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I am submitting herewith a dissertation written by Vijay Kumar Juneja entitled "Susceptibility of *Listeria monocytogenes* to Alkyl Esters of P-Hydroxybenzoic Acid and Sodium Chloride." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Food Technology and Science.

P. M. Davidson, Major Professor

We have read this dissertation
and recommend its acceptance:

Accepted for the Council:

Associate Vice Chancellor
and Dean of The Graduate School

SUSCEPTIBILITY OF *LISTERIA MONOCYTOGENES* TO ALKYL ESTERS OF P-HYDROXYBENZOIC ACID AND SODIUM CHLORIDE

A DISSERTATION PRESENTED FOR THE
DOCTOR OF PHILOSOPHY DEGREE
THE UNIVERSITY OF TENNESSEE, KNOXVILLE

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DEDICATION

This dissertation is dedicated to my wife, Poonam Juneja. With her love, and encouragement, anything would be possible.

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ABSTRACT

Lipid profile of nine strains of *Listeria monocytogenes* and its variation in the presence of antimicrobials (parabens and salt) and varying temperatures was determined. In addition, an attempt was made to develop *L. monocytogenes* cells with a defined fatty acid composition. The resistance of these cells and parent strains of *L. monocytogenes* to antimicrobials was compared. The effect of the antimicrobials on lipid synthesizing enzymes was carried out. Correlations between cytoplasmic membrane lipid composition, or enzymes, or both, and susceptibility of *L. monocytogenes* to antimicrobials were determined.

The average total lipid content was 6.65% (dry weight basis). The two major phospholipids found were phosphatidyl glycerol and cardiolipin. Phosphatidyl serine and phosphatidyl ethanolamine were detected only in strain Scott A and LM4C, respectively. Large percentages of branched chain fatty acids (C15:0br and C17:0br) were characteristic of all strains. The major differences among strains was the relative percentages of these fatty acids as well as variable presence of certain long chain fatty acids.

Phospholipid composition and fatty acid profiles of two strains of *L. monocytogenes* varied greatly in the presence of antimicrobials and at different temperature. The percentage of phosphatidyl glycerol increased and the cells secreted saturated fatty acids. Also, decreases in relative

percentages of branched chain fatty acids and variable changes in long chain fatty acids were found with increasing concentration of antimicrobials in the growth medium. Increasing temperature indicated similar results with the exception that most of the medium chain fatty acids decreased with temperature increased.

Cells adapted to saturated fatty acids (C14:0 and C18:0) were more resistant to antimicrobials than the parent strains. An opposite trend was observed for the cells adapted to unsaturated fatty acid (C18:1). The effect of the antimicrobials on fatty acid synthesizing enzymes indicated that antimicrobials altered enzyme activity.

The resistance of *L. monocytogenes* may be mediated by lipid composition. Results support a correlation between lipid composition and susceptibility of *L. monocytogenes*. Therefore, food preservation processes alter lipid composition of *L. monocytogenes* could result in changes in susceptibility to antimicrobials.

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CHAPTER I

INTRODUCTION

Listeria monocytogenes is widely distributed in nature and has been isolated from soil, sewage, animal feed, water and vegetation (Watkins and Sleath, 1981). The organism has emerged as a pathogen of concern to the food and dairy industries and was implicated in disease outbreaks involving food products (Fleming et al., 1985; Linnan et al., 1988). Of vital concern to the food industry is the psychrotrophic nature of *L. monocytogenes*. The organism grows rapidly at 4-7°C, which is the temperature range which was formerly thought to prevent growth of all foodborne pathogens.

A possible method of controlling the growth of *L. monocytogenes* during low temperature storage might be the use of antimicrobial agents. Many antimicrobials approved by the Food and Drug Administration for use in foods have shown little activity against *L. monocytogenes* (Davidson and Juneja, 1990). The methyl and propyl esters of p-hydroxybenzoic acid (parabens) are approved for use in the United States as antimicrobials in food and pharmaceutical products. In foods, a combination of the methyl and propyl esters may be added at 0.1%. Generally, the minimum inhibitory concentration against bacteria ranges from 1000-4000 μg/ml for the methyl ester and 50-1000 μg/ml for the propyl ester (Davidson and Juneja, 1990). The mechanism of action by which these compounds inhibit the growth of

microorganisms is not fully understood. Some research has suggested that they act on the cytoplasmic membrane resulting in leakage of intracellular constituents (Davidson, 1983). The antimicrobial effect of parabens was also shown to be correlated to total lipid content and phospholipid composition (Bargiota et al., 1987). Studies on a compound with a structure similar to the parabens, butylated hydroxyanisole, showed a significant effect on the activity of the membrane-bound adenosine triphosphatase of *Staphylococcus aureus* (Rico-Munoz et al., 1987). Therefore, the mechanism by which parabens inhibit microorganisms is most probably related to their direct or indirect (through interaction with lipids) effect on cellular enzymes systems located in the membrane. Since the mechanism of inhibition involved lipids, it was theorized that susceptibility of *L. monocytogenes* could be related to native lipid composition or concentration.

Sodium chloride (NaCl) has been used as an antimicrobial agent in foods for thousands of years. It has been shown that *L. monocytogenes* is particularly resistant to the antimicrobial effect of salt (Dje et al., 1990). While it is known that the antimicrobial mechanism of NaCl involves the reduction of water activity (Troller, 1980), little is known about the cellular mechanisms of resistance. In order to understand the mechanism by which *L. monocytogenes* resists the antimicrobial effects of salt, the

influence of the compound on cellular composition was examined.

The objectives of this study were to determine the lipid profile of *L. monocytogenes* (phospholipids, fatty acids and total lipids) and the effect on that profile by antimicrobials (methyl paraben, propyl paraben and NaCl) and varying temperatures. The purpose of this study was to determine whether the microorganism altered its lipid composition in a systematic way to resist the effect of the antimicrobials. In addition, an attempt was made to develop *L. monocytogenes* cells with a defined fatty acid composition. The resistance of these cells and parent strains of *L. monocytogenes* to parabens and sodium chloride was compared. The purpose of these experiments was to discover whether individual fatty acids could influence cellular susceptibility. In order to further elucidate the mechanism of susceptibility, the effect of the antimicrobials on lipid synthesizing enzymes was carried out. Correlations between cytoplasmic membrane lipid composition, or enzymes, or both, and susceptibility of *L. monocytogenes* to antimicrobials were determined.

CHAPTER II

LITERATURE REVIEW

LISTERIA MONOCYTOGENES

Taxonomy

Listeria monocytogenes was first described by Murray and co-workers in 1926 in rabbits and guinea pigs (Murray et al., 1926). Since it produced monocytosis as a symptom, the organism was designated *Bacterium monocytogenes*. In the following year, Pirie (1927) isolated an identical bacterium from the liver of infected gerbils (*Iatera lobenquiae*, African jumping mouse) and named it *Listerella hepatolytica*. For taxonomic reasons, the generic name was changed to *Listeria* in 1940 to honor the surgeon Lord Lister (Pirie, 1940).

After these early discoveries, several investigators attempted to characterize and classify this organism. The taxonomic position of the genus *Listeria* is far from clear. It was placed initially in the family *Corynebacteriaceae* as a single species *L. monocytogenes*. Later, *Listeria* was moved from the *Corynebacteriaceae* family and reclassified tentatively as Gram-positive, nonsporeforming, rod shaped bacteria (Welshimer, 1981).

In the 1984 edition of Bergey's Manual of Systematic Bacteriology (Seeliger and Jones, 1986), *Listeria* was classified as a regular, nonsporeforming, Gram-positive rod without designating a family affiliation. There are eight

species: *L. monocytogenes, L. ivanovii, L. denitrificans, L. grayi, L. murrayi, L. innocua, L. welshimeri, and L. seeligeri*. *Listeria denitrificans* does not belong to the genus *Listeria* (Jones, 1975) and has been made to a new genus, *Jonesia* (Jones and Seeliger, 1986). The similarities between *L. grayi* and *L. murrayi* have generated a proposal for the creation of a new genus, *Murraya*. This genus *Murraya* would include two subspecies, *M. grayi* ssp. *grayi* and *M. grayi* ssp. *murrayi* (Seeliger and Jones, 1986).

The characteristics that can be used to differentiate the seven accepted species are shown in Table 1. Only four species of *Listeria* have been reported to cause infections in animals and man, *L. monocytogenes, L. innocua, L. ivanovii, and L. seeligeri* (Mclauchlin, 1987). The others (*L. denitrificans, L. murrayi, L. gory,* and *L. welshimeri*) have not been implicated with naturally occurring infections.

Characteristics

Listeria monocytogenes is a Gram-positive, non-sporeforming, microaerophilic, short rod with rounded ends measuring 0.4-0.5 μm in diameter and 0.5-2 μm in length. It may be occasionally seen in young cultures as short chains, in parallel or a "V" shape. Older cultures present more filamentous forms and may be Gram variable. It is motile with four peritrichous flagella when grown at 20-25°C. These *L. monocytogenes* will display tumbling motility in a

Table 1. Differentiation of *Listeria* species.

Species	Hemolytic (Beta)	Nitrate reduction	Utilization			Sheep blood stab	Virulence (mouse test)
			Mannitol	Rhamnose	Xylose		
L. monocytogenes	+	-	-	+	-	+	+
L. ivanovii	+	-	-	-	+	+	+
L. innocua	-	-	-	V[a]	-	-	-
L. welshimeri	-	-	-	V[a]	+	-	-
L. seeligeri	+	-	-	-	+	+	-
L. grayi	-	-	+	-	-	-	-
L. murrayi	-	+	+	V[a]	-	-	-

[a] V, variable.

wet microscopic examination. Motility also can be confirmed by stabbing a tube of an appropriate medium. Growth along the stab line, spreading 3-5 mm below the surface of the medium in an umbrella fashion may be seen as early as 24 h. No motility is observed at 35°C (Seeliger and Jones, 1986). *Listeria monocytogenes* grows from 1°C to about 45°C, with optimum growth between 30°C and 37°C (Seeliger, 1961; Wilkins et al., 1972). At 37°C, the growth curve peak is reached in 16 to 18 hr. Although growth is slow at 3 to 4°C, turbidity in broth or growth on solid medium can be observed in 5 to 8 days. At 6°C, the log phase is reached in 10 to 11 days (Welshimer, 1960). The optimum pH range for *L. monocytogenes* is 6.0-9.0. It was reported that the organism could not grow below pH 5.6 (Seeliger, 1961); however, studies by Conner et al. (1986) revealed that listeriae can grow at pH 5.0. The organism is quite resistant to alkaline pH and can grow in liquid media at pH 9.6 (Seeliger, 1961). On solid media such as nutrient agar, after 24 hr of incubation at 37°C, *Listeria* colonies appear small, round, translucent, slightly raised and bluish-gray in color when viewed by 45° incident transmitted light. On blood agar at 37°C for 48 h, colonies may be 0.2-1.5 mm, dew-drop-like, and translucent, becoming grayish-white to opaque with age. Hemolysis may be observed on blood agar, but the zone of hemolysis may be so weak that it may not be detected without removing the colony from the agar surface.

In fluid media, *L. monocytogenes* produces faint clouding in 18 to 24 hr, depending on size and age of inoculum. Very small inocula may fail to grow. After several days, a thick, sticky, slimy precipitate forms. Continued incubation produces a precipitate which is virtually impossible to break up.

Listeria monocytogenes has 13 typed serovars (Table 2). Type 4b has been the primary serovar implicated in human listeriosis (McLauchlin, 1987).

Incidence

Since *Listeria monocytogenes* is commonly found in the environment, any human can become infected with this organism. However, listeriosis does not develop in each person who comes in contact with it. Pregnant women, newborns and infants, and adults with a compromised immune system are likely to develop disease (Seeliger and Finger, 1976). The mortality rate averages approximately 30% (Ralovich, 1984). Listeriosis is commonly characterized by formation of miliary granulomas (mass of inflamed tissue made up of many small lesions) and focal necrosis or by suppuration in the infected tissue (Seeliger and Finger, 1976).

The first listeriosis case was diagnosed in 1929 (Nyfeldt, 1929). On a yearly basis the following number of cases per 100,000 population have been reported (McLauchlin et al., 1986):

Table 2. Serology of *Listeria* species.

Species	Serotype
L. monocytogenes	1/2A, 1/2B, 1/2C, 3A, 3B, 3C, 4A, 4AB, 4B, 4C, 4D, 4E, "7"
L. ivanovii	5
L. innocua	4AB, 6A, 6B, Un[a]
L. welshimeri	6A, 6B
L. seeligeri	1/2B, 4C, 4D, 6B, Un[a]

[a]Un, unidentified.

Country	Percent of per 100,000 population Infected per year
Germany	0.00034
France	0.00033
USA	0.000066
Canada	0.000062

Even with a higher frequency of listeriosis, the route of transmission is difficult to determine. This is due to endemic sporadic cases with superimposed outbreaks and the tedious procedures required to isolate the organism. With the increase in the number of sporadic cases, the following possible routes of transmission of *L. monocytogenes* to humans have been listed (Marth, 1988).

Soil----------> Plant----------> Human

Animal----------> Soil----------> Plant--------> Human

Feed----------> Animal----------> Human

Animal----------> Human

Insects----------> Human

Dust (dirt)------> Air----------> Human

Food----------> Human

Human----------> Human

Foodborne Outbreaks

Several outbreaks associated with food have been reported in the literature (Table 3). There have been three well-documented listeriosis outbreaks involving foods in the

Table 3. Major Human Listeriosis Outbreaks.

Location	Year	Cases	Vehicles
[a]Germany	1966	279	Unknown
[b]France	1976	162	Unknown
[c]USA	1979	20	Milk/vegetables?
[d]New Zealand	1980	21	Seafood?
[e]Canada	1981	41	Coleslaw
[f]USA	1983	49	Milk
[g]Switzerland	1983-84	25	Unknown
[h]USA	1985	314	Soft Cheese

[a]Otrel. (1968), [b]Carbonnelle et al. (1978), [c]Ho et al. (1986) [d]Lennon et al. (1984), [e]Schlech et al. (1983), [f]Fleming et al. (1985), [g]Malinverni et al. (1986), [h]James et al. (1985).

1980's. The first occurred between March and September of 1981 in Nova Scotia, Canada. Coleslaw was implicated in this outbreak. The number of cases of perinatal listeriosis and adult listeriosis reported were 34 and 7, respectively. The mortality rate was 27% for the infant cases and 33% for the adult meningitis cases. *Listeria monocytogenes* Scott A type 4b was isolated from the patients and the coleslaw. The incriminated coleslaw was traced to a farm where the cabbage was grown in fields fertilized with manure from sheep infected with *Listeria*. The harvested cabbage was held in cold storage. The cold-storage temperatures allowed *Listeria* to thrive and contaminate the coleslaw (Schlech et al., 1983).

Between June and August 1983, 49 patients in Massachusetts were hospitalized for meningitis and septicemia. Seven cases occurred in fetuses or infants and 42 in immunocompromised persons. The mortality rate was 29%. This outbreak was associated with consumption of one brand of pasteurized milk (Fleming et al., 1985). Fourteen of the victims died during this outbreak. *Listeria monocytogenes* was not isolated from pasteurized milk, but 12% of raw milk samples from farms supplying the implicated milk processor were positive for *Listeria monocytogenes serotypes* 1a, 3b, 4ab, and 4b (Fleming et al., 1985).

The largest recognized foodborne outbreak due to *Listeria monocytogenes* infections in the United States

occurred in Los Angeles and Orange County, California in 1985. Three hundred fourteen cases of listeriosis were reported between January and November of 1985. Among the victims were 181 pregnant women and/or their children and 133 immunocompromised persons. The majority of patients cultured positive for *Listeria monocytogenes* serotype 4b. A Mexican-style soft cheese was implicated, and there was evidence that the cheese was made with unpasteurized milk (James et al., 1985). The outbreak serotype was isolated from the cheese and the manufacturing plant. The probable cause was mixture of raw milk with pasteurized milk or post-pasteurization contamination (Silliker, 1987).

Although the above mentioned infections are uncommon, the organism's ability to grow at refrigeration temperatures raises concerns about emerging technologies such as modified atmosphere packaging. All food products which come in contact with soil or fecal matter have the potential of carrying *L. monocytogenes*, though vegetable and dairy products have been largely implicated in outbreaks. Survival of *L. monocytogenes* in food processing environments presents a high risk of cross-contamination from raw to processed products.

A recent survey in the United States indicated that about 800 people are infected every year. The estimated mortality rate was 19.1% (Ciesielski et al., 1988).

Control

In view of the fact that this organism is commonly found in the environment, every effort should be directed to keeping the organism out of the food processing plant. A Hazard Analysis Critical Control ProGram (HACCP) should be developed to identify locations in the food processing plant at which *Listeria* may be controlled (WHO, 1988). Control of *Listeria* in food products should begin at the point of origin of the raw product. All prophylactic measures should be adopted to minimize the chances of contamination. Recyclable human and animal waste should not be used to produce crops that will be eaten without cooking or that become an ingredient of a product that will be eaten raw (Lovett, 1989). Trucks and containers used to transport food must be thoroughly cleaned and sanitized at frequent intervals to prevent contamination. Lopes (1986) demonstrated the efficiencies of six different types of commonly used dairy and food plant sanitizers on *Listeria* and found that quaternary ammonium compounds (QAC) were highly effective against *Listeria*.

At food processing plants, raw materials should be kept separate from finished products in order to eliminate chances of cross-contamination. Movement of workers within the plant should also be restricted. Raw materials, finished product, equipment and processing plant environment should be monitored constantly for microbiological

contamination. Sanitation of the entire plant must be under constant scrutiny and evaluation.

BACTERIAL STRUCTURE

Cell Membrane

It is now well documented that the major structural features (apart from size and shape) distinguishing prokaryotic bacterial cells from eukaryotic cells relate to the nature and organization of membrane systems, the nucleus, and the "packaging" of functions in membranous organelles (Salton, 1971). Anatomically, the majority of bacterial cells are relatively undifferentiated with respect to intracellular membrane-bound structures (Ghosh, 1974). Thus, the bacterial nucleus lacks a nuclear membrane, there are no separate mitochondrial organelles, and respiratory functions are localized in multifunctional plasma membranes (Van Iterson, 1965). According to Salton (1971), the cell membrane is the major site of functional differentiation in the bacterial cell, giving rise to a variety of internal membranes or membrane systems, including mesosomes, photosynthetic vesicles, and membranes involved in spore formation. The structure, chemical composition, and physical properties of the bacterial plasma membrane have been found to be similar to other types of biological membranes.

The bacterial cell is encased in an envelope structure

in both Gram-positive and Gram-negative organisms (Higgins and Shockman, 1971). Anatomical studies of bacteria by electron microscopy of thin sections have clearly established the relationships between walls and membranes in the Gram-positive bacteria. Surfaces of Gram-positive bacteria are bounded by a relatively thick (approximately 200-600 Å) cell wall structure and an underlying plasma membrane, the latter possessing the profile typical of cell membranes in general (i.e., alternate dense-light-dense layering as seen in thin sections) (Salton, 1967). The relationship of a thick cell wall to a plasma membrane has been shown in thin sections of *Micrococcus lysodeikticus* (Salton, 1964). There is usually fairly close contact between the inner surface of the wall and outer surface of the plasma membrane, except in regions containing pockets of membrane vesicles or membrane intrusions i.e., mesosomes (Fitz-James, 1960). In some bacteria, such as *L. monocytogenes* (Ghosh and Murray, 1967; and *Bacillus licheniformis* (Ghosh et al., 1969), the small peripheral vesicles between the plasma membrane and the wall may be quite numerous. Other investigators (Hurst and Stubbs, 1969) have suggested that the walls and membranes of *Streptococcus lactis* form highly integrated structures with regularly spaced points of attachment between the inner surface of the wall and outer surface of the membrane. They believe that some membrane sites may protrude through the

wall and act as receptors for *S. lactis* bacteriophage, which Oram and Reiter (1968) found adsorbs to the membrane. Strominger et al. (1959) stated that walls of Gram-positive bacteria, which represent about 20% of the dry weight of the cells, have relatively simple composition compared to more complex walls of Gram-negative organisms. Costerton et al. (1974) and Masayori (1979), studying bacterial envelopes, concluded that Gram-negative bacteria possess an additional external barrier which can exclude certain low molecular weight compounds and may have an important function in retention of some enzymes. They stated that this additional external barrier contributed to the ability of Gram-negative bacteria to live in extraordinarily diverse environments, to exclude certain toxic molecules, and to prevent the access of antibodies to the Gram-negative cell.

There have been several claims that bacterial membranes have a more complex layering than that seen in the classical, unit membrane profile. For example, a five layered structure has been suggested for *S. aureus* membranes by Suganuma (1966). Edwards and Stevans (1963) interpreted the plasma membrane of *L. monocytogenes* as consisting of three dense layers separated by two less-dense layers. However, Ghosh and Murray (1967) were unable to substantiate the existence of a five layered membrane in the latter organism.

The chemical composition of isolated membranes has

been subject of numerous investigations and various aspects of this topic have been reviewed by Gelman (1966); Kates (1964); and Kates (1966). The overall chemical composition of bacterial membranes is similar to that of other membrane systems in that they are composed of about 50 to 75% protein and 20 to 35% lipid (Salton, 1967). Washed plasma membrane, free of cytoplasmic material of an osmotically lysed protoplast of *Listeria* contained 55-60% protein, 1.5% ribonucleic acid, 0.1% deoxyribonucleic acid, 1.3-2.3% carbohydrate, 0.17-0.38% aminosugar, 0.2-0.4% rhamnose, 3.5-4.0% phosphorus, 10.5-12.0% nitrogen and 30-35% lipid (Ghosh and Carrol, 1968). Amino acid composition of the membrane differed from that of the whole cell in that sulfur-containing amino acids were not present. Glucose, galactose, ribose and arabinose were the membrane carbohydrates.

Overton (1895) first proposed that membranes were composed of lipids due to the ease with which lipid soluble substances penetrated the plasma membrane of the cell. Danielli and Davson (1935) proposed that lipids existed as a bilayer which was coated on both sides by proteins (Sherbet, 1978). Nonpolar fatty acyl chains were oriented inwards perpendicular to the membrane surface and polar groups of phospholipids occurred at the surface coated by proteins and polysaccharides. This concept of membrane structure was termed the "sandwich" or "unit" model (Sherbet, 1978).

The lipid bilayer concept did not account for the dynamic properties of lipid molecules. Lipid molecules were known to possess a rapid internal motion and a rotational motion along the axis of the molecule. Lateral movements of the molecules and exchange of lipids from one side of the bilayer to the other were reported (Singer and Nicolson, 1972). Recognition of these motional properties, conferring fluidity to the membrane, led to the development of the fluid mosaic model by Singer and Nicolson (1972). This model postulates that phospholipids of membranes are arranged in a bilayer to form a fluid, liquid-crystalline matrix or core. In this bilayer, individual lipid molecules can move laterally, endowing the bilayer with fluidity and flexibility. It also possesses a characteristically high electrical resistance and relative impermeability to highly polar molecules. Some globular protein molecules partially penetrate either surface of the lipid bilayer, while others penetrate through (Figure 1). The proteins are capable of lateral diffusion in the lipid bilayer. Thus, lipid bilayer is asymmetric with respect to the position of membrane-associated proteins. Proteins of the membrane are involved in enzymatic activity, transport of molecules and as receptor. The lipid bilayer provides the permeability barrier.

The cytoplasmic membrane has a number of major functions (Hughes, 1962; Kaback and Hong, 1973; Boyd and

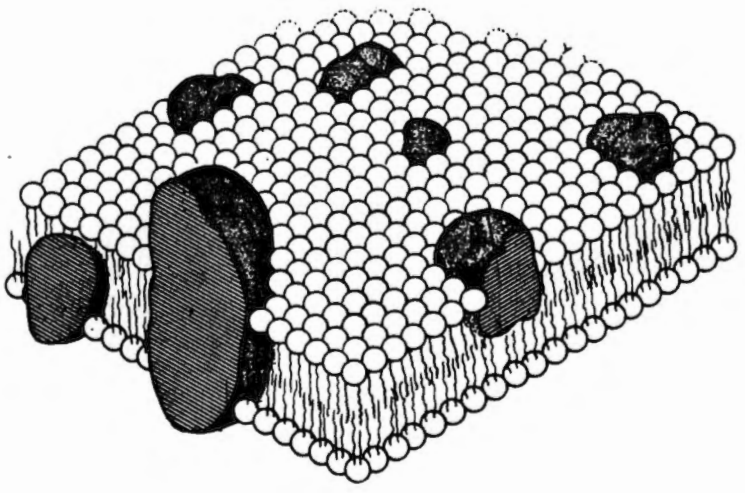


Figure 1. The Fluid Mosaic Model of Membrane Structure Consists of a Matrix of Fluid Lipid Bilayer Throughout Which Globular Integral Proteins are Dispersed.

Source: Lehninger, A.L. 1975. "Biochemistry," 2nd edition. Worth Publishers, Inc., New York.

Hoerl, 1981). These functions include: (1) respiratory electron transport by means of cytochromes, resulting in oxidative phosphorylation; (2) synthesis and export of precursors of peptidoglycan, teichoic acids, and outer membrane components; (3) secretion of extracellular and periplasmic enzymes and toxins; (4) regulating the segregation of chromosomal and plasmid DNA into daughter cells; and (5) numerous transport systems capable of generating gradients of up to 1000-fold for the accumulation of nutrients.

The bacterial cell membrane must maintain a specific fluidity in order to accomplish its functions (Silvius et al., 1980). Miller (1980) suggested that fluidity is controlled by homeostatic mechanisms. According to Fulco and Fujii (1980) and Kawagushi et al. (1980), this homeostasis can be accomplished by appropriate alterations of saturated and unsaturated fatty acid biosynthesis. Kates and Pugh (1980) suggested that phospholipid desaturases play an important role in control of membrane fluidity, whereas Russell and Sandercock (1980) found that regulation is achieved by modification of phospholipid fatty acyl chain length. Nozawa (1980) found both fatty acid and phospholipid composition altered membrane fluidity. It is clear that there are correlations between changes in phospholipid and/or fatty acid composition, enzymatic activities and membrane fluidity.

Bacterial Lipids

According to Lechevalier (1977), chemotaxonomy has played an important role in systematic classification. Identification of lipids is used as a taxonomic aid, especially when morphological, physiological or immunological data fail to lead to an unequivocal decision (Uchida and Mogi, 1973). Lipid composition is of primary taxonomic character in defining the generic boundaries of the *Nocardia-Mycobacterium-Corynebacterium* group (Lechevalier, 1977). Etemadi and Convit (1974), using lipid composition, were able to determine that lepromatous leprosy was caused by a *Mycobacterium* and not by a mixture of Corynebacteria, mycobacteria and other bacteria as generally accepted.

Lipid analysis of subcellular fractions of Gram-positive and Gram-negative bacteria have revealed marked differences in both content and distribution. According to Vorbeck and Marinetti (1965), Gram-positive bacteria contain less lipid than Gram-negative bacteria. In fact, Gram-positive cocci contain almost no lipid that is not in the membrane (Rogers and Perkins, 1968). Only small amounts (1-3%) of lipid have been found to be associated with isolated cell walls of Gram-positive bacteria. In contrast, Gram-negative cell walls have been reported to contain 10 to 20% lipid, mostly as lipoprotein and lipopolysaccharide complexes (Wiedel and Primosigh, 1958). A cell envelope

structure has been proposed for Gram-negative organisms which contains three layers of lipid material: the cytoplasmic membrane, a lipoprotein layer, and a lipopolysaccharide layer (Kellenberger and Ryter, 1958).

O'Leary (1962) reported total lipid content of most species of bacteria ranged between 1 and 10% of the dry cell weight while mycobacteria and certain allied forms such as corynebacteria of the diphtheria type and nocardiae, commonly have as much as 20% lipid by dry weight. Carrol et al. (1968) reported *L. monocytogenes* contained 6-7% chloroform-soluble lipid on a dry weight basis. It consisted of neutral and polar lipids in the ratio of about 1 to 4.

According to Mara et al. (1971) and Julak and Mara (1973) the lipid content of *L. monocytogenes* was higher when cultured in tryptic soy broth than in Todd-Hewitt or tryptose broth. The authors also indicated that the biological properties (virulence, hemolysin production and serotype) of different *Listeria* strains were directly related to lipid contents.

Studies by a number of researchers have suggested that lipid content of the bacterial cell envelope may contribute to antimicrobial resistance. Quantitative changes in individual lipids or an increase in the amount of surface lipids may alter the sensitivity of bacteria. Hugo and Stretton (1966) grew *S. aureus* in nutrient broth

supplemented with either glycerol (3%) or sodium oleate (20 μg/ml). They reported that induced appearance of surface lipid on cells was accompanied by an increase in the resistance to penicillin-type antibiotics. Hill et al. (1963) observed that naturally occurring cells of *Streptococcus pyogenes* possessing surface lipids were resistant to tetracycline. Brown and Watkins (1970) proposed that the amount and type of lipid in cells, especially cell envelopes, played a role in determining the drug sensitivity of many microorganisms. Extra lipid in the cell envelope of fattened cells (cells grown in the presence of glycerol) acted purely as a permeability barrier (Hamilton, 1968). Brown and Wood (1972) studied the influence of lipid content of cells and cell envelopes of *Pseudomonas aeruginosa* on their resistance to polymyxin. They used their results to support the theory that, within the cell envelope, there is a permeability barrier to antibiotics and the lipid content of cells was intimately involved in this barrier. Pechey et al. (1974) suggested that there was a redistribution of the total neutral lipid to the surface of the cell when cells were grown in the presence of gentamicin. Uptake of phenolic compounds was greater and leakage reduced in fattened *S. aureus* and *Bacillus subtilis* cells (cells grown in the presence of glycerol) compared to normal cells (Hugo and Franklin, 1968; Furr and Russell, 1972). They theorized that this was due

to phenolics being absorbed at the surface of high-lipid cells whereas they passed into the interior of normal cells. Similarly, Hugo and Davidson (1973) reported that lipid depleted cells of *S. aureus* were less resistant to a number of antimicrobial drugs, including phenolic compounds, than normal cells. Al-issa et al. (1984) inoculated a 14 h culture of *L. monocytogenes* into tryptone soy broth (TSB) containing 3% glycerol and incubated it at 37°C for 14 h. Repeated subculturing in TSB containing 3% glycerol caused a marked increase in cellular lipid. Fattened cells showed considerably greater resistance to butylated hydroxyanisole (BHA). It is possible that glycerol acted as a coating layer or, most probably, was involved in the synthesis of surface lipid, thereby offering the cells a non-specific mechanism for excluding the BHA. Supporting evidence was that when the fattened cells were re-subcultured in glycerol free medium, resistance to BHA was reduced (Al-issa et al., 1984).

Fatty Acids

Fatty acid constituents of bacterial lipids and membranes have been extensively investigated (Kates, 1964; O'Leary, 1967). In bacteria, most fatty acids are linked to larger molecules such as phospholipids, glycolipids, lipoproteins, lipopolysaccharides and lipoteichoic acids while free fatty acids and glycerides are minor (Lechevalier, 1977). The types of fatty acids occurring in

bacteria include: unsaturated, saturated straight chain, hydroxy, branched chain, and cyclopropane (Fulco, 1974; Kaneda, 1963). Asselineau (1966) reported that bacteria do not contain polyunsaturated fatty acids or sterols which are common in other living forms.

Certain types of fatty acids predominate in a given taxon and may be of importance in identifying microorganisms (Moss et al., 1974). Hydroxylated fatty acids are most frequently reported in Gram-negative bacteria (Shaw, 1974). They are found as components of lipopolysaccharides, ornithine-containing lipids, or the polymer, poly-ß-hydroxybutyrate. Differences in unique fatty acids permit the classification of a given genera (Lechevalier et al., 1971 and 1973). Raines et al. (1968) analyzed 33 strains representing ten serotypes of *L. monocytogenes*. A large percentage of methyltetradecanoic acid (C15:0br) was characteristic of each strain. Most strains (26) contained between 31 and 50% of this acid, but 3 contained between 28 and 30%, and 4 contained between 50 and 55%. Two isomeric forms of the methyltetradecanoic acid were present in each strain. Also, 12-methyltetradecanoic acid (C15:0 anteiso-form) was present in concentrations approximately four times that of 13-methyltetradecanoic acid (C15:0 iso-form). The next most abundant constituents were tetradecanoic (C14:0, myristic), hexadecanoic (C16:0, palmitic), and methylhexadecanoic (C17:0br, margaric) fatty acids. The

relative amount of these acids also varied among strains and were 2 to 25%, 6 to 24%, and 4 to 30%, respectively. Other saturated acids such as dodecanoic (C12:0, lauric), pentadecanoic (C15:0, no common name), heptadecanoic (C17:0, margaric) and decosanoic (C22:0, behenic) along with hexadecenoic (C16:1, palmitoleic) and an unidentified component were next in abundance at concentrations less than 10%. Other fatty acids were present in low or trace amounts. Carrol et al. (1968) reported that the fatty acids in *L. monocytogenes* consisted of straight- and branched-chain saturated acids, with C15:0 and C17:0 anteiso acids accounting for about 80% of the total. Al-issa et al. (1984) found that the most abundant acid was anteiso C-15:0 followed by anteiso C-17:0, iso C-15:0, and C-16:0 (hexadecanoic or palmitic). The relative amount of fatty acids varied among cultures. Cultures resistant to butylated hydroxyanisole had a higher proportion of saturated plus iso acids (higher melting points) and long chain acids (anteiso C-17:0 and iso C-17:0), and a lower proportion of medium-chain fatty acids (iso C-15:0 and anteiso C-15:0). Mara et al. (1971) detected an unknown fatty acid of more than 18 carbon atoms. Otherwise, their results were similar to those of Raines et al. (1968). Later, they demonstrated mycolic acid-like substances to be present in the lipids of *Listeria* (Mara and Michalec, 1977). Tadayon et al. (1969) studied the effect of growth

conditions on the fatty acid composition of *L. monocytogenes*. According to their results, fatty acid pattern was affected by growth temperature and growth medium. In particular, the proportion of C17:0br was much lower at 4°C than at higher temperatures and the difference was made up mainly by C15:0br. There was a high proportion of C18:1 during the early stages of the growth in trypticase soy broth while the proportion of C14:0 increased in stationary phase. None of these fatty acids were unique for *L. monocytogenes*. The general fatty acid composition was similar to that of certain *Propionibacterium* species (Moss and Cherry, 1968). One can conclude from the above findings that *L. monocytogenes* may be characterized by the presence of four major acids (C15:0br, C14:0, C16:0 and C17:0br). Variability among strains in the relative amounts of these acids (Raines et al., 1968) however, prohibits distinguishing among serotypes by fatty acid profiles.

In summary, several generalizations can be made concerning microbial fatty acid composition: (a) variation occurs due to media and growth conditions (Vaczi et al., 1967), (b) a high proportion of branched-chain fatty acids occur in aerobes such as *Bacillus* sp. and *M. lysodeikticus* (Cho and Salton, 1966), (c) an abundance of saturated fatty acids and low or virtual absence of polyunsaturated fatty acids (Salton, 1971), and (d) presence of cyclopropane fatty acids in some species (Salton, 1971).

Phospholipids

Phospholipids constitute the major fraction of membrane lipids and provide an essential "structural" function in the cell membrane (Salton, 1971). Phospholipids are basic elements of all cell membranes and the physical chemistry of their role in membrane structure and function has been reviewed in great detail (Chapman, 1968; Bolis and Pethica, 1968). Phospholipids are referred to as "polar" lipids because, although they are soluble in organic solvents, they have hydrophilic groups which serve to make one end of each molecule polar. The opposite end of the phospholipid molecule is substituted with fatty compounds such as fatty acids, fatty aldehydes, or alcohols and is thus hydrophobic (Cronan and Gelman, 1975). Two distinct roles have been elucidated: (1) structural- phospholipids impart permeability and provide a matrix in which membrane proteins are embedded; (2) physiological- phospholipids are required for the function of certain membrane associated enzymes (Cronan and Vagelos, 1972). White et al. (1971) reported that nearly all enzymes involved phospholipids biosynthesis are localized in the inner cytoplasmic membrane. Raetz (1978) observed a tight coupling of phospholipid and fatty acid synthesis in bacteria.

Bacterial phospholipids tend to be of the phosphatidyl glycerol type. The carbohydrates, inositol and glycerol, are common constituents of bacterial phospholipids.

Nitrogen containing compounds such as ethanolamine have also been detected (O'Leary, 1962; Kanfer and Kennedy, 1963). Phosphatidyl choline, prominent in animal cell membranes, occurs rather rarely in bacteria. Phospholipids most common to bacterial cells are phosphatidyl ethanolamine, phosphatidyl glycerol, diphosphatidyl glycerol (cardiolipin) and phosphatidyl serine (Thompson, 1980). Generally, one or two major phospholipids are present in a given species. For example, phosphatidyl ethanolamine in *B. megaterium* strain KM; phosphatidyl glycerol in *M. lysodeikticus*. Phosphatidyl ethanolamine is present in many bacilli but is absent or is a trace component in some micrococci. Extreme halophilic bacteria are quite unique in that they possess phospholipids with ether-linked phytanyl residues instead of fatty acids (Kates et al., 1963). Phosphatidyl glycerol is the major phospholipid in *L. monocytogenes* (Carrol et al., 1968; Ghosh and Carrol, 1968). However, Al-issa et al. (1984) reported phosphatidyl glycerol and diphosphatidyl glycerol as major fractions. The complexity of the phospholipid composition of the membrane lipids is undoubtly dependent upon a number of factors including species differences, age of culture, and nutritional environment during growth. Most available data relate to whole cells and not specifically to isolated membranes or envelopes (O'Leary, 1967).

