organism was present in 56.3% of chicken legs, 86.4% of ground meats, and in 20% of fermented sausages. Occurrence of *L. monocytogenes* in

meat and its role in foodborne illness has triggered the concern of food safety officials and prompted efforts to develop methods to control the growth of this pathogen in meat products.

B. General characteristics of *Listeria monocytogenes*

In the ninth edition of Bergey's Manual of Systematic Bacteriology (Seeliger and Jones, 1986), *L. monocytogenes* was described as a short, regular, Gram-positive rod that can occur singly, in chains, or the cells may be seen in 'V' form arrangements. These cells are 0.4-0.5 μm in diameter and 0.5-2.0 μm in length with rounded ends. They exhibit tumbling motility by a few peritrichous flagella when the cells are grown at 20-25°C. *Listeria* cells are not acid-fast, do not form capsules and do not produce spores. When grown on nutrient agar, they form colonies of 0.5-1.5 mm in diameter, round, translucent, dew drop in appearance, and low convex with fine texture and entire margin. These colonies appear bluish gray by normal illumination; however, they show characteristic blue-green sheen when exposed to 45° incident transmitted white light (Seeliger and Jones, 1986).

Listeria spp. are facultatively anaerobic. Under anaerobic conditions only hexoses and pentoses support growth; however, in the presence of air the cells metabolize maltose and lactose (Pine et al., 1989). Although their optimum growth is between 30 and 37°C, the cells are capable of growing at temperatures between 1 to 45°C (Seeliger and Jones, 1986). Due to its ability to grow at as low temperature as 1°C, *L. monocytogenes* is considered a psychrotroph. The bacterium is salt tolerant, and able to grow in media containing 10% (w/v) NaCl (Seeliger, 1961). *L. monocytogenes* can grow in a medium with a pH range of

5.6-9.8 (Seeliger and Finger, 1976). In addition, Blenden et al. (1987) reported that this bacterium was able to survive the harvesting process and to reproduce during storage of silage at pH of 4.0-4.5.

L. monocytogenes produces an extracellular protein, or hemolysin, that is recognized as its major virulence factor (Cossart and Mengaud, 1989). This hemolysin, also known as Listeriolysin O, or LLO, is a sulfhydryl (SH)-activated protein with a molecular weight of 60,000 daltons that is involved in the cytolytic action of the bacterium (Geoffroy et al., 1987). Listeriolysin O also produces a weak β- hemolysin on sheep blood agar plates. This hemolytic reaction can be detected by performing the CAMP test that utilizes *Staphylococcus aureus* (Christie et al., 1944). Geoffroy et al. (1987) demonstrated that LLO has properties similar to other bacterial sulfhydryl-activated toxins, such as: being inhibited by very low amounts of cholesterol, being activated by reducing agents (suppressed by oxidation), and having antigenic cross-reactivity with Streptolysin O (SLO). The maximum cytolytic activity of Listeriolysin O on erythrocytes from different animals occurs at low pH, around 5.5. Therefore it was suggested (Geoffroy et al., 1987) that the cytolytic action of this hemolysin is enhanced in acidic environments, such as within macrophage phagosomes, where *L. monocytogenes* is assumed to reproduce and destroy the infected macrophages.

C. Symptoms of the illness

L. monocytogenes causes listeriosis in both animals and humans, with the same clinical symptoms being displayed among animals (Seeliger, 1961). Human listeriosis can occur in different forms. Listeriosis during pregnancy is associated with "grippe"-like symptoms, such as headache, fever, and chills. This may lead

to infection of the fetus either during delivery or through the placenta, in which case it causes listeriosis of the newborn. Some of the neonatal listeric symptoms may include respiratory distress and failure of blood circulation that usually lead to the infant's death (Seeliger, 1961).

Other forms of listeriosis in man include the cutaneous, septicemic, oculoglandular, and cervicoglandular types, in which the infection occurs on the skin, in the leukocytes, in the conjunctiva of the eye, and in the cervical and submandibular lymph nodes (Seeliger, 1961). Listeriosis may also be involved with infection of the central nervous system which can result in meningitis, and cerebritis (Nieman and Lorber, 1980). It is also reported that immunocompromised individuals may be susceptible to different listeric infections that would lead to arthritis, spinal or brain abscesses, and peritonitis (Armstrong, 1985).

D. Foodborne outbreaks of listeriosis

L. monocytogenes is widely distributed in the environment. It has been isolated from such sources as vegetables, stream water, fish, sewage, and soil (Gray, 1963). In addition, the organism has been isolated from the intestinal tract of humans, and it is usually found in the gastrointestinal tract of ruminant animals (Gray and Killinger, 1966). Since *Listeria* is able to grow at low temperatures, a great concern over its ability as a post-process contamination agent for refrigerated foods has been raised (McLauchlin, 1987).

Three significant foodborne outbreaks due to *L. monocytogenes* occurred in the United States and Canada between 1981 and 1985 (Farber and Peterkin, 1991). The Canadian outbreak occurred in Nova Scotia between March and

September of 1981, in which consumption of contaminated coleslaw was associated with illness. This outbreak resulted in 41 cases of which 17 people died. The results of the survey in this case indicated presence of *L. monocytogenes* serotype 4b in the coleslaw, prepared with a cabbage grown in a field that had been fertilized with sheep manure (Farber and Peterkin, 1991; Schlech et al., 1983).

The second listeriosis outbreak, which also involved *L. monocytogenes* serotype 4b, was recorded in Boston, Massachusetts during summer of 1983 (Fleming et al., 1985). In this outbreak, which resulted in 14 deaths out of 49 cases, pasteurized milk was suspected to be the source of contamination.

Involvement of *L. monocytogenes* serotype 4b was also confirmed in a third outbreak in Los Angeles, California from January to August of 1985 (Linnan et al. , 1988). A thorough study of this case revealed a linkage between the consumption of Jalisco-brand Mexican-style soft cheese, that was prepared with contaminated milk, with the outbreak resulting in 48 deaths out of 142 cases. In addition to these three outbreaks, foodborne listeriosis was also reported in Philadelphia during the period of 1986 to 1987 (Schwartz et al., 1989). Contaminated ice cream, vegetables, and salami were suspected to be the cause of 16 deaths out of 36 cases.

E. Incidence of *Listeria monocytogenes* in foods

L. monocytogenes is a pathogen that has been isolated from both wild and domestic animals (Gray and Killinger, 1966; Seeliger, 1961), including raccoons, skunks, birds, cats, dogs, cattle and sheep. Therefore, it has been suggested that shedding of *Listeria* from infected animals with subsequent contamination of

silage produced from grasses, and reintroduction into mammals, can lead to contamination of milk and meat products (Wehr, 1987). Vegetables have also been shown to be source of contamination. As evidenced by the 1981- listeriosis outbreak in Canada, cabbage was found to be the vehicle for listerial contamination (Schlech et al., 1983). In addition, Conner et al. (1986) showed that cabbage juice could support the growth of *L. monocytogenes*. In a study that was conducted by Ho et al. (1986) the source of contamination that had caused listeric infection in several patients, celery, tomato, and lettuce were reported to be the vehicles of infection.

The results of surveys on raw milk for *L. monocytogenes* indicate that the occurrence of this organism is common in the U.S. (Lovett et al., 1987). Surveys done on raw milk in several countries show that from 0 to 7% of samples tested contained *L. monocytogenes* (Marth and Ryser, 1990). The first case of listeriosis that occurred in Germany in 1953 (Hird, 1987) involved a pregnant woman who drank raw milk from a cow with listerial mastitis that eventually led to child's stillbirth. Similarly, the 1983-listeriosis outbreak in Boston was also suspected to be due to contamination of pasteurized milk with *L. monocytogenes* (Fleming et al., 1985).

Presence of *L. monocytogenes* in dairy products other than milk has also been documented. Levels of this pathogen in soft and semi-soft cheese has been reported to be as high as 10^4-10^5 CFU/g product (Farber et al., 1987). Ryser and Marth (1987) found that contamination of Camembert cheese with this pathogen mainly occurred on the surface of the rind. Since a broad pH gradient develops in these cheeses during ripening, Farber and Peterkin (1991) suggested that the occurrence of *L. monocytogenes* on the surface of the soft cheese could be the

result of a pH effect. In addition to different variety of cheeses being the source of contamination, *L. monocytogenes* has also been shown to survive in products such as butter-milk, butter, and yogurt (Schacck and Marth, 1988; Siragusa and Johnson, 1988).

In addition to dairy products, a wide variety of meats are contaminated with *L. monocytogenes*. Studies conducted by Genigeorgis et al., (1989) indicated the presence of this organism in slaughter houses. In the processing plants, the raw product itself can become the source of contamination for the environment (Lovett and Twedt, 1988). Therefore, handling of meats during or after processing in such environments is suspected to allow the recontamination of the meat product with this organism.

In a survey that was done on various meat items, Farber et al. (1989) found *L. monocytogenes* to be present in 56.3% chicken legs, 86.4% of ground meats, and in 20% of fermented sausages. Presence of this pathogen was also reported in as high as 60% of raw poultry carcasses (Pini and Gilbert, 1988). Kwantes and Isaac (1971) isolated *L. monocytogenes* from 57% of fresh and frozen poultry samples. During a preliminary survey in the U.S., it was found that about 70% of ground beef, 43% of pork sausages, and 48% of poultry were contaminated with this organism (Brackett, 1988). In Yugoslavia, *L. monocytogenes* was isolated from 69% of minced meat (mixed pork and beef) samples (Buncic, 1991). In another study, Grau and Vanderlinde (1992) found *L. monocytogenes* to be present in 93 out of 175 vacuum-packaged processed meat samples tested. Glass and Doyle (1989) noticed that growth of *L. monocytogenes* on processed meat was dependent on the type and pH of the product. The organism had the tendency to grow on meat products with a pH value of around 6.0, while it grew poorly or

not at all on meats near or below pH 5.0.

Use of Packaging Atmosphere to Control the Growth of Bacteria in Meat

As early as 1882 a modified atmosphere with an elevated carbon dioxide (CO_2) concentration was known to extend the storage life of fresh meats by 4 to 5 weeks (Holland, 1980). The first application of modified atmospheres with high levels of carbon dioxide as a preservative method for the handling of fresh meat was during the 1930's, when beef carcasses were shipped from Australia and New Zealand to Great Britain (Silliker and Wolfe, 1980). Today, Modified Atmosphere Packaging, MAP, is used for packaging of fresh meat such as beef, lamb, and poultry for industrial and consumer uses in North America (Young et al., 1988).

Modified atmosphere packaging is defined by Young et al. (1988) as the "enclosure of food products in high gas barrier materials, in which the gaseous environment has been changed to slow respiration rates, reduce microbiological growth and retard enzymatic spoilage-with the intent of extending shelf life". In other words, modified atmosphere packaging (MAP) involves placing fresh meat in an environment in which the availability of oxygen has been changed. This is usually practiced either by removing the air (oxygen) by vacuum, or removing the air and then back-flushing with carbon dioxide, nitrogen, or a combination of the two (Brody, 1989)

A. Mode of action of different gases in MAP

Three main gases that are commonly used in MAP systems are oxygen, nitrogen, and carbon dioxide, although trace gases such as carbon monoxide, nitrous oxide, and sulfur dioxide have also been reported as possible gases for MAP of foods. In addition to inhibiting the growth of strictly aerobic bacteria, oxygen can also help to keep the meat myoglobin in its oxygenated form, oxymyoglobin. This would allow the bright red color, which most consumers associate with fresh red meat, to be maintained (Farber, 1991).

Nitrogen, on the other hand, is an inert filler gas that prevents packaging collapse when CO_2 dissolves in meat tissue (Huffman, 1974; Farber, 1991). It has no antibacterial activity of its own. However, by displacing the oxygen, nitrogen delays oxidation rancidity and, also, inhibits growth of aerobic bacteria (Farber, 1991).

Carbon dioxide is a lipid and water soluble gas that has bacteriostatic effect on microorganisms (Farber, 1991). In a MAP system, CO_2 is adsorbed on the surface of food and causes ionization of carbonic acid. As a result of this, the pH of the food will change. The bacteriostatic effect of carbon dioxide is, therefore, thought to be due to interference by the carbonic acid and pH with enzymes attached to the cell, and cellular dehydrogenating enzymes (Brody, 1989). In addition, King and Nagel (1975) reported that carbon dioxide may exert its inhibitory action on aerobic spoilage bacteria by interfering with decarboxylating enzymes through the mass action effect. Lastly, Sears and Eisenberg (1961) postulated that CO_2 can dissolve in the bacterial cell membrane, and cause disruption of the membrane and its function.

B. Effect of MAP on growth of spoilage organisms

Microorganisms are ubiquitous in nature and they are the major cause of deterioration for most foods. The most predominant spoilage bacteria in an aerobically-packaged meat are strains from *Pseudomonas, Moraxella,* and *Acinetobacter*. When stored at refrigeration temperatures, these Gram negative bacteria can grow faster than the competing species, and at higher temperatures, their growth rates become even faster. When meats are packaged under vacuum, or under some lower concentrations of oxygen, the *Pseudomonas* species are dominated by a combination of hetero- and homofermentative *Lactobacillus* species. In a CO_2-enriched environment, carbon dioxide inhibits the growth of Gram negative microorganisms like *Pseudomonas,* and promotes growth of lactic acid bacteria from such genera as *Streptococcus* and *Lactobacillus* (Brody, 1989). This shift from an initial Gram negative, aerobic spoilage organisms to a mostly Gram positive, facultatively anaerobic microflora is considered beneficial because the by-products from the lactobacilli metabolism are usually produced very slowly, and they are not offensive when compared to those produced by the pseudomonads (Farber, 1991).

C. Effect of MAP on growth of *Clostridium perfringens* and *Yersinia enterocolitica*

In a study conducted by Parekh and Solberg (1970), it was shown that at 43°C, *C. perfringens* had the same growth rate in fluid thioglycollate broth either in a 100% CO_2 or 100% N_2 atmosphere. On the other hand, Hintlian and Hotchkiss (1987) demonstrated that the outgrowth of this pathogen in cooked roast beef, preinoculated with *Pseudomonas fragi* , was prevented when samples

were packaged under 75% CO_2/25% O_2 and stored at 12.8°C. However, when the same samples were also inoculated with *Salmonella typhimurium* and *Staphylococcus aureus*, *C. perfringens* could not grow under air or 75% CO_2/10% O_2/15% N_2 during storage at 4°C.

While Johnston et al. (1982) recovered *Y. enterocolitica* from 66 out of 150 vacuum-packaged beef samples, Silliker and Wolfe (1980) reported that elevated levels of CO_2 had little effect on the growth of this pathogen in meat. Gill and Reichel (1989), on the other hand, found that growth of *Y. enterocolitica* in high-pH beef packaged under 100% CO_2 was influenced by the storage temperature. The pathogen was able to grow at 10°C; however, decreasing the storage temperature to 5°C prolonged the lag phase of the organism. Finally, the growth of *Y. enterocolitica* was totally inhibited during storage at 2°C or below.

D. Effect of MAP on survival of *L. monocytogenes*

Despite the numerous studies that have been done on the effect of MAP on survival of *Clostridium perfringens*, *Yersinia enterocolitica*, and *Pseudomonas fragi* very few studies have been conducted to investigate the effect of modified atmosphere packaging on the survival of *L. monocytogenes* in meat. Johnson et al. (1986) inoculated ground beef with *L. monocytogenes* at a concentration of 10^5 to 10^6 cells/g, and then packaged the samples in either oxygen-permeable or oxygen-impermeable bags before storing them at 4°C for two weeks. The number of *L. monocytogenes* in the ground beef remained constant during the incubation period and was not influenced by the oxygen permeability. Gill and Reichel (1989) found *L. monocytogenes* unable to multiply on beef with high pH and packaged in 100% CO_2 at 5°C or below, while

the pathogen was able to grow in CO_2-packaged meat held at 10°C, as well as on vacuum-packaged meat stored at 0, 2, 5, and 10°C. Similarly, Wimpfheimer et al. (1990) found that *L. monocytogenes* did not survive well in minced raw chicken packaged under the 75% CO_2/25% N_2 atmosphere, but the organism grew when oxygen was present, even at concentrations as low as 5%.