Each type of phospholipid has a specific function. For

example, diphosphatidyl glycerol has a structural role (Vaczi, 1973). Phosphatidyl glycerol is a precursor of the phospholipids diphosphatidyl glycerol and aminoesters of phosphatidyl glycerol. Vaczi (1973) believed that changes in environmental conditions altered the concentration of this phospholipid resulting in rapid structural alterations of the bacterial membrane. Phosphatidyl ethanolamine comprises the largest portion of phospholipids in many bacteria. Its main function is most probably structural (Ames, 1968). Amino acyl phosphatidyl glycerol has been reported to help regulation of membrane charge by increasing positively charged groups on the cell surface to give protection against the penetration of protons. Its esters may also have roles in synthesis of proteins, transport of amino acids, and biosynthesis of the cell wall (Vaczi, 1973). According to Lechevalier (1977), phosphatidic acid is the most important intermediate product of the biosynthesis of the phospholipids.

Glycolipids

Glycolipids are characterized by a combination of sugars such as glucose, galactose, arabinose, mannose, rhamnose, and trehalose with one or more fatty acids or alcohols. Shaw (1970) defined glycolipids as compounds that contain "carbohydrates in combination with long-chain aliphatic acids or alcohols and which are excreted into organic solvents without prior use of hydrolytic

procedures." Shaw and Baddiley (1968) stated that glycolipid in Gram-positive bacteria included: diglucosyl-, galactosylglucosyl-, dimannosyl-, and digalactosyl-diglycerides in addition to mono- and triglycosyl diglycerides. Although they have been found mainly in Gram-positive species, they contribute about 20% of the lipid of *Mycoplasma laidlawii* (Smith and Mayberry, 1968). In some Gram-positive species, glycolipids accounted for only a few percent of the total lipid (Vorbeck and Marinetti, 1965). Brundish et al. (1967) suggested that glycolipids can adopt a conformation in which hydroxyl groups are on one side of the molecule and lipophilic constituents are on the other side. These investigators raised the possibility of a "pore"-forming function for glycolipids. Hydrophilic regions of a number of glycolipid molecules could come together permitting passage of small ions and water-soluble metabolites through the pore. In *L. monocytogenes*, Carrol et al. (1968) reported that the neutral lipid fraction consisted mainly of diglycerides. This may have been an artifact since phosphatidyl glycerols tend to break down, liberating diglyceride as one of the products (Dehaas et al., 1966). This kind of decomposition may occur to some extent during column-chromatographic separations. Some features of the lipid composition, phospholipids and fatty acid constituents of the bacterial membranes are given in Tables 4 and 5.

Table 4. Features of the Lipid Constituents of Membranes or Envelops of Gram-Positive and Gram-Negative Bacteria.

1. Phospholipids: e. g., phosphatidyl glycerol, phosphatidyl ethanolamine, phosphatidyl cardiolipin (diphosphatidyl glycerol), phosphatidyl serine, glucosaminyl phosphatidyl glycerol, phytanyl glycerol diethers of phosphatidyl glycerol (in halophils).
2. Glycosyl diglycerides: e.g., mono-, di-, and triglycosyl diglycerides; diglycosyl diglycerides dominant class, e.g., mainly in *Mycoplasma* and Gram-positive bacteria.
3. Carotenoids, menaquinones, ubiquinones, polyisoprenoid derivatives: These accessory "lipid-soluble" compounds usually localized in membranes, envelopes, or particles derived from membranes. Polyisoprenoid alcohol phosphate as "wall" intermediates; squalene in halophil.
4. Sterols: Absent from bacterial membranes or envelopes except in *Mycoplasma* spp., where it can be taken up from exogenous source. Trace amounts in some blue-green algae.

Source: Salton, M.R.J. 1971. The bacterial membrane. Ch.1. In "Biomembranes." Vol.1. Manson, L.A. (Ed.) Plenum press, New York.

Table 5. Features of the Fatty Acid Composition of Bacterial Membranes.

Gram-positive	
Micrococci, Bacilli	High proportions of C_{15} and/or C_{17} branched-chain acids
Streptococci, Lactobacilli	Predominantly C_{16}, C_{16}-, and C_{18}-mono-olefinic (C_{18} and *cis*-vaccenic in some)
Gram-negative	
Predominantly C_{16} and C_{18} straight-chain fatty acids, saturated and unsaturated; cyclopropane acids in some; β-hydroxymyristate in lipopolysaccharides; C_{12} hydroxy acids in *Pseudomonas* lipopolysaccharides. Fatty acids only trace amounts in halophil envelopes.	
Polyunsaturated acids absent in most bacteria.	

Source: Salton, M.R.J. 1971. The bacterial membrane. Ch.1. In "Biomembranes." Vol.1. Manson, L.A. (Ed.) Plenum press, New York.

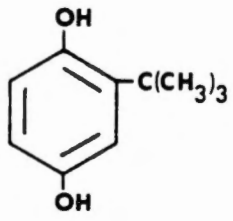
ANTIMICROBIAL AGENTS

Phenolic Compounds

Three phenolic compounds were used in the present study: methyl paraben, propyl paraben, and tertiary butylhydroquinone. Tertiary butylhydroquinone is used as an antioxidant in lipid-containing foods (Figure 2). Food antioxidant properties and the use of this compound have been reviewed (Sherwin and Thompson, 1967). While this compound is added primarily to prevent autoxidation of unsaturated lipids in foods, it is well known to have antimicrobial properties (Branen et al., 1980).

The alkyl esters of p-hydroxybenzoic acid are known as the "parabens" (Figure 3). They were first synthesized by Sabalitschka and co-workers in the 1920's (Prindle and Wright, 1977) as replacements for benzoic and salicylic acids which were effective only at acidic pH (Lueck, 1980). Parabens are effective at both acidic and alkaline pH. Esterification of the carboxyl group allows the molecule to remain undissociated up to pH 8.5 versus normal dissociation of benzoic acid at pH 5.0 (Busta and Foegeding, 1983).

Molecular weights of methyl paraben, propyl paraben and TBHQ are 152.14, 180.21 and 166.22, respectively. The solubility of parabens in water is low and is inversely related to the alkyl chain length. Tertiary butylhydroquinone is insoluble in water but fairly soluble



TBHQ

Figure 2. Structure of Tertiary Butylhydroquinone.



PARABENS

Figure 3. General Structure of the Alkyl Esters of p-Hydroxybenzoic Acid; R = CH_3 (Methyl), and C_3H_7 (Propyl).

in organic solvents. At 25°C, the aqueous solubilities (w/w) of the methyl and propyl esters, and TBHQ are 0.25g/100g, 0.05g/100g, and <1g/100g, respectively (Aalto et al., 1953; Lueck, 1980).

Methyl and propyl esters of p-hydroxybenzoic acid (parabens) are approved for use in the United States as antimicrobials in foods (Code of Federal Regulation, 1988). A combination of the methyl and propyl esters may be added at 0.1% (CFR21.184.1490 and 21.184.1670). The use of a combination of methyl and propyl paraben takes advantage of the higher water solubility of the methyl ester and the greater antimicrobial action of propyl ester (Chichester and Tanner, 1972). Actually, use depends on pH, solubility and their contribution to organoleptic properties (Lueck, 1980). Combination of methyl and propyl parabens (3:1) at the 0.03-0.06% may be used to increase the shelflife of fruit cakes, pie crusts, pastries (non-yeast), icing, toppings, and fillings such as fruit, jellies and creams (Davidson and Juneja, 1990). In certain countries, mixture of 5-15% methyl and propyl parabens in 5-15% alcohol is used to prevent mold growth in hard cheese. Methyl and propyl parabens (2:1) may be used as preservatives in jams and jellies (0.07%), salad dressings (0.1-0.13%), and wines (0.1%) (Davidson, 1983). Tertiary butylhydroquinone (21CFR172.185) has been approved for use in food products at a maximum concentration of 0.02% based upon the fat content

of the food (Code of Federal Regulation, 1988). Sherwin (1990) indicated that TBHQ interacts with free amines to give a red coloration that may preclude its use in some proteinaceous foods where such coloration is not desirable.

Parabens are, in general, more effective against yeast and molds than against bacteria. Against bacteria, they are more effective against Gram-positive than Gram-negative bacteria. Gram-negative bacteria have an outer cell membrane which apparently makes it difficult for parabens to penetrate the cell (Eklund, 1980). Generally, minimum inhibitory concentrations against bacteria range from 100-4000 μg/ml for the methyl ester and 50-1000 μg/ml for the propyl ester (Davidson and Juneja, 1990). Their inhibitory effects on various microorganisms have been determined by a number of researchers (Table 6).

Dje et al. (1989) found that 0.1% propyl paraben, was bacteriostatic against *L. monocytogenes* for 10 d at 4°C in non-fat milk. The ability of *L. monocytogenes* to survive in 15% salt brine and on hot dogs with and without parabens was also studied (Dje et al., 1990). It was found that *L. monocytogenes* remained at a constant level for 24 h in 15% salt brine at 4°C and addition of 0.1% propyl paraben had no effect on viability. In contrast, a combination of 0.1% methyl and propyl paraben (1:1) caused a decrease in *L. monocytogenes* cells. The antimicrobials did not significantly effect *L. monocytogenes* viability when the

Table 6. Antimicrobial Spectrum of Methyl, Propyl and Heptyl Esters of p-Hydroxybenzoic Acid Against Selected Bacteria, Yeasts and Molds.

Microorganism	Concentration for total inhibition[a]		
	Methyl	Propyl	Heptyl
Bacteria			
Aeromonas hydrophila	—	>200	—
Bacillus cereus	2000	125–400	12
Bacillus subtilis	2000	250	—
Clostridium botulinum type A	1000–1200	200–400	—
Enterobacter aerogenes	2000	1000	—
Escherichia coli	2000	400–1000	—
Klebsiella pneumoniae	1000	250	—
Lactococcus lactis	—	400	12
Listeria monocytogenes	>512	512	—
Pseudomonas aeruginosa	4000	8000	—
Pseudomonas fluorescens	1310	670	—
Pseudomonas fragi	—	4000	—
Salmonella typhosa	2000	1000	—
Salmonella typhimurium	—	>300	—
Staphylococcus aureus	4000	350–500	12
Vibrio parahemolyticus	—	50–100	—
Fungi			
Aspergillus flavus	—	200	—
Aspergillus niger	1000	200–250	—
Byssochlamys fulva	—	200	—
Candida albicans	1000	125–250	—
Penicillium chrysogenum	500	125–200	—
Rhizopus nigricans	500	125	—
Saccharomyces bayanus	930	220	—
Saccharomyces cerevisiae	1000	125–200	100

[a]Minimum inhibitory concentration in µg/mL (ppm).
Sources: Aalto et al. (1953); Bargiota et al. (1987); Davidson (1983); Dymicky and Huhtanen (1979); Jurd et al. (1971); Lee (1973); Marwan and Nagel (1986); Moustafa and Collins (1969); Payne et al. (1989); Pierson et al. (1980); Robach and Pierson (1978); Sokol (1952); Venugopal et al. (1984).

Obtained From: Davidson, P.M. and Juneja, V.K. 1990. Antimicrobial agents. Ch. 4. In "Food Additives", Branen, A.L., Davidson, P.M. and Salminen, S. (ed.) Marcel Dekker, Inc. New York.

organisms were inoculated onto the surface of hot dogs and dipped into salt brine containing parabens.

Branen et al. (1980) found that potassium sorbate in combination with TBHQ was synergistic against *S. aureus* and *Salmonella typhi*. In agar medium, TBHQ (0.003%) prevented the growth of *S. aureus* (Erickson and Tompkin, 1977). Davidson et al. (1981) reported that 25 ppm TBHQ was effective in preventing the growth of *S. aureus* in a nutrient broth. Rico-Munoz and Davidson (1982) indicated that 300 ppm TBHQ only delayed the lag phase of *P. fluorescens*. Also, TBHQ was found to have an inhibitory effect on aflatoxin production by aspergilli (Lin and Fung, 1983). The minimum inhibitory concentration (MIC) of TBHQ for eight strains of *L. monocytogenes* was reported to be 64 μg/ml (Payne et al., 1989). The antimicrobial effect of TBHQ against Gram-positive cocci was also studied by Raccach and Hemingsen (1981) with the finding of some inhibitory effects. The only drawback in using TBHQ as antimicrobial agents is its lipophilic character (Davidson, 1983). Solubilization of the antioxidant into the lipid phase nearly eliminates its activity against microorganisms which are present in the water phase.

Mode of action

The mechanism by which these compounds inhibit growth of microorganisms is not fully understood. There are three possible targets for the antimicrobial action of phenolic

compounds: (1) the cell membrane, (2) genetic material, and (3) enzymes (Davidson and Branen, 1981). Hamilton (1968) suggested phenolics have a common mechanism of action in which adsorption of the compound on the cell membrane is a critical step. Hugo and Franklin (1968) indicated that phenolic compounds were distributed between lipid and aqueous components of the cell in accordance with normal partition laws. When fattened cells (cells where the lipid content was increased due to the presence of glycerol in the growth medium) were used, phenols were partially distributed in the deposited lipid.

The effect of phenolic compounds on leakage was first studied by Vas (1953) who suggested that phenol attacked the cytoplasmic membrane of microorganisms, resulting in leakage of intracellular constituents. Furr and Russel (1972) showed that parabens inhibited the growth of *Serratia marcerens* and that they induced leakage of intracellular constituents from non-growing cells. They concluded that the parabens initially bind to the cell wall of the bacterial cells, but their primary effect was to cause damage to the cytoplasmic membrane. Eklund (1980) proposed that leakage of intracellular constituents was due to neutralization of normal membrane gradient by parabens. Davidson and Branen (1980), and Degre and Sylvestre (1983) suggested that inhibition and lethality against bacteria were at least partially due to leakage of intracellular

components following disruption of the cytoplasmic membrane when the phenolic antioxidant, butylated hydroxyanisole (BHA), was used. Bargiota et al. (1987) measured changes in total lipid content of four strains of *S. aureus* as related to resistance to the esters of p-hydroxybenzoic acid. The antimicrobial effect of parabens was shown to be correlated to total lipid content and phospholipid composition.

Some authors have investigated the effect of phenolic compounds on uptake of various nutrients. Freese et al. (1973) demonstrated that parabens inhibited serine uptake and ATP production in *Bacillus subtilis*. They concluded that parabens somehow uncoupled both substrate transport and oxidative phosphorylation from the electron transport system and partially inhibited the electron transport system itself. Parabens were also found to decrease the uptake of several amino acids, but not of glucose by membrane vesicles of *Escherichia coli*, *B. subtilis* and *P. aeruginosa* (Eklund, 1980). In addition, Eklund (1980) postulated that growth inhibition caused by parabens appeared to be a consequence of transport inhibition.

Several researchers studied the effect of phenolic compounds on enzymes (Fogg and Lodge, 1945; Roberts and Rahn, 1946; Rico-Munoz et al., 1987). Inactivation of enzymes disrupts membrane functions. Elter et al. (1974) suggested that compounds which perturb the lipids of membranes cause either decreased activity of membrane-bound

enzymes or put some enzymes in contact with substrates from which they are ordinarily restricted by means of cell geography, resulting in death of the cell. Studies on a compound with a structure similar to the parabens, butylated hydroxyanisole, showed a significant effect on the activity of the membrane-bound adenosine triphosphatase of *S. aureus* (Rico-Munoz et al., 1987).

The above-mentioned research provides evidence that phenolic compounds act on the cytoplasmic membrane. The actual mechanism by which parabens inhibit microorganisms is most probably related to their direct or indirect effect on cellular enzymes systems located in the membrane.

Sodium Chloride

Sodium chloride has been used as an preservative agent in foods for thousands of years (Jensen, 1954; Binkerd and Kolari, 1975; and Marsden, 1980). This compound is readily soluble in water at neutral pH values. A saturated solution of sodium chloride contains 26.5g/100g of water.

Sodium chloride lowers water activity, which results in unfavorable conditions for microbial growth. When available water is reduced, cells are subjected to osmotic shock and rapidly lose water through plasmolysis (Davidson and Juneja, 1990). Other mechanisms restricting the growth of microorganisms include dehydration, interference with enzymes, alteration of pH, toxicity of sodium and chloride ions, and loss of magnesium ions (Banwart, 1979).

Microorganisms vary in their tolerance to sodium chloride. In general, relatively low concentrations of sodium chloride stimulate microbial growth whereas high concentrations inhibit growth (Banwart, 1979). Ayres and co-workers (1980) reported that 3-5% NaCl is necessary to prevent the growth of most bacteria.

Some reports have indicated that *L. monocytogenes* is quite tolerant to sodium chloride, being capable of growing in 10% NaCl and surviving for 1 year in 16% NaCl at pH 6.0 (Seeliger, 1961). Dje et al. (1990) found that *L. monocytogenes* numbers remained constant for 24 hour in 15% salt brine at 4°C. Murray (1955) observed that this organism survived and remained fully virulent in 20% NaCl at 4°C for 8 weeks. At 30°C, *L. monocytogenes* could grow in cabbage juice containing 2% NaCl, but not 5% (Conner et al., 1986). *Listeria monocytogenes* strain CA was inactivated in 85 days in skim milk with 12% NaCl or 110 days in whey with 12% NaCl (Papageorgiou at al., 1989). In contrast, *L. monocytogenes* Scott A survived for 130 days in both 12% salted skim milk and whey. Shahamat et al. (1980) reported that *L. monocytogenes* could survive at 37°C for 15 days in 10.5% NaCl and 5 days in 20-30% NaCl. Survival time doubled when the temperature was lowered to 22°C. The organism was able to survive more than 100 days in 10.5-30% NaCl at 4°C. Thus, survival of the organism is a function of temperature. Other researchers also reported that lower temperature

enhanced survival (Welshimer, 1960; Dickgieber, 1980). This is an important factor in *Listeria's* significance in the food chain and a major concern to the food industry.

MODIFICATION OF LIPID COMPOSITION

Several methods have been used to manipulate lipid composition of prokaryotes and yeast (Sullivan et al., 1974) in order to elucidate the functional role or biochemical importance of lipids in membranes. Methods employed have included: use of fatty acid auxotrophs (Cronan and Gelman, 1975), altered growth conditions (Marr and Ingraham, 1962), ethanol supplementation and the use of 3-decynoyl-N-acetylcystamine, which inhibits synthesis of unsaturated fatty acids (Kass, 1968). In addition, an antilipogenic antibiotic, cerulenin, isolated from the culture filtrate of *Cephalosporium caerulens*, has been shown to be a specific inhibitor of lipid biosynthesis (Omura,1976). Cerulenin acts by inhibiting ß-Ketoacyl-acyl carrier protein synthetase, the enzyme which catalyzes the condensation reaction of fatty acid biosynthesis (Figures 4 and 5; more specifically, the enzyme which catalyzes the condensation of fatty acyl-acyl carrier proteins with malonyl-acyl carrier protein (melonyl-ACP); D'Agnolo, et al., 1973). Goldberg et al. (1973) reported the effect of cerulenin on metabolism and function of fatty acids in bacterial membranes. They found that cerulenin inhibited both saturated and

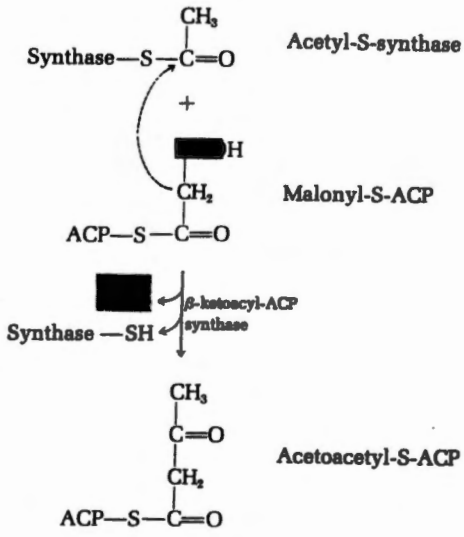


Figure 4. The Condensation Reaction in Fatty Acid Synthesis.

Source: Lehninger, A.L. 1975. "Biochemistry," 2nd edition. Worth Publishers, Inc., New York.

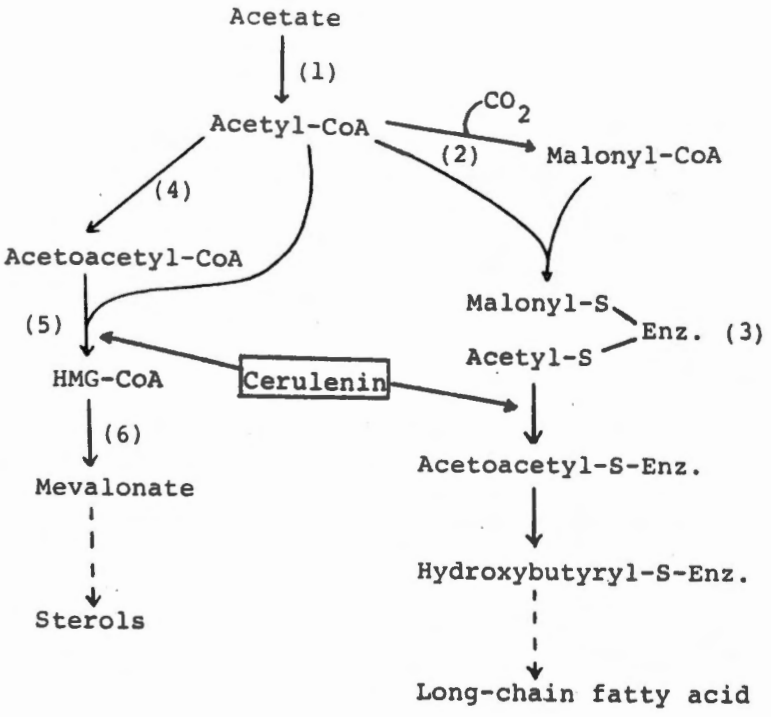


Figure 5. Targets of Cerulenin in Biosynthesis of Sterol and Fatty Acid. (1) Acetyl-CoA Synthetase; (2) Acetyl-CoA Carboxylase; (3) Fatty Acid Synthetase; (4) Acetoacetyl-CoA Thiolase; (5) HGM-CoA Synthase; (6) HGM-CoA Reductase.

Source: Omura, S. 1976. The antibiotic cerulenin, a novel tool for biochemistry as an inhibitor for fatty acid synthesis. Bacteriol. Rev. 40:681.

unsaturated fatty acid synthesis in *E. coli*. Prescott and Vagelos (1972) reported that the pathway of fatty acid biosynthesis in *E. coli* involves several enzymes and partial reactions (Figure 6). Reactions 4 to 7 are utilized in a cyclic fashion, with each successive addition of two carbon units being derived from malonyl-ACP.

Several studies have demonstrated that an exogenous supply of both saturated and unsaturated fatty acids or branched-chain saturated acid are required to restore fully or partially, the inhibition by cerulenin (Goldberg et al., 1973; Wille et al., 1975). Exogenous fatty acids are incorporated to different extents in the presence of cerulenin. Cerulenin treated *B. subtilis* cells were grown in the presence of palmitoleic acid (Krulwich et al., 1987). The authors observed that the cells restored this fatty acid in the membrane lipids to somewhat higher levels than was found in the parent strain. Esfahani et al. (1971) examined the fatty acyl content of the membrane phospholipids in *E. coli* and reported that linolenic acid was incorporated much less efficiently than was oleic acid. The above research provides sufficient evidence that exogenously supplied fatty acids can modify the lipid composition of bacterial membranes.

1. Acetyl CoA carboxylase	Acetyl-S-CoA + HCO_3^- + ATP $\rightleftharpoons$ malonyl-S-CoA + ADP + P_i
2. Acetyl CoA–ACP transacylase	Acetyl-S-CoA + HS-ACP $\rightleftharpoons$ acetyl-S-ACP + CoA-SH
3. Malonyl CoA–ACP transacylase	Malonyl-S-CoA + HS-ACP $\rightleftharpoons$ malonyl-S-ACP + CoA-SH
4. β-Ketoacyl synthetase	Acetyl-S-ACP + malonyl-S-ACP $\rightleftharpoons$ acetoacetyl-S-ACP + CO_2 + HS-ACP
5. β-Ketoacyl reductase	Acetoacetyl-S-ACP + NADPH + H^+ $\rightleftharpoons$ D(−)-β-hydroxybutyryl-S-ACP + $NADP^+$
6. β-Hydroxyacyl dehydrase	D(−)-β-Hydroxybutyryl-S-ACP $\rightleftharpoons$ crotonyl-S-ACP + H_2O
7. Enoyl reductase	Crotonyl-S-ACP + NADPH [or NADH (Weeks and Wakil, 1968)] + H^+ $\rightleftharpoons$ butyryl-S-ACP + $NADP^+$ [or NAD (Weeks and Wakil, 1968)]

Figure 6. The Pathway of Fatty Acid Biosynthesis in *Escherichia coli*.

Source: Prescott, D.J. and Vagelos, P.R. 1972. Acyl carrier protein. Adv. Enzymol. 36:269.

CHAPTER III.

MATERIALS AND METHODS

LISTERIA MONOCYTOGENES

Nine strains of *Listeria monocytogenes* (Scott A, LM4A, LM4C, LM4E, LM40, LM46, LM5069, F5027 and HD) were used in the lipid profile study. Two strains (Scott A and LM4A) were randomnly selected for the effect of antimicrobials and varying temperatures on lipid profile, and the modification of lipid composition studies. All strains were obtained from American Type Culture Collection (Rockville, MD) except for HD which was isolated from commercial hot dogs. All strains were grown on tryptose phosphate agar (TPA; Difco, Detroit, MI) slants at 35°C for 24 h and stored at 4°C. The cultures were transferred periodically to maintain viability. Tryptose phosphate broth (TPB; Difco) was used to grow cultures prior to all experiments. Peptone (0.1% w/v) was used as a diluent for cultures grown in TPB.

Lipid Profile of *Listeria monocytogenes*

Lipid extraction

A modification of the method described by Bligh and Dyer (1959) was used. *Listeria monocytogenes* cells were grown in TPB for 18 h at 35°C, and harvested by centrifugation at 14,000 x g for 20 min. The cell pellet was washed with peptone water (0.1%, w/v) and re-centrifuged for 20 min at 14,000 x g. Cells were weighed and suspended in 25 ml deionized water. Chloroform (ca. 25 ml) and

methanol (1:2) were added to the suspension followed by mixing. Chloroform (ca. 25 ml) was added and mixture shaken vigorously for 30 sec followed by ca. 25 ml water with further mixing for 30 sec. The homogenate was filtered through Whatman No. 1 filter paper on a Buchner funnel with slight suction. Filtrate was transferred to a 500 ml graduated cylinder and, after allowing a few minutes for complete separation, the volume of the chloroform layer recorded and the alcohol layer removed by aspiration. The chloroform layer contained the purified lipid. Chloroform was removed by evaporation under nitrogen and the fraction used phospholipid composition determination.

Determination of total lipids

A portion of the chloroform layer (10 ml) was added to a dried and weighed beaker, evaporated to dryness under nitrogen and placed in a vacuum desiccator. The beaker was heated at 83°C for 24 h and then re-weighed. Total lipid content per Gram of cells was determined on dry-weight basis.

Phospholipid determination

To separate and identify phospholipids, thin layer chromatography was used. Reference compounds were: phosphatidyl inositol, phosphatidyl glycerol, phosphatidyl ethanolamine, phosphatidyl serine, and diphosphatidyl glycerol. All reference compounds were obtained from Sigma Chemical Co. (St. Louis MO) and stored at -18°C. Lipid

extract from *Listeria monocytogenes* cells (50 mg) was dissolved in 1 ml chloroform and 10 μl portions spotted in 10 applications on pre-activated silica gel thin layer plates (20 x 20 cm, 250 μm thick; Whatman, Inc., Clifton, NJ). Plates were developed in tanks lined with No. 1 filter paper (Whatman) equilibrated for 10 min in mobile phase. The mobile phase consisted of chloroform : methanol : water (120:52.2:7.5; v/v/v). Plates were dried, sprayed with 50% sulfuric acid and charred at 130°C for 45 min. Qualitative phospholipid composition was determined by comparing R_f-values with standards. Quantitative determination (relative percentage) of individual phospholipids was determined by scanning the TLC plates with a spectrodensitometer.

Analysis of fatty acids

Fatty acid composition was determined using a modification of the MIDI Automated Microbial Identification System (MIS) (MIDI, no date). Cultures were grown for 24 h at 35°C on plates of tryptose phosphate agar (TPA; Difco). The cell mass from the surface of one plate was removed and transferred to a tube (13 x 100 mm) containing 1.0 ml of Reagent 1 (45 g sodium hydroxide, 150 ml methanol, 150 ml distilled water). Tubes were sealed with Teflon-lined caps and heated in a boiling water bath for ca. 5 min. Tubes was mixed for 5-10 sec and returned to the water bath to complete the 30 min heating. The tube was removed from the bath, cooled to room temperature, and 2 ml of Reagent 2 (325

ml 6.0 N HCl, 275 ml methyl alcohol) was added. The mixture was heated for 10 min at 80°C in a water bath, cooled and 1.25 ml of Reagent 3 (200 ml hexane, 200 ml methyl tert-butyl ether) added. The tube was gently tumbled on a clinical rotator for about 10 min and the aqueous (lower) phase discarded. About 3 ml of Reagent 4 (10.8 g NaOH in 900 ml of distilled water) was added to the organic phase and the tube mixed for 5 min. Two-thirds of the the organic phase was transferred to a vial, flushed with nitrogen and stored at -18°C. Methyl esters were analyzed using Hewlett Packard (HP; Kennett square, PA), 5890 Series II Gas Chromatograph on a SPB-1 fused silica capillary column (Supelco, Inc. Bellefonte, PA). Major fatty acids were identified by comparison of retention times with those of pure bacterial fatty acid methyl esters standard (Supelco, Inc.). The chromatographic conditions were as follows: column temperature programmed from 150°C to 250°C at 4°C/min; carrier gas flow rate linear velocity, 20 cm/sec of helium; injection temperature, 250°C; detector, flame ionization at 280°C; sample size, 5 μl of sample extract, 1 μl of standard.

Effect of Antimicrobials on Lipid Composition

Cultures were incubated at 7°C and 35°C in TPB containing various subminimal inhibitory concentrations of parabens (100 to 500 ppm) and sodium chloride (1% to 5%). Cells were incubated to an absorbance at 600 nm of 0.6.

Cells were harvested by centrifugation at 14,000 x g for 20 min, washed with peptone water (0.1%, w/v) and centrifuged again for 20 min at 14,000 x g. Phospholipid composition was determined as above. Also, the broth culture (1 ml) was spread-plated on TPA supplemented with the same level of parabens and sodium chloride and incubated for the same time as with TPB. The cells mass from the surface of TPA was removed with a sterile inoculating loop and processed for fatty acids as above.

Effect of Varying Temperatures on Lipid Composition

Cultures were incubated at various temperatures (7°C, 15°C, 24°C, 30°C and 35°C) in TPB. Cells were incubated to an absorbance at 600 nm of 0.6. Phospholipid and fatty acids composition were determined as above.

Modification of Lipid Composition

A system to create *Listeria monocytogenes* strains with altered fatty acid and phospholipid composition was developed in two ways. In method 1, bacterial cells were grown in TPB in the presence of exogenous fatty acids (e.g., C14:0, C18:0, C18:1) and harvested by centrifugation. The cell pellet was washed with peptone water (0.1%, w/v) containing 0.1% Tween 80. The pellet was broken with a sterile inoculating needle, followed by centrifugation. Cells were again washed with peptone water (without Tween 80) and centrifuged. This washing and centrifugation was repeated three times. Thereafter, cells were grown in TPB

(in the absence of exogenous fatty acid) for 24 hours. The cells were washed, harvested and processed for fatty acids and phospholipid composition. Method 2 involved the use of cerulenin. Cerulenin (Sigma) was dissolved in 95% ethanol. Sterile microtiter plates (120 mm x 80 mm) with 96 U-shaped wells were used to determine the minimum inhibitory concentration of cerulenin. Various concentrations of cerulenin, 10μl of bacterial inoculum containing about 10^5 cells, and TPB were added to wells. The total volume in each well was 300 μl. Plates were incubated at 35°C for 24 h. The first concentration of cerulenin that prevented growth (indicated by no turbidity) was termed the "minimum inhibitory concentration" (MIC).

Cells were grown in polypropylene micro centrifuge tubes (American Scientific Products; McGaw Park, IL.) containing TPB in the presence of subminimal inhibitory concentration (SIC; twofold dilution below the MIC) of cerulenin with exogenously supplied fatty acids. The cells were washed, harvested and processed for fatty acids.

Resistance of Lipid Adapted Strains

Listeria monocytogenes strains with altered lipid composition were evaluated for resistance to parabens and salt by minimum inhibitory concentration and inactivation over time.

Minimum inhibitory concentration

Cells were grown in TPB in the presence of exogenous

fatty acid and with or without cerulenin. Thereafter, cells were harvested and washed four times with peptone water (0.1%, w/v) with and without Tween 80. The MIC was determined using a broth dilution assay.

Inactivation over time

Cells were grown in TPB in the presence of exogenous fatty acid and with or without cerulenin. Cells were harvested and washed with peptone water (0.1%, w/v) four times with and without Tween 80 as above. Harvested cells were suspended in 0.1% peptone water containing methyl paraben (1500 ppm), propyl paraben (1000 ppm), methyl and propyl paraben (0.1%), sodium chloride (10%), or TBHQ (200 ppm). This was followed by incubation at 35°C. Samples were taken at 15 min interval for 24 h and at 3 h, serially diluted and plated on TPA. The plates were incubated at 35°C for 48 h for colony count determination.

Isolation of Fatty Acid Synthetase

Effect of parabens and sodium chloride on fatty acid synthetase enzyme activity at 7°C and 35°C was determined. A modification of the method described by Harder et al. (1974) was used. Cells were grown at 35°C, harvested in late log phase and washed twice with one culture volume of 0.01 M potassium phosphate (pH 7.0). Approximately 1 g of cells were suspended in 5 ml of 0.05 M potassium phosphate (pH 7.0) containing 1 mM dithiothreitol (DTT) and disrupted in a French pressure cell at 18,000 p.s.i. The supernatant

obtained by centrifuging the broken cell suspension at 4°C, 48,000 x g for 30 min were diluted in homogenization buffer to a protein concentration of 15 mg/ml measured by Bio-Rad protein assay (Bio-Rad, 1990). The solution was brought to 45% saturation with ammonium sulfate (0.285 g/ml, w/v) and allowed to stand for 10-15 min prior to centrifugation at 4°C, 40,000 x g for 10 min. The supernatant was brought to 80% saturation with ammonium sulfate (additional 0.226 g/ml) and the precipitate collected by centrifugation. The precipitate was frozen in liquid nitrogen and stored at -70°C.

Assay of fatty acid synthetase activity

In vitro fatty acid synthetase activity was assayed by modification of the method used by Kawaguchi (1982). All substrates were equilibrated at 7°C and 35°C (i.e., the activity was determined at two temperatures). The reaction mixture contained 0.1 M potassium phosphate buffer, pH 6.5, 2 mM EDTA, 10 mM cysteine, 0.2 mM NADPH, 0.05 mM acetyl-CoA, 0.08 mM malonyl-CoA, 0.3 mg of bovine serum albumin, and fatty acid synthetase complex, 10 μg of protein in a total volume of 1.0 ml. After measuring the blank for 3 min the reaction was started by the addition of 0.02 ml of 4 mM malonyl-CoA. The rate of NADPH oxidation was measured using Hewlet Packard, 8452A diode array spectrophotometer at 340 nm. To determine the effect of antimicrobials on the activity of the enzyme, 3 μl of a 1000 ppm of methyl

paraben, 3 μl of 500 ppm of propyl paraben, or 3 μl of 10% sodium chloride solution was added to the reaction mixture.

Statistical Analysis

All data were analyzed by analysis of variance using Statistical Analysis System (SAS) of the University of Tennessee Computer Center. The dependent variables were strains, temperatures, concentration of antimicrobials and replication. Tukey's Multiple-Range Test was used to separate significant differences ($p<0.05$) among means (Steel and Torrie, 1960).

CHAPTER IV

RESULTS AND DISCUSSION

Total Lipid Determination

The total lipid content of the nine strains of *L. monocytogenes* averaged 6.25% on a dry weight basis. The percentage of lipid ranged from a high of 7.5% for strain HD to 5.7% in strain LM5069 (Table 7). Statistical analysis of the results showed that there was significant difference among the strains. Carrol et al. (1968) reported *L. monocytogenes* contained 6-7% lipid on dry weight basis. It must be noted that the lipid content of bacteria varies with the environmental condition during growth of the organism. For example, Tadayon et al. (1969) indicated *L. monocytogenes* produces more lipid at lower temperature.

There are several reports in the literature correlating the amount of lipid possessed by bacterial cells and their resistance to antimicrobials (Hill et al. 1963; Hugo and Stretton, 1966; Bargiota, 1987). These studies indicated that cells with higher lipid content were more resistant to a variety of antimicrobial agents. Extra lipid in the cell envelope acts purely as a permeability barrier (Hamilton, 1968). Thus, within the cell envelope there is a permeability barrier to the antimicrobials and lipid content of the cells is intimately involved in this barrier. Hugo and Franklin (1968) and Furr and Russell (1972) theorized that antimicrobials are adsorbed to the surface of cells

Table 7. Percent Total Lipid[a] on a Dry Weight Basis of Nine Strains of *Listeria monocytogenes* Grown in Tryptose Phosphate Broth at 35°C.

Strains	Total Lipid Percentages	SD[k]
Scott A	6.70[f]	0.03
LM4C	6.95[e]	0.03
LM4A	6.55[g]	0.06
F5027	7.30[c]	0.04
LM5069	5.75[j]	0.04
LM46	6.09[h]	0.04
HD	7.50[b]	0.04
LM4E	7.24[d]	0.03
LM40	5.81[i]	0.01

[a]Means represent two replications.
[b-j]Means with different superscripts are significantly different ($p<0.05$).
[k]Standard deviation.

high in lipid whereas with normal cells many antimicrobials will pass into the interior of the cell. *Listeria monocytogenes* has been found to be relatively resistant to the effects of compounds such as sodium chloride, nitrite, sodium benzoate, sodium propionate, sorbic acid and parabens which normally inhibit the growth of foodborne pathogens (Conner et al. 1986; Janna et al. 1989; Moustafa et al. 1988). Therefore, it was logical to determine the influence of lipid content on resistance.