Recently, the effect of modified atmosphere of 20-40% CO_2 with or without O_2 on growth of *L. monocytogenes* serotype 4b in pork chops was determined. Manu-Tawiah (1991) found that numbers of *L. monocytogenes* in the pre-inoculated chops that were packaged under 20% CO_2/0% O_2 atmosphere were higher than, but not significantly different from, the numbers in the chops packaged in the 40% CO_2/0% O_2 gas mixture. Similarly, no difference in numbers of this pathogen was observed between chops packaged in the 40/0 gas mixture and those packaged in the 40% CO_2/10% O_2. Therefore, it was suggested that increasing the concentration of oxygen during packaging did not affect the number of *Listeria* on pork chops kept at 4°C. These findings also indicated that, in general, the numbers of *L. monocytogenes* in air or in vacuum-packaged samples were lower than, but not significantly different from, those packaged in the presence of gas. Manu-Tawiah (1991) also reported that although the number of *L. monocytogenes* did not change significantly under various gas combinations, the growth rate of the bacterium changed with the type of atmosphere present. When the CO_2 concentration was increased from 20 to 40%, there was no change in growth rate, but when 10% oxygen was added to the 40% CO_2 mixture, the growth rate of *L. monocytogenes* decreased significantly.

Use of Bacteriocins to Prevent Growth of Bacteria

Bacteriocins are proteinaceous substances that are antibacterial in nature, and are produced by a variety of microorganisms. According to Tagg et al. (1976), there are some criteria that can be used to identify antimicrobial compounds as bacteriocin: they are made of protein, they have bactericidal activity on a narrow spectrum of cells, and they require a specific receptor on the sensitive cells. Although these criteria have been used to identify bacteriocins such as colicins, produced by *Escherichia coli*, they may not be as complete for defining the bacteriocins from Gram positive bacteria. This is because the bacteriocins from Gram positive bacteria are very heterogeneous in their spectrum of activity. They affect a broader spectrum of microorganisms from different genera and species, and they are involved in nonspecific bacteriocin receptors on the host cells (Tagg et al., 1976; Klaenhammer, 1988).

Among the Gram positive microorganisms, Lactic Acid Bacteria (LAB) have been notorious for their ability to produce bacteriocins. Some examples of bacteria that are also used as starter cultures in food fermentation include: *Lactococcus lactis* (Jarvis and Farr, 1971), *Lactobacillus fermentii* (DeKlerk and Smit, 1976), *Lactobacillus helveticus* (Upreti and Hindsdill, 1975), *Lactobacillus acidophilus* (Barefoot and Klaenhammer, 1983), *Pediococcus pentosaceus* (Daeschel and Klaenhammer, 1985), and *Pedicoccus acidilactici* (Bhunia et al., 1987; Ray et al., 1989).

One of the earliest known bacteriocins produced by lactic acid bacteria is nisin. This bacteriocin, which is produced by a strain of *Lactococcus lactis*, was shown to inhibit the growth of spores from *Bacillus stearothermophilus* and

Bacillus coagulans in a variety of canned foods (Campbell et al., 1959). It has been suggested that nisin could reduce the thermal process that is required to stop the thermophilic spoilage in foods. Although in low pH-canned foods, such as tomato juice, tomato paste, pineapple, and pear, *C. botulinum* is not usually a major concern, Campbell et al. (1959) proposed that addition of nisin to such foods could be helpful in minimizing the thermal process required to stop the growth of this pathogen. In another study, Wheaton and Hays (1964) showed that nisin at a concentration of 2.5 ppm could stop the flat-sour spoilage in cream-style corn and chow mein. Today some European countries use nisin and nisin-producing starter cultures in food for their antibacterial properties during food processing and milk fermentation (Klaenhammer, 1988).

Recently some researchers have shown nisin to be inhibitory to *L. monocytogenes*. For example, Chung et al. (1989) showed that when nisin was added to beef samples at a concentration of 10^4 IU/ml, growth of *L. monocytogenes* Scott A was delayed for at least one day at room temperature. This study also indicated that the inhibitory effect of nisin was even greater at lower temperatures. For instance, when the meat samples, treated with the same concentration of nisin, were incubated at 5°C, growth of *L. monocytogenes* was delayed for more than two weeks.

The results from another study (Harris et al., 1991) indicated that nisin at a concentration of 10 µg/ml could decrease the initial concentration of *L. monocytogenes* (10^9 CFU/ml) by 6 to 7 logs. The sensitivity of this bacterium to nisin was shown to be intensified by addition of 2% NaCl or by reduction of the medium pH from 6.5 to 5.5 with lactic or hydrochloric acid. Similarly, El-Khateib et al. (1993) demonstrated that the addition of nisin ($4x10^4$ IU/ml) to cubical

pieces of beef (1 cm^3) that had been pre-inoculated with *L. monocytogenes* serotype 4b decreased listerial counts by 1.1 log CFU/6 cm^3 of meat surface within 48 hours of storage at 4°C.

Recently, some strains of *Pediococcus acidilactici* have been the focus of many studies due to their potential for antilisterial action. Pucci et al. (1988) found that a dried powder made from *Pediococcus acidilactici* PAC 1.0 culture supernatant supplemented with 10% milk powder contained a bacteriocin (pediocin PA-1) that was inhibitory to *L. monocytogenes*. Pediocin PA-1, which is known to be a protein with a molecular weight of 16,500 daltons (Gonzales and Kunka, 1987), was shown to be listericidal in APT broth within a pH range of 5.5 to 7.7 at 4 and 32°C. Pucci et al. (1988) also reported that pediocin PA-1 inhibited the growth of *L. monocytogenes* in some dairy products that had been pre-inoculated at an initial level of 10^2-10^4 CFU/g or ml sample at 4°C. The listericidal activity of pediocin PA-1 was not limited to dairy products. In a different study, Foegeding et al. (1992) demonstrated that this bacteriocin could help to maximize the safety of a fermented sausage by inhibiting the growth of *L. monocytogenes* both during the drying and fermentation steps.

Pediococcus acidilactici H is another example of a bacteriocinogenic organism among lactic acid bacteria that was originally isolated from a fermented sausage (Bhunia et al., 1987). Upon its purification, Bhunia et al. (1988) designated the name pediocin AcH to this bacteriocin and demonstrated that it was effective against many microorganisms, including both the spoilage and pathogenic bacteria such as *L. monocytogenes*. These investigators identified the bacteriocin as a peptide with a molecular weight of 2,700 daltons that was sensitive to proteolytic enzymes. They also found that pediocin AcH was

resistant to autoclaving conditions (121°C for 15 minutes), and that it was stable at pH values of 2.5 to 9.0. Production of this bacteriocin was shown to be encoded by a 33 kb fragment on an 11.4 kb plasmid in *P. acidilactici* H (Ray et al., 1992).

A. Qualitative method for detecting bacteriocin

There are different methods for determining bacteriocin activity. However, in the absence of a known chemical test that correlates with a specific biological activity of the bacteriocin, often demonstration of its antagonistic reaction is used to estimate the activity of the bacteriocin (Mayr-Harting et al., 1972; Tagg et al., 1976). One of the most well known methods of assay is the Critical Dilution Method. As defined by Mayr-Harting et al. (1972), this method consists of:

" (a) preparation of a series of dilutions of the sample (usually a two-fold series has been used);

(b) deposition of uniform drops from each dilution onto the surface of a plate of nutrient medium that has been seeded with a uniform and standard inoculum of the sensitive indicator strain (a double-layer plate has often been used);

(c) after a standardized period of incubation, examination of the degree of inhibition due to each drop, and the choice of an arbitrary end-point (usually the last dilution showing complete inhibition of the indicator strain)."

The Critical Dilution Method is simple to perform, and it is reproducible ,as long as the number of indicator strain per plate is kept constant and the incubation period is the same all the time (Goebel et al., 1955).

B. Mode of action of pediocin AcH

The mode of action of pediocin AcH on sensitive bacterial strains has been determined by Bhunia et al. (1991). These investigators hypothesized that the bactericidal effect of pediocin involves several steps. At first, the bacterium adsorbs the protein on the cell surface. This adsorption has been shown to occur on Gram positive but not on Gram negative bacteria. The receptor, or major site of attachment, was determined to be the lipoteichoic acid (LTA) portion of the cell wall which is only present in Gram positive bacteria. The binding of pediocin AcH was found to be rapid with a maximum occurrence at pH 6.0 both at 0 and 25°C. Chloride and phosphate salts were shown to compete with pediocin AcH molecules for binding to LTA sites, therefore they decrease the adsorption efficiency of this bacteriocin to sensitive and resistant Gram positive bacteria.

Upon the binding of pediocin AcH to specific receptors on the surface of the sensitive cells, the barrier property of the cell wall is believed to be disorganized. As a result, the pediocin comes in contact with the cytoplasmic membrane, causing the loss of potassium ions, U.V.-absorbing materials, and other small molecules. Bhunia suggested that in some strains the adsorption of pediocin AcH to the sensitive cells subsequently destroys the structural moiety of the cell membrane and the cell lyses.

C. Factors affecting the production of pediocin AcH

Perhaps the most complete work on determining the effect of different growth conditions on production of pediocin AcH is the one by Biswas et al. (1991). The result of this study showed that *P. acidilactici* H produced maximum

amounts of pediocin in TGE broth with the following composition: Trypticase (1%), glucose (1%), yeast extract (1%), tween 80 (0.2%), Mn^{2+} (0.033 mM), Mg^{2+} (0.02 mM) with pH 6.5 within 16 to 18 hours at 30 to 37°C. These results also indicated that a low final pH (3.6 to 3.7) and a large cell mass were necessary for production of high amounts of pediocin AcH.

D. Effect of pediocin AcH on *L. monocytogenes*

In addition to some spoilage organisms, Pediocin AcH has also been shown to be effective against some pathogenic bacteria such as *Yersinia enterocolitica* and *Listeria monocytogenes* (Bhunia et al., 1991). In fact, due to involvement of *L. monocytogenes* in foodborne outbreaks, coupled with the awareness of the listericidal property of pediocins, many researchers have conducted studies on the inactivation of *L. monocytogenes* by pediocin AcH and related pediocins (Pucci et al., 1988; Nielsen et al., 1990; Motlagh et al., 1992; Yousef et al., 1991; Degnan and Luchansky, 1992; El-Khateib et al., 1993).

Nielsen et al. (1990) grew a commercially-available strain of *P. acidilactici* in MRS broth supplemented with 2% yeast extract and studied the antimicrobial efficiency of the bacteriocin, produced by this organism, against *L. monocytogenes* Scott A and KC1714 in irradiated fresh beef. They found that when samples were originally inoculated with a low cell suspension (about 10^4/ml), a 1 to 2 $\log_{10}$ cell reduction occurred within 2 minutes with pediocin concentrations between 500 to 5000 AU/ml. However, when the meat samples were previously inoculated with a high number of *Listeria* cells (about 10^7/ml), about a 2-$\log_{10}$ cell reduction was observed at a pediocin concentration of 5000 AU/ml, with the highest reduction occurring during the first 2 minutes of

incubation. In this study, when 1000 AU/ml pediocin was used, the reduction was slightly more than 1 $\log_{10}$, but with the smallest concentration of the bacteriocin (500 AU/ml) the cell reduction was less than one log. These investigators did not observe any inactivation of the bacteriocin by the meat.

The listericidal effect of pediocin AcH has been demonstrated in meat, as well as in dairy products. In part of their study, Motlagh et al., (1992) conducted an experiment in which five pre-sterilized foods such as ground beef, sausage mix, cottage cheese, ice cream, and reconstituted dry milk were inoculated with about 10^7 CFU/ml *L. monocytogenes* Scott A or *L. ivanovii*. These foods were challenged with different concentrations of pediocin AcH ranging from 300 to 1,350 AU/ml at 4°C for 1 hour. The results of this experiment showed that, as the concentration of pediocin increased, the inhibition of strain Scott A increased from 10% at 300AU/ml to 91% at 1350 AU/ml. However for *L. ivanovii* the cell reduction was from more than 4 logs at 300 AU/ml to above 7 logs at 1350 AU/ml. It was also reported that the degree of inhibition of individual strain by pediocin was the same in all foods. In addition, it was concluded that the maximum inhibition by pediocin AcH occurred during 1 hour, and that it was not interrupted by the foods.

The inhibitory action of pediocin on *L. monocytogenes* was also demonstrated in processed meat. Yousef et al., (1991) applied pediocin AcH or an antibiotic resistant derivative of *P. acidilactici* H, known as *P. acidilactici* JBL1095, to challenge the growth of *L. monocytogenes* in wiener exudates at 4 or 25°C. The results from this experiment indicated the inactivation of the pathogen by both pediocin and the producer strain at either temperature. In addition, a rapid decrease in *Listeria* numbers were shown to occur in samples with pediocin

during the initial incubation hours at 4°C (0.74 $\log_{10}$ reduction in CFU/ml in 2 hours). The inactivation at 25°C, on the other hand, was more gradual (Yousef et al., 1991). It was also reported that when the producer strain was added to the sample, the pathogen grew during the first 64 hours of storage at 25°C and then decreased dramatically (5.84 $\log_{10}$/ml in 3 days).

Degnan and Luchansky (1992) compared the listericidal activity of pediocin AcH in beef tallow with that in beef muscle. After treating the samples with pediocin, they observed more bacteriocin recovery from tallow than muscle slurries. In addition, they found a higher saturation capacity of pediocin (100,000 AU) for muscle than for tallow (80,000-90,000 AU). The combination of these two results led the investigators to conclude that the higher loss of pediocin activity in muscle slurries might be due to higher protein content in muscles. Since pediocin AcH has the tendency to aggregate in the presence of proteins, while still having some antibacterial activity, this could also explain why less bacteriocin activity was recovered from muscle samples (Degnan and Luchansky, 1992).

Heat Shock Response

When bacteria are exposed to elevated sublethal temperatures, they respond by producing a small number of highly conserved proteins, known as heat shock proteins, or hsps (Lindquist, 1986; Burdon, 1986). This response, known as the heat shock response, is universal. It has been found in eubacteria, archaebacteria, as well as in plants and animals (Lindquist, 1986). Heat shock response was originally discovered in the fruit fly *Drosophila* (Ritossa, 1962), but

now it has been found to occur among many bacteria such as *Bacillus subtilis* (Streips and Polio, 1985), *Lactococcus lactis* (Whitaker and Batt, 1991), *Caulobacter crescentus* (Gomes, et al., 1986), *Salmonella typhimurium* (Spector et al., 1986; Morgan et al., 1986), and *Escherichia coli* (VanBogelen et al., 1987; Christopher and Fridovich, 1987).

The heat shock response involves production of a variety of proteins (hsps) in the organism. Streips and Polio (1985) grew different strains of bacilli in minimal growth medium at 37°C, and then exposed the cells to 48°C for different periods of time. As a result, cells responded by producing at least 12 proteins with molecular weights of 14,000 to 97,000 daltons. Neidhardt et al. (1984) identified about 17 heat shock proteins in *E. coli* with molecular weights of 10,000 to 94,000 daltons.

The induction of heat shock protein synthesis occurs at various temperatures in different organisms. However, in each case the temperature corresponds to the upper range of the natural growth temperature of the organism. Induction of these proteins enables the cells to be tolerant to toxic effects of heat and other forms of stress (Lindquist, 1986). In organisms that grow within a broad range of temperatures, the maximum response usually occurs at 10-15°C above the optimum growth temperature. In organisms that grow over a narrow range, the maximum response takes place at about 5°C above the optimum (Lindquist, 1986).

Pre-exposure of cells to a sublethal elevated temperature enables the cells to withstand a higher temperature treatment. Yamamori and Yura (1982) showed that incubation of *E. coli* at 42°C before exposing them to 55°C led to slower rate of cell death than a direct switch from 30°C to 55°C. This

thermotolerance was found to be transient. It allowed the cells to withstand 55°C for up to 30 minutes, and after 60 minutes it disappeared. Similarly, Mackey and Derrick (1987) found that when a suspension of *Salmonella thompson* in minced beef was heat shocked at 48°C for 30 minutes, the cells became resistant to 54 and 60°C. As a result, the respective D values for the control (37°C) and heat-shocked cells were 10.5 and 25 minutes at 54°C and 0.46 and 1.26 minutes at 60°C. This study indicated that, upon a prior heat shock at 48°C for 30 minutes, the D values for the cells at 54 and 60°C increased 2.4 and 2.7 times, accordingly.

In addition to acquiring thermotolerance, heat shock proteins may also protect the cells against other stresses. In *E. coli*, for instance, a cross-resistance between heat and ethanol has been reported. Cells that were grown at 42°C , rather than at 37°C, became more resistant to a subsequent exposure to 55°C or ethanol (Neidhardt et al.,1984). In another study, Christopher and Fridovich (1987) showed that when the midlog cells of *E. coli* B, growing aerobically at 37°C, were transferred to 48°C for 1 hour, the organism responded by sythesizing large quantity of manganese-containing superoxide dismutase (MnSOD). These results indicated that heating increased the concentration of superoxide ($\cdot O_2$), and of hydrogen peroxide-producing autoxidations in *E. coli*, by disrupting the electron transport system of the cytoplasmic membrane, and the high $\cdot O_2$ production, increased synthesis of MnSOD (Christopher and Fridovich, 1987).

A. Functions of heat shock proteins

The specific functions of hsps are not totally clear; however their increased synthesis at high temperatures indicate that they are somehow involved in protection of cells at the elevated temperatures. For example, large amounts of

gro EL in *E. coli* have been found associated with the unfolded chromosome therefore preventing further unfolding (Neidhardt et al., 1984). Another heat shock protein, *lon* protease, plays a major role in degrading abnormal proteins (Neidhardt et al., 1984; Burdon, 1986). Bensaude et al. (1990) proposed that heat shock denatures some cellular proteins. These damaged or "abnormal" proteins then unravel themselves so that their hydrophobic internal domains become exposed. Heat shock proteins then become insoluble in the cell and bind to "abnormal " proteins through the hydrophobic region. This binding serves two functions: it stops aggregation of the unfolded proteins, and it protects the cellular proteins from further heat damage.

B. Heat shock response in *L. monocytogenes*

Heat shock response has also been observed in *L. monocytogenes*. Fedio and Jackson (1989) grew this organism in Trypticase Soy Broth with 0.6% Yeast Extract (TSBYE) at 37°C for 24 hours, and then exposed the cells to a preheating condition of 48°C for 1 hour. Both the heat-shocked and nonheat-shocked cells (37°C) were further heated at 60°C. Aliquots from both cultures were plated on TSAYE and LPM (Lithium chloride Phenylethanol Moxalactam) at every 5-minute interval. A comparison of the growth on the nonselective (TSAYE) with that on the selective (LPM) media indicated more than 3-$\log_{10}$ higher survivor difference for the heat-shocked cells after 10 to 20 minutes. When the heat shock experiment was done in ultra high temperature (UHT) milk, the number of survivors became slightly smaller than when the experiment was done in TSBYE.

Knabel et al. (1990) studied the effect of heat shock (43°C), in combination

with aerobic or anaerobic plating, on survival of stationary *L. monocytogenes* F5069 to 62.8°C. The results from this study indicated a 6-fold higher D-value at 62.8°C in sterile milk for cells that had been grown at 43°C for 18 hours and enumerated anaerobically than cells that were held at 37°C and then heated at 62.8°C and plated aerobically. It was also proposed that, based on the conditions of this study, high concentrations of *L. monocytogenes* might survive the minimum low-temperature, long-time treatment required by the U.S. Food and Drug Administration for pasteurizing milk.

Bunning et al. (1990) speculated on the effect of different heat shock temperatures (42, 48, and 52°C) for 5 to 60 minutes on the survival of *L. monocytogenes* to either 52 or 57.8°C. The results of this experiment indicated that when cells were heat shocked at 42°C or 48°C, they showed 1.1-1.4 fold higher D-value at 57.8°C than nonheat-shocked cells (35°C). It was also reported that this acquired thermal tolerance remained in the heat shocked cells for up to 4 hours. However, when the heat-shocked cells were transferred back to 35°C, the thermal tolerance disappeared in about 1 hour.

The effect of prior heat shock on survival of *L. monocytogenes* in meat to higher temperature was determined by Farber and Brown (1990) . In this experiment a large number of the organism (10^7/g) was initially inoculated in a fermented sausage mix (66% pork, 33% beef). Meat samples were then sealed under vacuum and kept overnight at 4°C. After 24 hours, the pre-inoculated meat samples were subjected to heat shock temperatures of 40, 44, 48, and 52°C for up to 120 minutes, followed by a heat treatment of either 62°C for 0, 2, 7.5, 15, 25, and 35 minutes or 64°C for 0, 2, 4, 6, and 8 minutes. Enumeration of the cells were done by plating aliquots onto TSAYE plates and incubating them at 25 to

30°C for 7 days.

The results from this experiment indicated that the optimum temperatures for induction of the highest thermotolerance in *L. monocytogenes* were 44 and 48°C. Therefore, heat shocking temperature of 48°C was selected for further heat treatment of the cells. These results also showed that when cells were heat shocked at 48°C for only 30 minutes, there was no significant difference between the D-values of the control and heat shocked cells at both temperatures (62 and 64°C). In contrast, when the heat shock time was increased from 30 minutes to 60 and 120 minutes, the $D_{64°C}$ values became significantly different, especially with the 2-hour heat shock time inducing the greatest amount of protection for the cells (more than 2-fold higher D-value for the heat-shocked cells than for the control cells).

Farber and Brown (1990) also studied the effect of the incubation period between the heat shock (48°C for 1 hour) and the heat treatment (64°C) on the resistance of *L. monocytogenes* in meat to heat. They noticed that when the meat was held at 4°C for 24 hours, the resistance to final temperature (64°C) decreased in both the heat-shocked and control cells. However, the ratios between the recovered control and recovered heat-shocked cells at each time (0 h/4°C and 24 h/4°C) remained constant. As a result of this, it was concluded that the acquired thermotolerance in heat-shocked cells could last for at least 24 hours at the refrigeration temperature. Farber and Brown (1990) found the results of this experiment to be important to the meat industry in two ways: meats that are heat processed slowly, such as in the Sous-Vide type products, may have microorganisms that could be heat shocked during processing and, hence, acquire thermal tolerance. Also meats that are kept on warming trays before

being heated again might provide the opportunity for certain bacteria to become heat resistant.

In another study, Linton et al. (1990) heat shocked the log phase cells of *L. monocytogenes* Scott A in TSYE broth at 40, 44, and 48°C for 3, 10, and 20 minutes at each temperature before the cells were heated again at 55°C for 50 minutes. At each time interval, the cells were enumerated by placing aliquots onto TSAYE (nonselective) and McBride *Listeria*, or ML (selective) solid media, followed by incubation of the plates at 37°C for 48 hours. The results from this study indicated that maximum thermotolerance occurred in cells that were heat shocked at 48°C for 20 minutes, where the D-value at 55°C was about 2.2 (during enumeration on TSAYE plates) or 1.6 (during enumeration on ML plates) times higher for the heat-shocked cells than for the nonheat-shocked cells. However, due to a higher inconsistency of the results obtained from the heat shock condition of 48°C for 20 minutes, the investigators proposed the heat shock condition of 48°C for 10 minutes to be the optimum heat shock criteria for maximum thermotolerance in *L. monocytogenes*. This optimum heat shock condition corresponded to a D-value at 55°C that was 2.3 (when enumerated on TSAYE) or 1.5 (when enumerated on ML) times higher for the heat-shocked cells compared to nonheat-shocked cells.

In a separate study, Linton et al. (1992) exposed the log phase cells of *L. monocytogenes* Scott A in TSYE broth to a heat shock condition of 48°C for 10 minutes, followed by a heat treatment of 55°C for up to 50 minutes. Enumeration of the cells was done on TSAYE and ML plates under aerobic and anaerobic atmospheres. The results from this study indicated that while using the nonselective medium (TSAYE), the D-values at 55°C for heat-shocked cells

were 2.1 (during aerobic incubation) and 2.2 (during anaerobic incubation) times higher than the ones for the nonheat-shocked cells. Similarly, when the D-values of both cells were compared on the selective medium (ML), it was found that the heat-shocked cells had a 1.4-fold higher $D_{55°C}$ value under aerobic condition. However, no growth was detected on the selective medium under anaerobic environment. In addition, it was noticed that after heating at 55°C for 50 minutes, the nonheat-shocked cells on TSAYE medium that were incubated anaerobically had a higher number of survivors than the same kind of cells that were plated on TSAYE but had been incubated aerobically. Also the results showed that the heat-shocked cells had a 3-$\log_{10}$ higher survivors on nonselective than on selective medium under aerobic condition. The nonheat-shocked cells, on the other hand, had a lesser number of survivors (only 2 $\log_{10}$ higher) on TSAYE than on McBride *Listeria* agar plates under aerobic condition. Linton et al. (1992) concluded that, similar to the results obtained by Knabel et al. (1990), their study also indicated that anaerobic incubation of the nonheat-shocked cells on the nonselective medium gave rise to a higher number of cells than when the cells were enumerated under aerobic conditions.

In addition to production of thermotolerance in *L. monocytogenes*, heat shock can also increase production of Listeriolysin O by these cells. In a study conducted by Sokolovic and Goebel (1989), it was shown that when cells of *L. monocytogenes* SV1/2a strain SLCC5764 were heat shocked at 48°C for 30 minutes, the cells started producing 12 to 14 different heat shock proteins with molecular weights between 20 to 120 kilodaltons. After separation of these bands through sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by the immunoblotting of the protein bands with antibiotics against

Streptolysin O (SLO), it became evident that Listeriolysin O (LLO) with the molecular weight of 58 kilodaltons was the only major heat shock protein that had been secreted extracellularly during the heat shock.

The enhanced production of Listeriolysin O, which is present in all virulent strains of *Listeria*, under heat shock conditions was also demonstrated by Kim (1992). In this study, the stationary phase cells of *L. monocytogenes* serotype 1 were heat shocked at 48°C for 2 hours, followed by a heat treatment at 62°C for 10 minutes. A comparison between the bacterial counts on the TSAYE (nonselective) and OXA, or Oxford Agar, (selective) indicated higher number of survivors for the heat-shocked cells on the former. However, no hemolysin activity (due to Listeriolysin O production) was detected immediately after heat shock or heat treatment. It was suggested that this decrease in production of LLO could be due to cell injury caused by the heat shock, or it could be because of inactivation of the Listeriolysin O by the heat treatment. On the other hand, when the heat-shocked cells were incubated at 37°C for 4 hours, there was a 40-fold increase in production of LLO as compared to the 2-fold increase for the nonheat-shocked cells.

As it was explained, *L. monocytogenes* is a potential foodborne pathogen that has been involved in several outbreaks of foodborne illness in humans. The organism is prevalent in nature, and it is associated with domestic livestock. This provides an opportunity for the pathogen to contaminate the meat in slaughter houses and processing plants. Since *L. monocytogenes* is a psychrotroph, refrigeration of meat alone cannot guarantee its growth inhibition in such a product. Therefore, additional preventive actions should be taken in order to eliminate this organism from fresh meat. In recent years, the use of

"natural biopreservatives" such as bacteriocins have been shown to greatly reduce the number of *Listeria* in different foods. In addition, vacuum and modified atmosphere packaging have been very effective in reducing the growth of this pathogen in meat. The objectives of this study were to determine the effect of heat shock, pediocin, and packaging atmosphere on survival of *L. monocytogenes* in fresh pork, as well as to determine the effect of pediocin on meat color, odor, and texture.

MATERIALS AND METHODS

Bacterial cultures and growth conditions: *Listeria monocytogenes* Scott A (serotype 4b) and *Listeria ivanovii* ATCC 19119 were obtained from the Iowa State University Department of Food Science and Human Nutrition (Ames, IA), and American Type Culture Collection (Rockville, MD), respectively. *Pediococcus acidilactici* was kindly provided by Dr. J. S. Dickson (Meat Animal Research Center, Agricultural Research Service, U.S. Department of Agriculture, Clay Center, NE). Cultures of *Listeria* were maintained on Trypticase Soy Agar (BBL Microbiological Systems, Cockeysville, MD) slants plus 0.6% Yeast Extract (Difco Laboratory, Detroit, MI). *P. acidilactici* was maintained in MRS broth (Difco) with 2% Yeast Extract (Difco). All bacterial cultures were kept at 4°C, and transferred on a monthly basis.

Preparation of "crude" bacteriocin: Extraction of pediocin was performed by a modified method of Nielsen et al. (1990). Briefly, *P. acidilactici* was grown in 100 mls of MRS plus 2% Yeast Extract (MRSYE) for 16 hours at 37°C. The cells were removed via centrifugation at 8,000 x g (Beckman Instruments, Inc., Palo Alto, CA) for 15 minutes at 4°C. The supernatant was first neutralized with 8N NaOH and then heated for approximately 5 minutes to inactivate the proteolytic enzymes. After cooling it down at 4°C, the supernatant was filter-sterilized through a 0.45 µm-pore- size membrane (Costar Corporation, Cambridge, MA) and stored at 4°C.

Determination of bacteriocin activity: The Arbitrary Units (AU) of activity of the pediocin was determined using a 5 ml soft TSAYE (Trypticase soy broth with 0.75% agar and 0.6% yeast extract) overlay containing 10^6 CFU *L. ivanovii*

(as the indicator bacterium). The bacteriocin was serially 2-fold diluted in 0.1% peptone (Difco) water. five µl from each dilutions of pediocin was spotted on the seeded plates. All plates were incubated at 37°C for 24 hours. The reciprocal of the highest dilution of pediocin that produced a visible zone of inhibition (≥ 2 mm) on the plates was used to calculate the AU of activity of pediocin per ml of the original stock solution.

Statistical analysis of the results: Throughout this study, survival of *L. monocytogenes* and *P. acidilactici* in pork chops, as well as the pH, color ,and texture of the meat were analyzed by comparing the means and standard deviations obtained from different observations. The results from sensory evaluations were studied by performing Analysis of Variance (ANOVA), using the SAS. The statistical model was based on the randomized complete block, where replication was considered as block. The results from ANOVA were compared by performing a t-test (alpha = 0.05) among samples.