Lechevalier (1977) reported that the total lipid content of most bacteria is quite low, usually less than 5% by dry weight. Four strains of *S. aureus* were found to contain an average of 3.3% total lipid (Bargiota, 1987). In this previous study, a significant correlation ($p<0.05$) was found between the amount of lipid in the cells and the resistance to propyl paraben ($r=0.77$) or methyl and propyl paraben mixture ($r=0.89$). Dunnick and O'Leary (1970) reported that the antibiotic resistant and sensitive strains of *S. aureus* contained 5.45% and 3.41% lipid, respectively. Freshly isolated pathogenic streptococci possessing more cell lipid than laboratory strains showed higher resistance to tetracycline (Hill et al., 1963). This provides sufficient evidence that the amount of total lipid is directly related to resistance of microorganisms. Thus, the increased resistance of *L. monocytogenes* to a number of antimicrobials may be attributed to high lipid content.

Researchers have attempted to increase the cellular lipid content of *L. monocytogenes* by repeated subculturing in the presence of glycerol (Al-issa et al. 1984). In this study, fattened cells showed considerably greater resistance to butylated hydroxyanisole.

The fact that several antimicrobial agents have shown an increase in their antimicrobial activity when their lipid solubility increased has led to the suggestion that cellular lipid may be a factor in determining the susceptibility of bacteria to these compounds. For example, lipid solubility of parabens, as measured by their chloroform-buffer distribution (Baranowski and Nagel, 1983), was suggested to be of equal importance in the resistance of cells as the amount of cellular lipid.

Phospholipid Determination

The relative percentage of phospholipid in the nine strains of *L. monocytogenes* was determined from the total extractable lipid using thin layer chromatography. The two major phospholipids found in each of the nine strains examined were phosphatidyl glycerol (RF=0.39) and diphosphatidyl glycerol (RF=0.41). Also, phosphatidyl serine (RF=0.24) and phosphatidyl ethanolamine (RF=0.29) were detected in strain Scott A and LM4C, respectively (Table 8). Strain Scott A had a higher percentage ($p<0.05$) of phosphatidyl glycerol than LM4C and less than ($p<0.05$) LM4A, F5027, LM5069, LM46, HD, LM4E, and LM40. For strains

Table 8. Relative Percentage (± Standard Deviation) of Phospholipid Fractions of Nine Strains of *Listeria monocytogenes* Grown in Tryptose Phosphate Broth at 35°C.

Strain	Mean Relative Percentage[a]			
	Phosphatidyl Glycerol	Phosphatidyl Glycerol	Phosphatidyl Ethanolamine	Phosphatidyl Serine
Scott A	40.3[d]±3.25	29.5[d]±1.55	ND[f]	30.2±1.70
LM4C	31.7[e]±1.98	28.8[d]±2.97	39.5±0.99	ND
LM4A	52.0[c]±3.81	48.0[b]±3.82	ND	ND
F5027	59.8[b]±3.39	40.2[c]±3.39	ND	ND
LM5069	63.2[b]±3.11	36.8[c]±3.11	ND	ND
LM46	57.1[bc]±3.25	42.9[bc]±3.25	ND	ND
HD	61.4[b]±1.55	38.6[c]±1.55	ND	ND
LM4E	57.6[bc]±2.40	42.4[bc]±2.40	ND	ND
LM40	62.1[b]±1.83	37.9[c]±1.84	ND	ND

[a]Means represent two replications.
[b-c]Means in columns with different superscripts are significantly different ($p<0.05$).
[f]Not detectable.

Scott A and LM4C, the relative percentage of diphosphatidyl glycerol was less ($p<0.05$) than the other strains. This may be attributed to the fact that strain Scott A and LM4C contained additional phospholipid i.e., phosphatidyl serine (strain Scott A) and phosphatidyl ethanolamine (strain LM4C).

The major phospholipids found in the nine strains were in agreement with results from previous researchers. Carrol et al. (1968) reported phosphatidyl glycerol as the major phospholipid in *L. monocytogenes*. Al-issa et al. (1984) reported phosphatidyl glycerol and diphosphatidyl glycerol as the major phospholipid fractions of *L. monocytogenes*.

Phospholipid composition was determined because it may be related to resistance of bacteria. Bargiota (1987) reported that *Staphylococcus aureus* strain LP, which was more resistant to antimicrobials, had the highest amount of phosphatidyl glycerol. Van Deenen (1972) showed that the permeability of a membrane is partially determined by its lyso-phosphatidyl glycerol (a phosphatidyl glycerol derivative) content. Vaczi (1973) stated that two phospholipids can be derived from phosphatidyl glycerol, cardiolipin and amino acid esters of phosphatidyl glycerol. The possibility that phospholipids with two opposite charges could be synthesized from the same precursor could insure a rapid structural alteration of the bacterial membrane to adverse conditions or environmental conditions.

The role of phosphatidyl serine and phosphatidyl ethanolamine, detected in Scott A and LM4C, respectively, has not been correlated to resistance in the literature. Most probably, the biological role of these phospholipids in bacteria is either structural or unknown.

Determination of Fatty Acid Composition

The fatty acid composition of the nine strains of *L. monocytogenes* was surveyed to find if differences existed among the types or relative percentages of individual fatty acids. The objective of this experiment was to determine the variability of *L. monocytogenes* strains. This variability could be important since gas-liquid chromatography of fatty acid methyl esters is used to identify *L. monocytogenes*. The most abundant fatty acids for each of the nine strains of *L. monocytogenes* were C15:0 branched chain fatty acids (13-methyltetradecanoic acid, i-15:0; 12-methyl- tetradecanoic acid, a-15:0; Table 9). These fatty acids accounted for an average 48.9% of the total. The percentage of these fatty acids ranged from a high of 61.4% for strain LM40 to 37.1% in strain HD. The next most abundant, with an average of 32.6%, were C17:0 branched chain fatty acids (15-methylhexadecanoic acid, i-17:0; cis-9,10-methylene hexadecanoic acid, C17:0Δ). Strain LM46 had the greatest percentage of this fatty acid (43.7%) and strain LM4C had the lowest (22.9%). The remaining fatty acids detected in order of prevalence were (mean relative

Table 9. Fatty Acid Composition (Mean Relative Percentages from two Replications) of 9 Strains of *Listeria monocytogenes*, Grown on Tryptose Phosphate Agar at 35°C.

Fatty Acid	*Listeria monocytogenes* strain								
	Scott A	LM4C	LM4A	F5027	LM5069	LM46	HD	LM4E	LM40
2-OH 10:0	.11^{e}	.16de	.10^{e}	.47^{b}	.16de	.27^{c}	.69^{a}	.47^{b}	.22^{cd}
12:0	.25^{g}	.37^{f}	.17^{g}	1.14^{b}	.24^{g}	.67^{d}	1.26^{a}	.93^{c}	.45^{e}
13:0	.03^{e}	.07^{e}	.45^{b}	-	.32^{b}	-	-	.71^{a}	-
14:0	.67^{e}	.65^{e}	.53^{e}	1.13^{a}	.53^{e}	.63^{e}	1.24^{a}	.93^{b}	.56^{e}
2-OH 14:0	1.89^{b}	.90^{d}	2.15^{a}	2.19^{a}	1.66^{b}	1.88^{b}	1.65^{b}	1.13^{c}	1.86^{b}
br-15:0^{h}	49.85^{c}	61.15^{a}	40.66^{f}	39.96^{f}	47.89^{d}	43.19^{e}	37.10^{g}	59.34^{b}	61.36^{a}
15:0	.18^{ab}	.17^{ab}	.16^{ab}	-	.11^{b}	.24^{a}	-	-	-
16:0	2.77^{d}	2.55^{e}	2.27^{f}	5.07^{a}	2.05^{g}	2.60^{e}	4.75^{b}	3.15^{c}	1.67^{h}
16:1	-	-	.09^{a}	-	.14^{a}	-	-	-	-
2-OH 16:0	.07^{b}	.12^{b}	-	.26^{a}	.13^{b}	-	-	-	-
br-17:0^{i}	32.99^{c}	22.91^{f}	32.71^{c}	33.42^{c}	37.18^{b}	43.73^{a}	29.19^{e}	30.60^{d}	31.03^{d}
17:0	-	-	.17^{b}	-	.33^{a}	-	-	-	-
18:0	.69de	1.08^{c}	.25^{f}	1.83^{a}	.78^{d}	.59^{e}	1.50^{b}	-	.54^{e}
18:1	.86^{d}	1.90^{bc}	.38^{e}	2.40^{a}	.94^{d}	.92^{d}	2.13^{ab}	1.62^{c}	.66de
18:2	.19^{b}	-	-	.59^{a}	-	.15^{b}	-	-	-
19:0 Δ	.12^{d}	.40^{cd}	2.20^{a}	.67^{c}	1.10^{b}	.31^{d}	1.26^{b}	-	-
Unk^{j} 1	.13^{b}	-	-	-	.90^{a}	.34^{b}	-	-	-
Unk 2	.44	-	-	-	-	-	-	-	-
Unk 3	.14^{e}	1.39^{d}	3.68^{b}	1.64^{c}	1.33^{d}	-	7.97^{a}	-	-
20:0	.25^{c}	.10^{d}	.40^{b}	.45^{ab}	-	.41^{b}	.45^{ab}	.55^{a}	.16^{cd}
Unk 4	1.95^{c}	2.02^{c}	5.65^{a}	4.33^{b}	1.73^{c}	-	5.83^{a}	-	.21^{d}
Unk 5	1.92^{a}	-	-	-	-	.81^{a}	-	-	-
Unk 6	2.96^{b}	2.96^{b}	5.30^{a}	3.89^{b}	1.37^{c}	2.99^{b}	3.91^{b}	-	.57^{c}
TOTALS	98.46	98.90	97.32	99.44	98.89	99.73	98.93	99.43	99.29

$^{a-g}$Means in rows with different superscripts are significantly different ($p < 0.05$).
^{h}i-15:0 and a-15:0.
^{i}i-17:0 and 17:0Δ.
^{j}Unknown fatty acid.

percentage): C16:0 (3%), 2-OH-14:0 (1.7%), C18:1 (1.3%), C18:0 (0.8%), C14:0 (0.8%), C19:0Δ (0.7%), C12:0 (0.6%), C20:0 (0.3%), and 2-OH-C10:0 (0.3%). Trace amounts of C13:0 (0.2%), C15:0 (0.1%), C18:2 (0.1%), 2-OH-C16:0 (0.1%), C17:0 (0.1%), and C16:1 (0.03%) were also detected. Thus, the relative percentage of each fatty acid varied among the strains. At least 6 unknown fatty acids with retention times greater than that for C19:0Δ were present in most strains with relative percentages as high as 6%. A few fatty acids were found to be unique to one or two strains.

The major fatty acids identified for the nine strains of *L. monocytogenes* generally agreed with results of previous researchers. Raines et al. (1962) found C15:0-saturated branched-chain fatty acid as the most abundant fatty acid of *L. monocytogenes*, followed by C14:0, C16:0 and C17:0 branched-chain fatty acids. Other researchers (Carrol et al., 1968) reported straight- and branched-chain saturated acids, with C15:0 and C17:0 anteiso acids as the most abundant fatty acids. Al-issa et al. (1984) found that the most abundant acid was anteiso C-15:0 followed by anteiso C-17:0, iso C-15:0 and C-16:0. Slight differences observed in these studies could be due to environmental conditions present during growth i.e., pH, atmosphere, temperature and growth medium.

In the present study, the major differences among strains was in relative percentages of saturated branched

chain fatty acids (C15:0br and C17:0br) as well as variable presence of certain long chain fatty acids. The results of this study indicate that it would be difficult to use gas-liquid chromatography to identify *L. monocytogenes* strains. For example, strain HD, which was isolated from contaminated hot dogs, had much lower relative percentages of several branched chain fatty acids than some clinical strains.

Effect of Antimicrobials and Varying Temperatures on Lipid Composition

Effect of antimicrobials on phospholipid composition

Phospholipid composition of *L. monocytogenes* strains Scott A and LM4A was determined at 35°C and 7°C in TPB containing 100 - 500 μg/ml methyl paraben, 100 - 500 μg/ml propyl paraben or 1 to 5% sodium chloride (Tables 10-15). The objectives of this experiment were: (1) to determine if a relationship existed between the types or relative percentages of individual phospholipids and the presence of antimicrobials and (2) to determine if a relationship existed between types and quantity of phospholipid and incubation temperature.

For strain Scott A at 35°C, phosphatidyl glycerol increased from 40.3% in the control to a maximum at 2% salt (70.3%) (Table 10). The percentage at 5% salt (52.0%) was significantly greater than the control. At 7°C, this phospholipid increased from 29.4% (control) to 48.3% (5% salt) (Table 11). The relative percentage of diphosphatidyl

Table 10. Relative Percentage (± Standard Deviation) of the Phospholipid Fractions of 2 Strains of *Listeria monocytogenes* Grown in Tryptose Phosphate Broth at 35°C with Five Sodium Chloride Concentrations.

Sample	Mean Relative Percentage[a]		
	Phosphatidyl Glycerol	Diphosphatidyl Glycerol	Phosphatidyl Serine
Strain Scott A			
Control	40.3^{e}±1.70	29.5^{d}±0.99	30.2±0.71
1% Sodium Chloride	69.8^{b}±1.70	30.2^{d}±1.70	ND[f]
2% Sodium Chloride	70.3^{b}±3.39	29.7^{d}±3.39	ND
3% Sodium Chloride	62.1^{bc}±2.54	37.9^{cd}±2.54	ND
4% Sodium Chloride	56.2^{cd}±1.27	43.8^{bc}±1.27	ND
5% Sodium Chloride	52.0^{d}±1.84	48.0^{b}±1.84	ND
Strain LM4A			
Control	52.0^{bc}±1.84	48.0^{bc}±1.84	ND
1% Sodium Chloride	53.8^{bc}±2.97	46.2^{bc}±2.97	ND
2% Sodium Chloride	48.2^{c}±2.26	51.8^{b}±2.26	ND
3% Sodium Chloride	56.6^{bc}±1.55	43.4^{bc}±1.55	ND
4% Sodium Chloride	51.9^{bc}±2.40	48.1^{bc}±2.40	ND
5% Sodium Chloride	59.9^{b}±1.98	40.1^{c}±1.98	ND

[a]Means represent two replications.
[b-c]Means in columns within strains with different superscripts are significantly different ($p<0.05$).
[f]Not detected.

Table 11. Relative Percentage (± Standard Deviation) of the Phospholipid Fractions of 2 Strains of *Listeria monocytogenes* Grown in Tryptose Phosphate Broth at 7°C with Five Sodium Chloride Concentrations.

Sample	Mean Relative Percentage[a]		
	Phosphatidyl Glycerol	Diphosphatidyl Glycerol	Phosphatidyl Serine
Strain Scott A			
Control	29.4^{c}±2.69	36.2^{d}±1.70	34.4^{b}±0.99
1% Sodium Chloride	37.2^{cdc}±1.13	29.9^{d}±2.26	32.9^{b}±1.13
2% Sodium Chloride	44.1^{bc}±1.84	30.8^{d}±2.69	25.1^{b}±4.52
3% Sodium Chloride	40.7^{bcd}±1.55	59.3^{bc}±1.55	ND[f]
4% Sodium Chloride	33.3^{de}±3.11	66.7^{b}±3.11	ND
5% Sodium Chloride	48.3^{b}±1.84	51.7^{c}±1.84	ND
Strain LM4A			
Control	49.9^{c}±2.12	50.1^{b}±2.12	ND
1% Sodium Chloride	51.1^{c}±2.40	48.9^{b}±2.40	ND
2% Sodium Chloride	56.9^{dc}±0.85	43.1^{bc}±0.85	ND
3% Sodium Chloride	63.3^{cd}±2.54	36.7^{cd}±2.54	ND
4% Sodium Chloride	72.9^{b}±3.25	27.1^{c}±3.25	ND
5% Sodium Chloride	66.7^{bc}±1.98	33.3^{dc}±1.98	ND

[a]Means represent two replications.
[b-c]Means in columns within strains with different superscripts are significantly different ($p<0.05$).
[f]Not detected.

glycerol increased from 29.5% (control) to 48.0% (5% salt) at 35°C (Table 10). The percentage at 7°C increased from 36.2% to a greatest value of 66.7% at 4% salt (Table 11). The percentage at 5% salt (51.7%) was lower than at 4% but significantly higher than the control. Phosphatidyl serine was not detected in the presence of sodium chloride at 35°C (Table 10). However, this phospholipid persisted up to 2% salt at 7°C (Table 11). For strain LM4A, phosphatidyl glycerol increased slightly from 52.0% (control) to 59.9% (5% sodium chloride) at 35°C (Table 10). This increase was not statistically significant. In contrast, at 7°C, the percentage of phosphatidyl glycerol increased significantly from 50.0% to 66.7% (Table 11). Diphosphatidyl glycerol decreased at 35°C and 7°C from 48.0% (control) to 40.1% (5% salt) and 50.1% to 33.3%, respectively. Only the results at 7°C were significant.

As with sodium chloride, addition of methyl paraben to the growth medium resulted in altered phospholipid composition of *L. monocytogenes* strain Scott A and LM4A (Tables 12 and 13). Increasing the concentration of methyl paraben from 100 μg/ml to 500 μg/ml resulted in increases in phosphatidyl glycerol and diphosphatidyl glycerol for strain Scott A at 35°C and 7°C. Only phosphatidyl glycerol at 35°C increased with increased methyl paraben present in strain LM4A (Table 12). For strain Scott A at 35°C, phosphatidyl glycerol increased significantly from 40.3% (control) to

Table 12. Relative Percentage (± Standard Deviation) of the Phospholipid Fractions of 2 Strains of *Listeria monocytogenes* Grown in Tryptose Phosphate Broth at 35°C with Five Methyl Paraben Concentrations.

Sample	Mean Relative Percentage[a]		
	Phosphatidyl Glycerol	Diphosphatidyl Glycerol	Phosphatidyl Serine
Strain Scott A			
Control	40.3^{e}±1.70	29.5^{d}±0.99	30.2±0.71
100ppm Methyl Paraben	62.9^{bc}±2.97	37.1^{cd}±2.97	ND[f]
200ppm Methyl Paraben	66.7^{b}±1.84	33.3^{d}±1.84	ND
300ppm Methyl Paraben	50.5^{d}±1.55	49.5^{b}±1.55	ND
400ppm Methyl Paraben	55.2^{cd}±2.69	44.8^{bc}±2.69	ND
500ppm Methyl Paraben	48.4^{de}±1.27	51.6^{b}±1.27	ND
Strain LM4A			
Control	52.0^{c}±1.84	48.0^{b}±1.84	ND
100ppm Methyl Paraben	55.1^{de}±3.25	44.9^{bc}±3.25	ND
200ppm Methyl Paraben	61.5^{cd}±2.40	38.5^{cd}±2.40	ND
300ppm Methyl Paraben	64.9^{bc}±1.70	35.1^{de}±1.70	ND
400ppm Methyl Paraben	69.3^{bc}±2.12	30.7^{de}±2.12	ND
500ppm Methyl Paraben	71.4^{b}±2.26	28.6^{e}±2.26	ND

[a]Means represent two replications.
[b-c]Means in columns within strains with different superscripts are significantly different ($p<0.05$).
[f]Not detected.

Table 13. Relative Percentage (± Standard Deviation) of the Phospholipid Fractions of 2 Strains of *Listeria monocytogenes* Grown in Tryptose Phosphate Broth at 7°C with Five Methyl Paraben Concentrations.

Sample	Mean Relative Percentage[a]		
	Phosphatidyl Glycerol	Diphosphatidyl Glycerol	Phosphatidyl Serine
Strain Scott A			
Control	29.4[c]±2.69	36.2[c]±1.70	34.4±0.99
100ppm Methyl Paraben	56.5[b]±1.84	43.5[bc]±1.84	ND[f]
200ppm Methyl Paraben	49.5[b]±2.97	50.5[b]±2.97	ND
300ppm Methyl Paraben	50.6[b]±2.69	49.4[b]±2.69	ND
400ppm Methyl Paraben	50.0[b]±3.96	50.0[b]±3.96	ND
500ppm Methyl Paraben	55.8[b]±0.99	44.2[bc]±0.99	ND
Strain LM4A			
Control	49.9[b]±2.12	50.1[b]±2.12	ND
100ppm Methyl Paraben	52.8[b]±1.98	47.2[b]±1.98	ND
200ppm Methyl Paraben	54.3[b]±2.54	45.7[b]±2.54	ND
300ppm Methyl Paraben	57.8[b]±0.56	42.2[b]±0.56	ND
400ppm Methyl Paraben	56.5[b]±2.97	43.5[b]±2.97	ND
500ppm Methyl Paraben	54.9[b]±2.40	45.1[b]±2.40	ND

[a]Means represent two replications.
[b-c]Means in columns within strains with different superscripts are significantly different ($p<0.05$).
[f]Not detected.

66.7% at 200 μg/ml methyl paraben (Table 12). Thereafter, the relative percentages of this phospholipid decreased. At 500 μg/ml, the relative percentage (48.4%) was not significantly different from the control. At 7°C, phosphatidyl glycerol increased from 29.4% (control) to 55.8% at 500 μg/ml methyl paraben (Table 13). The relative percentage of diphosphatidyl glycerol increased from 29.5% (control) to 51.6% (500 μg/ml methyl paraben) at 35°C (Table 12). At 7°C, diphosphatidyl glycerol increased from 36.2% (control) to a maximum 50.5% at 200 μg/ml methyl paraben (Table 13). Thereafter, the percentage stabilized until at 500 μg/ml (44.2%) when it was not significantly different from the control. Phosphatidyl serine was not detected in the presence of methyl paraben at 35°C or 7°C (Tables 12 and 13).

For strain LM4A at 35°C, phosphatidyl glycerol increased from 52.0% (control) to 71.4% at 500 μg/ml methyl paraben (Table 12). In contrast, the relative percentage of this phospholipid did not change significantly at 7°C (Table 13). Diphosphatidyl glycerol decreased significantly from 48.0% (control) to 28.6% (500 μg/ml methyl paraben) at 35°C (Table 12). Again, no significant change was detected at 7°C (Table 13).

As with sodium chloride and methyl paraben, phospholipid composition of two strains of *L. monocytogenes* was altered in the presence of propyl paraben at 35°C and

7°C (Table 14 and 15). For strain Scott A at 35°C, phosphatidyl glycerol increased from 40.3% (control) to 66.1% (200 μg/ml propyl paraben) (Table 14). Above 200 μg/ml propyl paraben, the relative percentage of this phospholipid decreased. At 7°C, phosphatidyl glycerol increased from 29.4% (control) to 57.1% with the addition of 100 μg/ml propyl paraben (Table 15). The quantity remained the same with increasing propyl paraben. The relative percentage of diphosphatidyl glycerol increased from 29.5% (control) to 57.6% (500 μg/ml propyl paraben) at 35°C (Table 14). At 7°C, this phospholipid increased from 36.2% (control) to 48.0% at 200 μg/ml propyl paraben (Table 15). The percentage at 500 μg/ml (40.5%) was not significantly different from the control. No detectable level of phosphatidyl serine was observed in the presence of propyl paraben.

For strain LM4A at 35°C, phosphatidyl glycerol increased from 52.0% (control) to a maximum 69.9% at 200 μg/ml propyl paraben (Table 14). The relative percentage at 500 μg/ml (57.2%) was not significantly different from the control. At 7°C, phosphatidyl glycerol increased from 49.9% (control) to 60.6% (500 μg/ml propyl paraben) (Table 15). The highest percentage (74.7%) of phosphatidyl glycerol was detected at 200 μg/ml propyl paraben. Diphosphatidyl glycerol decreased to a minimum (30.1%) at 200 μg/ml at 35°C but was not different from the control at 500 μg/ml propyl

Table 14. Relative Percentage (± Standard Deviation) of the Phospholipid Fractions of 2 Strains of *Listeria monocytogenes* Grown in Tryptose Phosphate Broth at 35°C with Five Propyl Paraben Concentrations.

Sample	Mean Relative Percentage[a]		
	Phosphatidyl Glycerol	Diphosphatidyl Glycerol	Phosphatidyl Serine
Strain Scott A			
Control	40.3^{e}±1.70	29.5^{f}±0.99	30.2±0.71
100ppm Propyl Paraben	61.0^{bc}±2.55	39.0^{de}±2.55	ND[g]
200ppm Propyl Paraben	66.1^{b}±3.39	33.9^{ef}±3.39	ND
300ppm Propyl Paraben	52.9^{cd}±1.84	47.1^{cd}±1.84	ND
400ppm Propyl Paraben	44.5^{de}±1.13	55.5^{bc}±1.13	ND
500ppm Propyl Paraben	42.4^{e}±2.40	57.6^{b}±2.40	ND
Strain LM4A			
Control	52.0^{d}±1.84	48.0^{b}±1.84	ND
100ppm Propyl Paraben	64.3^{bc}±2.83	35.7^{cd}±2.83	ND
200ppm Propyl Paraben	69.9^{b}±1.84	30.1^{d}±1.84	ND
300ppm Propyl Paraben	63.2^{bc}±2.40	36.8^{cd}±2.40	ND
400ppm Propyl Paraben	63.6^{bc}±3.11	36.4^{cd}±3.11	ND
500ppm Propyl Paraben	57.2^{cd}±0.99	42.8^{bc}±0.99	ND

[a]Means represent two replications.
[b-f]Means in columns within strains with different superscripts are significantly different ($p<0.05$).
[g]Not detected.

Table 15. Relative Percentage (± Standard Deviation) of the Phospholipid Fractions of 2 Strains of *Listeria monocytogenes* Grown in Tryptose Phosphate Broth at 7°C with Five Propyl Paraben Concentrations.

Sample	Mean Relative Percentage[a]		
	Phosphatidyl Glycerol	Diphosphatidyl Glycerol	Phosphatidyl Serine
Strain Scott A			
Control	29.4^{c}±2.69	36.2^{c}±1.70	34.4±0.99
100ppm Propyl Paraben	57.1^{b}±2.97	42.9^{bc}±2.97	ND[l]
200ppm Propyl Paraben	52.0^{b}±2.26	48.0^{b}±2.26	ND
300ppm Propyl Paraben	58.8^{b}±2.54	41.2^{bc}±2.54	ND
400ppm Propyl Paraben	56.5^{b}±1.55	43.5^{bc}±1.55	ND
500ppm Propyl Paraben	59.5^{b}±3.25	40.5^{bc}±3.25	ND
Strain LM4A			
Control	49.9^{c}±2.12	50.1^{b}±2.12	ND
100ppm Propyl Paraben	71.4^{b}±2.26	28.6^{c}±2.26	ND
200ppm Propyl Paraben	74.7^{b}±1.55	25.3^{c}±1.55	ND
300ppm Propyl Paraben	68.6^{bc}±1.13	31.4^{dc}±1.13	ND
400ppm Propyl Paraben	57.5^{dc}±2.12	42.5^{bc}±2.12	ND
500ppm Propyl Paraben	60.6^{cd}±3.39	39.4^{cd}±3.39	ND

[a]Means represent two replications.
[b-c]Means in columns within strains with different superscripts are significantly different ($p<0.05$).
[l]Not detected.

paraben (Table 14). At 7°C, the minimum (25.3%) was again at 200 μg/ml but the percentage at 500 μg/ml was significantly lower than the control (Table 15). Diphosphatidyl glycerol decreased from 48.0% (control) to 42.8% (500 μg/ml propyl paraben) at 35°C (Table 14) and 50.1% to 39.4 % at 7°C (Table 15).

Changes in phospholipid composition in response to growth in the presence of sodium chloride and parabens may be due to protective measures initiated by the cell or toxic effects of the compounds. The concentration of phosphatidyl glycerol increased with the increasing concentration of antimicrobials in both the strains. A parallel decrease of diphosphatidyl glycerol percentage in strain LM4A was observed. In strain Scott A, disappearance of phosphatidyl serine contributed to increases in diphosphatidyl glycerol percentage as compared to the control. Phosphatidyl glycerol, by their ability to form phospholipids with opposite charges, may control the permeability of the membrane.

It is well known that each type of phospholipid has a specific function. For example, diphosphatidyl glycerol nas a structural role (Vaczi, 1973). Phosphatidyl glycerol is a precursor of diphosphatidyl glycerol and the aminoesters of phosphatidyl glycerol. Vaczi (1973) believed that changes in the concentration of this phospholipid could result in rapid structural alterations of the bacterial membrane

depending on environmental conditions. Changes in phospholipids also may affect the activity of membrane-bound enzymes and proper functioning of associated enzyme systems.

Ingram (1977) reported on the changes in phospholipid composition of *E. coli* cells grown in the presence of sublethal concentrations of a variety of organic solvents and food additives. She theorized that changes in individual phospholipid components could be expected to alter the charge distribution of the membrane. Davidson and Branen (1980) reported no significant differences in the proportion of phospholipids in *Pseudomonas fluorescens* between the control and a culture treated with 50 μg/ml BHA. However, Post (1982) found in *P. fluorescens*, the relative percentages of phosphatidyl inositol and phosphatidyl glycerol increased and phosphatidyl ethanolamine decreased with increasing concentration of BHA in the growth medium. *Salmonella typhimurium* grown in the presence of sodium deoxycholate and sodium benzoate resulted in altered phospholipid composition (Tomlins et al., 1982). In the latter study, phosphatidyl glycerol and cardiolipin increased and phosphatidyl ethanolamine decreased.

Several researchers reported that the relative percentages of negatively charged phospholipids (phosphatidyl glycerol and diphosphatidyl glycerol) is greater in cultures grown in the presence of salt (Komaratat and Kates, 1975; Ohno et al., 1979). Hanna (1984) reported

in *Vibrio costicola*, phosphatidyl glycerol content markedly increased whereas that of phosphatidyl ethanolamine decreased, with increasing salt concentration in the growth medium. In contrast, *S. aureus* (Kanemasa et al., 1972) and *S. epidermidis* (Komaratat and Kates, 1975) showed opposite effect, i.e., phosphatidyl glycerol decreased and diphosphatidyl glycerol increased with increasing salinity. Phospholipid composition changes may be interpreted as being a mechanism for counteracting the increase in Na^+ concentration (excess cationic charge) at the membrane surface. High content of negatively charged phospholipids may contribute to the regulation of the permeability mechanisms of cations. Hiramatsu et al. (1980) postulated that the increased content of anionic phospholipids may serve to neutralize excess Na^+ ions at the membrane surface and/or regulate the ionic permeability of the membrane. Thus, relative increase in phosphatidyl glycerol may be a necessary physiological response for adaptation to increased salt present. Phospholipid changes may be mediated by the effects of osmotic pressure in the presence of sodium chloride. Since the phospholipid synthesizing enzymes are located in the cytoplasmic membrane (Raetz, 1978), the osmotic effects could be mediated by lateral stretching of the membrane caused by plasmolysis. In fact, cells in the presence of NaCl are exposed not only to high concentration of ions but also to osmotic stress.

Effect of varying temperatures on phospholipid composition

Increasing the temperature from 7°C to 35°C altered phospholipid composition for *L. monocytogenes* strains Scott A and LM4A (Table 16). For strain Scott A, phosphatidyl glycerol was lowest ($p<0.05$) at 7°C (29.4%), intermediate at 35°C and highest at 15°C, 24°C and 30°C. The relative percentages of diphosphatidyl glycerol were highest at 15°C (44.7%) and decreased with increasing temperature. Phosphatidyl serine was only detected at 7°C and 35°C. The relative percentage of this phospholipid was significantly lower (30.2%) at 35°C than 7°C (34.4%). If the effect of phosphatidyl serine was removed at 35°C, the percentages of phosphatidyl glycerol and diphosphatidyl glycerol were 57.7 and 42.3%, respectively.

For strain LM4A, the relative percentage of phosphatidyl glycerol ranged from 63.8% at 30°C to 49.9% at 7°C (Table 16). The percentage at 35°C (52.0%) was not significantly different from the percentage (49.9%) at 7°C. In contrast, diphosphatidyl glycerol decreased from 50.1% at 7°C to 36.2% at 30°C. As with phosphatidyl glycerol, the relative percentage (48.0%) of this phospholipid at 35°C was not significantly different from 50.1% at 7°C. *Listeria monocytogenes* appears to initiate adaptive measures in response to changes in temperature.

The temperature induced alteration of the proportions

Table 16. Relative Percentage (± Standard Deviation) of the Phospholipid Fractions of 2 Strains of *Listeria monocytogenes*, Grown on Tryptose Phosphate Broth at Five Temperatures.

Temperature (°C)	Mean Relative Percentage[a]		
	Phosphatidyl Glycerol	Diphosphatidyl Glycerol	Phosphatidyl Serine
Strain Scott A			
7	29.4^{e}±2.69	36.2^{c}±1.69	34.4^{b}±0.99
15	55.3^{c}±1.84	44.7^{b}±1.84	ND[f]
24	63.5^{bc}±2.40	36.5^{c}±2.04	ND
30	64.3^{b}±1.56	35.7^{c}±1.56	ND
35	40.3^{d}±1.70	29.5^{c}±0.99	30.2^{c}±0.71
Strain LM4A			
7	49.9^{d}±2.12	50.1^{b}±2.12	ND
15	58.9^{bc}±2.97	41.1^{cd}±2.97	ND
24	61.1^{b}±1.70	38.9^{d}±1.70	ND
30	63.8^{b}±2.26	36.2^{d}±2.26	ND
35	52.0^{cd}±1.84	48.0^{bc}±1.84	ND

[a]Means represent two replications.
[b-c]Means in columns within strains with different superscripts are significantly different ($p<0.05$).
[f]Not detected.

of individual phospholipids seems to be similar to that induced by antimicrobials in the growth medium. Ohno et al. (1979) observed in *Pseudomonas halosaccharolytica* that increasing the temperature from 16°C to 30°C caused a marked increase in phosphatidyl glycerol and a concomitant decrease in diphosphatidyl glycerol. Miller (1985) examined the phospholipid composition of a Gram-positive halotolerant *Planococcus* sp. as a function of growth temperature. In that study, an increase in the relative percentage of phosphatidyl ethanolamine was detected at increased growth temperature with a corresponding decrease in cardiolipin. Similar trends in phospholipid composition as a function of growth temperature have been detected in *Bacillus caldotenax* (Hasegawa et al., 1980) and *B. megaterium* (Rilfors et al., 1980). Phosphatidyl ethanolamine content has been reported to decrease, however, within a thermophilic bacillus (Souza et al., 1974) and within a strain of *B. megaterium* (Eisenberg and Corner, 1978) when the growth temperature was increased. It appears from these studies that alterations of phospholipid composition in response to temperature change may differ markedly in character among bacterial strains. It is therefore difficult to generalize the effects of such alterations. However, the phospholipid composition changes suggest a temperature regulated mechanism which permits proper membrane function. By such metabolic adaptation, the cell strives to maintain membrane

order and viability.

Effect of antimicrobials on fatty acids composition

The fatty acid composition of *Listeria monocytogenes* strains Scott A and LM4A at 35°C and 7°C grown on tryptose phosphate agar containing 100 - 500 μg/ml methyl paraben, 100 - 500 μg/ml propyl paraben or 1 - 5% sodium chloride was determined. The actual data obtained is given in appendix 2-15. The objectives of these experiments were to establish if a relationship existed between the types or relative percentages of individual fatty acids and the concentration of antimicrobials present and to determine the effect of temperature on fatty acid profile of the two strains.

The relative percentages of a-C15:0 and i-C15:0 have been combined and indicated as C15:0br. Similarly, i-C17:0 and C17:0Δ have been reported as C17:0br. Increasing the concentration of sodium chloride from 1.0 to 5.0% resulted in an overall decrease in the major branched chain fatty acids (C15:0br and C17:0br) for both strains at 35°C and 7°C (Figures 7-10). For strain Scott A, the C15:0br fatty acids decreased from 62.4% in the control to 50.2% at 5.0% salt and from 75.7% to 63.2% at 35°C and 7°C, respectively (Figures 7 and 8). For strain LM4A at 35°C and 7°C, the same fatty acids decreased from 56.4% to 38.4% and from 79.8% to 72.9%, respectively (Figures 9 and 10). The C17:0br fatty acids increased at 3% and 5% salt for strain Scott A at 35°C (Figure 7). The relative percentages of

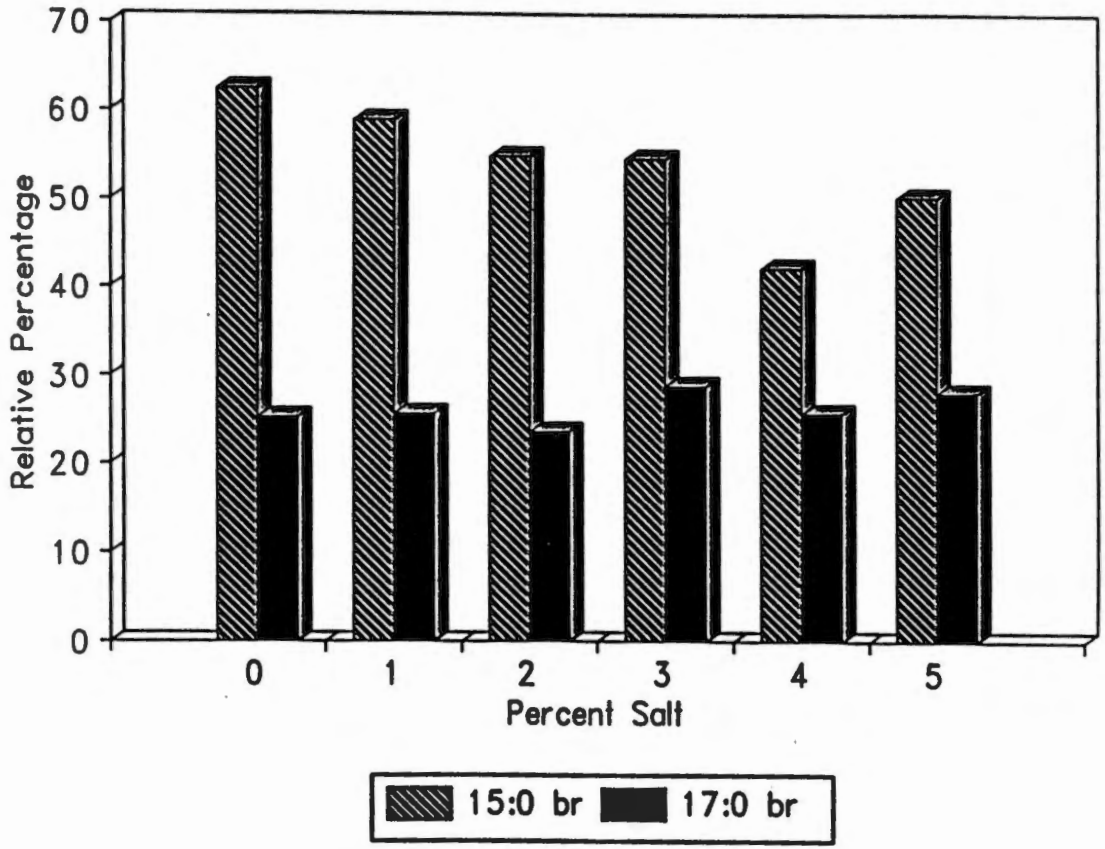


Figure 7. Mean Relative Percentage of Branched Chain Fatty Acids Found in *Listeria monocytogenes* Strain Scott A at Five Sodium Chloride Concentrations, Grown on Tryptose Phosphate Agar at 35°C.