Effect of Heat Shock on Survival of *L. monocytogenes* Scott A to Pediocin

A. Heat shock procedure

L. monocytogenes was grown in Trypticase Soy Broth with 0.6% Yeast Extract (TSBYE) for 6 hours at 37°C. A dilution of the culture in the same broth with approximately 10^6 CFU/ml was prepared. Cells were heat-shocked by inoculating 2 mls of the *Listeria* culture in 10 mls of sterile TSBYE, preheated at 48°C in a water bath (model 730, Fisher Scientific, Pittsburgh, PA). The cell suspension was heat-shocked at 48°C for 10 or 20 minutes. The internal temperature of the broth was monitored with an iron-constantan thermocouple

(Omega Engineering, Inc., Stamford, CT) and datalogger (model LI-1000, LI-cor, Lincoln, NE). The nonheat-shocked or control cells were prepared by inoculating 2 mls of the *Listeria* suspension in 10 mls of pre-warmed TSBYE and incubating it at 37°C.

B. Pediocin treatment and enumeration of *Listeria*

After heat shocking *Listeria*, the cultures were immediately transferred to an ice-water bath for 30 seconds. Two-ml aliquots from each of the heat-shocked and nonheat-shocked cell suspensions were transferred to separate sterile tubes. One ml pediocin (204,800 AU) was added to half of the tubes when necessary. The other half received 1 ml of sterile TSBYE in order to adjust their volumes with those from the pediocin-treated samples. The tubes were transferred to a 37°C-water bath for 24h. At different time intervals, 1 ml from each reaction mixture was serially diluted in 0.1% peptone water and plated onto TSAYE (non-selective) and OXA (selective medium, Oxoid, Ogdenburg, NY). Enumeration of the *Listeria* colonies was performed by using the colony counter (Quebec darkfield colony counter, Reichert-Jung, Fisher Scientific, Pittsburgh, PA) after incubating the plates at 37°C for 48 h.

Effect of *P. acidilactici* and Pediocin on Survival of *L. monocytogenes* Scott A in Pork Chops under Different Atmospheric Conditions

A. Preparation of *Pediococcus* inoculum

P. acidilactici was grown in 100 ml of MRSYE broth for 16 h at 37°C. The cells were pelleted via centrifugation (8,000 x g), washed once with 0.1% peptone

water, and resuspended in the same solution to the original volume. The crude extraction of the pediocin and determination of its concentration in terms of Arbitrary Units (AU) of activity were performed as previously described.

B. Preparation of *Listeria* inoculum

L. monocytogenes was grown in TSBYE at 37°C for 6 h. The cells were pelleted by centrifugation (8,000 x g), washed, and resuspended to the original volume using 0.1% peptone water. Further dilutions of the culture were also performed in peptone water to obtain the appropreate concentrations of *Listeria*.

C. Preparation of the meat samples

Pork chops were obtained from a local retailer. The meat was aseptically cut into cubes of 25 g each. Meat samples were soaked in a 50-ml. suspension of *Listeria* in sterile beakers for 20 minutes to allow the attachment of approximately 10^4 CFU/g. Excess liquid was drained from the meat samples by placing them on sterile racks for 2 minutes. Meat pieces were further inoculated on the surface with either 10^6 CFU/g of *P. acidilactici* or 204,800 AU of pediocin. The meat samples were then sealed in plastic bags (Curlon 861, Curwood Inc., Oshkosh, WI) under air, vacuum or modified atmosphere, using a vacuum packaging machine (Model A300 Fresh vac, CVP systems Inc., Downers Grove, IL). The modified atmosphere consisted of 40% CO_2 and 60% N_2 (Freshpak, Air products and Chemicals Inc., Allentown, PA). The samples were stored at 4°C for 10 days.

D. pH measurement and enumeration of bacteria in meat

At different time intervals (days 0, 1, 5, 10), the pork chops were examined for pH and bacterial counts. The pH of the meat was determined by placing the pH meter (Model 910, Fisher Scientific, Pittsburgh, PA) probe on the surface of the meat. Enumeration of *L. monocytogenes*, *P. acidilactici*, total aerobic and total facultatively anaerobic bacteria was performed regularly by blending the samples in 0.1% peptone water in a Stomacher (Tekmar Co., Cincinnati, OH) for 2 minutes. After making the appropriate dilutions, 0.1 ml. aliquots were surface-plated onto OXA and MRSYE to enumerate *Listeria* and *Pediococcus*, respectively. TSAYE was also used for enumeration of total aerobic and total facultatively anaerobic bacteria. All plates were incubated aerobically at 37°C for 48 hours before the colonies were enumerated. *P. acidilactici* was identified based on its colony morphology on MRSYE agar and by performing a Gram stain. Presence of *Listeria* in the sample (if less than 2 $\log_{10}$) was detected through the use of a commercially-available Listeria ELISA kit (Listeria-Tek ELISA, Organon Teknika, Durham, NC). Further identification of *Listeria* colonies was confirmed by using the Listeria Micro ID test system (Organon Teknika), accompanied by Gram stain.

Effect of Heat Shock and Packaging Atmosphere on Survival of *L. monocytogenes* to Pediocin in Ground Pork

L. monocytogenes was heat-shocked at 48°C for 10 or 20 minutes in TSBYE as previously described. The heat-shocked cells were transferred to an ice-water bath for 30 seconds, and were immediately used for inoculation of ground pork.

Crude extraction of the pediocin was performed following the procedure discussed previously.

A. Preparation of meat samples

Ground pork (85% lean) was obtained in frozen packages from the Meat Laboratory at the Iowa State University. Frozen meat was thawed in the refrigerator (4°C) overnight prior to the experiment. On the day of the experiment, the meat was aseptically removed from the packages and placed in a large sterile beaker to make a homogenious meat mixture. Ground pork was transferred to several stomaching bags (Stomacher 400 closure bags, Seward medical, London, UK). Each bag received 10^3 CFU/g of heat-shocked or nonheat-shocked (37°C) *L. monocytogenes*. Meat samples were further inoculated with 8192 AU/g pediocin or equal volume of sterile peptone water (for the controls). Meat mixtures were first hand-massaged from the outside of the bags for 30 seconds, and then were stomached for 5 minutes. Twenty-five grams of the inoculated ground pork were transferred to several vacuum packaging pouches (Curlon 861, Curwood). The bags were sealed under air, vacuum, and modified atmosphere (40% CO_2, 60% N_2), and were stored at 4°C for 24 hours.

B. Enumeration and identification of *L. monocytogenes*

The samples were examined for the survival of *Listeria* after one day. Meat samples were blended with 0.1% peptone water in a stomacher for 2 minutes. Aliquots of the diluted mixture were spread-plated onto OXA, and the plates were incubated aerobically at 37°C for 48 hours. Presence of *L. monocytogenes* in meat was confirmed by using the Listeria ELISA kit and

Listeria Micro ID test system (Organon Teknika). Other complementary identification procedures performed on colonies from OXA medium were the Gram stain, catalase, and cytochrome oxidase tests.

Effect of Pediocin on the Color and Texture of Pork Chops

A. Preparation of meat samples

Pork chops were cut into cubes of 25 grams each. The samples were inoculated on the surface with 51200 AU of pediocin. Both the bacteriocin-treated and controls (without pediocin) were packaged in different plastic bags (Curlon 861, Curwood) under air. Meat samples were incubated at 4°C for 0, 1, 5, and 10 days. These samples were analyzed for their color and texture qualities at the end of each period.

B. Color and texture analysis of pork chops

Five samples were assigned per treatment throughout this experiment. Four readings were made from each cube of meat during the color analysis with the Hunter Color Lab (Hunter associate laboratory Inc., Reston,VA). Two samples from each cube of meat were also prepared by cutting the pork chops with a metal coring device of 12.7 mm in diameter during the texture analysis. The tensile force, or the amount of force necessary to shear the cored meat samples, was recorded with an Instron (model 4502, Canton, MA).

Sensory Analysis of Pork Chops

The pediocin-treated and control (without pediocin) pork chops were also examined by a trained-sensory panel of 11 members, following the procedure for a scaled descriptive analysis (Larmond, 1982). During the color evaluation, the anchor words used were "undesirable color" and "desirable color", from left to right. For the odor analysis, a separate descriptive line was labeled as "no off odor" and "off odor" from left to right (see the appendix). One meat sample from each treatment per day was used during either analysis. The panel members were asked to evaluate the color and odor of randomly-assigned meat samples by labeling the line based on the intensity of each attribute. The distance between the marked point and the beginning of the line for each sample was recorded and used for statistical analysis.

RESULTS

In this study, the effect of environmental conditions on survival of *L. monocytogenes* exposed to a bacteriocin was determined. The conditions tested were heat shock (48°C for 10 or 20 minutes) and packaging atmosphere. In addition, the effect of bacteriocin on quality of the meat, including color, texture and odor was examined.

Effect of Heat Shock on Survival of *L. monocytogenes* Exposed to Pediocin

The bacterial counts on both OXA (selective medium) and TSAYE (nonselective medium) plates were very similar during this study. Therefore, only numbers obtained from the counts on the latter are reported here. Heat shocking of *Listeria* at 48°C for 10 or 20 minutes resulted in lower number of survivors when compared to control cells within the first 2 hours of incubation at 37°C (Figure 1). *Listeria* cells heat shocked for 20 minutes showed slightly lower number of survivors as opposed to the cells that were heat shocked for only 10 minutes. Both the control and heat-shocked *Listeria* started increasing in numbers after 2 hours, and the difference ($\log_{10}$ CFU/ml) between the two decreased during the subsequent hours.

Addition of pediocin (204,800 AU, as obtained by following the procedure described in the Materials and Methods section) to *Listeria* cultures resulted in approximately 2 $\log_{10}$ reduction in control and heat-shocked cells during the first two hours. Both the heat-shocked and control cells multiplied in numbers during further incubation at 37°C, with no appreciable difference in the number

of survivors between them.

Effect of *P. acidilactici* and Pediocin on Survival of *L. monocytogenes* in Pork Chops Packaged Under Different Atmospheric Conditions

Growth of *Listeria* in meat inoculated with this organism was not affected by the type of atmosphere used during storage at 4°C (Figure 2).

Addition of *P. acidilactici* to pork chops previously inoculated with *L. monocytogenes* reduced the number of the latter by about 2 $\log_{10}$ within the first 24 hours of storage at 4°C (Figure 3). The atmospheric conditions for packaging of the samples had no impact on survival of the pathogen. Total number (log CFU/g) of *Listeria* survivors did not change during further storage under air, vacuum, or modified atmosphere.

Similar results were obtained when pediocin was added to pork chops (Figure 4). The bacteriocin brought about a 2-$\log_{10}$ reduction of *Listeria* in the meat during the first 24 hours of storage at 4°C. The $\log_{10}$ number of *Listeria* survivors did not change any further during subsequent storage, regardless of the packaging atmosphere.

A. Effect of *L. monocytogenes* and packaging atmospheres on survival of *P. acidilactici*

Growth of *Pediococcus* in meat inoculated only with this organism did not change throughout storage at 4°C, regardless of packaging atmosphere (Figure 5). Presence of *L. monocytogenes* in the pork chops did not affect the survival of *Pediococcus*, regardless of the incubation periods and packaging atmospheres

(Figure 6).

B. Total plate counts

The total numbers of aerobic bacteria (log_{10} CFU/g) in pork chops after inoculation with *Listeria*, *Pediococcus*, or pediocin over ten days of storage at 4°C are shown in Figure 7. Both the uninoculated (control) and pediocin-treated samples showed a slight increase in total bacterial counts after 24 hours. When meat samples were inoculated with *Listeria* or a combination of *Listeria* and pediocin, there was a slight decrease in the total counts after 24 hours. However when either *Pediococcus* alone, or *Pediococcus* along with *Listeria* were added to pork chops, a decrease in total counts of about 1.5 log_{10} was observed after 24 hours. The total bacterial counts increased in all samples until it reached 10^6 CFU/g after five days of storage. No further change in bacterial counts was observed for any of the samples from day 5 to day 10.

Figure 8 shows the total facultatively anaerobic bacterial counts from pork chops, previously inoculated with *Listeria*, *Pediococcus*, or pediocin, that were packaged under vacuum and plated aerobically. Similar to the results shown for aerobic bacteria, Figure 7, presence or absence of pediocin in fresh pork did not decrease the total bacterial counts after 24 hours. The total number of bacteria in uninoculated samples (controls), and in samples that only received pediocin, increased constantly until the number reached 10^4 CFU/g after ten days of storage. Inoculation of meat samples with *Listeria* or a combination of *Listeria* and pediocin did not lower the total bacterial counts significantly within 24 hours. The total number of facultative anaerobes did not increase as rapidly as the total aerobes (Figure 7) from day 1 to day 10. The former reached only to 10^4-

10^5 CFU/g (1.5 $\log_{10}$ less than the aerobes) by the end of day 10. Pork chops that received either *Pediococcus* or a combination of *Pediococcus* and *Listeria* exhibited 1.5 $\log_{10}$ reduction in total counts after 24 hours. However, no further change in total number of facultative anaerobes was seen in either of the samples during subsequent storage. The total bacterial counts in these samples remained at 10^6 CFU/g by the end of the incubation period.

The total number of bacteria from pork chops previously treated with pediocin or different microorganisms, packaged under modified atmosphere (40% CO_2, 60% N_2), and plated aerobically, are shown in Figure 9. The results from this experiment were very similar to those obtained for facultative anaerobes (Figure 8) in that presence or absence of pediocin did not influence the total bacterial counts after 24 hours. In addition, the total number of bacteria in pork chops did not increase as rapidly as the total aerobes (Figure 7) from day 1 to day 10. As with the facultative anaerobes (Figure 8), the total number of bacteria in pork chops packaged under modified atmosphere was 10^4-10^5/g by the end of day 10 (1.5 $\log_{10}$ lower than the aerobes). Moreover, presence of *Pediococcus* in pork chops brought about 1.5 $\log_{10}$ reduction in total counts after 24 hours. However, the total number of bacteria in these samples remained relatively constant throughout the rest of the storage period, so that by the end of day 10, the total bacterial count was 10^6 CFU/g.

C. Effect of *Listeria*, *Pediococcus*, pediocin, and different packaging atmospheres on pH of pork chops

Figure 10 shows the pH values from meat samples, inoculated with either of the bacteria or with pediocin, and packaged under air, vacuum, or modified

atmosphere. The pH of the meat remained the same throughout the ten-day incubation period at 4°C, regardless of the packaging atmosphere. Similarly, addition of a combination of bacteria and pediocin to pork chops did not influence the meat pH under air, vacuum, or modified atmosphere (Figure 11).

Effect of Heat Shock and Packaging Atmosphere on Survival of *L. monocytogenes* to Pediocin in Ground Pork

Heat-shocking of *Listeria* at 48°C for 10 or 20 minutes did not greatly affect its survival in aerobically packaged samples after 24 hours (Figure 12). Addition of pediocin to ground pork, previously inoculated with *Listeria*, caused approximately 1.5 $\log_{10}$ reduction in numbers of survivors both for the nonheat-shocked (controls) and heat-shocked cells. Similar results were obtained in the inoculated meat samples that were packaged under vaccum (Figure 13), or modified atmosphere (Figure 14).

Effect of Pediocin on Color and Texture of Pork Chops

The overall color quality (L, a, and b values) of the meat samples , previously treated with pediocin, did not change greatly when compared to those that did not receive the bacteriocin (Figure 15). Furthermore, the incubation period did not influence the color of the pediocin-treated and control pork chops during storage at 4°C.

In analyzing the samples for texture differences, the tensile forces (also known as the shear forces) that were necessary to cut the cored meat samples

from the pediocin-treated and control pork chops were compared (Figure 16). Presence of bacteriocin in meat did not change the texture quality of the samples when compared to the controls. In addition, the texture of all pork chops remained relatively unchanged throughout the ten-day storage at 4°C.

Sensory Analysis of the Pork Chops

Results from the analysis of variance on the color and odor of the pediocin-treated and control (without pediocin) pork chops are summarized in Table 1 and Table 2, respectively. These results are based on 3 separate replications. Statistical analysis was performed on a randomized complete block design, with replication being considered as the block. Data from the sensory evaluation were analyzed by compared t-test across the means for distances between the beginning of the scaled descriptive line and the points that were labled on the line by the sensory panel.

The analysis of variance on color quality of pork chops showed no significant difference between the control and bacteriocin-treated pork chops within each day (Table 1). The overall t-test across the paired samples (control and pediocin-treated) from different days showed significant differences among the groups during the ten-day storage at 4°C.

The statistical analysis on odor of pork chops indicated a significant difference between the control and bacteriocin-treated samples from day 0 (Table 2). However, no significant differences between the samples were observed within each subsequent days. An overall t-test across the paired samples from different days revealed samples from day zero not to be significantly different

from those from day 1. On the other hand, the 5- and 10-day-old samples were significantly different from one another and from the 1-day old samples.

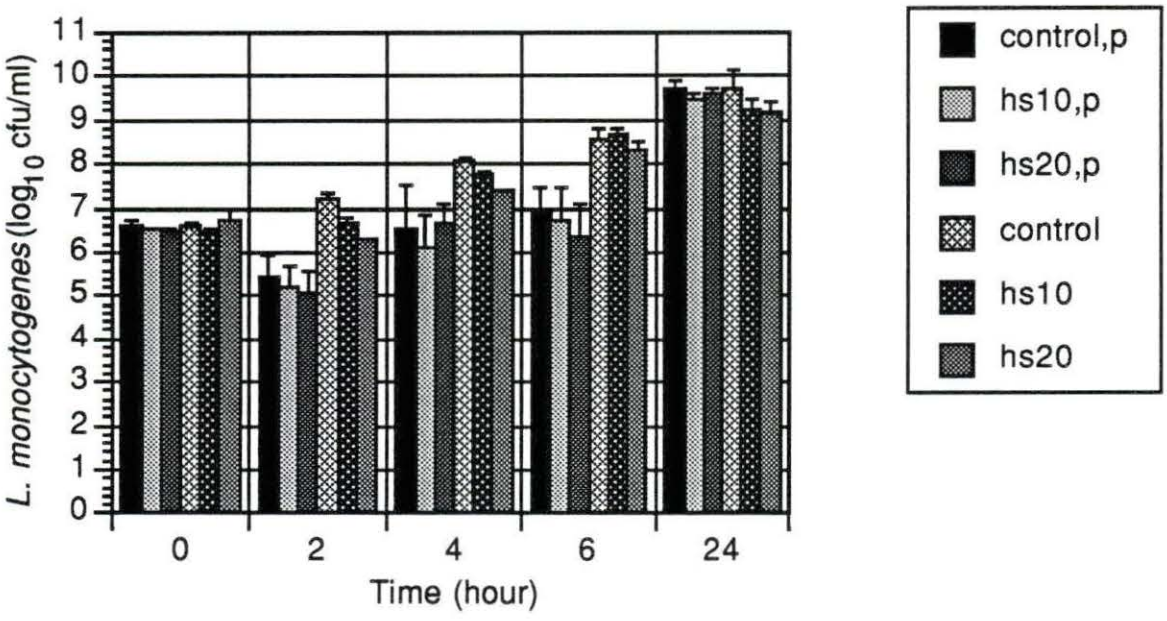

Figure 1. Survival of *L. monocytogenes* exposed to pediocin after heat shock at 48°C for 10 and 20 minutes (on TSAYE). Control=*Listeria* incubated at 37°C, hs10 = cells heat shocked for 10 minutes, hs20 = cells heat shocked for 20 minutes. The error bars represent standard deviations of 2 replications

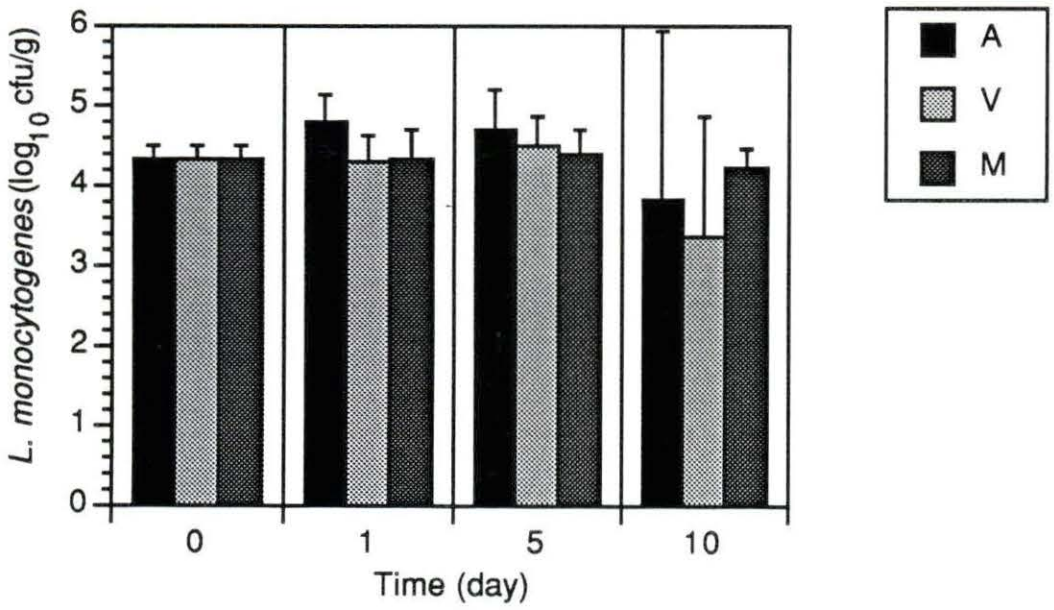

Figure 2. Survival of *Listeria* in meat packaged under different atmospheric conditions at 4°C. A=air; V=vacuum; M=modified atmosphere (40% CO_2, 60% N_2). The error bars represent standard deviations of 3 replications

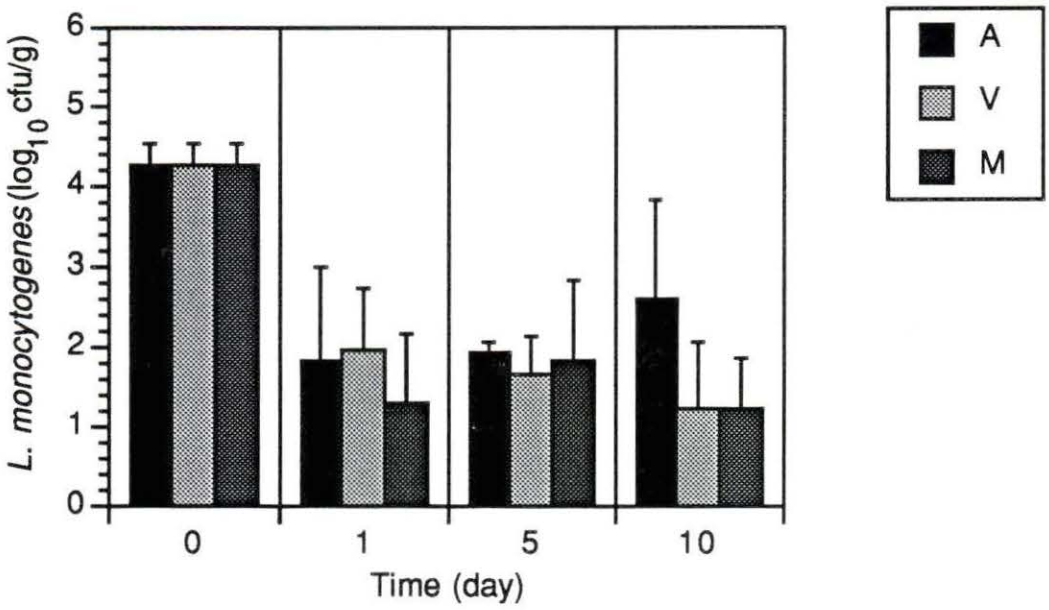

Figure 3. Survival of *Listeria* in meat (in the presence of *P. acidilactici*) packaged under different atmospheric conditions at 4°C. A=air; V=vacuum; M=modified atmosphere (40% CO_2, 60% N_2). The error bars represent standard deviations of 3 replications

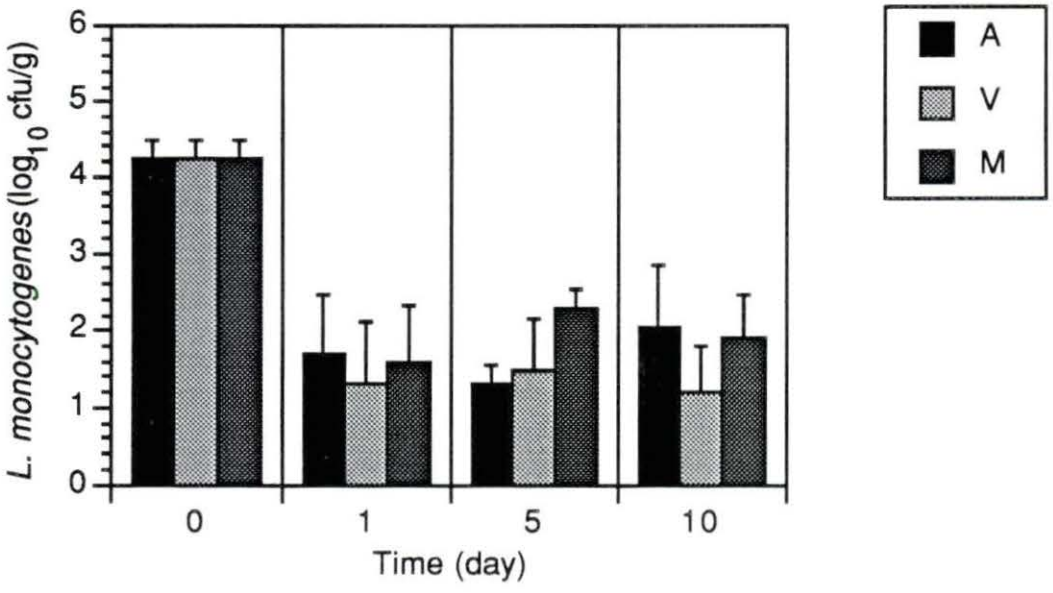

Figure 4. Survival of *Listeria* in meat (in the presence of pediocin) packaged under different atmospheric conditions at 4°C. A=air; V=vacuum; M=modified atmosphere (40% CO_2, 60% N_2). The error bars represent standard deviations of 3 replications

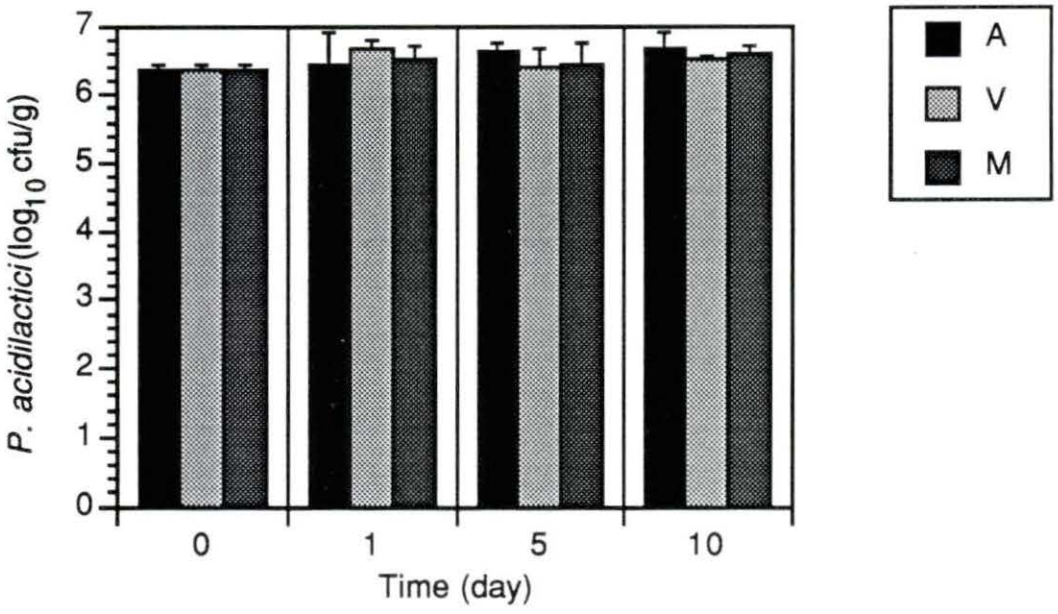

Figure 5. Survival of *Pediococcus* in meat packaged under different atmospheric conditions at 4°C. A=air; V=vacuum; M=modified atmosphere (40% CO_2, 60% N_2). The error bars represent standard deviations of 3 replications

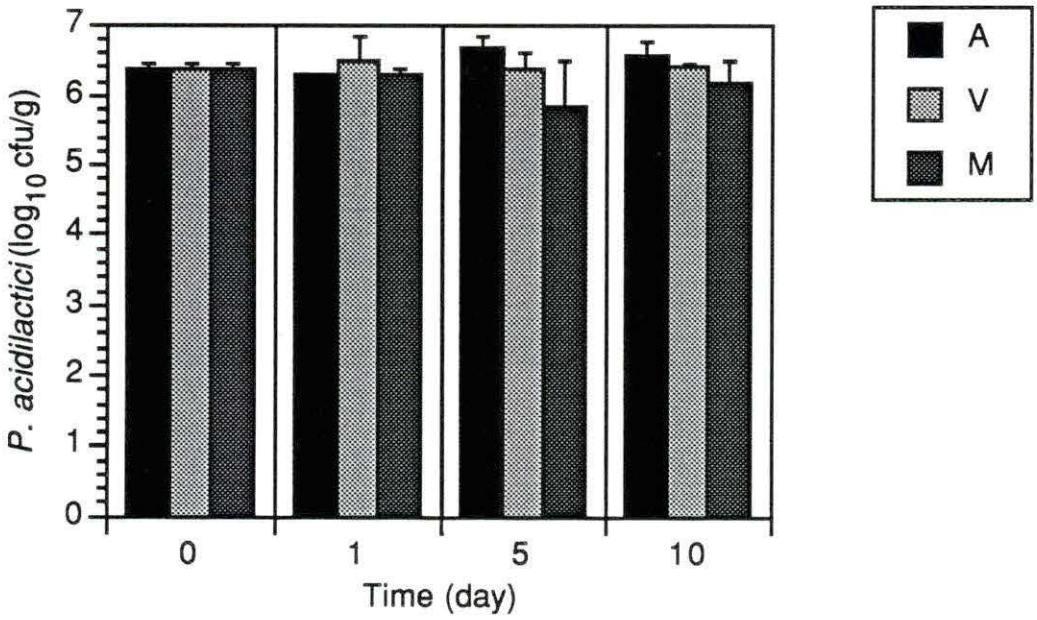

Figure 6. Survival of *Pediococcus* in meat (in the presence of *L. monocytogenes*) packaged under different atmospheric conditions at 4°C. A=air; V=vacuum; M=modified atmosphere (40% CO_2, 60% N_2). The error bars represent standard deviations of 3 replications