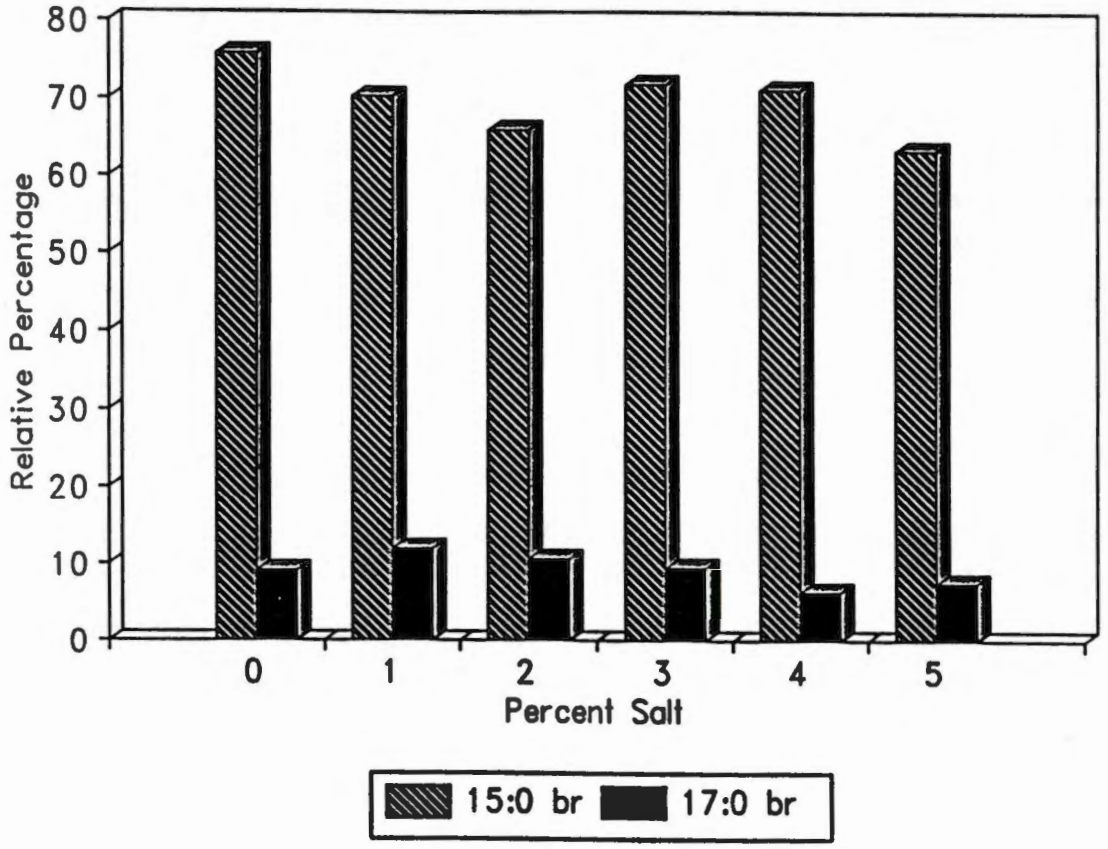


Figure 8. Mean Relative Percentage of Branched Chain Fatty Acids Found in *Listeria monocytogenes* Strain Scott A at Five Sodium Chloride Concentrations, Grown on Tryptose Phosphate Agar at 7°C.

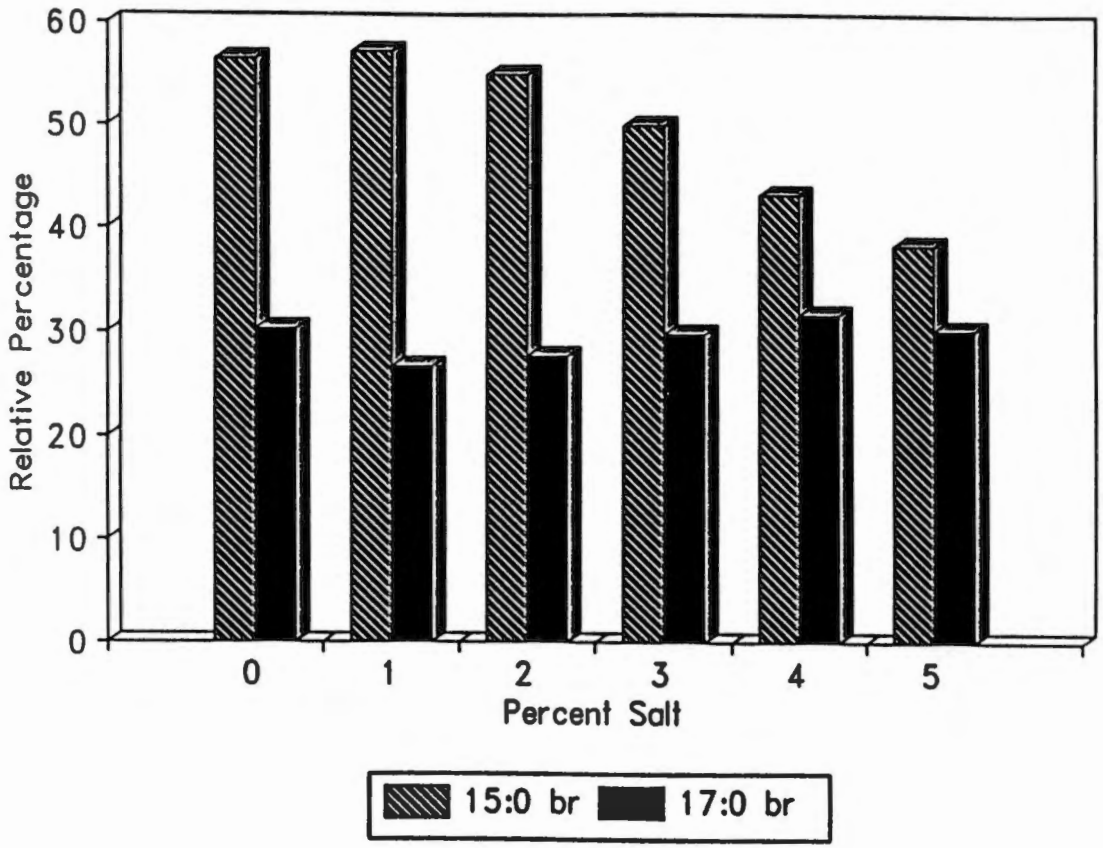


Figure 9. Mean Relative Percentage of Branched Chain Fatty Acids Found in *Listeria monocytogenes* Strain LM4A at Five Sodium Chloride Concentrations, Grown on Tryptose Phosphate Agar at 35°C.

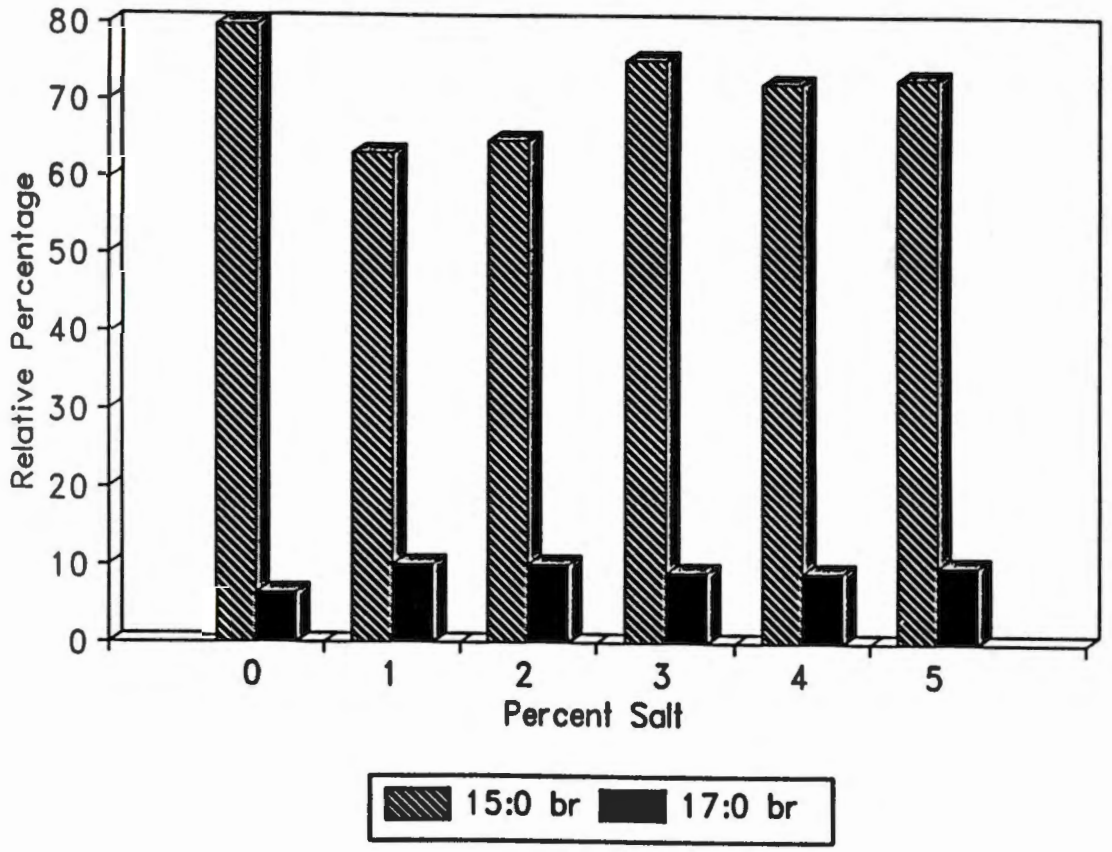


Figure 10. Mean Relative Percentage of Branched Chain Fatty Acids Found in *Listeria monocytogenes* Strain LM4A at Five Sodium Chloride Concentrations, Grown on Tryptose Phosphate Agar at 7°C.

these acids were 25.3% (control) and 28.0% (5% sodium chloride). At 7°C, these fatty acids decreased from 9.1% to 7.4% (Figure 8).

The C17:0br fatty acids remained approximately constant for LM4A strain at 35°C and 7°C as the sodium chloride concentration increased except for a significant decrease at 1% and 2% salt at 35°C (Figure 9). In the LM4A control and 5% sodium chloride at 35°C, the relative percentages for C17:0br were 30.3% and 30.2%, respectively. At 7°C, the relative percentages of these acids were increased from 6.4% (control) to 10.0% (5% salt; Figure 10).

In contrast to branched chain fatty acids, relative percentages of unknown fatty acid 1, C12:0, C14:0, C15:0, OH-C14:0, and C16:0 increased with increasing sodium chloride concentration in both strains at 35°C and 7°C (Figures 11-14). The total of the relative percentages of these 6 fatty acids increased from 6.4% (control) to 12.3% (5% sodium chloride) in Scott A at 35°C (Figure 11), 8.6% to 12.5% in Scott A at 7°C (Figure 12), 8.4% to 14.3% in LM4A at 35°C (Figure 13), and 7.9% to 9.7% in LM4A at 7°C (Figure 14). For Scott A strain, no detectable levels of C15:0 at 4% and 5% salt at 35°C (Figure 11) or 1%, 3%, 4% or 5% salt at 7°C (Figure 12) were observed. For LM4A strain at 7°C, neither C15:0 nor C12:0 at 1% salt were detected (Figure 14). For strain Scott A and LM4A, 2-OH-C10:0 was detected at only 35°C (Appendix 2,3), however C11:0 was detected at

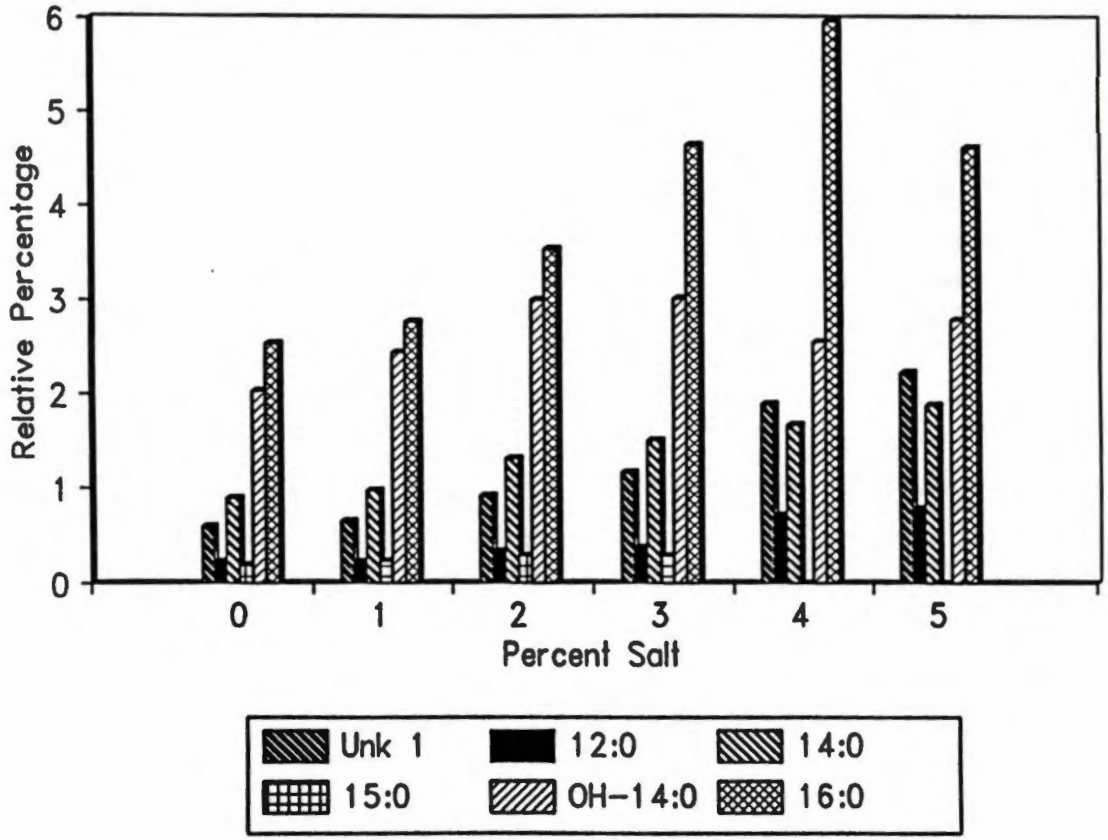


Figure 11. Mean Relative Percentage of Selected Medium Chain Fatty Acids Found in *Listeria monocytogenes* Strain Scott A at Five Sodium Chloride Concentrations, Grown on Tryptose Phosphate Agar at 35°C.

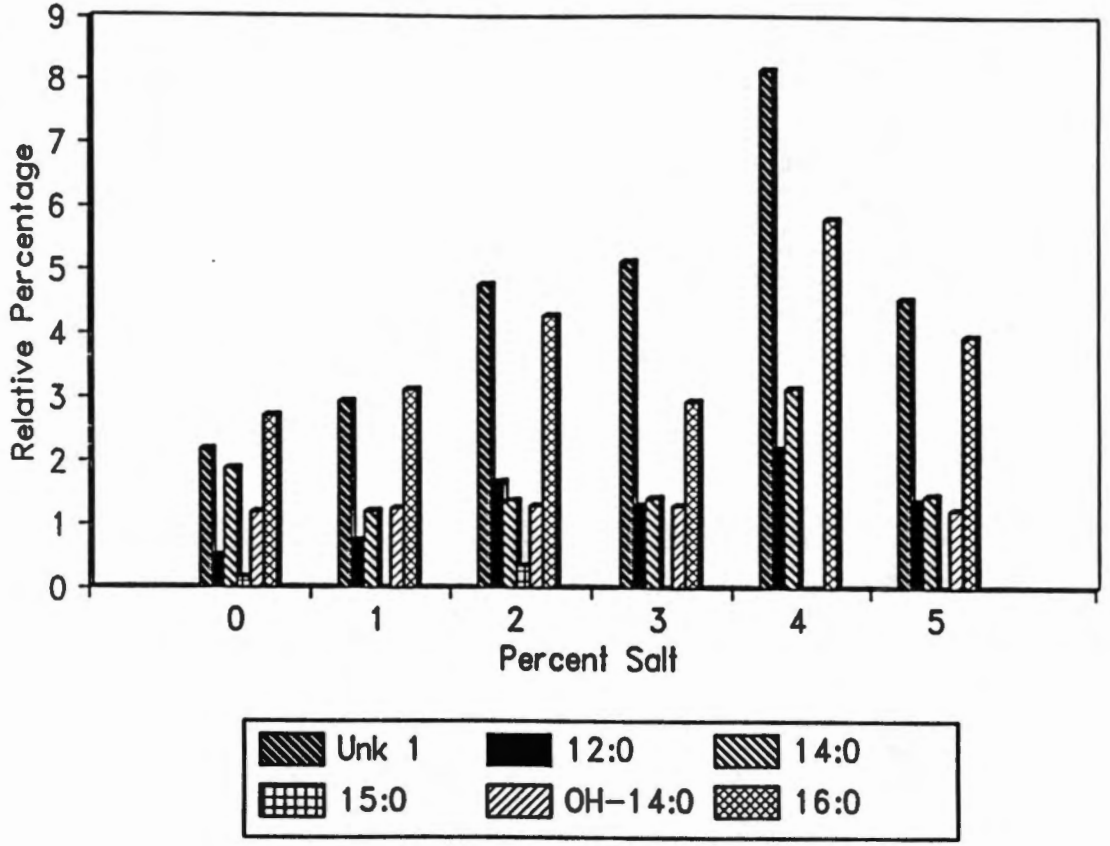


Figure 12. Mean Relative Percentage of Selected Medium Chain Fatty Acids Found in *Listeria monocytogenes* Strain Scott A at Five Sodium Chloride Concentrations, Grown on Tryptose Phosphate Agar at 7°C.

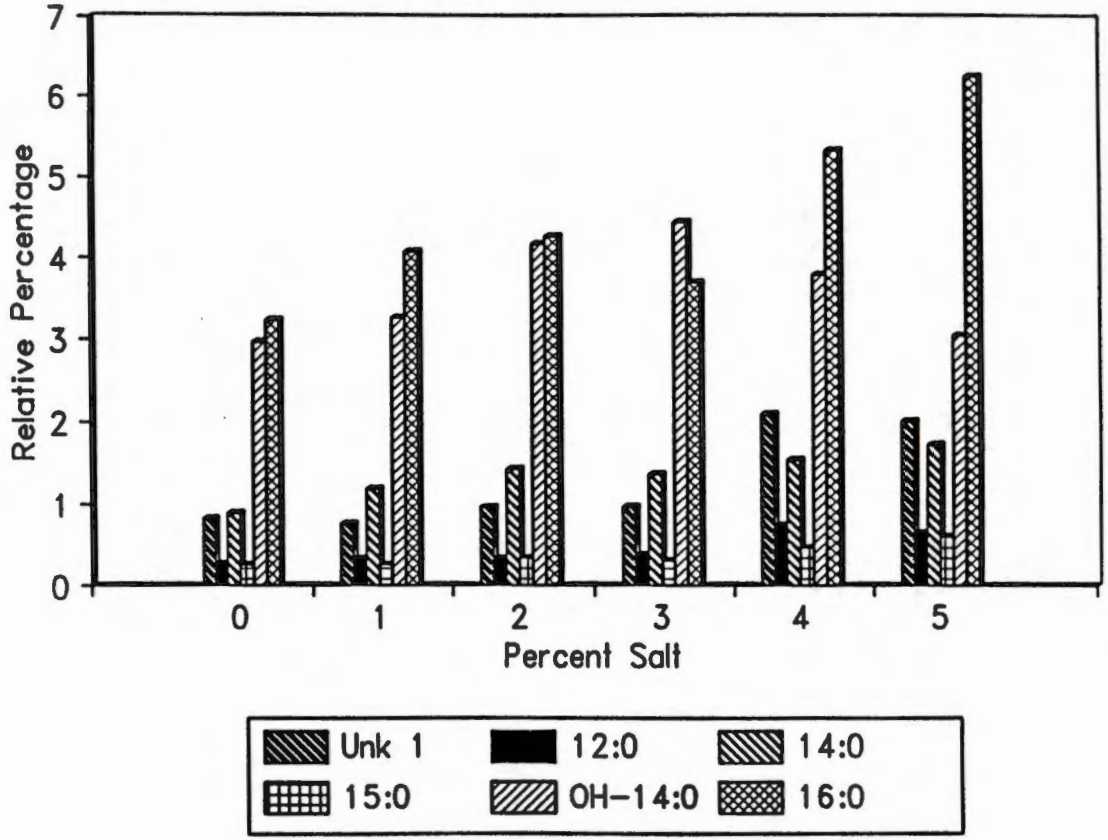


Figure 13. Mean Relative Percentage of Selected Medium Chain Fatty Acids Found in *Listeria monocytogenes* Strain LM4A at Five Sodium Chloride Concentrations, Grown on Tryptose Phosphate Agar at 35°C.

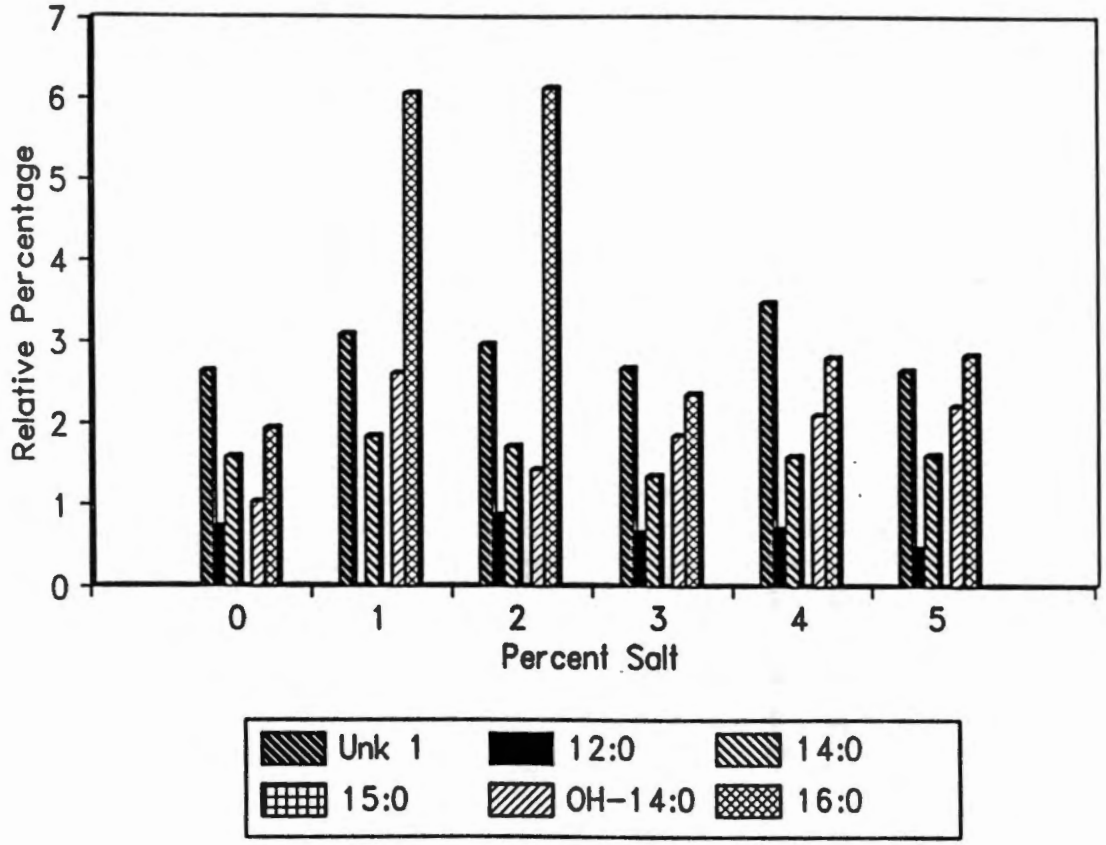


Figure 14. Mean Relative Percentage of Selected Medium Chain Fatty Acids Found in *Listeria monocytogenes* Strain LM4A at Five Sodium Chloride Concentrations, Grown on Tryptose Phosphate Agar at 7°C.

7°C in both strains (Appendix 4,5). Unknown fatty acids 2 and 3 with retention times less than that of 2-OH-C12:0 were detected at 7°C and 35°C, respectively, in strain LM4A (Appendix 3,5). Unknown fatty acid 4 and 5 (retention times < C14:0) were detected in both strains at only 7°C (Appendix 4,5). With the increase of sodium chloride in the growth medium, the percentage of unknown fatty acid 3 and 4 remained relatively constant but unknown fatty acid 5 increased.

There was great variation in the types and relative percentages of long chain fatty acids between the two strains in the presence of sodium chloride at 35°C and 7°C (Figures 15-18). The total of relative percentages of C18:0, C18:1, C18:2, C19:0Δ, C19:0 and C20:0 generally increased with increased sodium chloride. For strain Scott A at 35°C, the total relative percentage of these long chain fatty acids was greatest at 4% salt (10.1%) and decreased at 5% salt (7.0%, Figure 15). At 7°C, these fatty acids were not detected at 3% and 4% salt concentration. In fact, C19:0Δ and C19:0 were only detected at 5% salt (Figure 16). Strain LM4A at 35°C was more consistent having a total of 1.7% long chain fatty acids in the control which increased to 8.5% in 5% sodium chloride (Figure 17). However, C19:0Δ and C19:0 were not detected. In addition, a few fatty acids such as C18:2, C18:1 and C20:0 were not detected at differing salt concentrations. At 7°C, long chain fatty

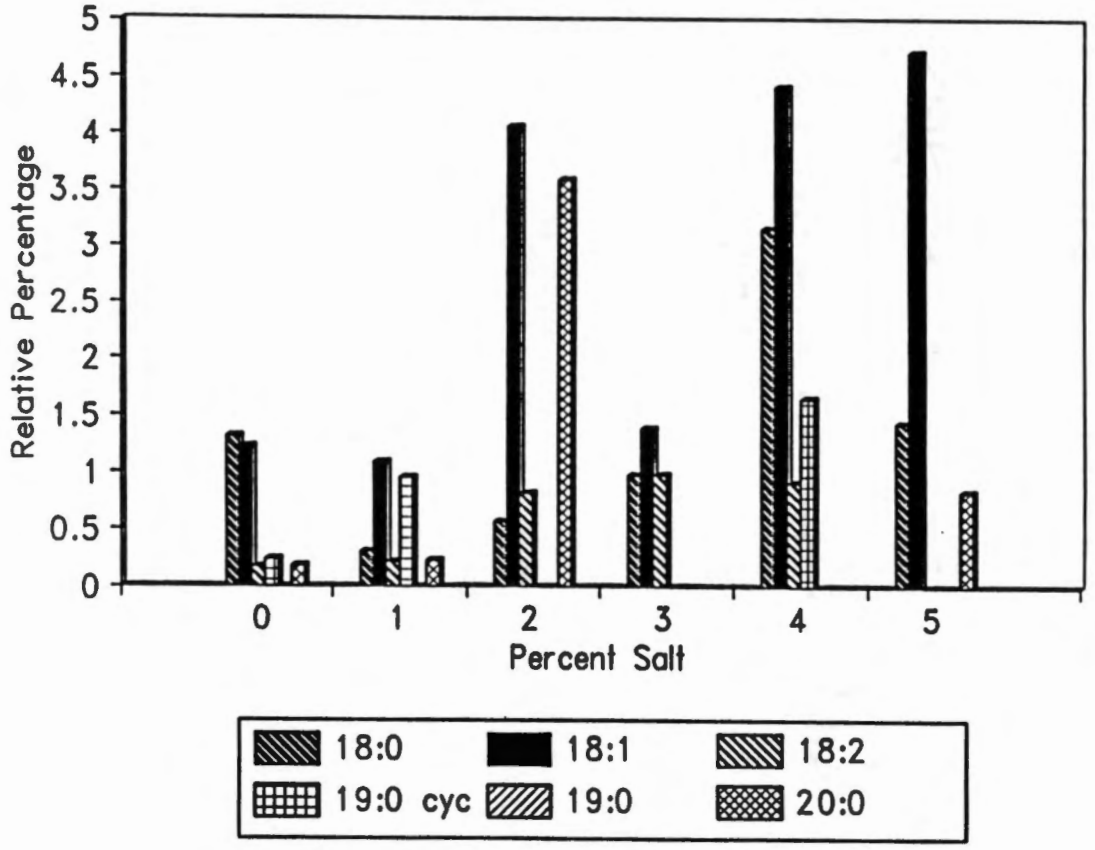


Figure 15. Mean Relative Percentage of Long Chain Fatty Acids Found in *Listeria monocytogenes* Strain Scott A at Five Sodium Chloride Concentrations, Grown on Tryptose Phosphate Agar at 35°C.

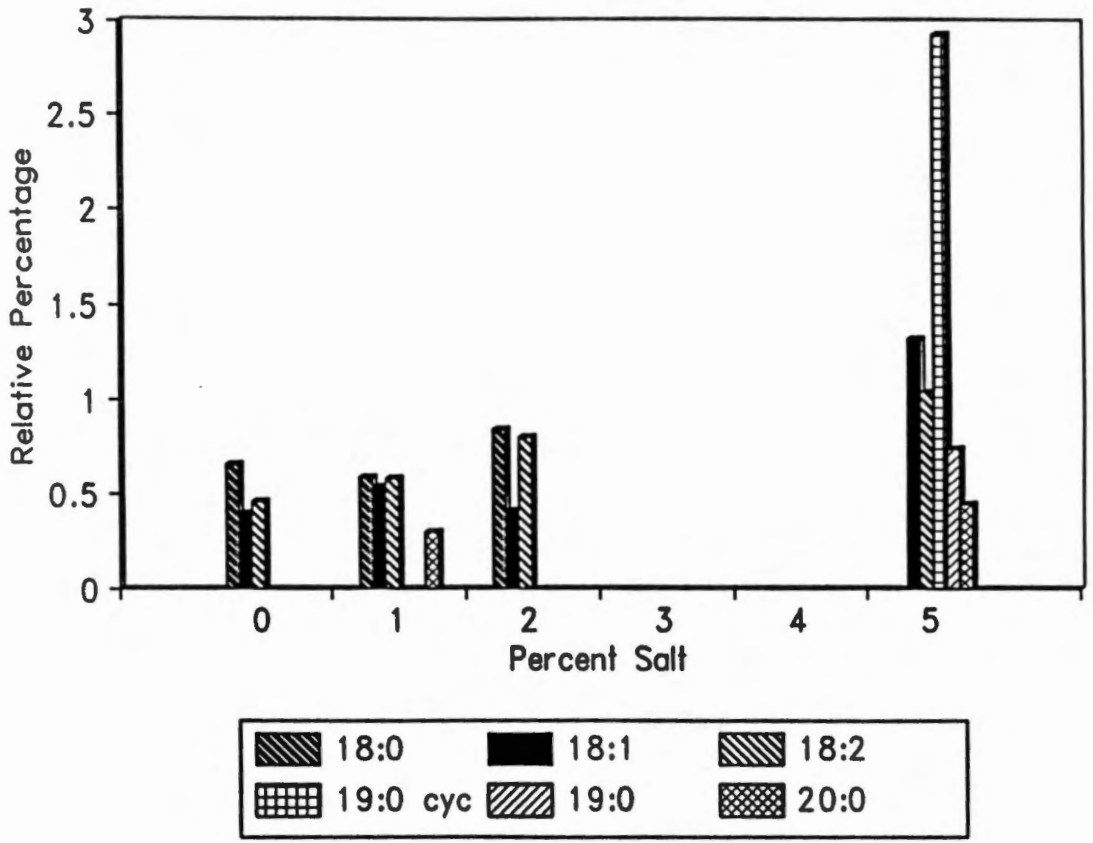


Figure 16. Mean Relative Percentage of Long Chain Fatty Acids Found in *Listeria monocytogenes* Strain Scott A at Five Sodium Chloride Concentrations, Grown on Tryptose Phosphate Agar at 7°C.

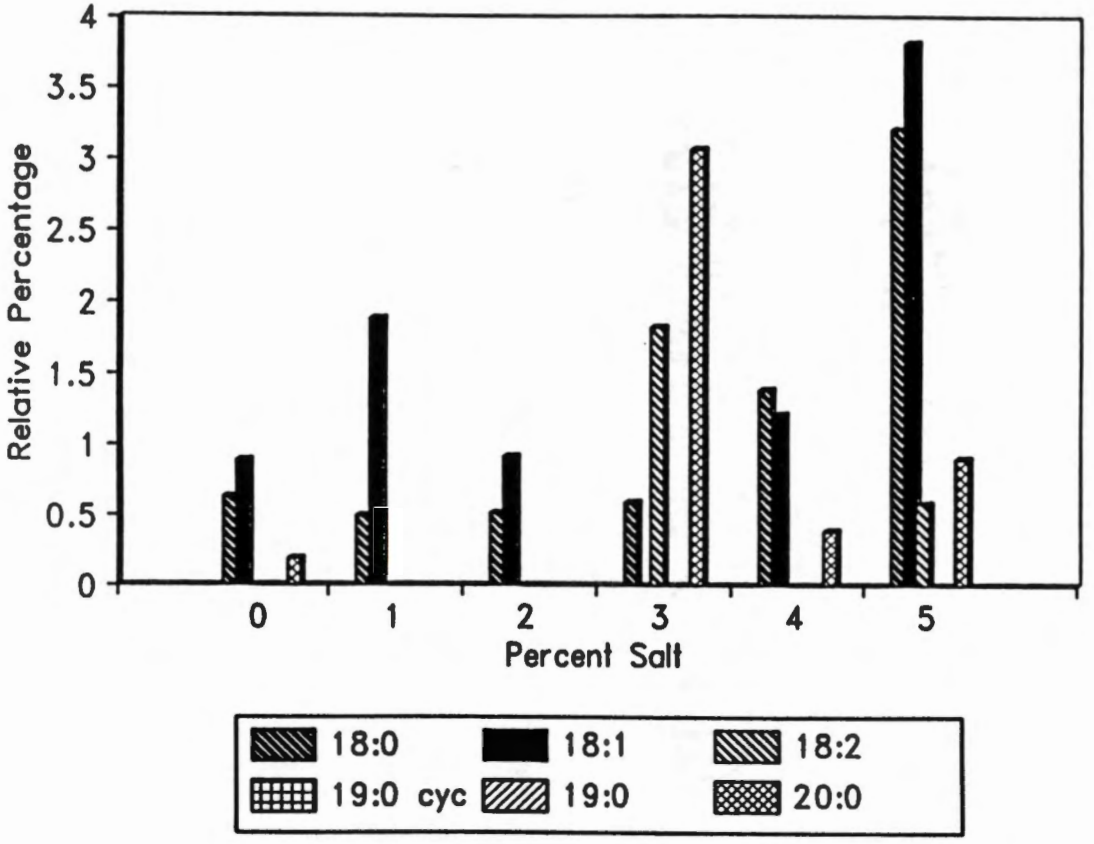


Figure 17. Mean Relative Percentage of Long Chain Fatty Acids Found in *Listeria monocytogenes* Strain LM4A at Five Sodium Chloride Concentrations, Grown on Tryptose Phosphate Agar at 35°C.

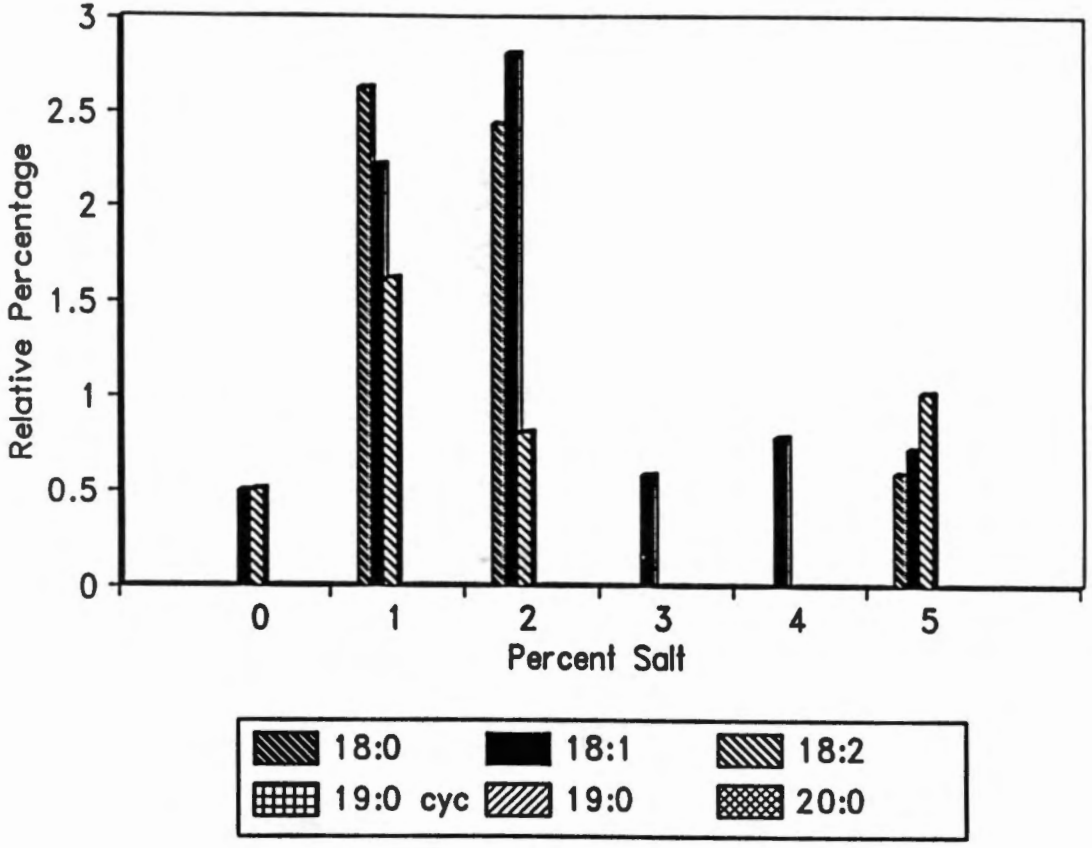


Figure 18. Mean Relative Percentage of Long Chain Fatty Acids Found in *Listeria monocytogenes* Strain LM4A at Five Sodium Chloride Concentrations, Grown on Tryptose Phosphate Agar at 7°C.

acids increased from 1% (control) to 2.3% (5% salt) for strain LM4A but were highest at 1 and 2% salt (Figure 18). No detectable levels of C19:0Δ, C19:0 and C20:0 were observed. Also, C18:0 and C18:2 were not detected at some salt concentrations. Unknown long chain fatty acids with retention times greater than that of C18:0 were observed in both strains at 35°C and 7°C at some sodium chloride concentrations.

The present results are comparable with reports published previously. Hanna (1984) observed, in *Vibrio costicola*, that total monounsaturated fatty acids were highest at the optimal salinity for growth (1.0 M NaCl) but then decreased in 2.0 and 3.0 M sodium chloride grown cells, whereas total saturated fatty acids showed an opposite effect. When Gram-positive halotolerant *Planococcus* sp. was grown in media containing increased concentration of salt, the relative percentage of the major fatty acid C15:0br (anteiso) increased (Miller, 1985). Similar increases in the relative level of C15:0br in response to increased salt concentration has also been observed in *S. aureus* (Kanemasa et al., 1972) and *S. epidermidis* (Komaratat and Kates, 1975). In the present study, decreases in relative percentages of C15:0br and increases in saturated fatty acids in the presence of sodium chloride likely decreased membrane fluidity. The changes in fatty acid composition occurring in the presence of sodium chloride may be

triggered and/or mediated by the effects of osmotic pressure. Cells may synthesize saturated or high melting point fatty acids as a defense mechanism so that the physical state of the membrane is less fluid in the presence of salt. Low melting point fatty acids (i.e., unsaturated and branched-chain fatty acids) increase membrane fluidity (Welker, 1976) whereas high melting point fatty acids (i.e., saturated, long and straight chain fatty acids) decrease membrane fluidity (McElhaney, 1976). The changes in fatty acid profiles may be attributed to the protective measures initiated by the cells in response to the presence of sodium chloride. In *E. coli*, the only change observed owing to increased salt was an increase in cyclopropane fatty acids (McGarrity and Armstrong, 1975). Considering that the melting point of cyclopropane fatty acids is between the corresponding saturated and monounsaturated fatty acids, regulation of cyclopropanization may contribute to maintaining suitable membrane integrity in a salty environment.

As with sodium chloride, addition of methyl paraben to the growth medium resulted in altered fatty acid composition for *L. monocytogenes* strain Scott A and LM4A at 35°C and 7°C. For strain Scott A at 35°C, the presence of methyl paraben resulted in an increase in C15:0br from 49.8% (control) to 57.5% (100 μg/ml methyl paraben, Figure 19). However, the percentage of these fatty acids was 39.1% at

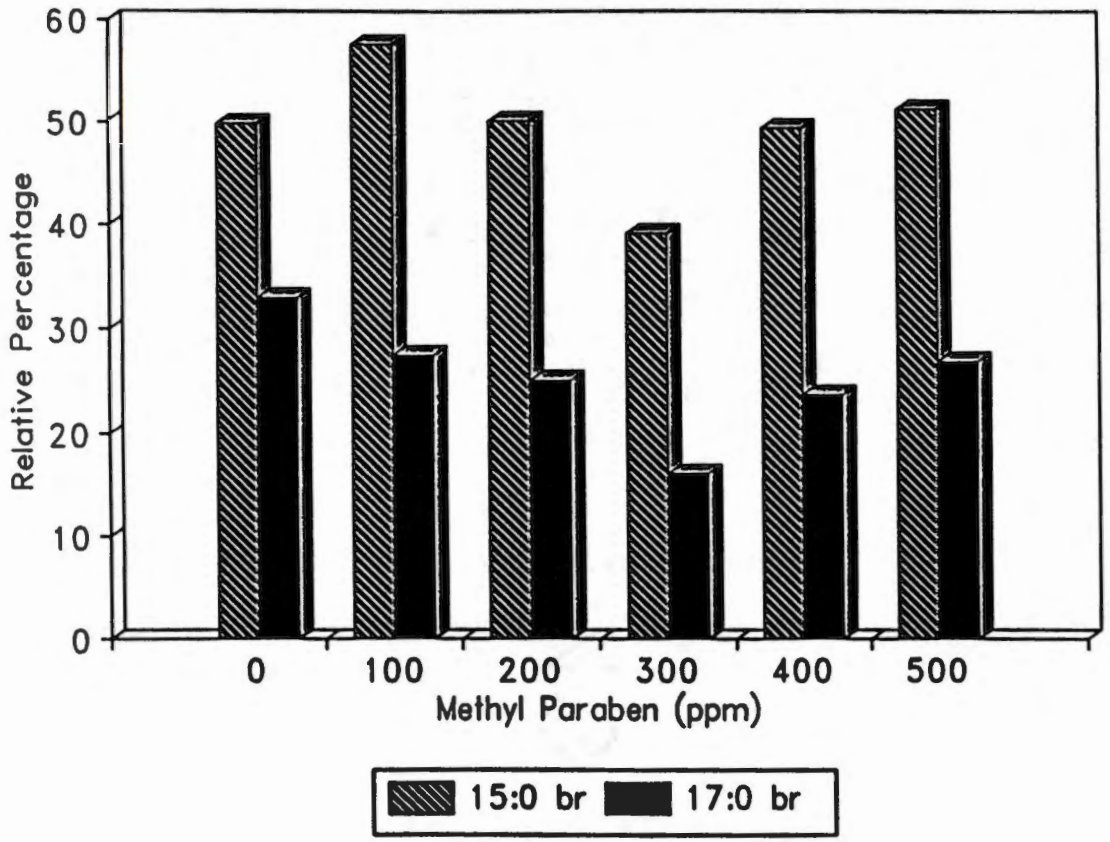


Figure 19. Mean Relative Percentage of Branched Chain Fatty Acids Found in *Listeria monocytogenes* Strain Scott A at Five Methyl Paraben Concentrations, Grown on Tryptose Phosphate Agar at 35°C.

300 μg/ml methyl paraben. For strain Scott A at 7°C, C15:0br decreased from 75.7% in the control to 68.8% at 500 μg/ml methyl paraben (Figure 20). For strain LM4A at 35°C, the initial trend of C15:0br was similar to that of strain Scott A. Above 100 μg/ml, the relative percentage of this fatty acid decreased (Figure 21). However, the percentage at 500 μg/ml (52.7%) was higher than the control (40.7%). For strain LM4A at 7°C, C15:0br decreased from 79.8% in the control to 75.1% at 500 μg/ml methyl paraben (Figure 22). For strain Scott A at 35°C, the C17:0br fatty acids decreased up to 300 μg/ml methyl paraben and then increased (Figure 19). However, percentage of this acid in control was significantly higher (33%) than at 500 μg/ml methyl paraben (26.8%). At 7°C, C17:0br remained constant as the methyl paraben concentration increased (Figure 20). In contrast, for strain LM4A at 35°C (Figure 21), C17:0br remained constant as the methyl paraben concentration increased. At 7°C, this fatty acid declined from 6.3% (Control) to 3.0% (500 μg/ml methyl paraben, Figure 22).

As with sodium chloride, relative percentages of unknown fatty acid 1, C12:0, C14:0, C15:0, OH-C14:0, and C16:0 increased with increasing methyl paraben concentration in both strains at 35°C and 7°C. The total of the relative percentages of these 6 fatty acids increased from 6.0% (control) to 10.1% (500 μg/ml methyl paraben) in Scott A at 35°C (Figure 23), 8.6% to 22.2% in Scott A at 7°C

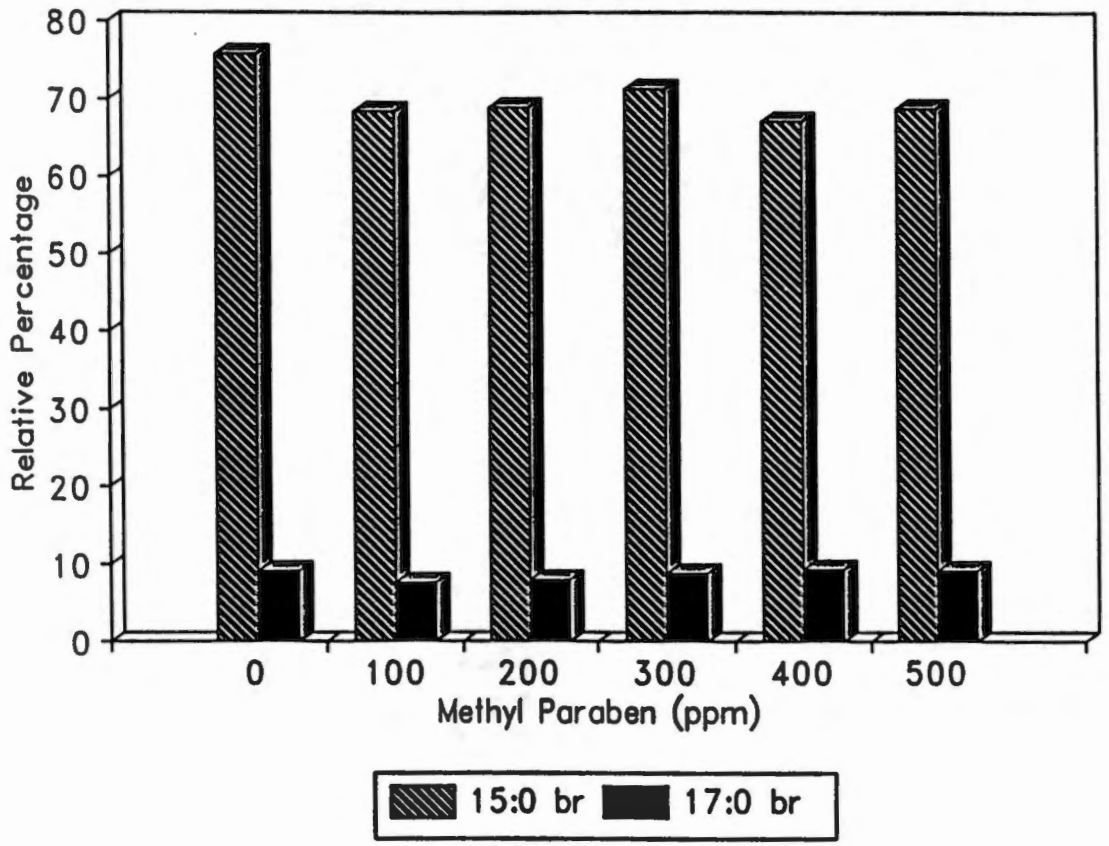


Figure 20. Mean Relative Percentage of Branched Chain Fatty Acids Found in *Listeria monocytogenes* Strain Scott A at Five Methyl Paraben Concentrations, Grown on Tryptose Phosphate Agar at 7°C.

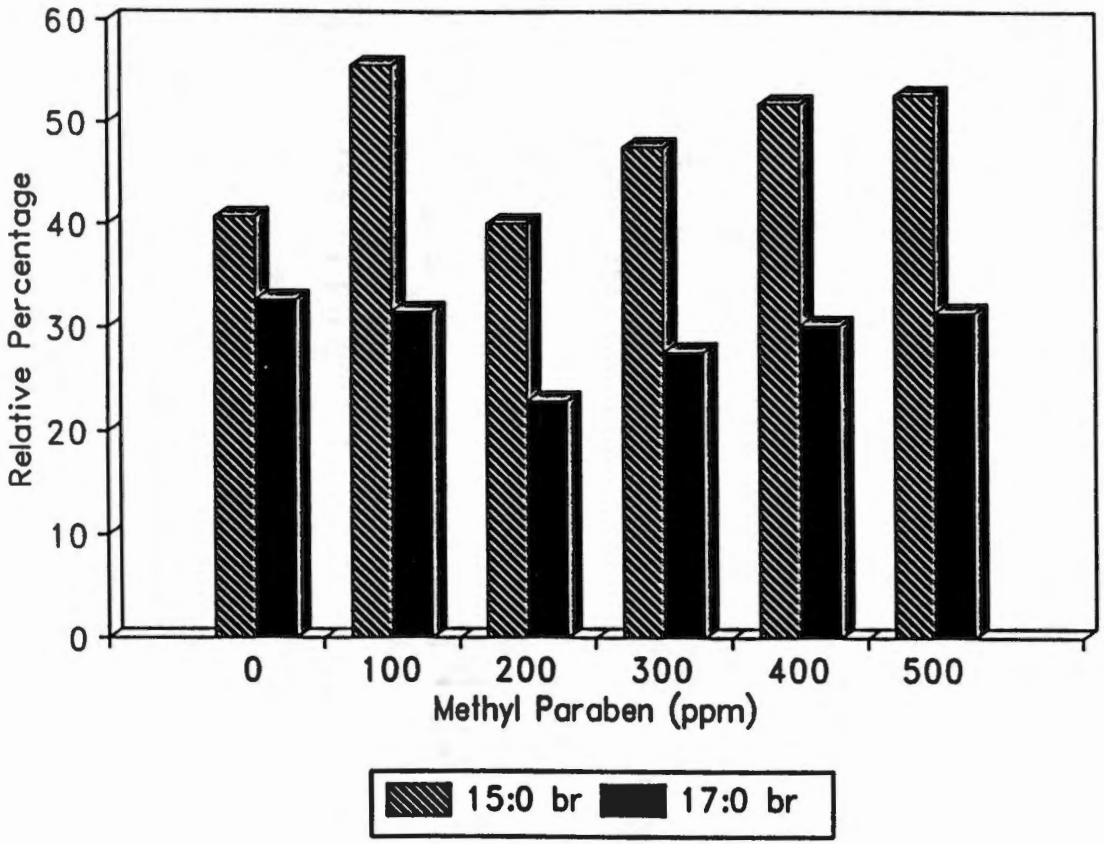


Figure 21. Mean Relative Percentage of Branched Chain Fatty Acids Found in *Listeria monocytogenes* Strain LM4A at Five Methyl Paraben Concentrations, Grown on Tryptose Phosphate Agar at 35°C.

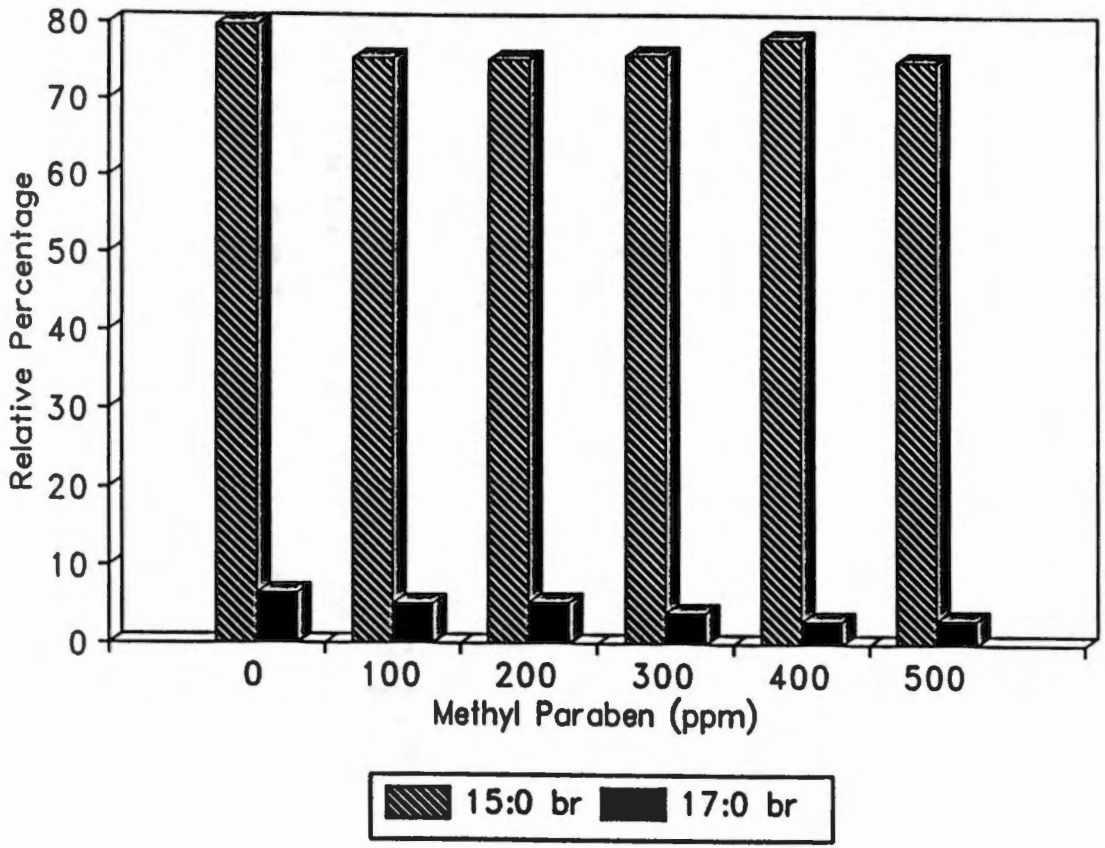


Figure 22. Mean Relative Percentage of Branched Chain Fatty Acids Found in *Listeria monocytogenes* Strain LM4A at Five Methyl Paraben Concentrations, Grown on Tryptose Phosphate Agar at 7°C.

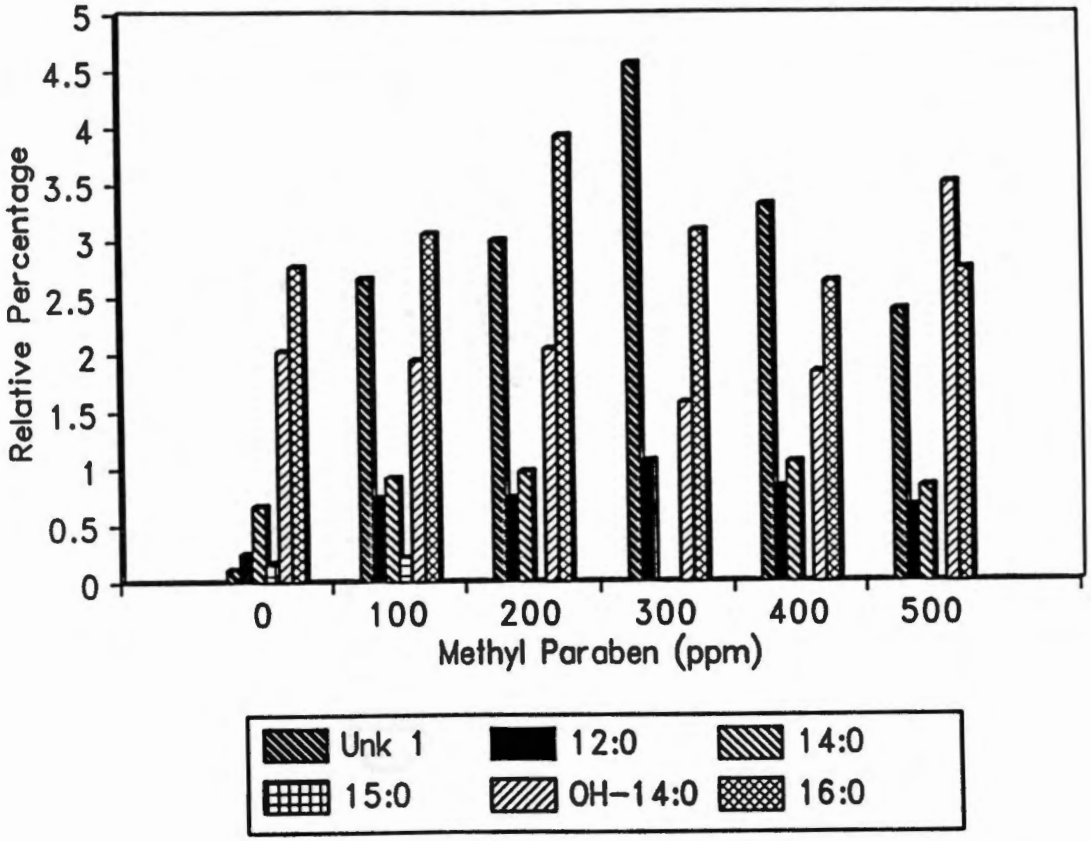


Figure 23. Mean Relative Percentage of Selected Medium Chain Fatty Acids Found in *Listeria monocytogenes* Strain Scott A at Five Methyl Paraben Concentrations, Grown on Tryptose Phosphate Agar at 35°C.

(Figure 24), 5.2% to 10.5% in LM4A at 35°C (Figure 25) and 7.9% to 16.7% in LM4A at 7°C (Figure 26). For strain Scott A strain at 35°C and 7°C, C15:0 was not detected in the presence of methyl paraben with the exception of trace amount (0.2%) at 100 μg/ml methyl paraben at 35°C (Figure 23). At 7°C, C12:0 and OH-C14:0 were not detected at 500 μg/ml methyl paraben (Figure 24). For strain LM4A at 7°C, C12:0 was not detected in lower (100 μg/ml to 300 μg/ml) methyl paraben concentrations (Figure 26). For strain Scott A and LM4A at 35°C and 7°C, C11:0 was not detected in control but appeared in the presence of methyl paraben (Appendix 6, 7, 8, 9). The only exception was in strain Scott A at 7°C where this fatty acid was also not detected at 100 μg/ml and 500 μg/ml but was considerably increased at 400 μg/ml (2.1%). The fatty acid C13:0 was not detected at 7°C in either strain. Instead, unknown fatty acid 4 and 5 appeared at 7°C (Appendix 7, 9). The relative percentage of these fatty acids was considerably high in strain LM4A at 400 μg/ml methyl paraben.

There was great variation in the types and relative percentages of long chain fatty acids between the two strains in the presence of methyl paraben at 35°C and 7°C. Total relative percentages of C18:0, C18:1, C19;0Δ, C19:0 and C20:0 increased from 1.9% (control) to 6.9% (500 μg/ml methyl paraben) in strain Scott A at 35°C (Figure 27). At 7°C, C19:0Δ, C19:0 and C20:0 were not detected (Figure 28).

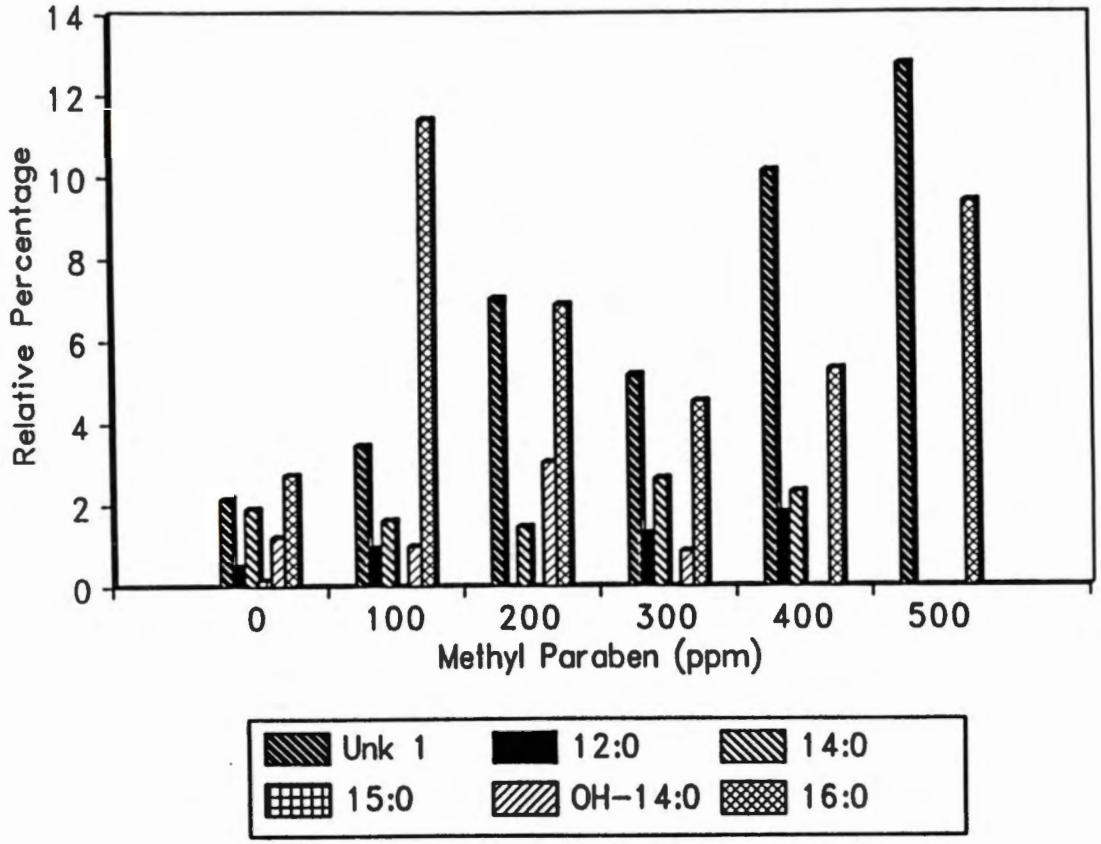


Figure 24. Mean Relative Percentage of Selected Medium Chain Fatty Acids Found in *Listeria monocytogenes* Strain Scott A at Five Methyl Paraben Concentrations, Grown on Tryptose Phosphate Agar at 7°C.

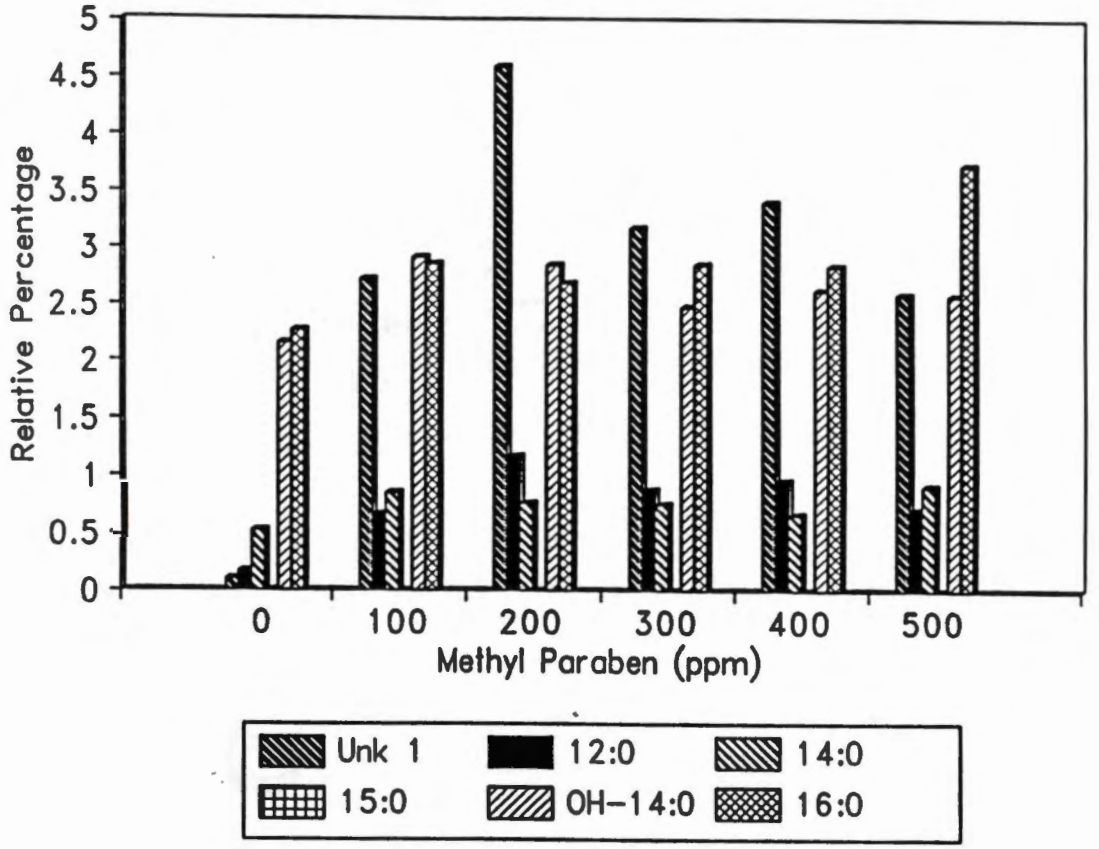


Figure 25. Mean Relative Percentage of Selected Medium Chain Fatty Acids Found in *Listeria monocytogenes* Strain LM4A at Five Methyl Paraben Concentrations, Grown on Tryptose Phosphate Agar at 35°C.

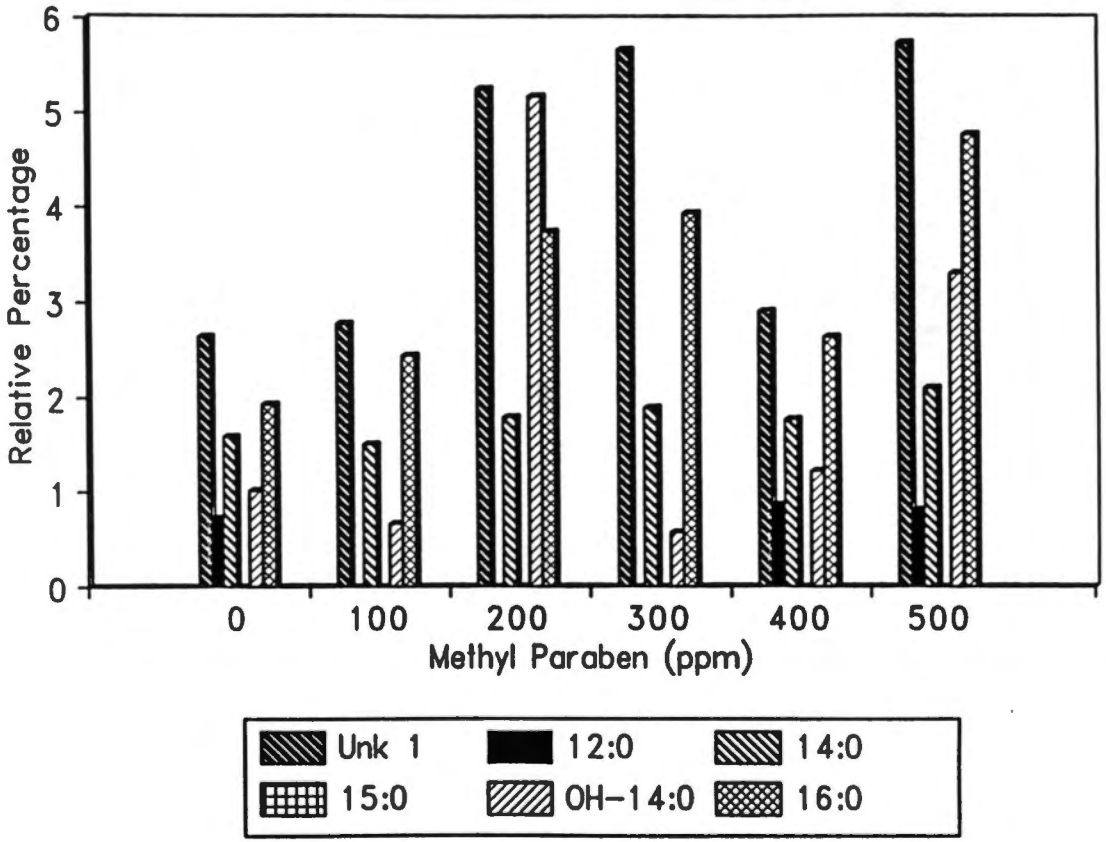


Figure 26. Mean Relative Percentage of Selected Medium Chain Fatty Acids Found in *Listeria monocytogenes* Strain LM4A at Five Methyl Paraben Concentrations, Grown on Tryptose Phosphate Agar at 7°C.

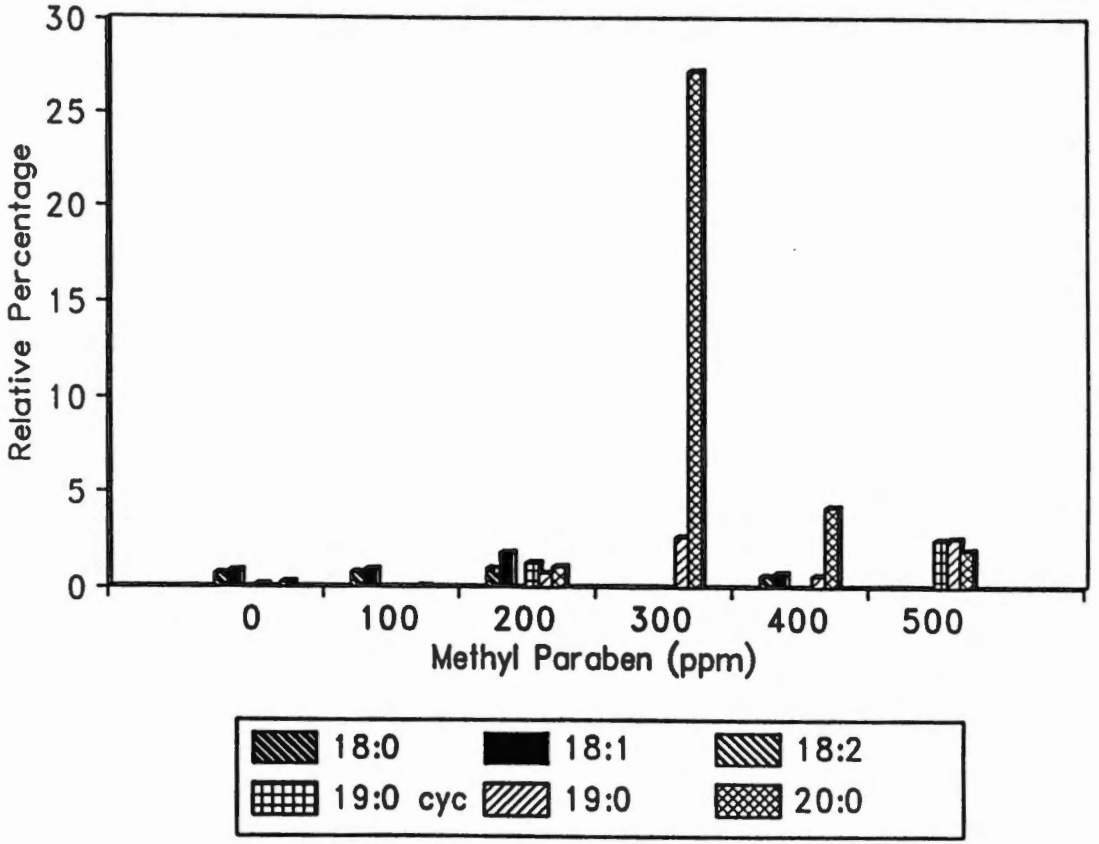


Figure 27. Mean Relative Percentage of Long Chain Fatty Acids Found in *Listeria monocytogenes* Strain Scott A at Five Methyl Paraben Concentrations, Grown on Tryptose Phosphate Agar at 35°C.

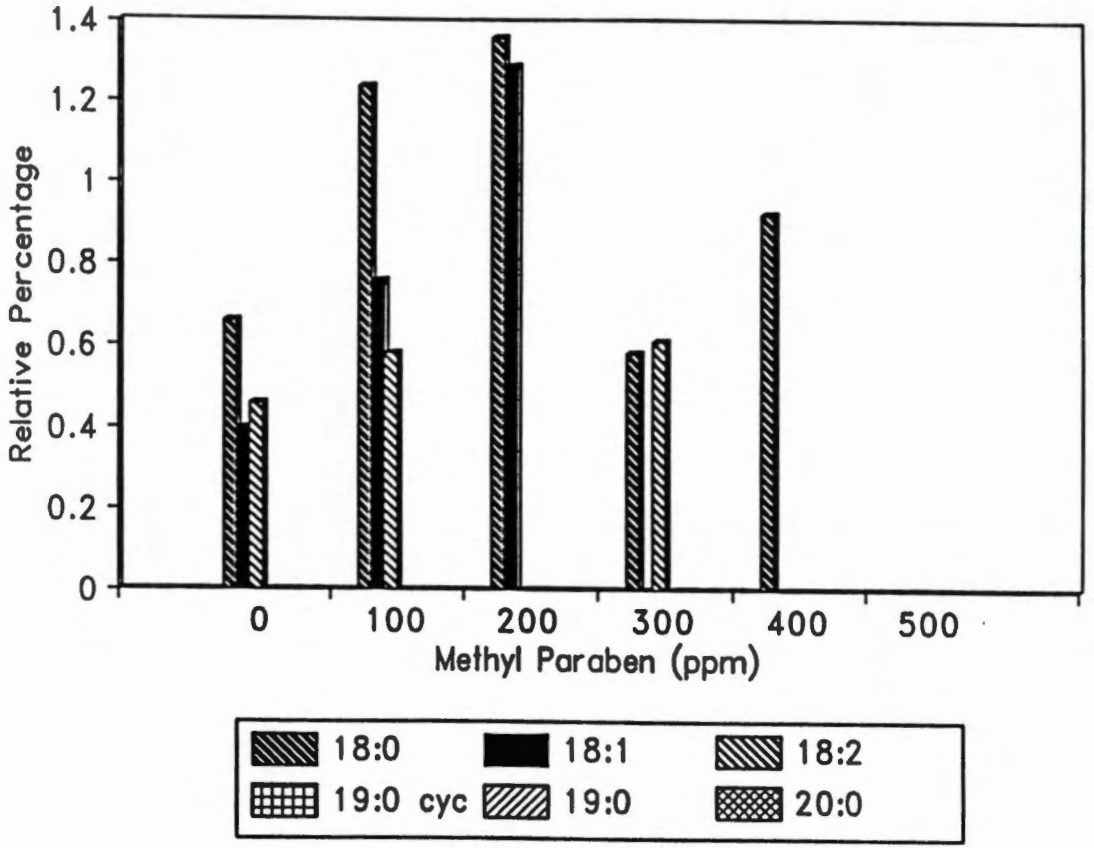


Figure 28. Mean Relative Percentage of Long Chain Fatty Acids Found in *Listeria monocytogenes* Strain Scott A at Five Methyl Paraben Concentrations, Grown on Tryptose Phosphate Agar at 7°C.

Total relative percentages of long chain fatty acids such as C18:0, C18:1 and C18:2 was greatest at 100 μg/ml methyl paraben (2.6%) however they were not detected at several methyl paraben concentrations (Figure 28). In fact, no detectable level of these 3 fatty acids was observed at 500 μg/ml methyl paraben. In contrast to strain Scott A, a trace amount of C18:2 (0.5%) was detected at 500 μg/ml methyl paraben in strain LM4A at 35°C while C19:0 was not detected (Figure 29). The total relative percentage of C18:0, C18:1, C19:0Δ, C20:0 decreased from 3.2% (control) to 2.2% (500 μg/ml methyl paraben) in LM4A. These fatty acids were not detected at 100 μg/ml or 400 μg/ml methyl paraben with the exception of C20:0 (Figure 29). This fatty acid increased from 0.40% (control) to 4.76% at 400 μg/ml methyl paraben. For strain LM4A at 7°C, the total relative percentage of these long chain fatty acids was greatest at 100 μg/ml (2.86%; Figure 30). At 500 μg/ml methyl paraben, they were not detected. Unknown fatty acids 9-14 with retention times greater than that of C18:0 were detected in strain Scott A and LM4A only at 35°C (Appendix 6,8). For strain LM4A, these fatty acids were either decreased or not detected in the presence of methyl paraben. The only exception was unknown fatty acid 13 which was present at 200 μg/ml methyl paraben (5.62%).

Fatty acid profiles of the two strains of *L. monocytogenes* was altered in the presence of propyl paraben.

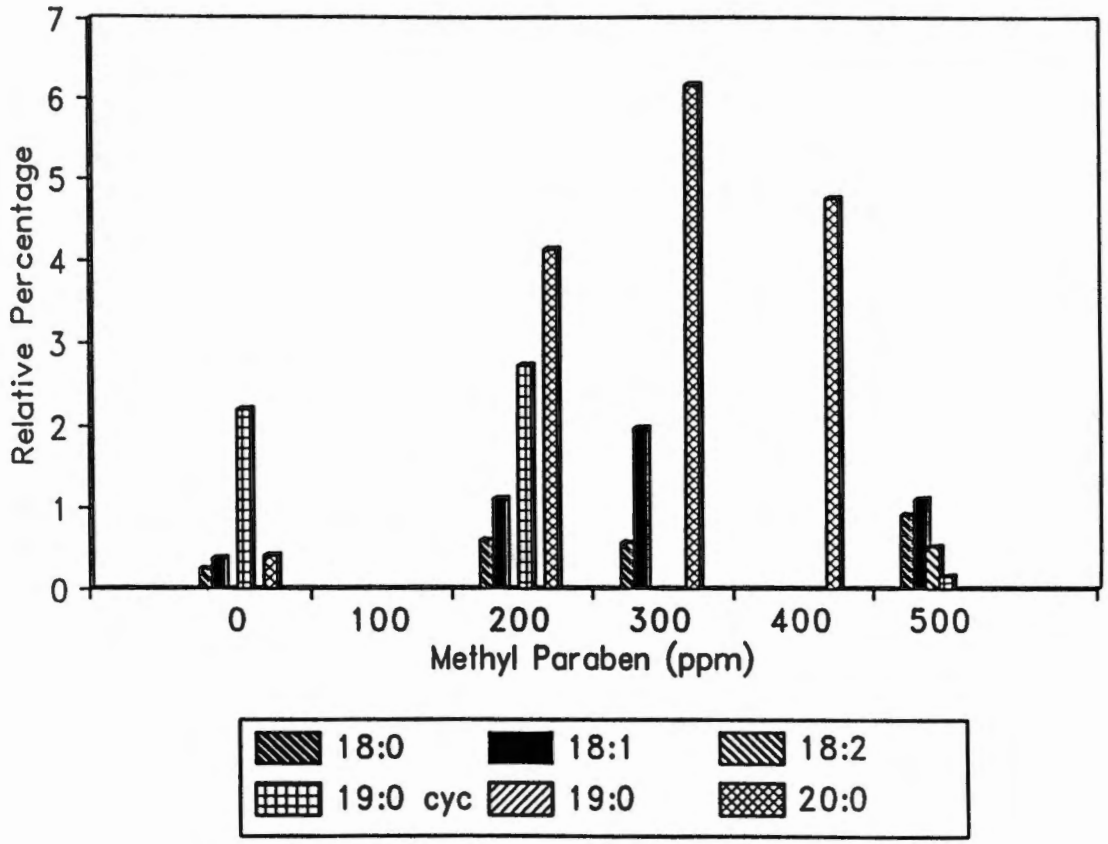


Figure 29. Mean Relative Percentage of Long Chain Fatty Acids Found in *Listeria monocytogenes* Strain LM4A at Five Methyl Paraben Concentrations, Grown on Tryptose Phosphate Agar at 35°C.

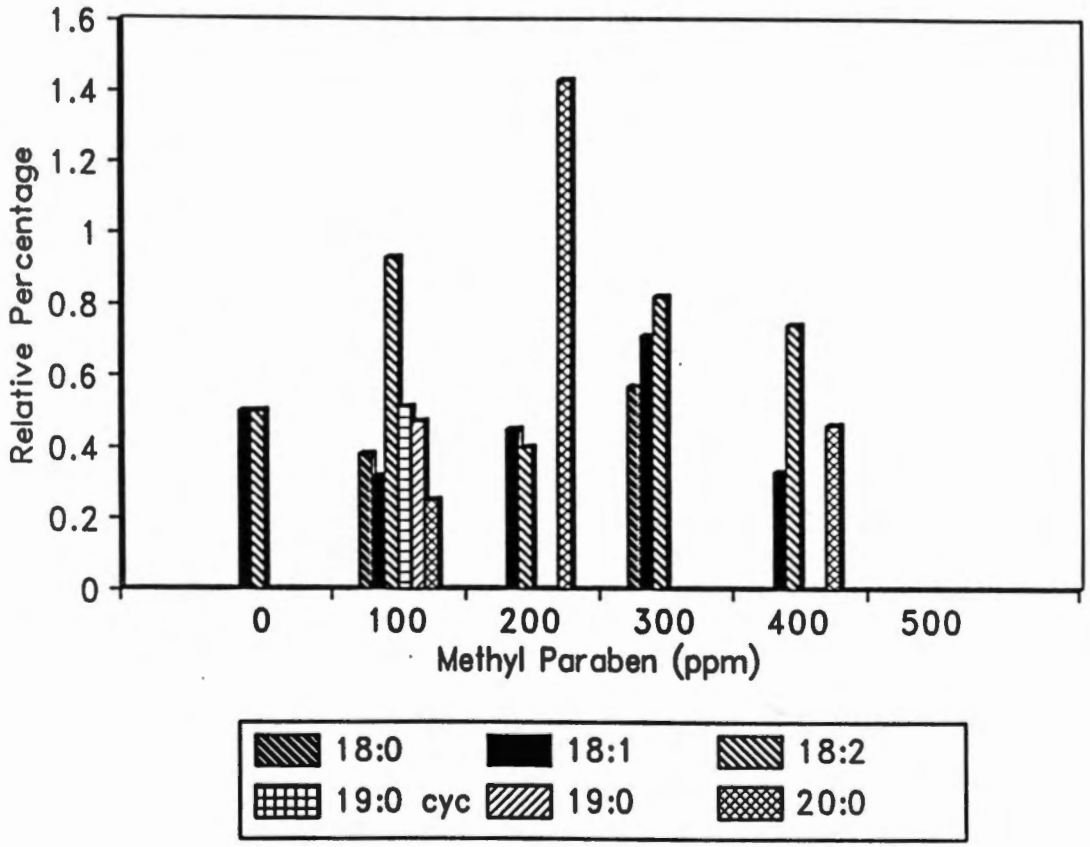


Figure 30. Mean Relative Percentage of Long Chain Fatty Acids Found in *Listeria monocytogenes* Strain LM4A at Five Methyl Paraben Concentrations, Grown on Tryptose Phosphate Agar at 7°C.

Increasing the concentration of propyl paraben from 100 μg/ml to 500 μg/ml resulted in a general decline of C15:0br fatty acids. However, at 35°C, an increase from 49.8% (control) to 54.0% (100 μg/ml propyl paraben) in Scott A occurred (Figure 31). The percentage of this fatty acid was 3.1% in Scott A at 500 μg/ml propyl paraben. For strain Scott A at 7°C, the C15:0br fatty acids decreased from 75.7% in the control to no detectable levels at 500 μg/ml propyl paraben (Figure 32). As in strain Scott A, C15:0br increased from 40.7% (control) to 53.0% (100 μg/ml) in LM4A at 35°C (Figure 33). No detectable levels of C15:0br was observed at 500 μg/ml. For strain LM4A at 7°C, C15:0br decreased from 79.8% (control) to 1.73% at 500 μg/ml (Figure 34). Therefore, for both strains at 35°C and 7°C, C17:0br decreased with increased concentration of propyl paraben present. This fatty acid was not detected at 500 μg/ml in either strains at 35°C or 7°C.

Relative percentages of unknown fatty acid 1, C12:0, C14:0, C15:0, OH-C14:0 and C16:0 increased with increasing concentration of propyl paraben in both strains at both temperatures. Total relative percentages of these 6 fatty acids increased from 5.9% (control) to 47.9% (500 μg/ml propyl paraben) in strain Scott A at 35°C (Figure 35) and 8.6% to 39.65% at 7°C (Figure 36). For strain LM4A, the fatty acids increased from 5.5% (control) to 26.8% (500 μg/ml propyl paraben) at 35°C (Figure 37) and 7.9% to 59.6%

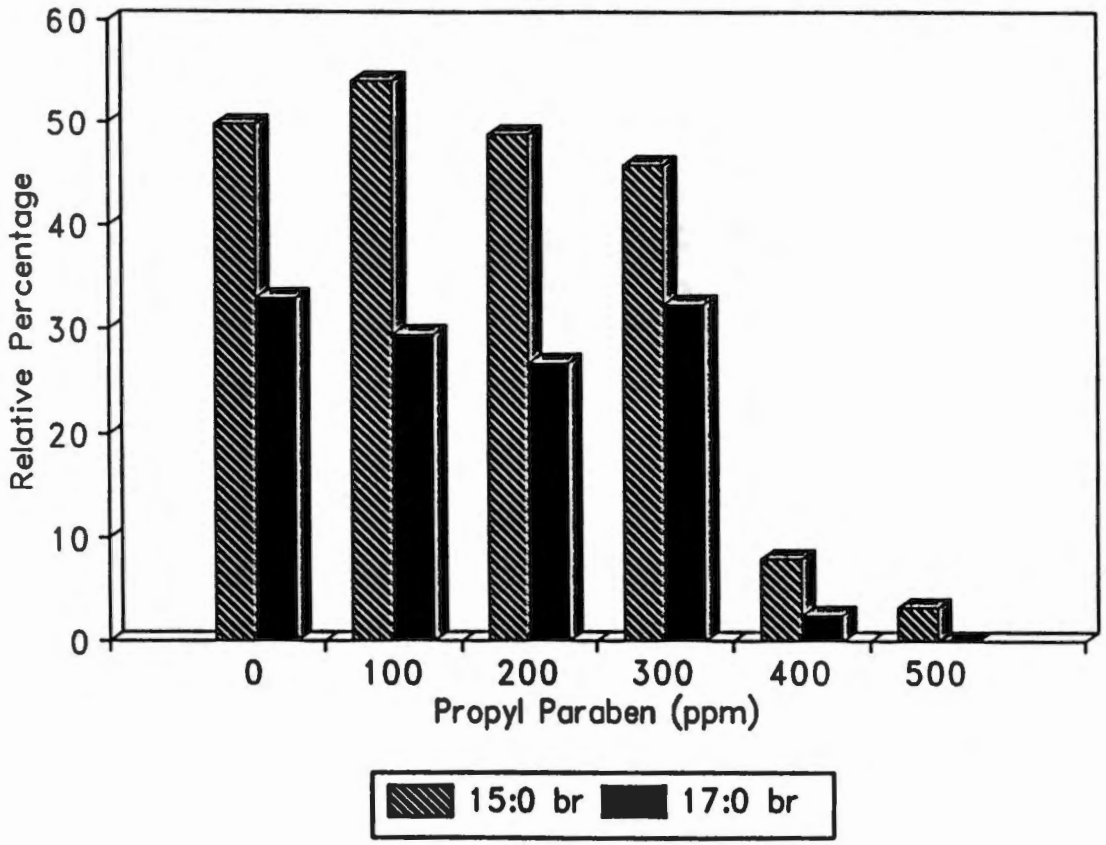


Figure 31. Mean Relative Percentage of Branched Chain Fatty Acids Found in *Listeria monocytogenes* Strain Scott A at Five Propyl Paraben Concentrations, Grown on Tryptose Phosphate Agar at 35°C.

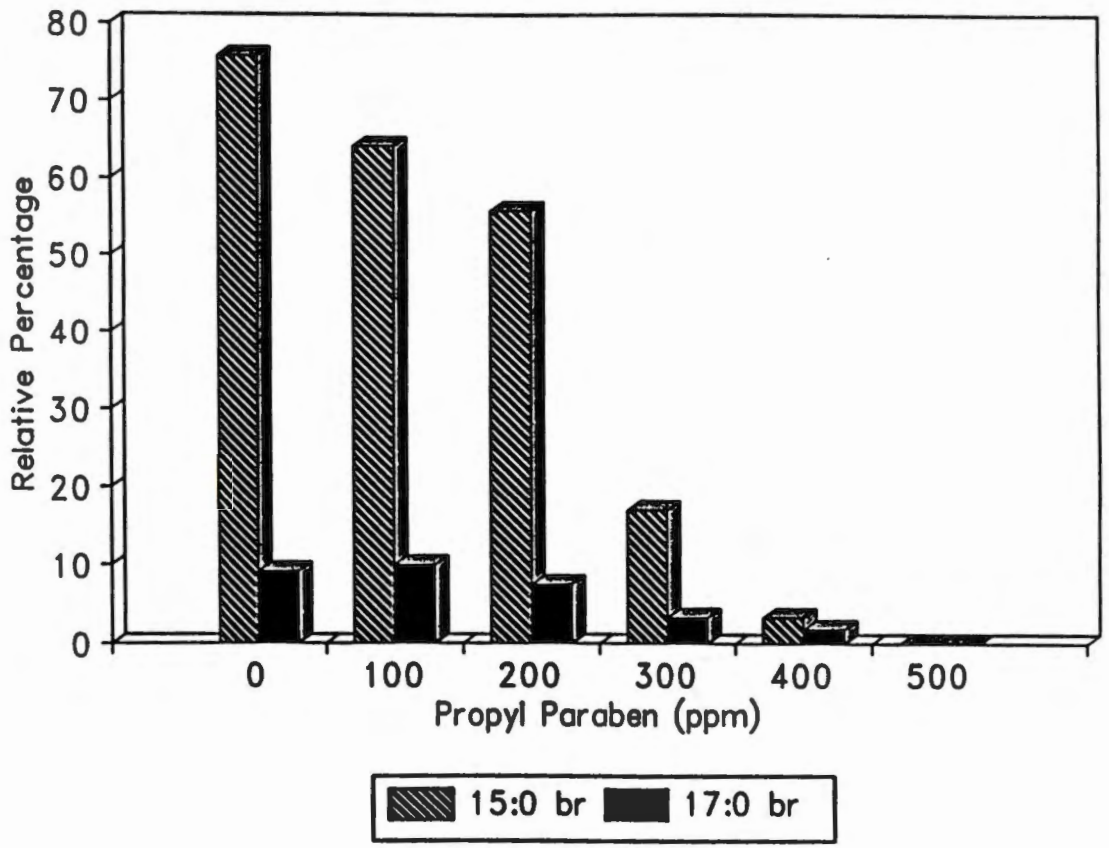


Figure 32. Mean Relative Percentage of Branched Chain Fatty Acids Found in *Listeria monocytogenes* Strain Scott A at Five Propyl Paraben Concentrations, Grown on Tryptose Phosphate Agar at 7°C.

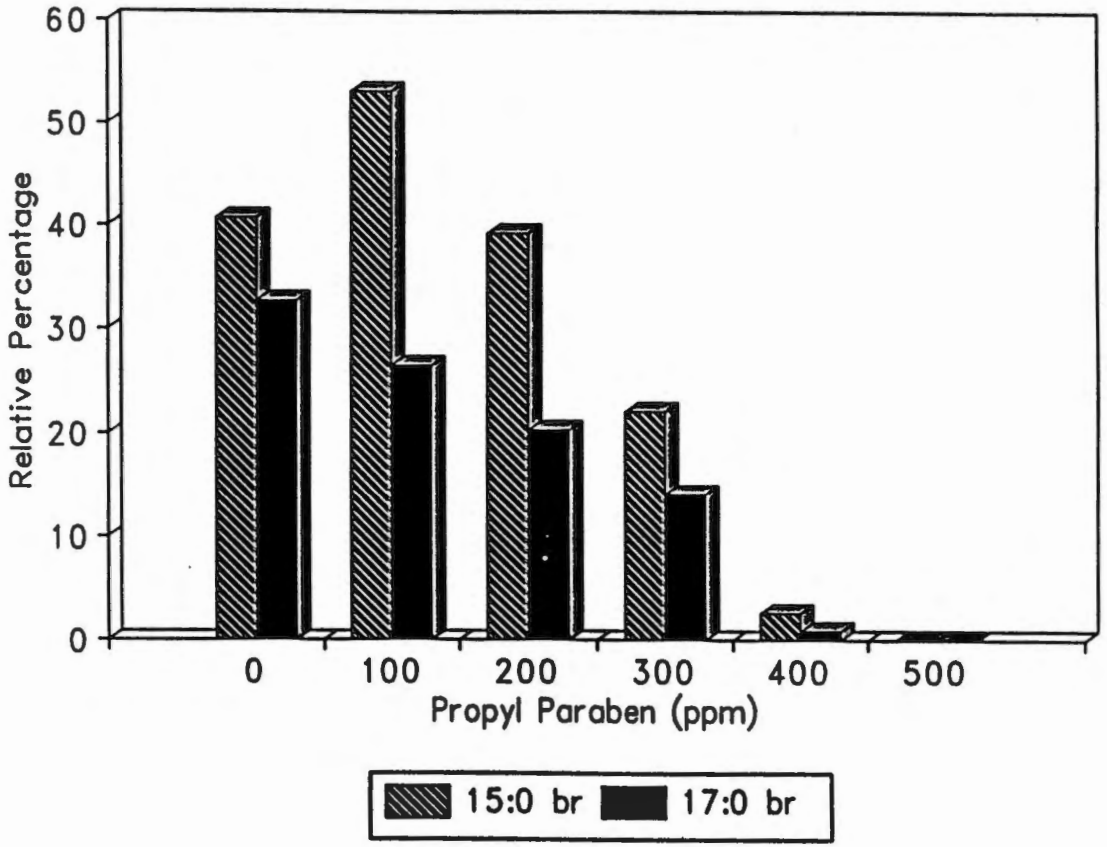


Figure 33. Mean Relative Percentage of Branched Chain Fatty Acids Found in *Listeria monocytogenes* Strain LM4A at Five Propyl Paraben Concentrations, Grown on Tryptose Phosphate Agar at 35°C.

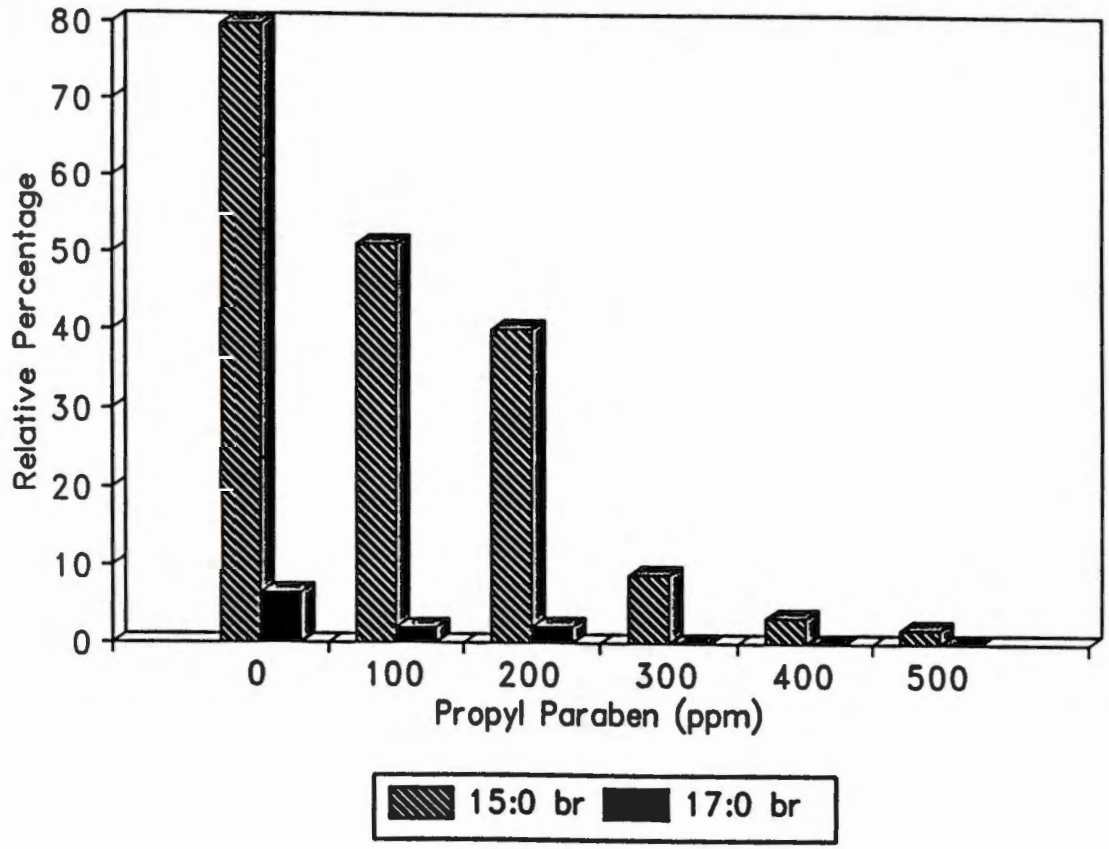


Figure 34. Mean Relative Percentage of Branched Chain Fatty Acids Found in *Listeria monocytogenes* Strain LM4A at Five Propyl Paraben Concentrations, Grown on Tryptose Phosphate Agar at 7°C.

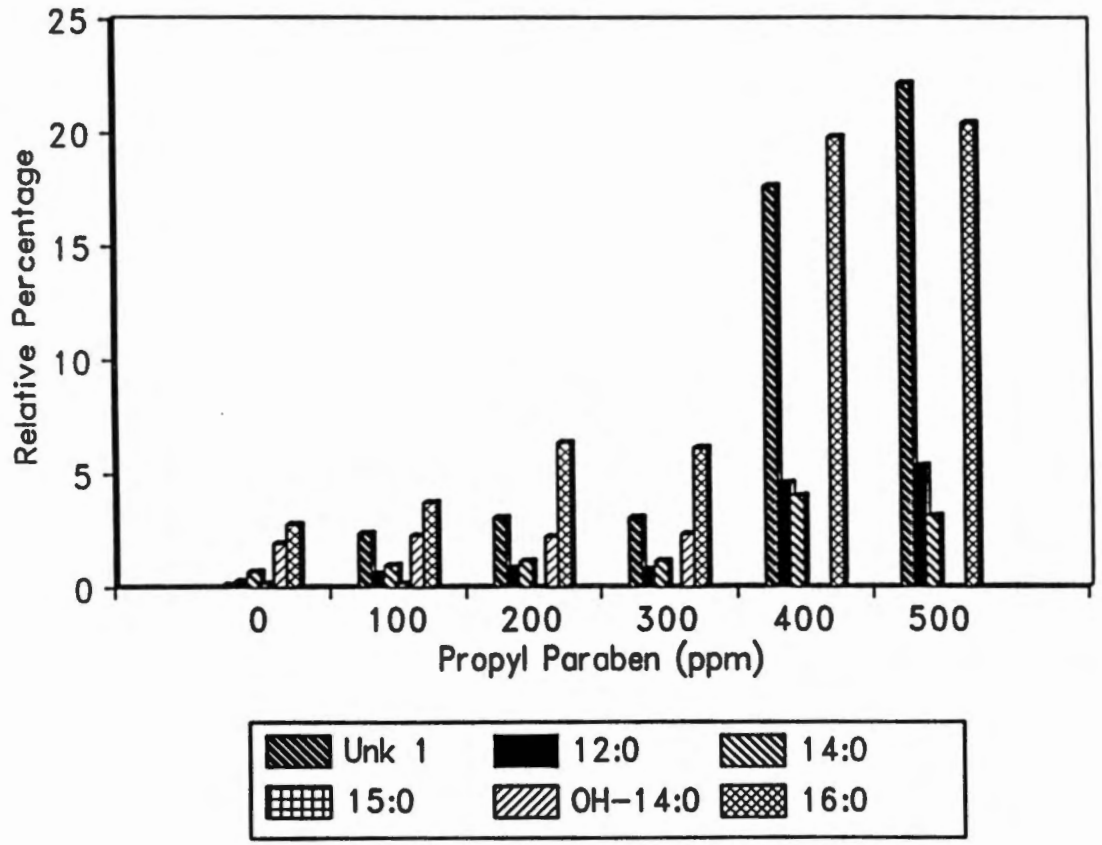


Figure 35. Mean Relative Percentage of Selected Medium Chain Fatty Acids Found in *Listeria monocytogenes* Strain Scott A at Five Propyl Paraben Concentrations, Grown on Tryptose Phosphate Agar at 35°C.

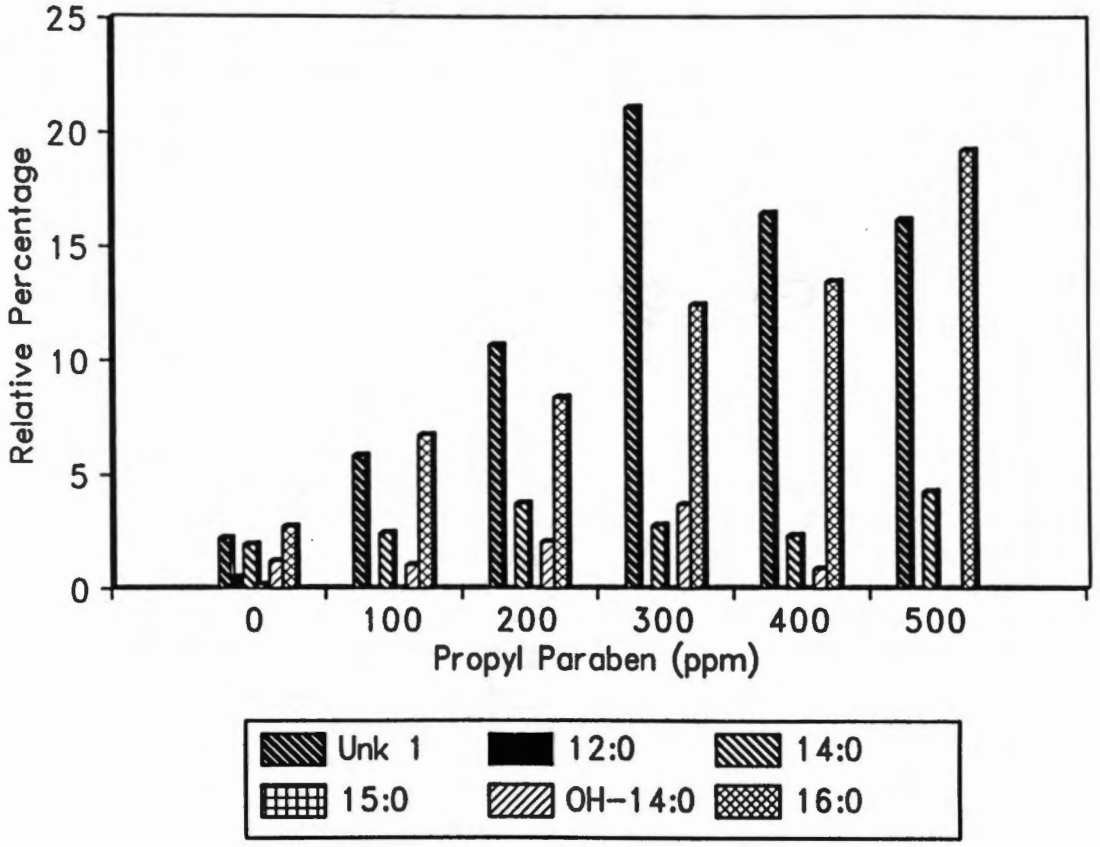


Figure 36. Mean Relative Percentage of Selected Medium Chain Fatty Acids Found in *Listeria monocytogenes* Strain Scott A at Five Propyl Paraben Concentrations, Grown on Tryptose Phosphate Agar at 7°C.

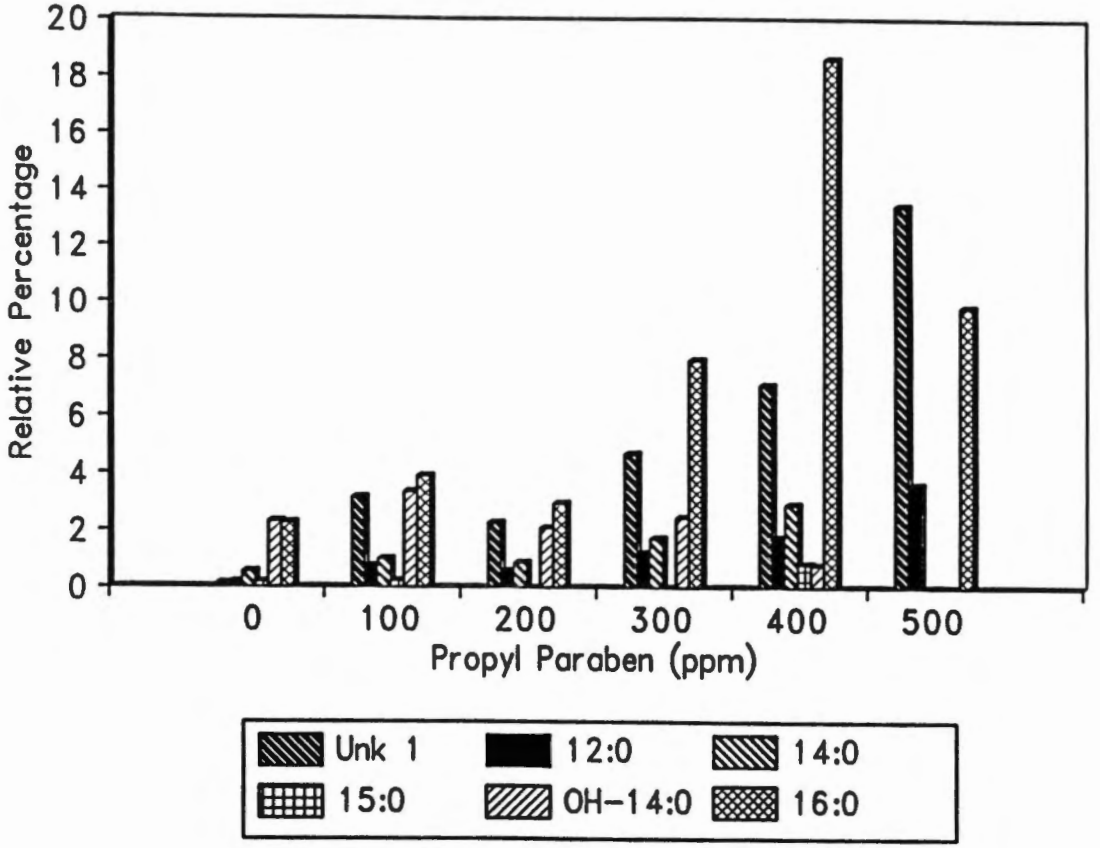


Figure 37. Mean Relative Percentage of Selected Medium Chain Fatty Acids Found in *Listeria monocytogenes* Strain LM4A at Five Propyl Paraben Concentrations, Grown on Tryptose Phosphate Agar at 35°C.

at 7°C (Figure 38). For Scott A strain at 35°C and 7°C,C15:0 was not detected or at very low levels with propyl paraben present (Figures 35 and 36). Trace amount of C12:0 in the control (0.5%) disappeared in the presence of propyl paraben at 7°C (Figure 36). For strain LM4A at 35°C, C14:0, C15:0 and OH-C14:0 were not detected at 500 μg/ml propyl paraben (Figure 37). At 7°C, no detectable levels of C15:0 were observed in control as well as in the presence of propyl paraben (Figure 38). Fatty acids C11:0 and C13:0 were detected in strains Scott A and LM4A at only 35°C in the presence of propyl paraben (Appendix 10, 12). The relative percentage of C11:0 increased with increasing concentration of propyl paraben. C11:0 was not detectable in control while 3.77% and 2.44% were found in strain Scott A and LM4A, respectively, at 500 μg/ml propyl paraben. Fatty acid 2-OH-C12:0 was detected in both strains at 35°C and 7°C at various propyl paraben concentrations (Appendix 10,11,12,13). Unknown fatty acids 2 to 5 were detected in both the strains at only 7°C (Appendix 11,13).

Total relative percentages of C18:0, C18:1, C18:2, C19:0Δ, C19:0 and C20:0 for Scott A increased from 2.1% (control) to 27.7% (500 μg/ml propyl paraben) at 35°C (Figure 39) and 1.5% (control) to 30.3% (500 μg/ml propyl paraben) at 7°C (Figure 40). For strain LM4A, increases in the sum total of relative percentages of the same fatty acids were 3.23% (control) to 70.7% (500 μg/ml propyl

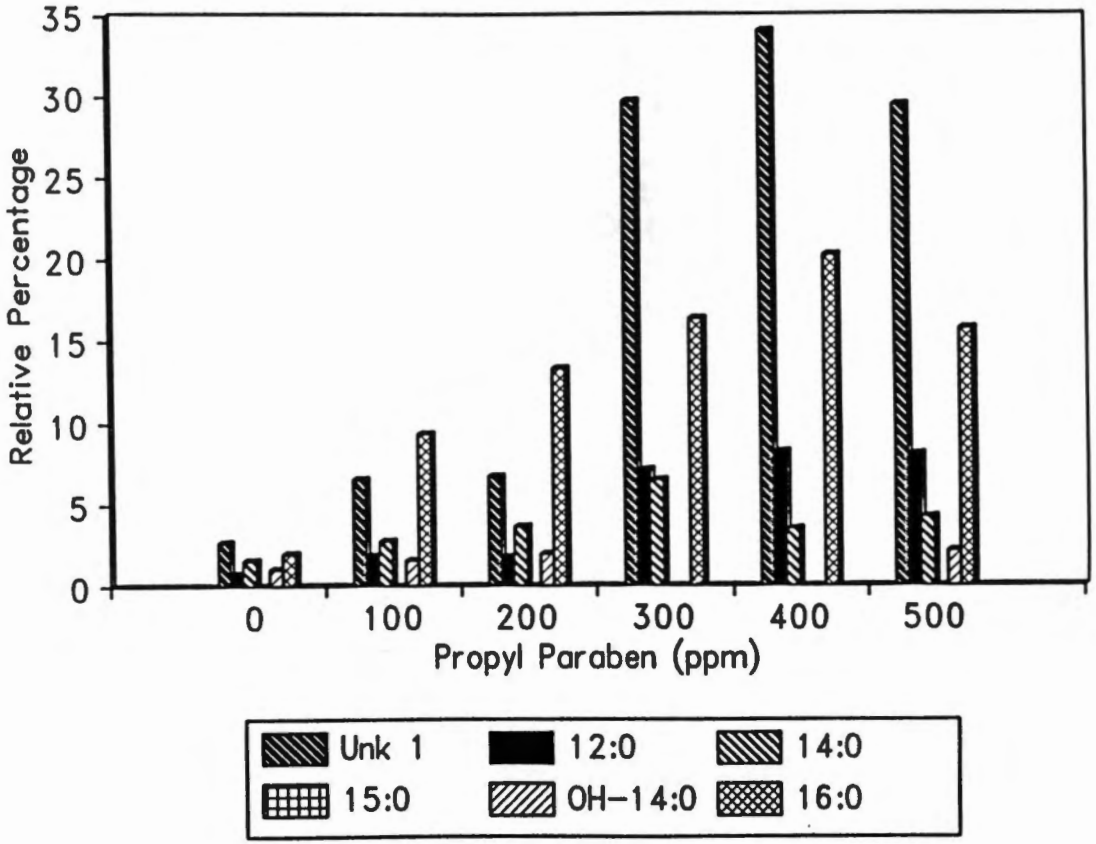


Figure 38. Mean Relative Percentage of Selected Medium Chain Fatty Acids Found in *Listeria monocytogenes* Strain LM4A at Five Propyl Paraben Concentrations, Grown on Tryptose Phosphate Agar at 7°C.

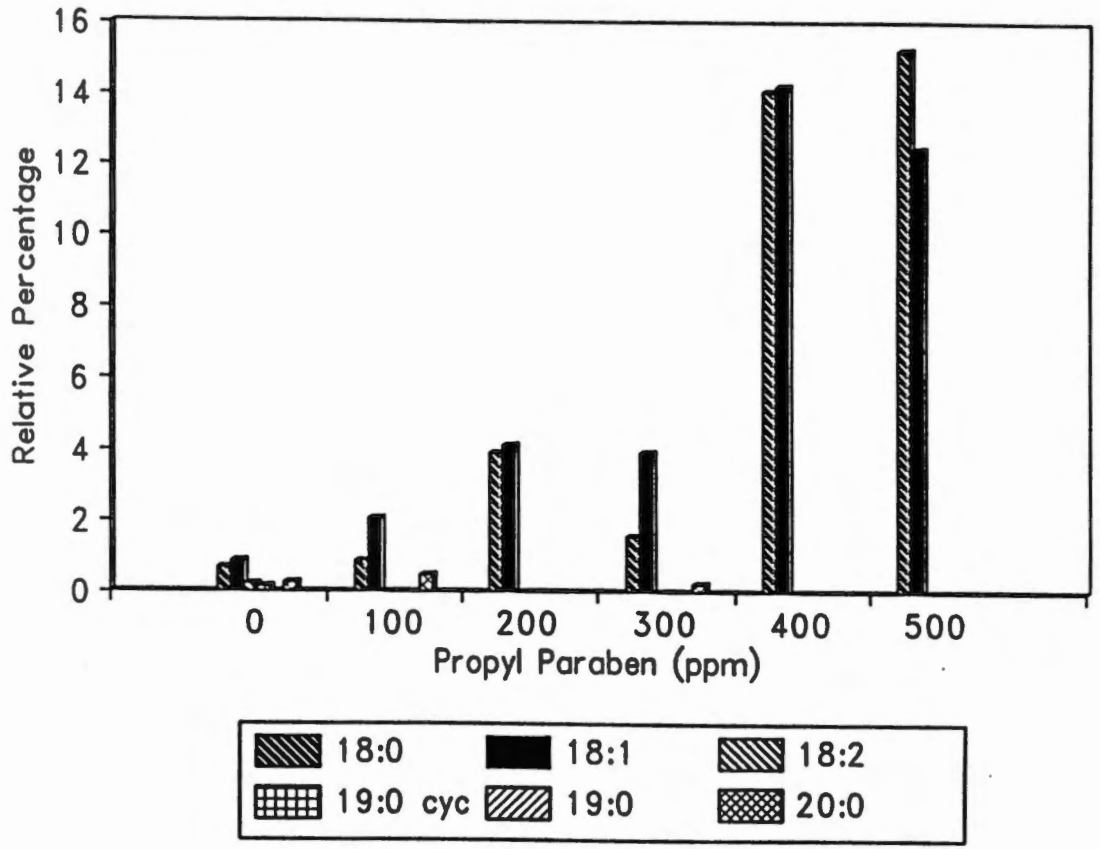


Figure 39. Mean Relative Percentage of Long Chain Fatty Acids Found in *Listeria monocytogenes* Strain Scott A at Five Propyl Paraben Concentrations, Grown on Tryptose Phosphate Agar at 35°C.

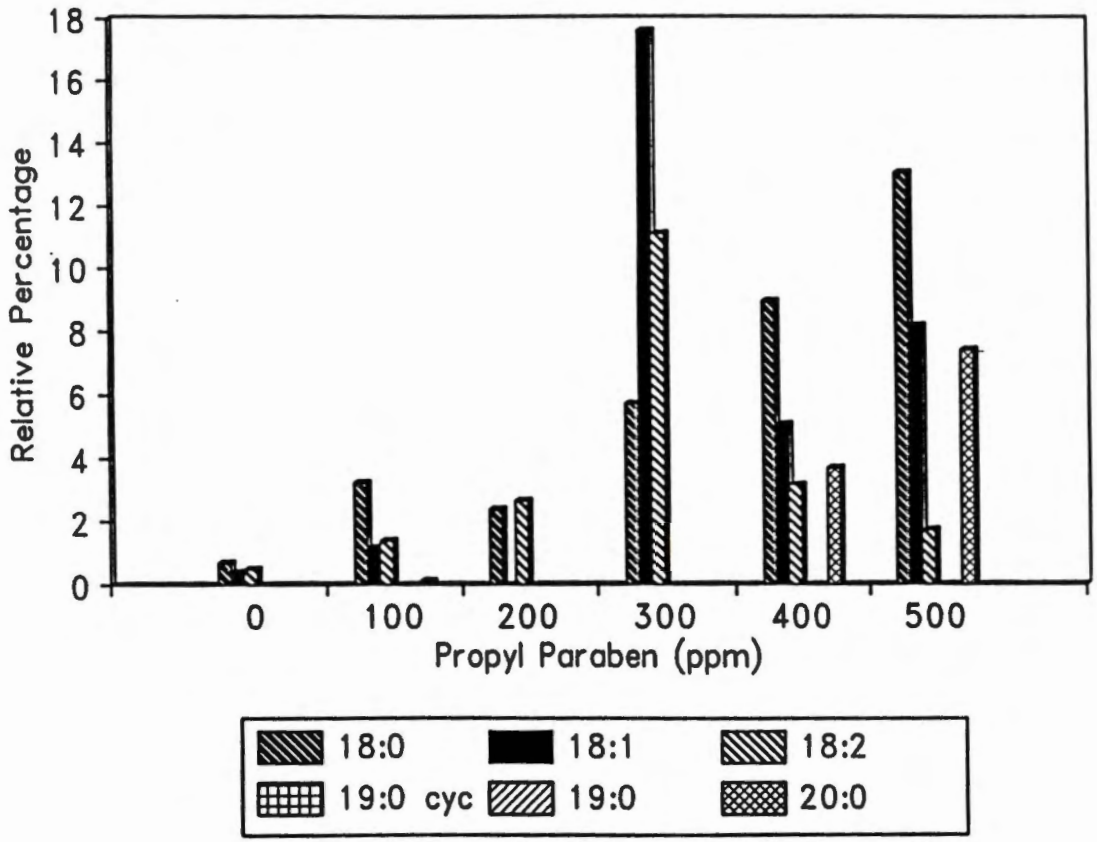


Figure 40. Mean Relative Percentage of Long Chain Fatty Acids Found in *Listeria monocytogenes* Strain Scott A at Five Propyl Paraben Concentrations, Grown on Tryptose Phosphate Agar at 7°C.

paraben) at 35°C (Figure 41) and 1% (control) to 31.7% (500μg/ml propyl paraben) at 7°C (Figure 42). For strain Scott A at 35°C, trace amounts of C18:2, C19:0Δ detected in the control disappeared in the presence of propyl paraben (Figure 39). Fatty acid C19:0 was not detected at 35°C or 7°C. For strain LM4A at 35°C, C20:0 was not detected at 500 μg/ml propyl paraben and C19:0 and C18:2 were only present at higher propyl paraben concentrations (Figure 41). At 7°C, C19:0Δ, C19:0 were not detected and C20:0 was only present at 100 μg/ml propyl paraben (Figure 42). A few of the unknown long chain fatty acids 9-14 were also detected in both the strains at 35°C and 7°C at various propyl paraben concentrations (Appendix 10, 11, 12, 13).

Results of this study clearly demonstrate that the fatty acid profiles of the two strains of *L. monocytogenes* were altered under conditions imposed by sodium chloride and parabens. General decreases in relative percentages of branched chain fatty acids, increases in medium chain fatty acids and variable changes in long chain fatty acids were found with increasing concentration of sodium chloride and parabens in the growth medium. In addition, antimicrobial treated cells displayed variations in the types and relative percentages of individual fatty acids.

In the presence of 100 μg/ml parabens at 35°C, the relative percentage of C15:0br increased (Figures 19, 21, 31 and 33). In contrast, no such increase in C15:0br was

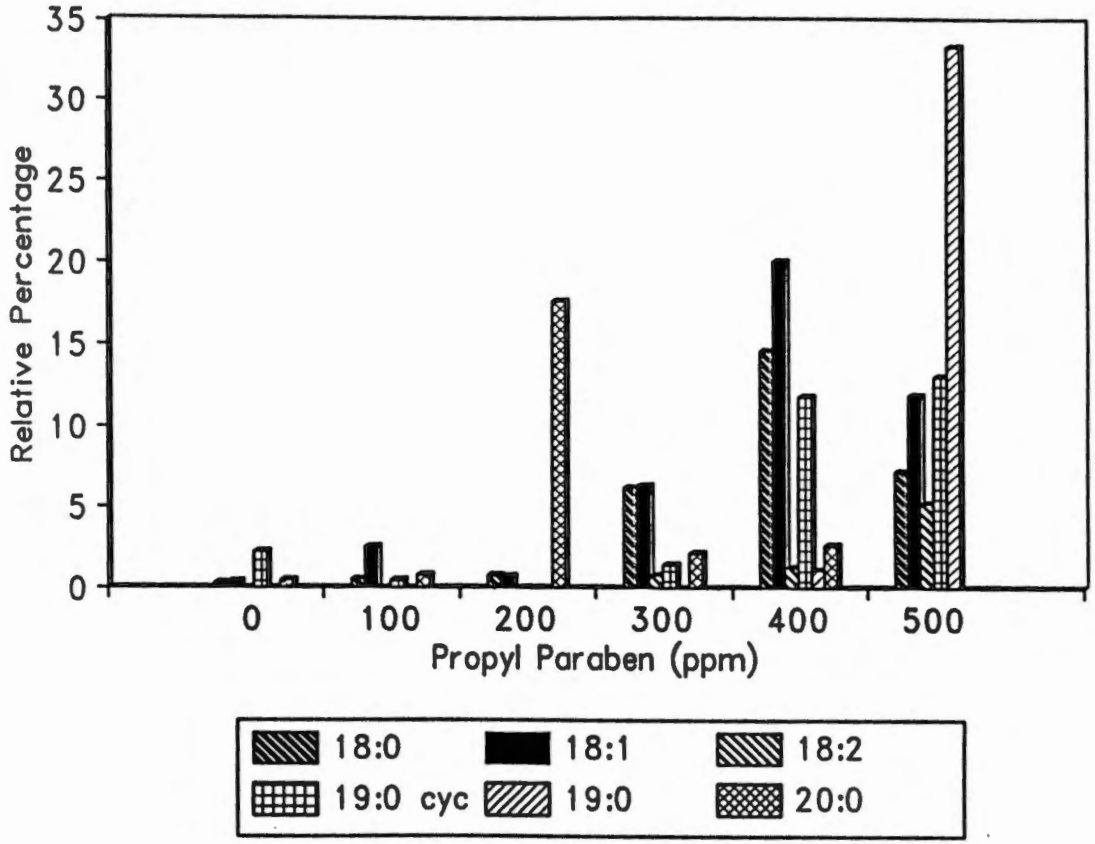


Figure 41. Mean Relative Percentage of Long Chain Fatty Acids Found in *Listeria monocytogenes* Strain LM4A at Five Propyl Paraben Concentrations, Grown on Tryptose Phosphate Agar at 35°C.

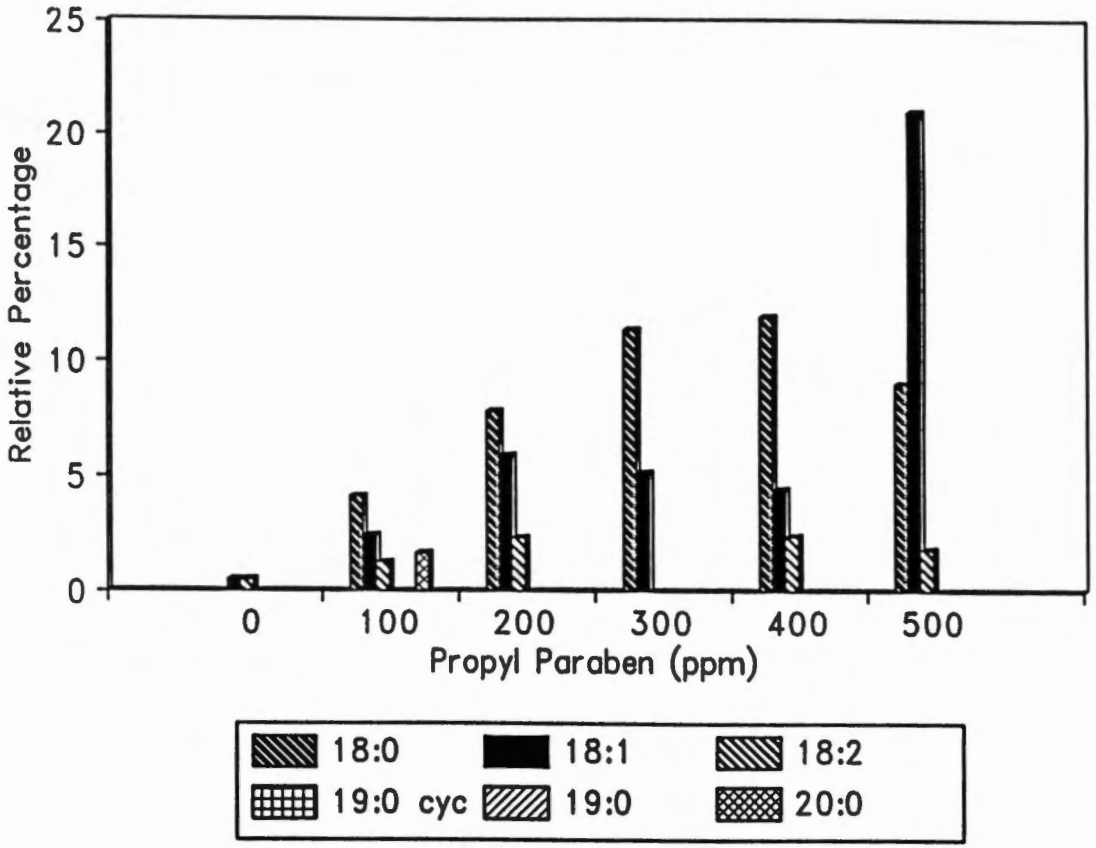


Figure 42. Mean Relative Percentage of Long Chain Fatty Acids Found in *Listeria monocytogenes* Strain LM4A at Five Propyl Paraben Concentrations, Grown on Tryptose Phosphate Agar at 7°C.

observed in the presence of sodium chloride (Figures 7 and 9). The synthesis of C15:0 results from the utilization of propionyl-ACP as a primer for fatty acid synthesis instead of acetyl-ACP (Ingram, 1977). This depends upon the conformation of the enzyme beta-Keto acyl ACP synthetase. One conformation of the enzyme might display greater affinity for acetyl-CoA, while the other displays greater affinity for propionyl-CoA (Canonica and Pisano, 1988). Thus, the increase in the relative percentage of C15:0br in the presence of 100 μg/ml parabens may be attributed to the fact that the conformation of the enzyme was such that the synthesis of C15:0br was enhanced. The high relative percentage of C15:0br fatty acid observed at lower concentration of parabens likely increased membrane fluidity. However, increasing concentration of parabens resulted in decreased branched chain fatty acids, indicating decreased membrane fluidity. Thus, cells may resist the antimicrobial effects of parabens by decreasing the relative percentages of branched chain fatty acids.

Increases in the relative percentages of saturated fatty acids was observed with increasing concentration of sodium chloride and parabens. This may be due to the inhibition of fatty acid desaturating enzyme in the presence of antimicrobials. In fact, desaturating enzyme is involved in the dehydrogenation of long-chain saturated fatty acids. This indicates that the cells resist the affect of

antimicrobials by synthesizing saturated fatty acids, thereby decreasing membrane fluidity. The changes in fatty acid profiles may be attributed to the protective measures initiated by the cells or toxic effects of the compounds. The results of this portion of the study tend to support a relationship between types and/or relative percentages of individual or groups of fatty acids and the resistance of *L. monocytogenes* to sodium chloride and parabens used in the study.

Cells may synthesize saturated or high melting point fatty acids as a defense mechanism so that the physical state of the membrane is less fluid in the presence of antimicrobials. The present results agree with similar reports published previously. Ingram (1977) working with *E. coli* reported that cells synthesize and add increased amount of saturated fatty acids to the membranes in order to maintain the proper fluid state as a protective measure when exposed to phenolic compounds.

Effect of varying temperatures on fatty acids composition

Increasing the temperature from 7°C to 35°C altered the fatty acid profiles of the two strains of *L. monocytogenes* (Figures 43 and 44). For strain Scott A, C15:0br decreased with the increase of temperature from 75.7% at 7°C to 49.8% at 35°C (Figure 43) and 79.8% to 40.7% for strain LM4A (Figure 44). In contrast, C17:0br increased from 9.1% to

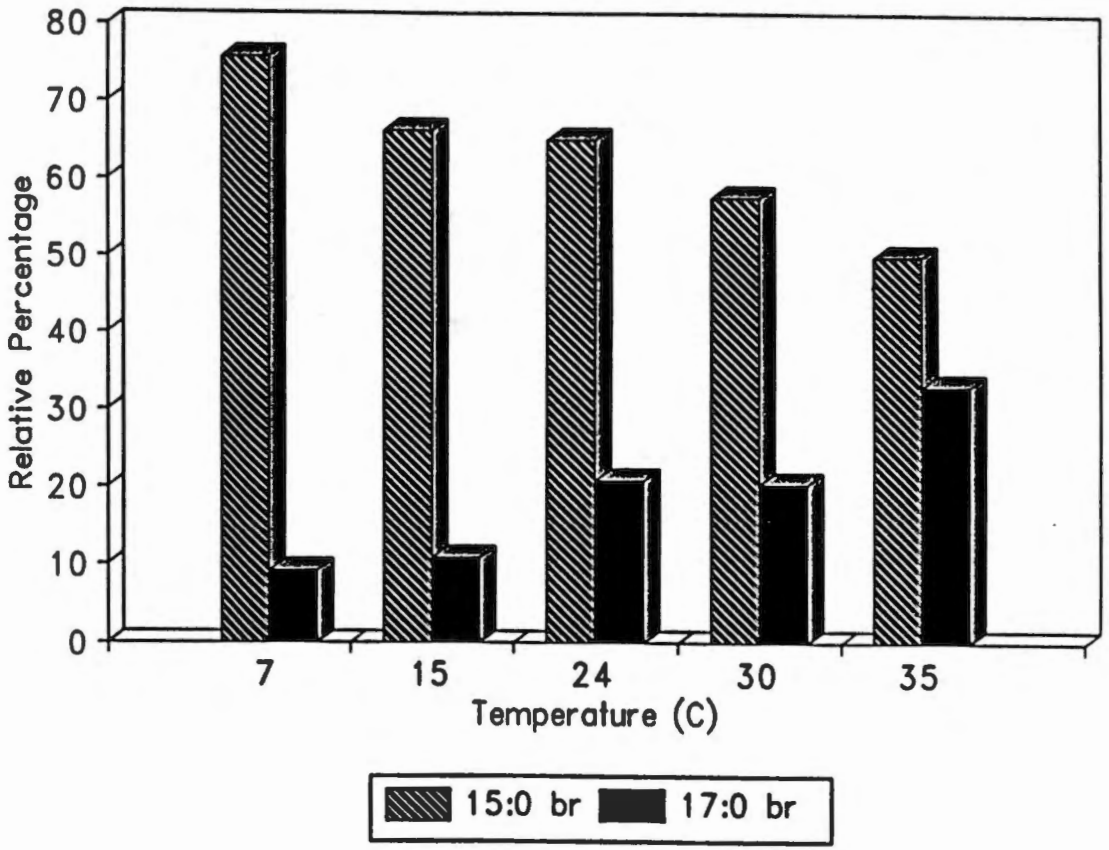


Figure 43. Mean Relative Percentage of Branched Chain Fatty Acids Found in *Listeria monocytogenes* Strain Scott A, Grown on Tryptose Phosphate Agar at Five Temperatures.

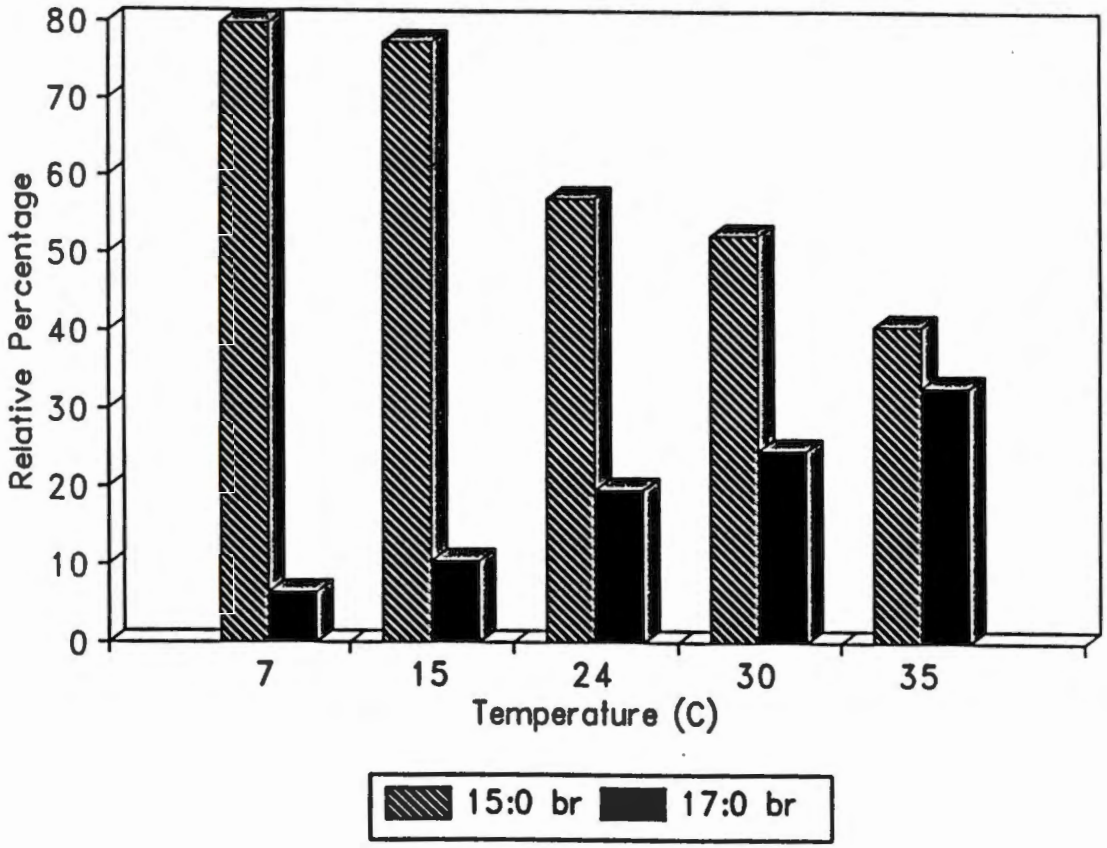


Figure 44. Mean Relative Percentage of Branched Chain Fatty Acids Found in *Listeria monocytogenes* Strain LM4A A, Grown on Tryptose Phosphate Agar at Five Temperatures.

33% for strain Scott A (Figure 43) and 6.3% to 32.7% for strain LM4A (Figure 44).

The branched chain fatty acid pattern agreed with the results of other researchers. Tadayon et al. (1969) reported in *L. monocytogenes* that the proportion of C17:0br was much lower at 4°C than at higher temperatures and the difference was made up mainly by C15:0br. In the present study, decrease in relative percentage of C15:0br with increase in temperature likely decreased membrane fluidity. Changes in fatty acids may be attributed to adaptations to different temperature.

The relative percentages of unknown fatty acid 1, C12:0, C14:0, C15:0, OH-C14:0, and C16:0 overall decreased with increased temperature (Figures 45 and 46). For strain Scott A, these fatty acids decreased from 8.6% at 7°C to 6% at 35°C and 7.9% to 5.5% for strain LM4A. Fatty acid C12:0 was not detected in strain LM4A at 15°C or 24°C while unknown fatty acids 4 and 5 were detected (Figure 46). These fatty acids were also detected in strain Scott A (Figure 45). The relative percentages of these fatty acids decreased with temperature increase (Appendix 14,15). In contrast to the fatty acid profiles in the presence of antimicrobials, most of the medium chain fatty acids decreased with increase in temperature.

There were great variation in the types and relative percentages of long chain fatty acids such as C18:0, C18:1,

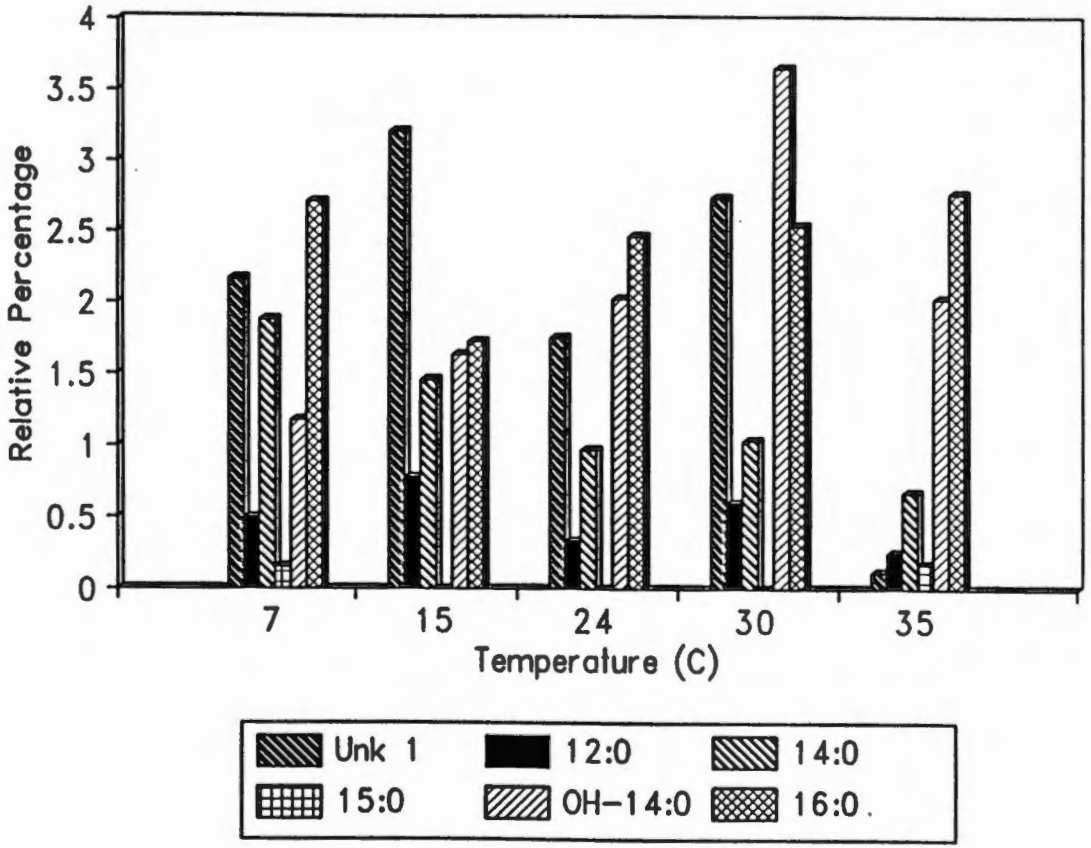


Figure 45. Mean Relative Percentage of Selected Medium Chain Fatty Acids Found in *Listeria monocytogenes* Strain Scott A, Grown on Tryptose Phosphate Agar at Five Temperatures.

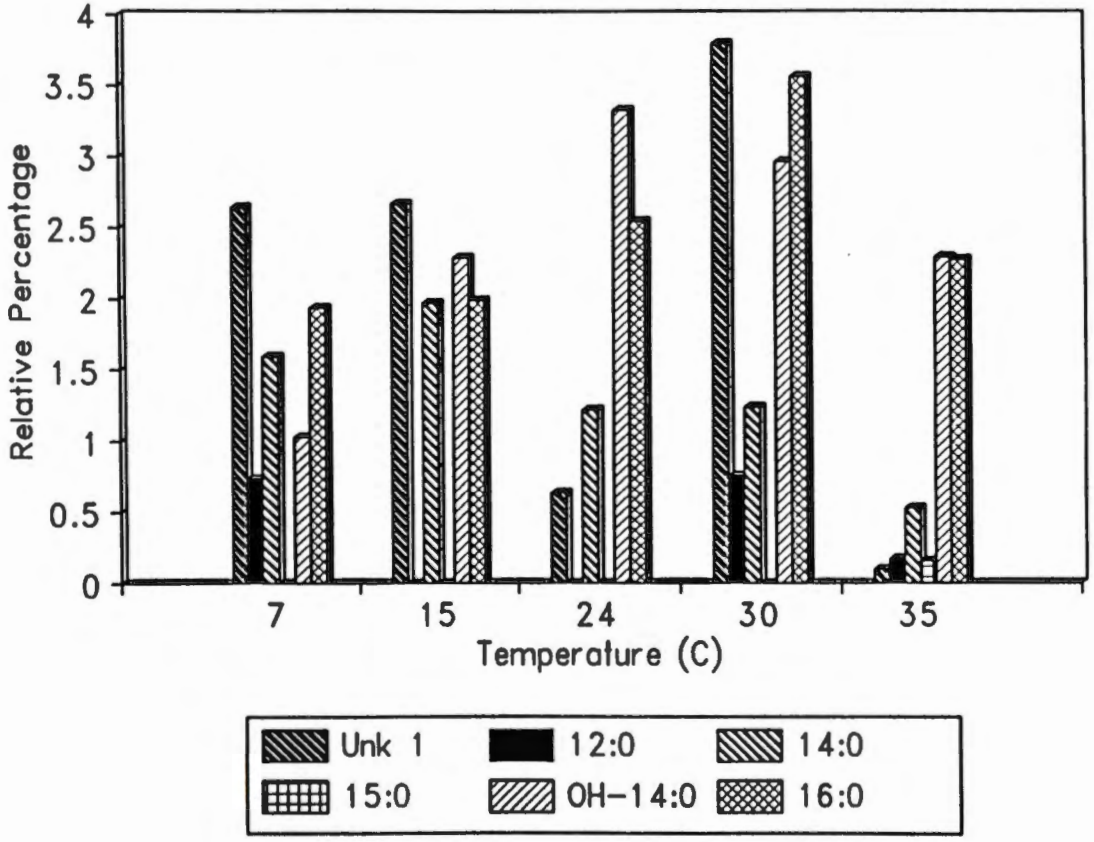


Figure 46. Mean Relative Percentage of Selected Medium Chain Fatty Acids Found in *Listeria monocytogenes* Strain LM4A, Grown on Tryptose Phosphate Agar at Five Temperatures.

C18:2, C19:0Δ, and C20:0 between the two strains at different temperatures. Fatty acid C19:0 was not detected (Figures 47 and 48). For strain Scott A, the total relative percentage of long chain fatty acids was greatest at 15°C (4.74%), though C18:0, C18:1, and C18:2 were not detected (Figure 47). The percentage decreased at 35°C to 2.09%. For strain LM4A, the total relative percentage of long chain fatty acids was greatest at 24°C (4.1%), followed by 3.23% at 35°C (Figure 48). No detectable levels of C18:0, C18:1, C18:2, C19:0Δ or C20:0 were observed in LM4A at 7°C, 15°C or 30°C with the exception of significantly high percentage of C20:0 at 30°C and trace amounts of C18:1 and C18:2 at 7°C (Figure 48). Unknown long chain fatty acids 9 to 13 were detected in both strains at various temperatures (Appendix 14, 15). The relative percentage of these fatty acids was greatest in strain Scott A (7.07%) and LM4A (13.15%) at 35°C (Appendix 14). None of these fatty acids were observed at 7°C or 15°C in strain LM4A (Appendix 15).

Temperature induced-fatty acid alterations have been extensively studied (McGarrity and Armstrong, 1975; Marr and Ingraham, 1962; Khuller and Goldfine, 1974; Miller, 1985) and have been attributed to adaptations to environments of different temperature. The changes in fatty acid profiles are consistent with the hypothesis that cells adapt their membrane lipids to compensate for the alteration in the temperature of incubation. This is an indication of

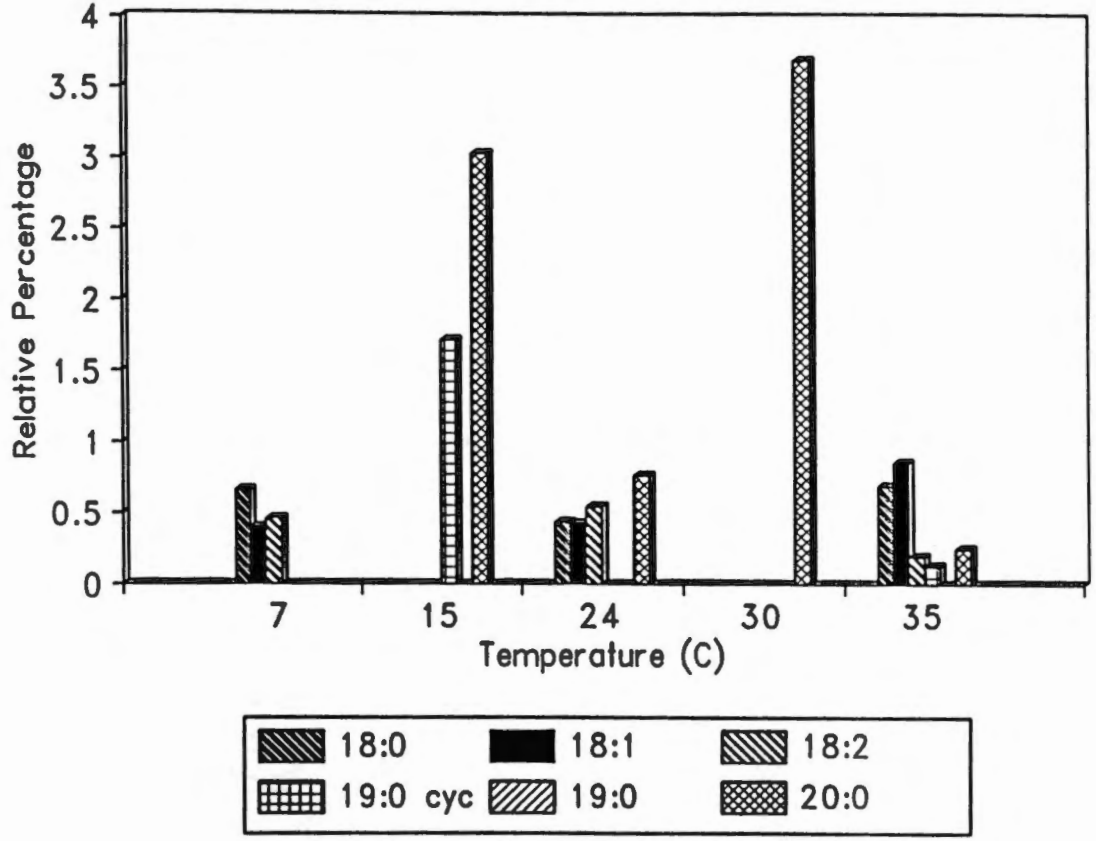


Figure 47. Mean Relative Percentage of Long Chain Fatty Acids Found in *Listeria monocytogenes* Strain Scott A, Grown on Tryptose Phosphate Agar at Five Temperatures.

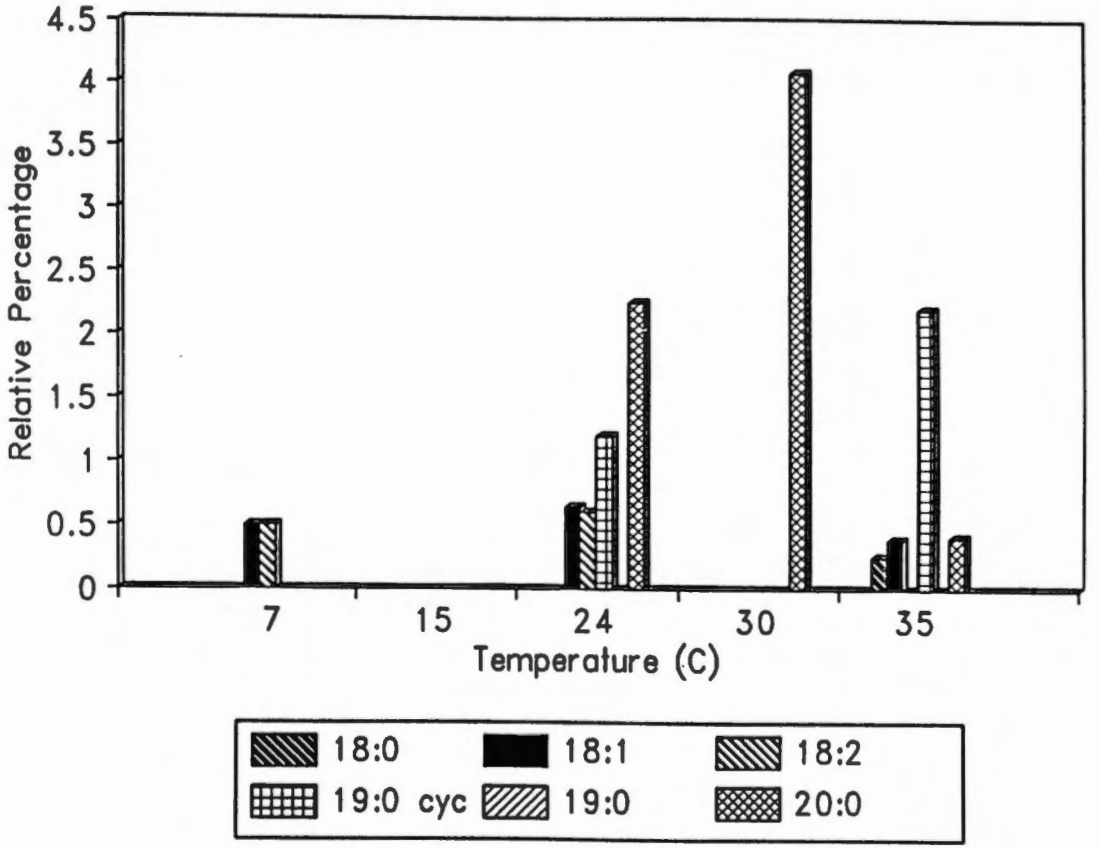


Figure 48. Mean Relative Percentage of Long Chain Fatty Acids Found in *Listeria monocytogenes* Strain LM4A, Grown on Tryptose Phosphate Agar at Five Temperatures.

temperature-regulated mechanism that modifies membrane fluidity. Sinensky (1974) suggested that an appropriate level of membrane fluidity must be maintained by bacteria in response to temperature change. Homeoviscous adaptation has been used to describe this mechanism by which organisms attempt to avoid excessive order or fluidization of membrane lipids. The change is brought about substantially by alteration of the relative abundance of membrane saturated and unsaturated fatty acids (Sinensky, 1974). Jackson and Cronan (1978), however, indicated that the measured average membrane fluidity level of the bacterial membrane may not be as important as the need for a minimal amount of fluid lipid domain within the membrane.