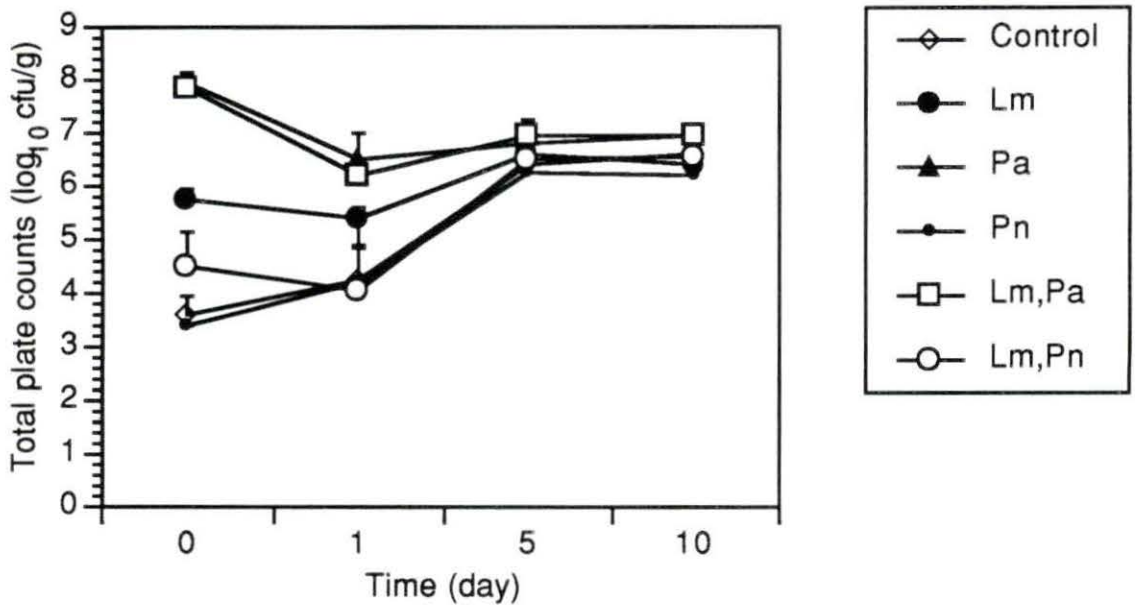

Figure 7. Total bacterial counts on pork chops inoculated with *Listeria* (Lm), *Pediococcus* (Pa), pediocin (Pn), *Listeria* and *Pediococcus* (Lm,Pa), or *Listeria* and pediocin (Lm,Pn), packaged and plated aerobically. Control = uninoculated meat. The error bars represent standard deviations of 3 replications

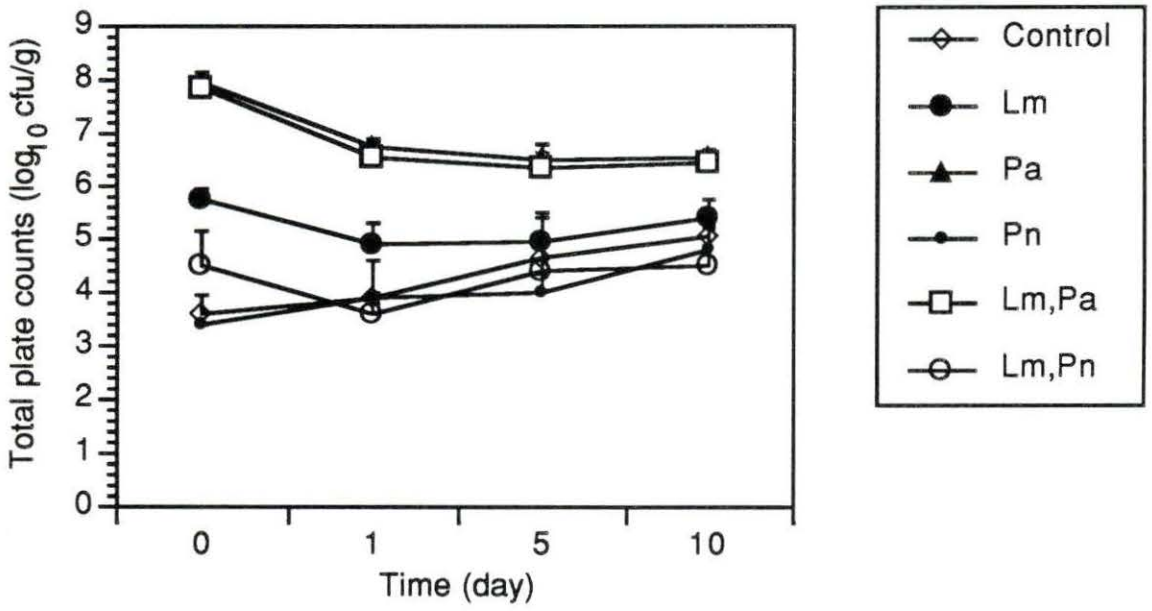

Figure 8. Total bacterial counts on pork chops inoculated with *Listeria* (Lm), *Pediococcus* (Pa), pediocin (Pn), *Listeria* and *Pediococcus* (Lm,Pa), or *Listeria* and pediocin (Lm,Pn), packaged under vacuum and plated aerobically. Control = uninoculated meat. The error bars represent standard deviations of 3 replications

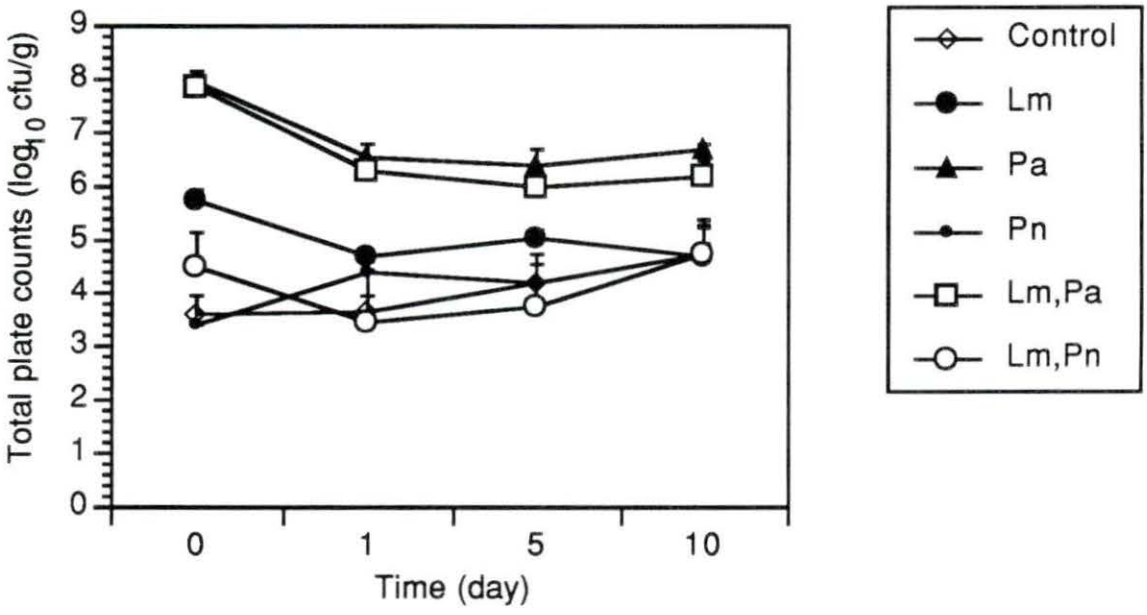

Figure 9. Total bacterial counts on pork chops inoculated with *Listeria* (Lm), *Pediococcus* (Pa), pediocin (Pn), *Listeria* and *Pediococcus* (Lm,Pa), or *Listeria* and pediocin (Lm,Pn), packaged under modified atmosphere (40% CO_2, 60% N_2) and plated aerobically. Control = uninoculated meat. The error bars represent standard deviations of 3 replications

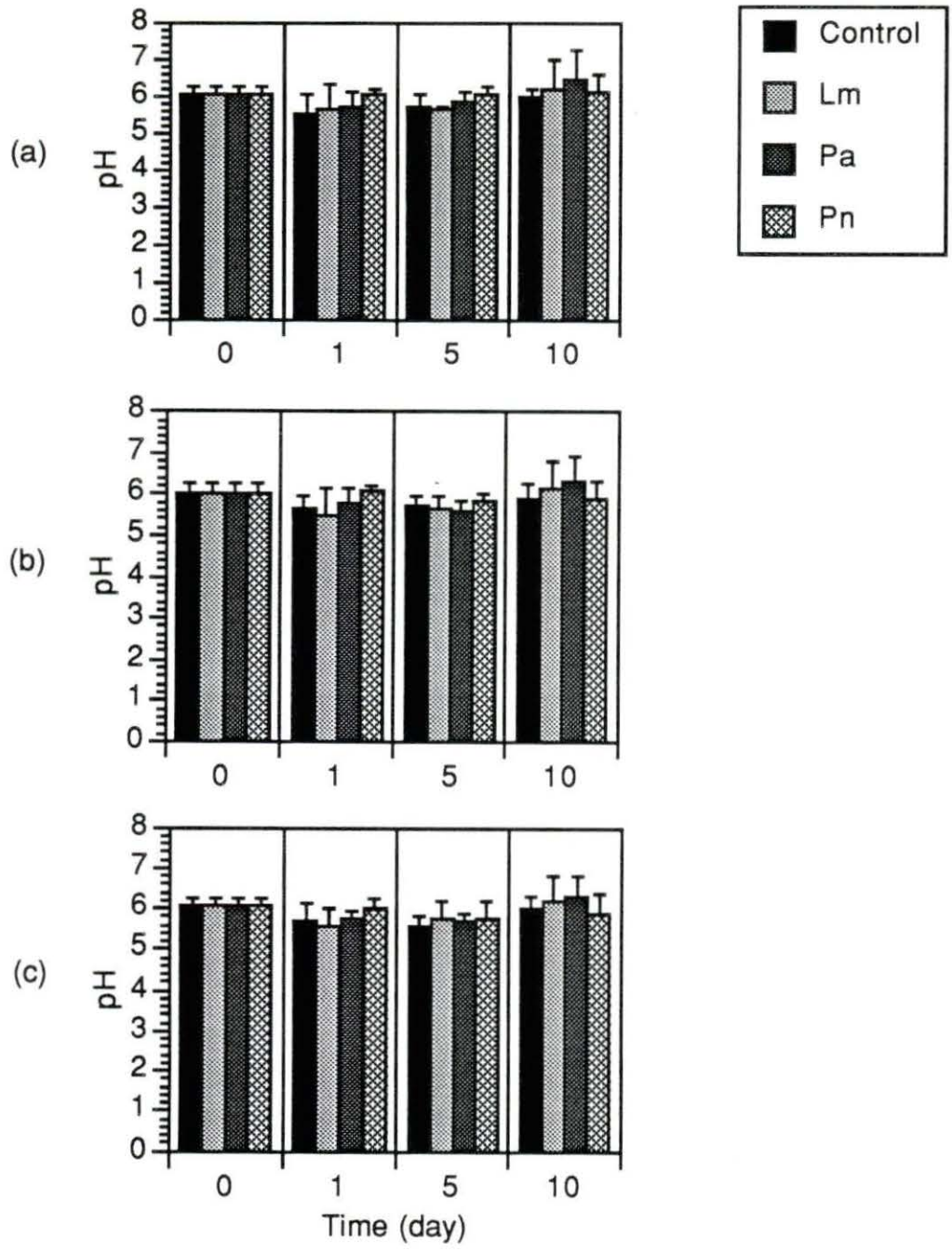

Figure 10. Effect of *Listeria* (Lm), *Pediococcus* (Pa), or pediocin (Pn) on pH of the meat packaged under air (a), vaccum (b), or modified atmosphere (c) at 4°C. Control = uninoculated meat. The error bars represent standard deviations of 3 replications

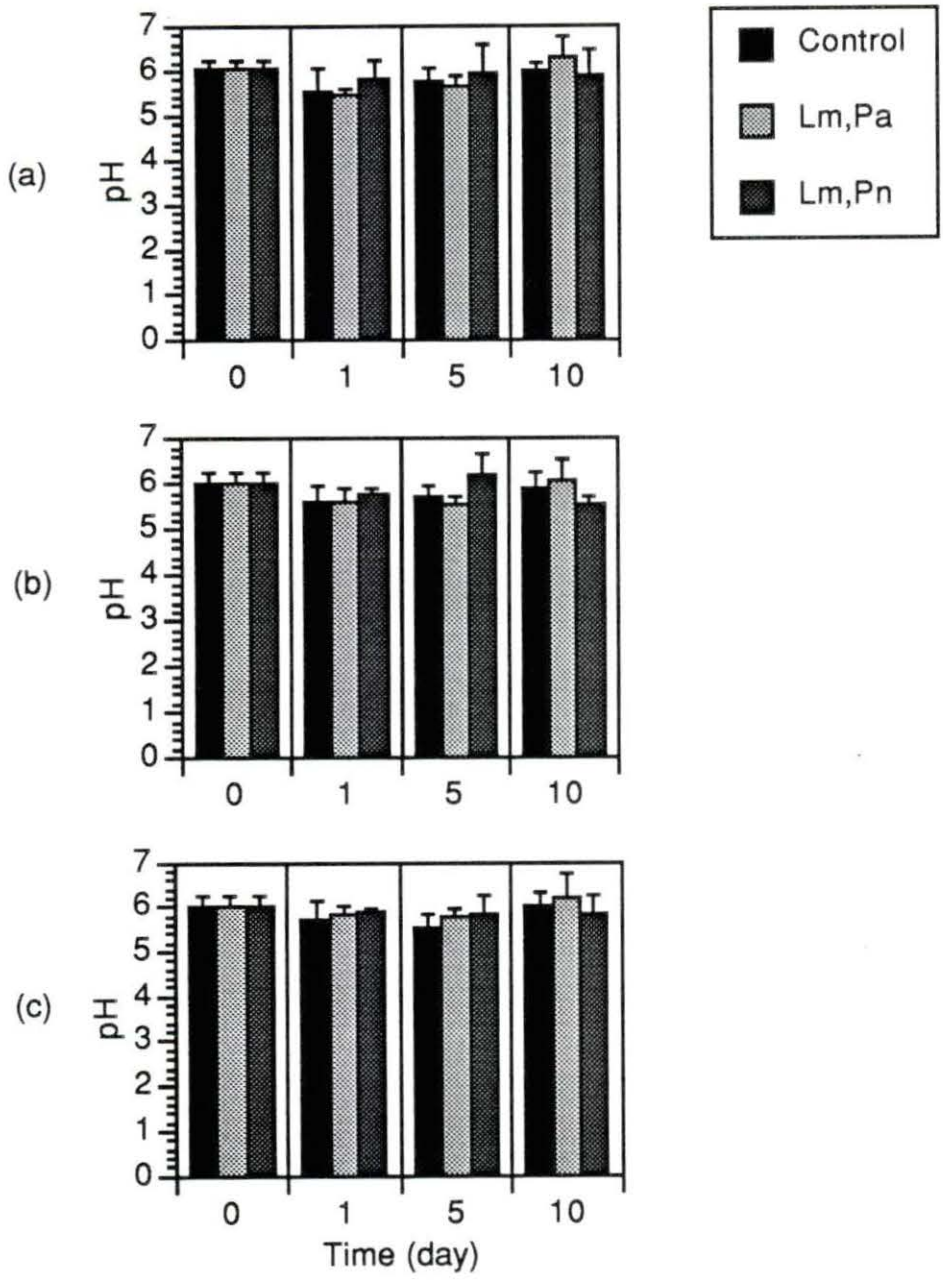

Figure 11. Effect of *Listeria* and *Pediococcus* (Lm,Pa), or *Listeria* and pediocin (Lm,Pn) on pH of the meat packaged under air (a), vaccum (b), or modified atmosphere (c) at 4°C. Control = uninoculated meat. The error bars represent standard deviations of 3 replications

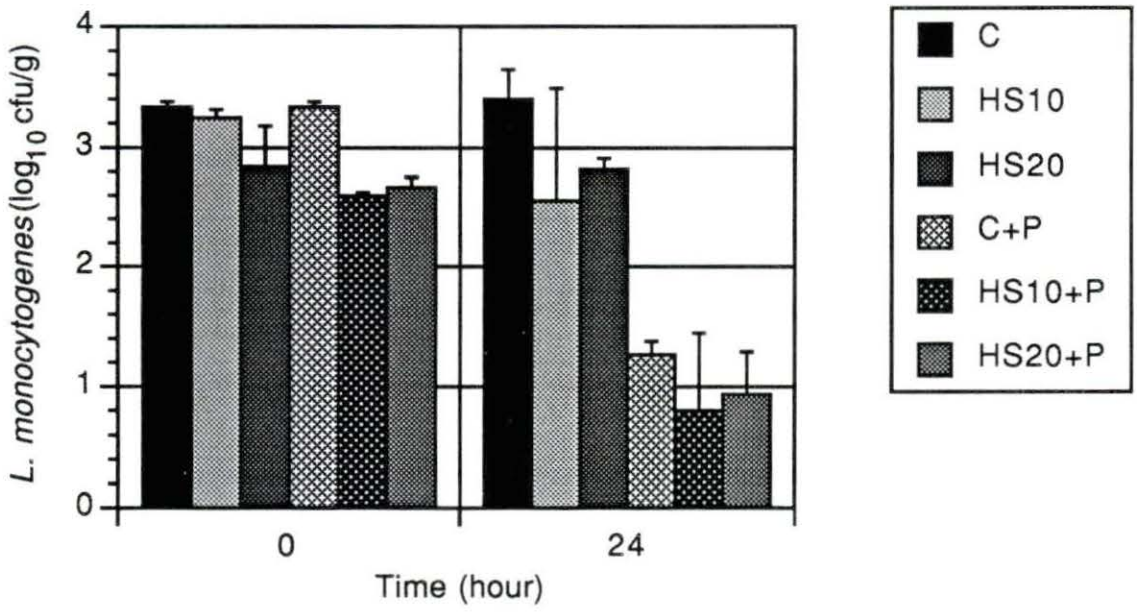

Figure 12. Effect of heat shock (48°C) and pediocin on survival of *Listeria* in ground pork packaged in air and stored at 4°C for 24 hours. C = control cells (37°), HS10 = cells heat shocked for 10 minutes, HS20 = cells heat shocked for 20 minutes, P = pediocin present. The error bars represent standard deviations of 3 replications

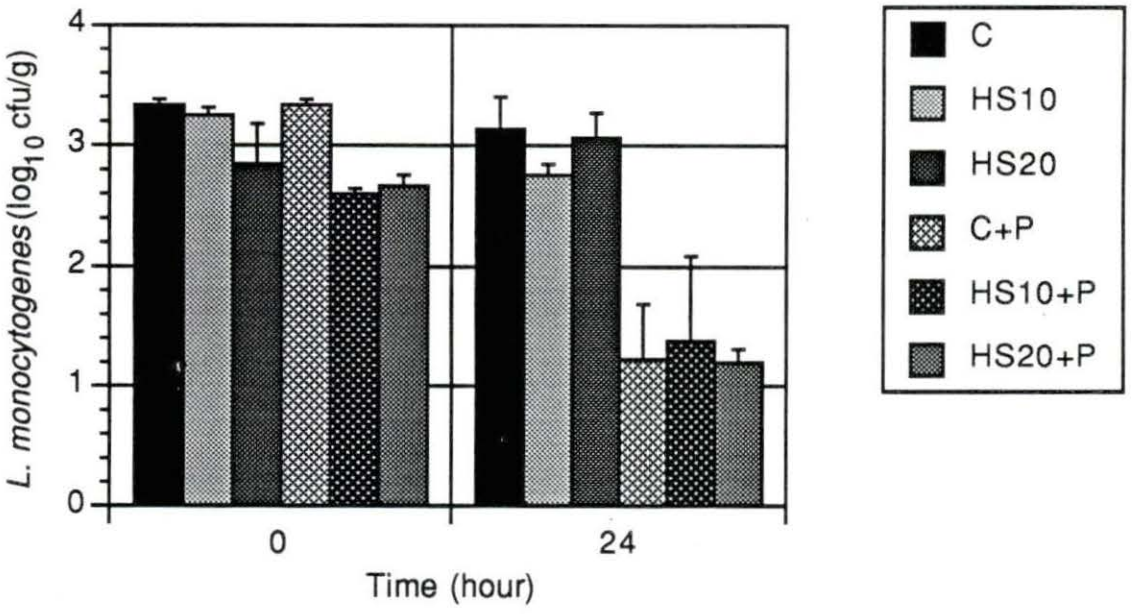

Figure 13. Effect of heat shock (48°C) and pediocin on survival of *Listeria* in ground pork packaged under vacuum and stored at 4°C for 24 hours. C = control cells (37°), HS10 = cells heat shocked for 10 minutes, HS20 = cells heat shocked for 20 minutes, P = pediocin present. The error bars represent standard deviations of 3 replications

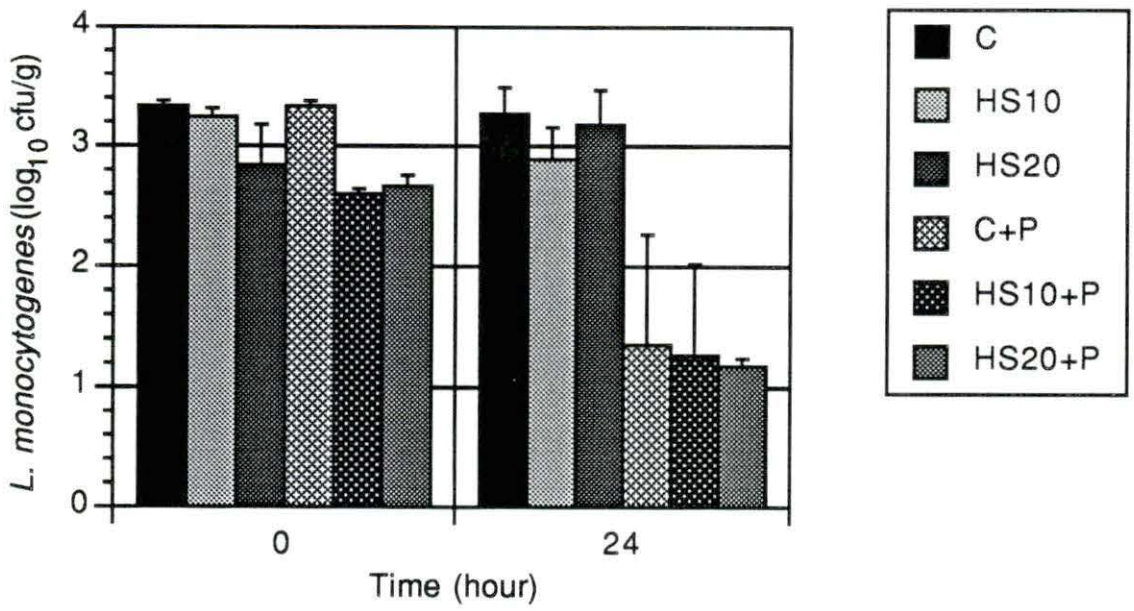

Figure 14. Effect of heat shock (48°C) and pediocin on survival of *Listeria* in ground pork packaged under modified atmosphere (40% CO_2, 60% N_2) and stored at 4°C for 24 hours. C = control cells (37°), HS10 = cells heat shocked for 10 minutes, HS20 = cells heat shocked for 20 minutes, P = pediocin present. The error bars represent standard deviations of 3 replications

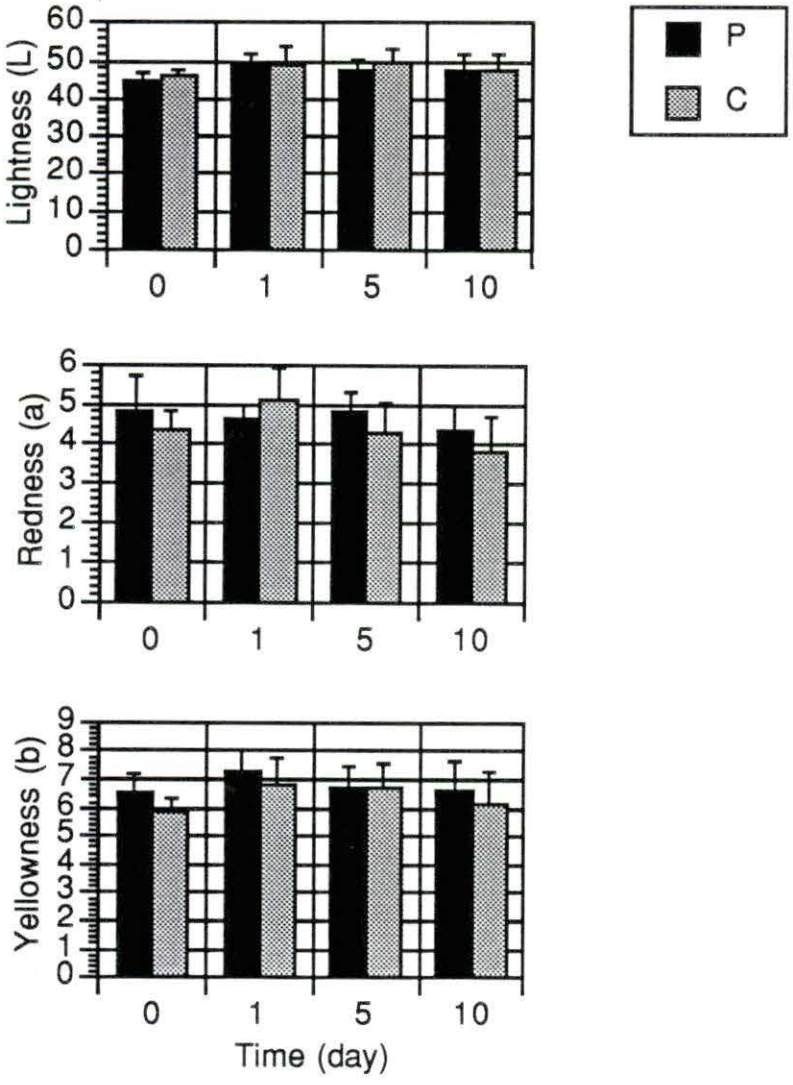

Figure 15. Colorimetric analysis of pork chops treated (P), or untreated (C) with pediocin by the Hunter Color Lab method. L = lightness (100 = perfect lightness, 0 = black); a = (redness [+], gray [0], greenness [-]); b = (yellowness [+], gray [0], blueness [-]). The error bars represent standard deviations of 3 replications

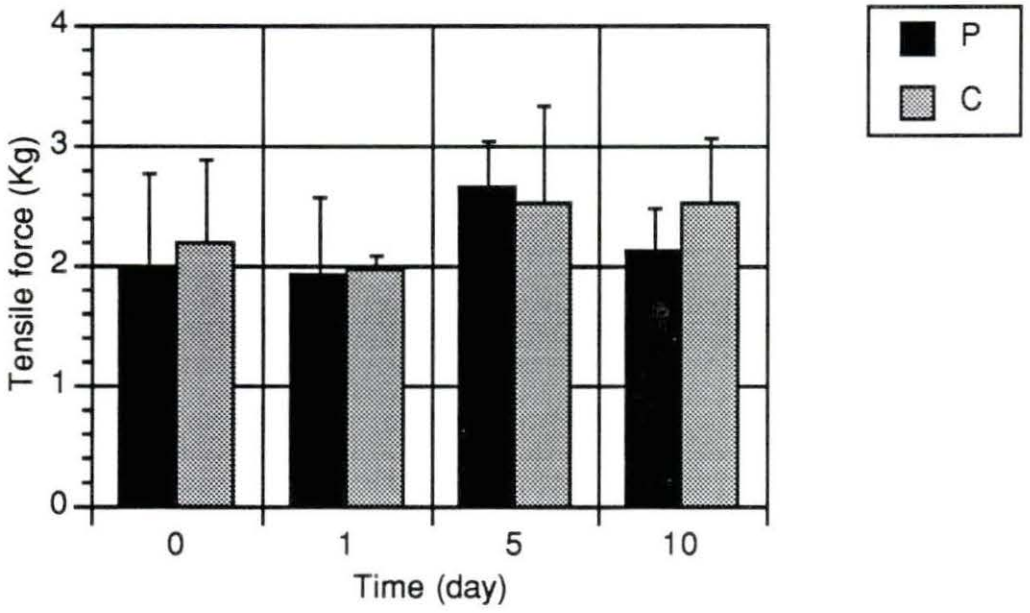

Figure 16. Texture analysis (shear force) of pork chops treated (P) or untreated (C) with pediocin by Instron Universal Testing Machine. The error bars represent standard deviations of 3 replications

Table 1. Analysis of variance (ANOVA) procedure for variable color performed as a t-test between the pediocin-treated and control pork chops, using the SAS system

Day	N[1]	Treatment[2]	Mean[3] (mm)	Mean[4] (mm)
0	3	p	25.97[a]	
	3	c	27.45[a]	26.71[e]
1	3	p	47.84[b]	
	3	c	36.12[b]	41.98[f]
5	3	p	83.45[c]	
	3	c	90.06[c]	86.75[g]
10	3	p	104.18[d]	
	3	c	101.72[d]	102.95[h]

[1] Number of samples for total of 3 replications.

[2] Pork chops treated with pediocin are designated as 'P', and the non-treated samples (controls) are shown as 'C'.

[3] Mean for the distance from the left end of the 150 mm-long scaled, descriptive line. Larger numbers indicate more undesirable color for the particular samples tested. The t-test was performed separately for each day at alpha = 0.05. Means with the same letter within each day are not significantly different.

[4] The overall t-test was performed across the means of paired samples (P and C together) from different days at alpha = 0.05. Means with the same letter are not significantly different.

Table 2. Analysis of variance (ANOVA) procedure for variable odor performed as a t-test between the pediocin-treated and control pork chops, using the SAS system

Day	N[1]	Treatment[2]	Mean[3] (mm)	Mean[4] (mm)
0	3	p	82.90[a]	
	3	c	29.09[b]	56.00[f]
1	3	p	70.42[c]	
	3	c	23.94[c]	47.18[f]
5	3	p	72.51[d]	
	3	c	79.33[d]	75.92g
10	3	p	115.97[e]	
	3	c	112.39[e]	114.18[h]

[1] Number of samples for total of 3 replications.

[2] Pork chops treated with pediocin are designated as 'P', and the non-treated samples (controls) are shown as 'C'.

[3] Mean for the distance from the left end of the 150 mm-long scaled, descriptive line. Larger numbers indicate more off odor for the particular samples tested. The t-test was performed separately for each day at alpha = 0.05. Means with the same letter within each day are not significantly different.

[4] The overall t-test was performed across the means of paired samples (P and C together) from different days at alpha = 0.05. Means with the same letter are not significantly different.

DISCUSSION

L. monocytogenes is an intracellular pathogen that, because of its economic and public health impacts, has become one of the most important foodborne organisms of the decade (Lovett, 1989). Although the optimum growth temperature of this bacterium is between 30 and 37°C, the organism can survive higher temperatures after it is heat-shocked at some elevated sublethal temperatures (Knabel et al., 1990; Bunning et al., 1990; Farber and Brown, 1990). Linton et al. (1990) demonstrated that exposure of *L. monocytogenes* Scott A to a heat-shock temperature of 48°C for 10 and 20 minutes in TSBYE resulted in an increased 2-fold resistance of the organism to 55°C.