Khullar and Goldfine (1974) generalized the changes in fatty acid composition in bacteria in response to the temperature of environment and reported that when the growth temperature is lowered, the composition of the fatty acids is modified in the direction of lower average melting points. In Gram-negative organisms, this adaptation is usually accomplished by an increased proportion of unsaturated and in some cases cyclopropane fatty acids at the expense of saturated fatty acids at lower growth temperatures (Marr and Ingraham, 1962). In certain Gram-positive bacteria there are increased amounts of lower-melting branched-chain fatty acids at the expense of the higher melting branched and straight-chain fatty acids at

lower growth temperatures (Weerkamp and Heinen, 1972). In addition, a fatty acid desaturating enzyme system is induced in some bacilli at lower growth temperatures (Fulco, 1967). These changes have been shown by a number of workers to result in a lowering of the temperature at which the bulk of the membrane lipids change from a melted or fluid state to a frozen or condensed state (Overath et al., 1970). Marr and Ingraham (1962) suggested the mechanism by which a change in temperature regulates the synthesis of fatty acids. According to them, the effect might be (1) a direct effect of temperature on the relative rates of two or more enzymes which synthesize saturated and unsaturated fatty acids, respectively, (2) an indirect effect resulting from a change in growth rate, or (3) an indirect effect resulting from a change in the concentration of intermediates.

Modification of Lipid Composition

A system to create *L. monocytogenes* strains Scott A and LM4A with altered fatty acid and phospholipid composition was developed. Bacterial cells were grown in the presence of exogenous fatty acids with and without cerulenin. Both methods were used because initially it was not known which would prove to be a successful. Cerulenin is a fatty acid synthetase inhibitor. It was expected that inhibition of fatty acid synthesis might facilitate incorporation of exogenous fatty acids into the cell membrane of *L. monocytogenes*. Cells grown in the presence of exogenous

fatty acids were washed with peptone water (0.1%, w/v) containing 0.1% Tween 80 in order to remove exogenous fatty acids not incorporated in the membrane.

The concentration of each exogenous fatty acid (C14:0, C16:0, C18:0, and C18:1) used was 50 μg/ml. Results indicated that modification of the lipid composition was achieved in the presence of exogenous fatty acids with or without cerulenin. Therefore, the simple method i.e., cells grown in the presence of exogenous fatty acids without cerulenin was used in subsequent studies.

The relative percentages of exogenously supplied fatty acids were greatly increased compared to the control (Table 17). In contrast, the mean relative percentage of phospholipid fractions was not altered (Table 18).

Resistance of Lipid Adapted Strains

Resistance of *L. monocytogenes* strains Scott A and LM4A with altered lipid composition to parabens and salt was determined by minimum inhibitory procedure and inactivation over time study.

Minimum inhibitory concentration

Parent strains and lipid adapted cells were grown in the presence of methyl paraben, propyl paraben and TBHQ using intervals of 25 μg/ml. Sodium chloride was at intervals of 0.5% (w/v). The range for methyl paraben, propyl paraben, TBHQ and salt were 1000 - 2000 μg/ml, 350 - 1000 μg/ml, 25 - 300 μg/ml and 6 - 15%, respectively. The

Table 17. Mean Relative Percentages[a] of Fatty Acids in *Listeria monocytogenes* strain Scott A, Grown in Tryptose Phosphate Broth Containing Exogenous Fatty Acids[b] at 35°C and Subsequently Washed with Peptone Water Containing 0.1% Tween 80, Only Major Fatty Acids are Shown.

	Exogenous Fatty Acid				
Fatty Acid	Control	C14:0	C16:0	C18:0	C18:1
14:0	0.97	14.03	-	-	-
Br-15:0	41.86	48.39	22.98	41.90	34.98
16:0	2.32	-	30.92	-	1.31
Br-17:0	27.72	32.05	7.47	24.69	31.83
18:0	0.81	-	3.42	16.30	-
18:1	0.72	-	-	-	27.90

[a]Means represent two replications.
[b]Cells grown in the presence of 50 μg/ml exogenous fatty acids.

Table 18. Mean Relative Percentage[a] of Phospholipid Fractions Found in *Listeria monocytogenes* strain Scott A, Grown in Tryptose Phosphate Broth Containing 50 ppm Exogenous Fatty Acids at 35°C and Subsequently Washed with Peptone Water Containing 0.1% Tween 80.

	Exogenous Fatty Acid				
Phospholipid	Control	C14:0	C16:0	C18:0	C18:1
Phosphatidyl Glycerol	40.3	41.8	42.2	42.6	40.2
Diphosphatidyl Glycerol	29.5	28.5	28.4	28.5	29.5
Phosphatidyl Serine	30.2	29.7	29.4	28.9	30.3

[a]Means represent two replications.

objective of this experiment was to determine the minimum amount of antimicrobial necessary to inhibit growth of parent strains and lipid adapted cells. The data would be used to determine if individual fatty acids play a role in the resistance of *L. monocytogenes*.

For *L. monocytogenes* strain Scott A, the MIC for methyl paraben was significantly increased from the control (1412.5 μg/ml) to 1637.5 μg/ml and 1900 μg/ml in the cells adapted to C14:0 and C18:0 fatty acids, respectively. In contrast, the MIC of methyl paraben for the cells grown in the presence of C18:1 was significantly decreased (1162.5 μg/ml; Table 19). The same pattern was observed for propyl paraben, TBHQ, and sodium chloride. The only exception was the MIC for TBHQ for the cells grown in the presence of 18:1. In this case, the decrease in MIC was slight but not significant. Similar results were obtained with another strain of *L. monocytogenes* i.e., LM4A strain (Table 20).

Results for MIC studies of the parent strains of *L. monocytogenes* were comparable to those reported by Payne et al. (1989). In Payne's study, methyl paraben, propyl paraben and TBHQ had MIC's of >512 μg/ml, 512 μg/ml and 64 μg/ml, respectively, for *L. monocytogenes*. The slight differences in the MIC values between the present study and those reported previously were, most probably, due to the differences in the growth conditions or the concentration intervals of the test compounds. The present results were

Table 19. Minimum Inhibitory Concentrations[a] of antimicrobials against Parent Strain and Lipid Adapted Cells of *Listeria monocytogenes* Scott A, Grown in Tryptose Phosphate Broth, after incubation at 35°C for 24 hr.

	Exogenous Fatty Acid			
	Control	C14:0	C18:0	C18:1
Antimicrobial	Minimum Inhibitory Concentration[c]			
Methyl Paraben	1412[c]±18	1637[b]±18	1900[a]±0	1162[d]±18
Propyl Paraben	537[c]±18	712[b]±18	812[a]±18	400[d]±0
TBHQ[f]	87[c]±18	162[b]±18	237[a]±18	50[c]±00
Sodium Chloride	10[c]±0	12[b]±0	13[a]±0	8[d]±0

[a]Means represent two replications.
[b-d]Means in rows with different superscripts are significantly different ($p<0.05$).
[c]Data expressed in ppm for methyl paraben, propyl paraben, and TBHQ; and in percentage for sodium chloride.
[f]Tertiary butylhydroquinone.

Table 20. Minimum Inhibitory Concentrations[a] for antimicrobials against Parent Strain and Lipid Adapted Cells of *Listeria monocytogenes* LM4A, Grown in Tryptose Phosphate Broth, after incubation at 35°C for 24 hr.

	Exogenous Fatty Acid			
	Control	C14:0	C18:0	C18:1
Antimicrobial	Minimum Inhibitory Concentration[c]			
Methyl Paraben	$1400^{c}\pm0$	$1625^{b}\pm35$	$1912^{a}\pm18$	$1137^{d}\pm18$
Propyl Paraben	$550^{c}\pm0$	$712^{b}\pm18$	$812^{a}\pm18$	$412^{d}\pm18$
TBHQ[f]	$75^{c}\pm0$	$175^{b}\pm0$	$262^{a}\pm18$	$50^{c}\pm0$
Sodium Chloride	$10^{c}\pm0$	$12^{b}\pm0$	$13^{a}\pm0$	$8^{d}\pm0$

[a]Means represent two replications.
[b-d]Means in rows with different superscripts are significantly different ($p<0.05$).
[c]Data expressed in ppm for methyl paraben, propyl paraben, and TBHQ; and in percentage for sodium chloride.
[f]Tertiary butylhydroquinone.

also in accordance with those obtained by Al-issa et al. (1984). They found that BHA resistant cultures of *L. monocytogenes* had greater saturated plus iso acids (higher melting points). Moreover, they had lower proportions of short-chain fatty acids (i.e., iso C-15:0 and anteiso C-15:0) and a higher proportions of long chain acids (i.e., anteiso C-17:0 and iso C-17:0).

The increased resistance of cells grown in the presence of exogenous fatty acids (C14:0, C18:0) and decreased resistance of the cells grown in the presence of C18:1 was likely due to incorporation of exogenous fatty acids in the cell membranes. Low melting point fatty acids (i.e., unsaturated and branched-chain fatty acids) increase membrane fluidity (Welker, 1976) whereas high melting point fatty acids (i.e., saturated, long and straight chain fatty acids) decrease membrane fluidity (McElhaney, 1976). In resistant lipid adapted cells, the physical state of the fatty acid mix was less fluid due to increased saturated fatty acids (C14:0 and C18:0). These saturated or high melting point fatty acids acted as a defense mechanism and decreased the ability of antimicrobials to react with or pass through the membrane. Thus, increased level of saturated fatty acids conferred protection. In contrast, cells adapted to increased level of unsaturated fatty acid (C18:1) were more susceptible to antimicrobials.

The bacterial cell membrane must maintain a specific

fluidity in order to accomplish its functions (Silvius et al., 1980). Miller (1980) suggested that fluidity is controlled by homeostatic mechanisms within close tolerances. According to Fulco and Fujii (1980) and Kawagushi et al. (1980), this homeostasis can be accomplished by appropriate alterations of the relative rates of saturated and unsaturated fatty acid biosynthesis. Kates and Pugh (1980) suggested that phospholipid desaturases play an important role in control of membrane fluidity whereas Russell and Sandercock (1980) found that regulation is achieved by modification of phospholipid fatty acyl chain length. Nozawa (1980) found both fatty acid and phospholipid composition altered membrane fluidity. Thus, there is a correlation between changes in phospholipid and/or fatty acid composition, membrane fluidity and enzymatic activities.

These experiments suggest that the susceptibility of *L. monocytogenes* is mediated by changes in individual or classes of fatty acids. These changes would affect membrane fluidity and consequently membrane function. As there is a correlation between fatty acid composition, membrane fluidity, and enzymatic activities, changes in membrane fluidity would affect the enzymes responsible for membrane organization. White et al. (1971) reported that nearly all enzymes involved in the synthesis of phospholipids and fatty acids are localized in the inner cytoplasmic membrane.

Raetz (1978) observed a tight coupling of phospholipids and fatty acid synthesis in bacteria. The antimicrobials would either be trapped or passed through the membrane depending upon the fluidity. Ability of antimicrobials to react with or pass through the membrane is likely decreased with increased length of saturated fatty acids.

Determination of inactivation over time

Listeria monocytogenes strains Scott A and LM4A were grown in the presence of exogenous fatty acids (C14:0, C18:0, C18:1) and subsequently suspended in 0.1% peptone water. They were exposed to antimicrobials at concentrations above the MIC: methyl paraben (1500 μg/ml), propyl paraben (1000 μg/ml), methyl and propyl paraben (0.1%), sodium chloride (10%) or TBHQ (200 μg/ml). This was followed by incubation at 35°C. Samples were taken at 15 min intervals up to 1 hr and at 3 hr and enumerated by pour plating. The objective of this experiment was to determine the inactivation of the bacteria over time and its relationship to fatty acid composition.

No inactivation of the control was achieved in the presence of methyl paraben, propyl paraben, methyl and propyl paraben, or sodium chloride. The number of cells of the parent strain remained constant during the 3 hr incubation period at 35°C. This was most likely due to the slow-acting nature of these compounds. In contrast, the control was rapidly inactivated in the presence of TBHQ (200

μg/ml) (Figures 49 and 50). This allowed comparison of the parent strain and lipid adapted cells. Parent strain Scott A decreased from (log_{10}) 7.88 CFU/ml to (log_{10}) 5.94 CFU/ml in 45 min (Figure 49). Another decline was observed after 1 hr of incubation and the number of survivors at 3 hr was (log_{10}) 3.65 CFU/ml. Cells grown in the presence of C18:1 appeared to be more susceptible than the control. The number of cells decreased from (log_{10}) 7.23 CFU/ml to (log_{10}) 2.98 CFU/ml in 3 hr. In fact, a sharp decline (99%) in the number of survivors was observed in 15 min. Conversely, cells grown with C14:0 and C18:0 fatty acids were equal or more resistant to 200 μg/ml TBHQ than the control. For cells treated with C14:0, the number of survivors were decreased from (log_{10}) 6.97 CFU/ml to (log_{10}) 4.30 CFU/ml in 3 hr. Cells grown with C18:0 displayed the greatest resistance. A slight decrease in the number of cells was observed over a period of 3 hr ((log_{10}) 6.94 CFU/ml to (log_{10}) 5.82 CFU/ml).

A similar pattern was observed for strain LM4A (Figure 50). For the control, the number of survivors decreased from (log_{10}) 7.86 CFU/ml to (log_{10}) 5.04 CFU/ml in 45 min. As with strain Scott A, a sharp decline was observed after 1 hr of incubation. The number of survivors at 3 hr was (log_{10}) 3.51 CFU/ml. For cells grown in the presence of C18:1, a decline in viable cells from (log_{10}) 7.86 CFU/ml to (log_{10}) 5.66 CFU/ml was observed in 15 min. After 3 hr,

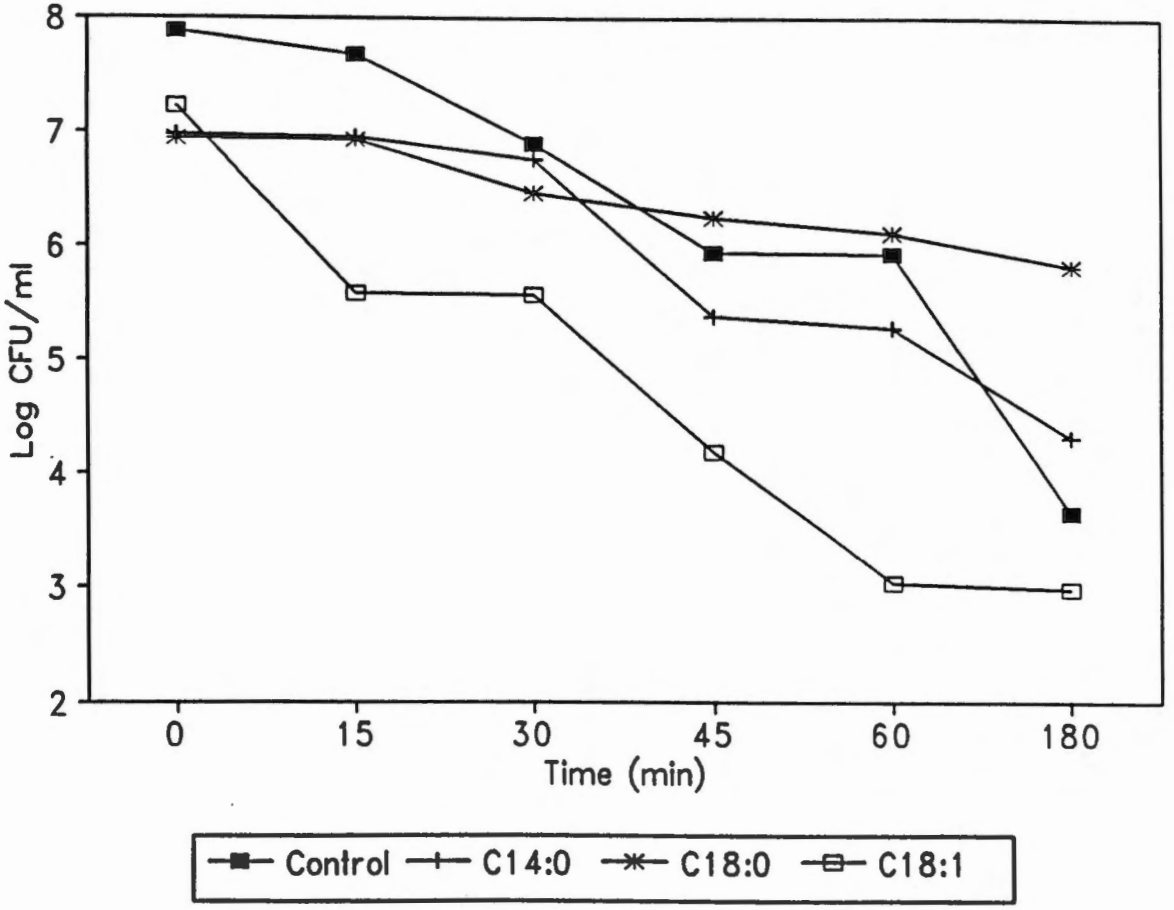


Figure 49. Inactivation over time of Parent Strain and the Lipid Adapted Cells of *Listeria monocytogenes* Strain Scott A, Suspended in 0.1% Peptone Water (w/v) Containing 200 ppm Tertiary Butylhydroquinone at 35°C, Where Each Point Represents the Mean of Two Replicates.

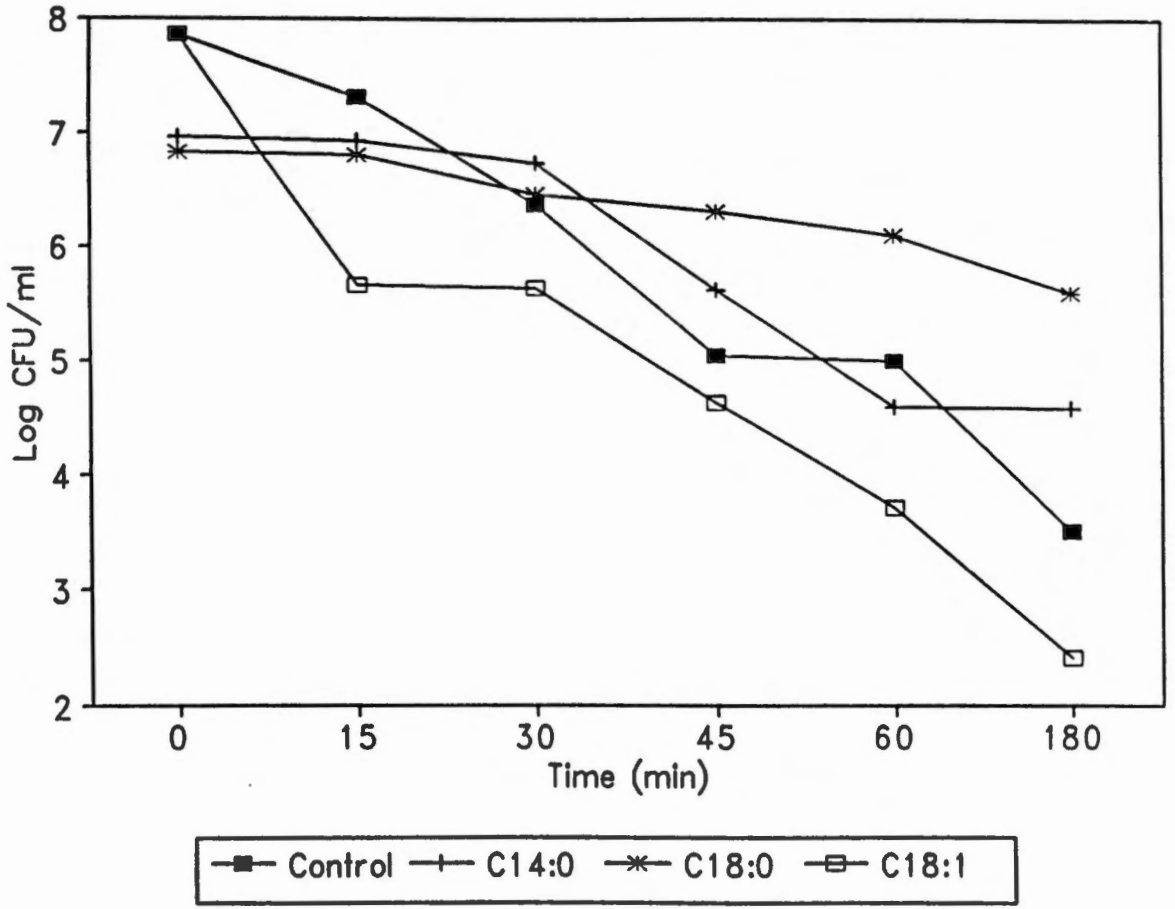


Figure 50. Inactivation over time of Parent Strain and the Lipid Adapted Cells of *Listeria monocytogenes* Strain LM4A, Suspended in 0.1% Peptone Water (w/v) Containing 200 ppm Tertiary Butylhydroquinone at 35°C, Where Each Point Represents the Mean of Two Replicates.

(log_{10}) 2.41 CFU/ml remained. In contrast, cells treated with C14:0 displayed slightly higher resistance than the parent strain of LM4A. For cells treated with C14:0, the number of survivors decreased from (log_{10}) 7.85 CFU/ml to (log_{10}) 4.57 CFU/ml in 3 hr. As with Scott A, LM4A treated with C18:0 was most resistant. Viable cells were reduced only 2 logs ((log_{10}) 7.84 CFU/ml to (log_{10}) 5.59 CFU/ml) in 3 hr.

In MIC procedure, cells grown with C14:0 and C18:0 fatty acids showed a linear increase in resistance. In contrast, cells adapted to C14:0 and C18:0 were equal or more resistant to TBHQ than the control in the inactivation study. No linear increase in resistance was observed. The inactivation over time study and MIC procedure were two different methods to determine the resistance of the parent strains and lipid adapted cells of *L. monocytogenes*. Both procedures had their weak and strong points. In the case of MIC, the results were read as turbidity or no turbidity in a broth dilution assay. Inactivation over time was a more detailed study where the survival of the parent strains and lipid adapted cells was observed over a short period. Therefore, the resistance or susceptibility of the controls and treated cells was known for any time within the limits of the inactivation study. Although inactivation over time study was more detailed, it was restricted to the sufficiently high concentration studied. The inactivation

study was conducted, apart from MIC, as another means to determine the resistance or susceptibility of the lipid adapted cells as compared to the parent strains of *L. monocytogenes*.

Assay of Fatty Acid Synthetase Activity

Fatty acid synthetase activity of *L. monocytogenes* strain Scott A was assayed by spectrophotometric method at 7°C and 35°C. The rate of NADPH oxidation was followed at 340 nm and the slope was used to demonstrate the relative enzyme activity in the presence of antimicrobials. The objective of this phase of the experiment was to determine if parabens and sodium chloride have an effect on fatty acid synthetase enzyme, an enzyme responsible for membrane organization.

Effect of antimicrobials on fatty acid synthetase enzyme activity is shown in figures 51 and 52. Enzyme activity was low at 7°C as compared to 35°C. For controls, the rate of oxidation of NADPH slowed after 12 min and 16 min at 7°C and 35°C, respectively. Presence of antimicrobials decreased the activity of the enzyme. At 35°C, the rate of oxidation of NADPH was slowed at 10 min in the presence of methyl paraben, propyl paraben and sodium chloride. At 7°C, the rate was decreased at 6 min in case of methyl paraben and sodium chloride and 4 min in the presence of propyl paraben.

Researchers in the past have suggested that compounds

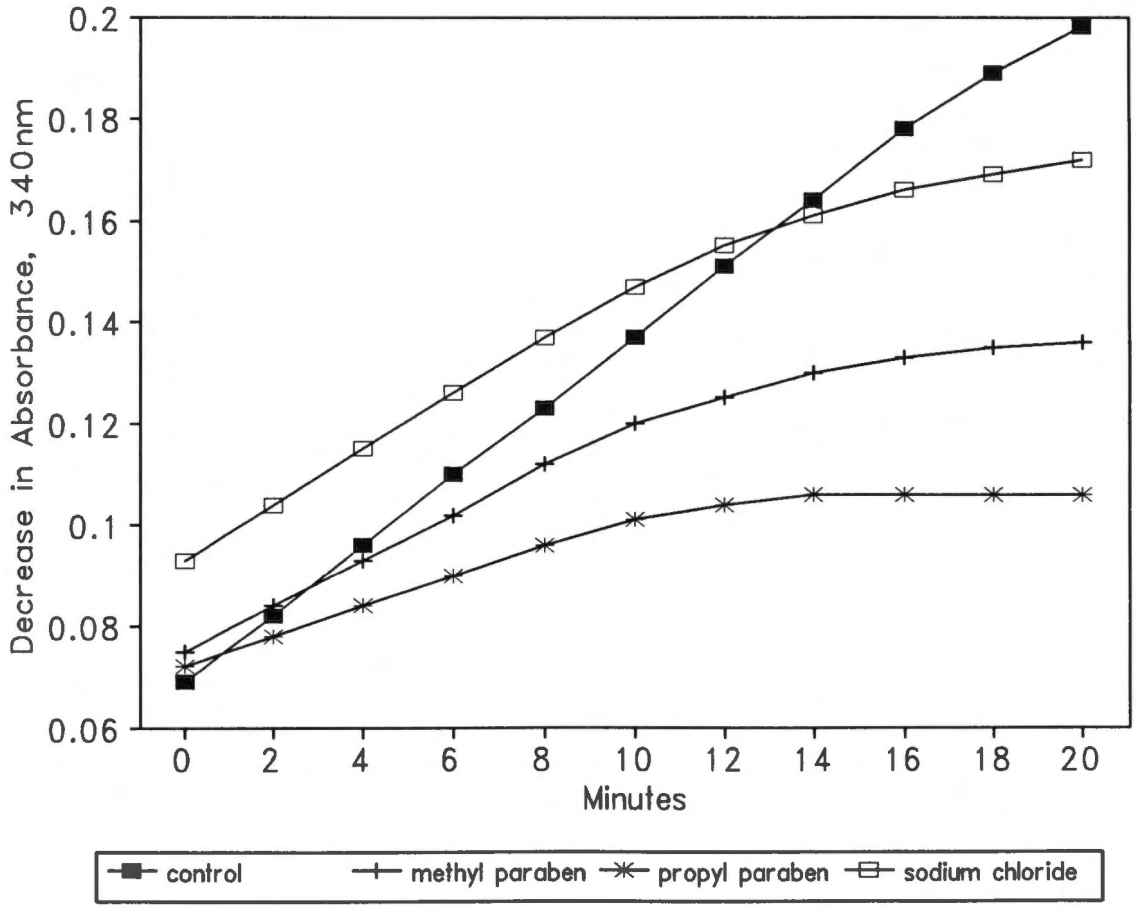


Figure 51. Effect of Antimicrobials on Fatty Acid Synthetase activity at 35°C.

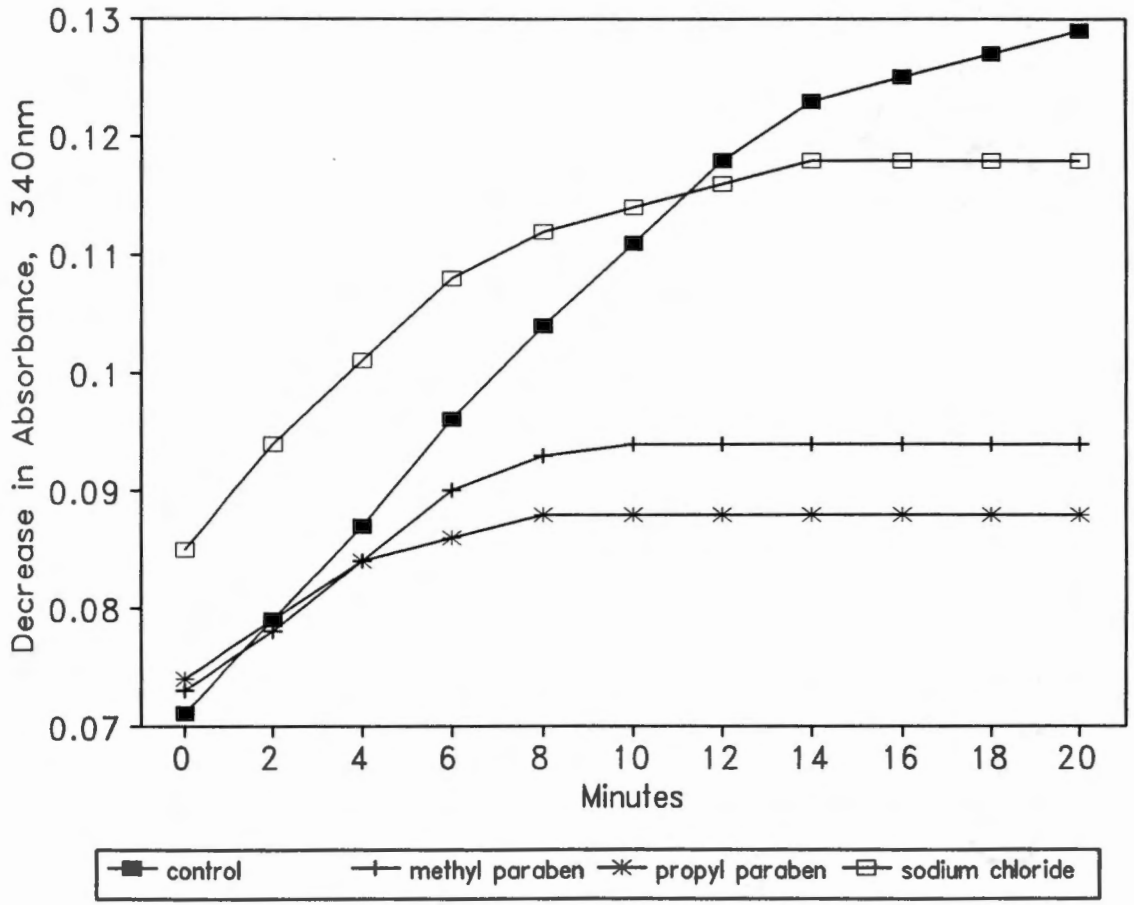


Figure 52. Effect of Antimicrobials on Fatty Acid Synthetase activity at 7°C.

which perturb lipids of membranes cause either decreased activity of membrane-bound enzymes or put some enzymes in contact with substrates from which they are ordinarily restricted by means of cell geography, resulting in death of the cell (Eletr et al. 1974). Several researchers suggested the effect of phenolic compounds on enzymes (Fogg and Lodge 1945; Roberts and Rahn, 1946). Inactivation of enzymes disrupts membrane functions. Studies on a compound with a structure similar to the parabens, butylated hydroxyanisole, showed a significant effect on the activity of the membrane-bound adenosine triphosphatase of *S. aureus* (Rico-Munoz et al. 1987).

The present study clearly indicated that parabens and sodium chloride affect the enzymes responsible for membrane organization. Thus, the actual mechanism by which parabens and salt inhibit *L. monocytogenes* is probably related to their direct effect on cellular enzymes systems located in the membrane.

Several factors might lead to increased resistance of *L. monocytogenes* to antimicrobials. This organism may synthesize negatively charged phospholipids and saturated or high melting point fatty acids as a defense mechanism. The former, by their ability to form phospholipids with opposite charges, can control the permeability of the membrane. The latter, by influencing the membrane fluidity, may affect the ability of antimicrobials to react with or pass through the

membrane. As there is a correlation between phospholipid and/or fatty acid composition, membrane fluidity and enzymatic activities, changes in membrane fluidity would affect the enzymes, involved in the synthesis of phospholipids and fatty acids, localized in the inner cytoplasmic membrane.

Phospholipid and fatty acid composition of *L. monocytogenes* depends on the environmental conditions during growth. Therefore, the environmental conditions may be manipulated to achieve a state where synthesis of high melting point fatty acids and anionic phospholipids is inhibited. This would provide a potential to increase the susceptibility of *L. monocytogenes* to antimicrobials.

In a food system, several environmental factors including pH and a_w may be manipulated. These factors may interact directly or synergistically to result in an environmental condition that would alter the membrane enzyme activity. This could result in changes in susceptibility to antimicrobials.

CHAPTER V

CONCLUSIONS

High lipid content and abundance of phosphatidyl glycerol in the nine strains of *L. monocytogenes* provide evidence for the mechanism of increased resistance of this organism to a number of antimicrobials. The amount of total lipid and phosphatidyl glycerol has been shown to be directly related to resistance of microorganisms. Variable percentages of certain fatty acids and singular presence of a few fatty acids in one or two strains indicate that it would be difficult to use gas-liquid chromatography to identify *L. monocytogenes* strains.

Phospholipid composition and fatty acid profiles of two strains of *L. monocytogenes* varied greatly in the presence of antimicrobials (sodium chloride and parabens) and at different temperature. The results of this study tend to support a relationship between variation in phospholipids, types and/or relative percentages of individual or groups of fatty acids and resistance of *L. monocytogenes* to antimicrobials and different temperatures. It seems logical that resistance of the organism may be mediated by lipid composition. *Listeria monocytogenes* may synthesize phosphatidyl glycerol and saturated fatty acids as a defense mechanism. The ability of phosphatidyl glycerol to be converted to phospholipids with opposite charges, may control the permeability of the membrane.

Saturated fatty acids may influence membrane fluidity affecting the ability of antimicrobials to react with or pass through the membrane. Changes in phospholipid and fatty acid composition may affect protein-lipid interactions as well as membrane organization.

An attempt to develop a system to create *L. monocytogenes* cells with altered fatty acid composition was successful with exogenous fatty acids, with or without cerulenin. Cells adapted to saturated fatty acids (C14:0 and C18:0) were more resistant to antimicrobials than the parent strains. This seems logical because the physical state of the fatty acid mix was less fluid due to increased saturation and thus, decreased the ability of antimicrobials to react with, or pass through, the membrane. Of course, an opposite trend was observed for the cells adapted to unsaturated fatty acid (18:1). The lipid adaptation likely influenced membrane fluidity. Changes in membrane fluidity can affect enzymes involved in the synthesis of phospholipids and fatty acids localized in the cytoplasmic membrane. In this study, the effect of the antimicrobials on fatty acid synthesizing enzymes indicated that both parabens and salt alter enzyme activity. However, there is no concrete evidence that these compounds influence membrane bound enzymes *in vivo*, only indications to that effect. The inhibitory effect of antimicrobials may be mediated through direct alterations of individual fatty acids or may occur as

a result of alteration in the nonspecific interaction between membrane integral proteins and surrounding lipids.

Furr and Russell (1972) showed that the uptake of parabens was the result of a general dissolution of the esters on the "cell surface." From the non-specific physicochemical nature of this adsorption they suggested that inhibition was caused by changes in the membrane structure, resulting from the breakage of hydrogen bonds, etc., rather than loss or inactivation of specific enzymes.

It is reasonable to suppose that the parabens would distribute themselves between the lipid and the aqueous components in the cell, in accordance with the normal distribution laws. Due to their hydrophobic and hydrophilic groups the parabens tend to orientate in an oil/water interface, if such an interface is available. In the case of cells containing high levels of saturated fatty acid or the cells in which the physical state of the fatty acid mix is less fluid, it is likely that phenol is immobilized with the alkyl side immersed in the fatty acid mix or the cell lipid and the phenolic hydroxy-group projecting into the aqueous environment. Therefore, it could well be that many antimicrobial molecules are thus trapped and sensitive areas in the cytoplasmic membrane are protected.

The working hypothesis proposed would be that the parabens and salt are adsorbed onto the cell membrane. The amount of the antimicrobial adsorbed and its rate of

adsorption may be a relatively non-specific physicochemical phenomenon. Resistance results from a greatly decreased penetration of the antimicrobial substance to the membrane. In the present study, ability of antimicrobials to react with or pass through the membrane was likely decreased with increased length of saturated fatty acids.

The results tend to support a relationship between lipid composition and susceptibility of *L. monocytogenes*. Food safety in the food industry may be assured by inhibiting microbial growth in a product. Hurdles or barriers to growth including acidity, water activity, and redox potential may be built into a particular food product. These hurdles may interact to alter the lipid composition of *L. monocytogenes*, resulting in changes in susceptibility to antimicrobials.

REFERENCES

REFERENCES

Aalto, T.R., Firman, M.C. and Rigler, N.E. 1953. p-Hydroxybenzoic acid esters as preservatives. I. Uses, antibacterial and antifungal studies, properties and determination. J. Am. Pharm. Assoc. (Sci.Ed.) 42:449.

Al-issa, M., Fowler, D.R., Seaman, A. and Woodbine, M. 1984. Role of lipid in butylatedhydroxyanisole