Given that *L. monocytogenes* could acquire thermotolerance upon a previous heat shock, one of our objectives was to determine whether heat shocking *Listeria* would also make the cells more resistant to pediocin in a broth system, as well as in a meat system. A comparison between addition of pediocin or of its producer strain, *P. acidilactici*, on survival of *L. monocytogenes* was also pursued. A third objective was to determine effect of packaging atmosphere on survival of *L. monocytogenes* in fresh pork. Finally, we sought to study whether addition of pediocin to fresh pork would affect the color, texture, and odor of the meat during aerobic storage at 4°C.

The results from our study indicate that heat shocking *Listeria* at 48°C for 10 or 20 minutes did not make the cells more sensitive or resistant to bacteriocin (Figure 1). Addition of pediocin to *Listeria* suspension brought about approximately 2 $\log_{10}$ reduction in both the control and heat-shocked cells during the first two hours. The number of *Listeria* in both cultures increased

within subsequent hours until it reached 10^9 CFU/ml in all cultures (with or without bacteriocin) after 24 hours.

The fact that the number of *Listeria* in the pediocin-treated samples increased after 2 hours, may indicate that the concentration of *Listeria* could have been higher than the number of pediocin molecules in the mixture. Therefore, the cells that were not affected by pediocin had the opportunity to multiply within the subsequent hours. These results also indicate that pediocin, at the concentration applied in this experiment, is capable of reducing the number of *Listeria* by only 2 logs, and that this inhibition is not intensified or blocked by the prior heat shocking of the target cells.

It is also important to note that all reaction mixtures (with or without pediocin) were incubated at the optimum growth temperature for *Listeria* (37°C). This would provide additional support for growth of *Listeria* during subsequent hours. Nevertheless, knowing that this organism is capable of growing even at temperatures as low as 1°C, it seems unlikely to expect a significantly different growth pattern for *Listeria* if the experiment were to be conducted at room temperature (25°C), or below.

Many researchers have documented that heat shocking an organism triggers production of a series of highly-conserved heat shock proteins in the cells (Lindquist,1986; Neidhardt et al., 1984; Sokolovic and Goebel, 1989). In some bacteria, production of these proteins can cross-protect the cells against other stresses. In *E. coli* , for instance, a cross-resistance between heat and ethanol has been reported. Cells that are grown at 42°C, become more resistant to subsequent exposure to 55°C or ethanol (Neidhardt et al., 1984). Similarly, Morgan et al. (1986) demonstrated that in *Salmonella typhimurium*, a 28°C to 42°C

temperature shift induced 13 different heat shock proteins, of which five were co-induced by exposure of the cells to 60 μM of hydrogen peroxide. In our study, both the heat-shocked and control cells showed similar growth pattern in the presence of pediocin during this experiment. Therefore, it can be concluded that none of the heat shock proteins involved in resistance to higher temperature in *L. monocytogenes* overlapped with proteins that could bring about protection against pediocin. Furthermore, we may infer that the specific site of action for the induced heat shock proteins may be different from the site of action of pediocin molecules in the cell.

It is possible that, in addition to causing resistance to a higher temperature, a mild heat shock can also injure the bacterial cell membrane (Hurst, 1977). Bhunia et al. (1991) suggested that binding of pediocin AcH to certain receptors on the surface of the sensitive bacteria, changes the integrity of the cell wall, allowing more pediocin molecules to come in contact with the cytoplasmic membrane. As a consequence, the structural moiety of the cell membrane is destroyed and the cell may lose its viability. In our experiment, both heat shocking and exposure to pediocin, which are believed to make the cell membrane more vulnerable, were applied to *Listeria*. It is possible that exertion of two stresses on the same cells may have resulted in a lack of any protection against the bacteriocin.

In addition to pediocin, effect of packaging atmospheres on survival of *L. monocytogenes* in the meat system was examined. When pork chops previously inoculated with *Listeria*, were packaged under air, vacuum or modified atmosphere, the organism was able to survive all packaging conditions without any appreciable decrease in numbers (Figure 2). The incubation period and

temperature (4°C) did not influence the survival of *Listeria*. This finding is in agreement with previous reports that have indicated the psychrotrophic nature, and hence survival, of this organism at refrigeration temperatures (Seeliger and Jones, 1986; Leung et al., 1992).

Manu-Tawiah (1991) found that numbers of *L. monocytogenes* in the preinoculated pork chops that had been packaged under 20% CO_2/0% O_2 atmosphere were higher than, but not significantly different from, the numbers in the chops packaged in the 40% CO_2/0% O_2 gas mixture after 7 days of storage at 4°C. Moreover, addition of 10% O_2 to 40% CO_2- containing samples did not affect the number of the organisms in pork chops compared to those samples packaged under 40% CO_2/0% O_2. Lastly, it was reported that, in general, the numbers of *L. monocytogenes* in the samples that were packaged under air or vacuum were lower than, but not significantly different from, the ones packaged in the gas atmospheres.

Other investigators have conducted experiments with *Listeria* that show different results. Buncic et al. (1991) demonstrated that the total number of *Listeria* increased 30-fold after 10 days and by 420 times after 20 days in frankfurters packaged under vacuum and stored at 4°C. Gill and Reichel (1989) found *L. monocytogenes* incapable of multiplying on high-pH beef packaged under 100% CO_2 at 5°C. However, the pathogen was able to grow under vacuum. Wimpfheimer et al. (1990), on the other hand, reported that under modified atmosphere packaging (75% CO_2/25% N_2) *L. monocytogenes* did not survive well in raw chicken. However, the organism was able to grow when oxygen was present at concentrations as low as 5%.

A comparison between the results obtained by different investigators

indicate that survival of *L. monocytogenes* in preinoculated meats can be decreased by applying vacuum or modified atmosphere to the sample with little or no oxygen present. However, the degree of inhibition can be dependent on the type of meat to which *Listeria* is added. For instance, Glass and Doyle (1989) demonstrated that under vacuum, growth of *L. monocytogenes* was more pronounced in poultry products than in beef and ham samples. In our study, pork chops were used, and presence or absence of air did not have any impact on survival of this organism. Since *L. monocytogenes* is a facultative anaerobe, it should be able to grow both in the presence and absence of oxygen.

Addition of 10^6 CFU/g *P. acidilactici* to pork chops, previously inoculated with 10^4 CFU/g *Listeria*, reduced the number of the latter by about 2 $\log_{10}$ within the first 24 hours of storage at 4°C (Figure 3). The packaging atmosphere had no effect on the survival of the pathogen. In addition, total number ($\log_{10}$ CFU/g) of *Listeria* did not change during further storage under air, vacuum or modified atmosphere. Similar results were also reported by Berry et al. (1991). These investigators demonstrated that high levels of *P. acidilactici* (ca. 10^7 CFU/g) were necessary to inhibit the growth of *Listeria*, initially present at 10^4 CFU/g, in frankfurters packaged under vacuum and stored at 4°C. It was also reported that *L. monocytogenes* grew in the samples by approximately 2 $\log_{10}$ in the absence of *Pediococcus* and after 60 days of storage. Therefore, the authors speculated that the antilisterial activity of *P. acidilactici* was due to bacteriocin production. Berry et al. (1991) also reported that aerobic or anaerobic incubation did not result in any difference in the growth pattern of *Listeria* when *Pediococcus* was present. Instead, it was only in the absence of *P. acidilactici* during aerobic storage that enhanced growth of *L. monocytogenes* was observed.

We observed similar inhibition of *Listeria* when pediocin was added to pork chops (Figure 4). The bacteriocin brought about a 2-$\log_{10}$ reduction of the pathogen in the meat during the first 24 hours of storage at 4°C. The number of *Listeria* ($\log_{10}$ CFU/g) did not change any further during subsequent storage, regardless of the packaging atmosphere. This finding also indicates that the storage atmosphere did not affect the activity of pediocin. The bacteriocin caused the same amount of reduction in *Listeria* cells under air as it did under vacuum or modified atmosphere.

In our study, absence or presence of *Listeria* (Figures 5 and 6, respectively) did not have any effect on survival of *P. acidilactici* in pork chops. Furthermore, the type of packaging atmosphere did not influence the number ($\log_{10}$ CFU/g) of *Pediococcus* over 10 days of storage at 4°C. This could be due to the fact that *Pediococcus* is a facultative anaerobe or microaerophile (Seeliger and Jones, 1986). Therefore, presence or absence of air should not and did not affect its growth.

Results from total aerobic counts are shown in Figure 7. In most samples, the total number of aerobic bacteria did not change greatly during the first 24 hours of storage. However, addition of *Pediococcus* caused approximately 1.5 $\log_{10}$ reduction in number of aerobic bacteria. The total aerobic counts reached its highest (10^6 CFU/g) in all samples after 5 days, with no further increase by the end of the storage period. The limited degree of inhibition of aerobic bacteria caused by pediocin can be the result of the type of organisms that are usually present in meat. The normal flora of meat contains strains of *Pseudomonas, Moraxella, Acinetobacter,* and *Aeromonas* (Lambert et al., 1991), all of which are Gram negative. Since pediocin has been shown to be effective mainly against

Gram positive bacteria (Bhunia et al., 1991), one may not expect the total number of bacteria present in meat to be greatly affected by this bacteriocin. In our study, *Pediococcus* was unable to cause further decrease in bacterial counts after 24 hours.

Similar results were also obtained for the total facultatively anaerobic organisms in pork chops (Figure 8). However, the number of these bacteria did not increase as rapidly as the total aerobic bacteria throughout the storage. Under vacuum packaging, the total number of facultative anaerobes only reached 10^4-10^5 CFU/g (about 1.5 $\log_{10}$ lower than the aerobes) by day 10. The same effects were also observed when pork chops were packaged under modified atmosphere (Figure 9).

The results from the pH measures of pork chops with *Listeria*, *Pediococcus*, or pediocin and from various packaging atmospheres (Figures 10 and 11) indicate that the pH of the meat remained around 6, and that it was not affected by the type of inoculum, storage temperature, incubation period, or the packaging atmosphere. This implies that inhibition of *L. monocytogenes* in pork chops that had been further inoculated with *P. acidilactici* or pediocin was mainly due to the listericidal activity of the bacteriocin, rather than a pH effect.

Addition of pediocin to ground pork, containing heat-shocked cells of *L. monocytogenes* (3 $\log_{10}$/g), also resulted in 1.5 $\log_{10}$ reduction of the pathogen in the samples within 24 hours of storage at 4°C (Figures 12-14). The cell reduction was independent of the packaging atmosphere. This implies that, regardless of packaging atmosphere, the use of bacteriocin at the level applied in this experiment could be sufficient to completely eliminate *Listeria* from meat. Our results also indicate that a prior heat shock on *Listeria* did not make the cells

more sensitive to pediocin. Instead, both heat-shocked and control cells were equally susceptible to bacteriocin.

It is important to note that although ground pork was inoculated with about 3 $\log_{10}$/g of *Listeria* in this experiment, contamination of meat with this pathogen does not usually reach 10^2 cells/g. Therefore, the use of pediocin, with the concentration employed during this study, should still be sufficient to eliminate *Listeria* from meat. However, in a situation where the food is suspected to be contaminated with a higher number of this organism, the use of a more concentrated and purified form of pediocin should be considered.

In pursuing our fourth objective, we found out that the surface inoculation of pork chops with pediocin did not change the 'L', 'a', and 'b' values of the meat when compared to the control samples (Figure 15). This finding was consistent throughout the storage for 10 days. Knowing that color is usually one of the main factors in consumer decision-making regarding acceptability of fresh meat, our results should provide assurance to the meat industry , as well as to consumers, that addition of pediocin to fresh meat will not change its color quality.

In addition to not affecting the fresh color of pork chops, pediocin also did not alter the texture of the meat (Figure 16). This is another positive consideration for using the bacteriocin in foods, since we have shown that it may lower bacterial hazards in fresh pork while maintaining both its color and texture qualities.

The color analysis of pork chops performed by the sensory panel (Table 1) showed some similar results to those obtained by the Hunter Color Lab method (Figure 15). The panel did not notice any significant difference in color between

the control and pediocin-treated samples within each day. However, there appeared to be a positive correlation between the age of the pork chops and their corresponding distances on the line, that were defined by the panel. As the meat samples became older, the panel members perceived them as having more undesirable color, regardless of presence or absence of pediocin in the meat. This finding is believed to be due to growth of more spoilage bacteria over time, since our previous results (Figures 7-9) indicated progressive growth of such organisms in both the control and bacteriocin-treated samples.

When the same pork chops were analyzed for their odor quality, the sensory panel evaluated the pediocin-containing samples from day 0 to have more undesirable odor than the corresponding controls (Table 2). This could be due to the fact that in the pediocin-treated samples, the panel were smelling the odor of the bacteriocin, rather than the fresh meat itself. The panel did not think there was any significant odor difference between the controls and those that contained pediocin within the subsequent days. However, as the pork chops became older, the sensory panel thought that the samples had more off odor, regardless of the treatment. This is very similar to the finding from the color analysis (Table 1), and would indicate that, as pork chops became older, the spoilage process, which was initiated between 1 and 5 days, also accelerated. Therefore, it resulted in masking the smell of the fresh meat.

SUMMARY

Listeria monocytogenes is known as one of the major foodborne pathogens of the past decade. The bacterium has been involved in several outbreaks during the 1980's. The wide distribution of the organism in nature, as well as its association with domestic livestock have been the leading factors for contamination of fresh meat with this pathogen.

In recent years, *Pediococcus acidilactici* has been a subject of many studies for its antibacterial activity and its use in meat fermentation. Both the organism and its bacteriocin have been shown to inhibit the growth of *L. monocytogenes* in a variety of meats. In addition to *P. acidilactici* and its pediocin, vacuum- and modified atmosphere-packaging of fresh meat have been alternative methods for controlling the growth of *L. monocytogenes* in such a product.

The results from this study indicate that both *Pediococcus* and pediocin reduced the number of *L. monocytogenes* in fresh pork by 2 $\log_{10}$ under air, vacuum, or gas mixture (40% CO_2/60% N_2). Simultaneous application of multibarriers, such as pediocin, vacuum-, and modified atmosphere-packaging in pork chops, previously inoculated with *L. monocytogenes*, did not inhibit the growth of this pathogen any further. Moreover, a prior heat shock treatment of *Listeria* at 48°C for 10 or 20 minutes did not make the cells more sensitive or resistant to pediocin under any atmosphere.

The texture of pork chops was not affected by pediocin. The bacteriocin also did not have any significant impact on the overall color and odor of the meat. However, the storage period had an effect on the color and odor of pork chops. Meat samples that were stored aerobically for longer periods of time

showed more undesirable color and odor. This was probably due to more spoilage of the meat in older samples, rather than to any effect by the presence of pediocin.

Since pediocin is considered a "natural biopreservative", its use in meat to inhibit *Listeria* should be more acceptable to consumers than the use of synthetic chemical preservatives. More research needs to be done regarding purification and application of pediocin in meat to determine the concentrations of pediocin that can be used so that maximum inhibition of not only *Listeria*, but also of other common foodborne pathogens, can be achieved.

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APPENDIX

Name: _____ Date: _____

SENSORY SCORECARD

Place a vertical line on the horizontal line to indicate your rating of the **odor** of each sample. Label each line with the code number of the sample it represents.

no off-odor off-odor

Comments:

Place a vertical line on the horizontal line to indicate your rating of the **color** of each sample. Label each line with the code number of the sample it represents.

desirable color undesirable color

Comments: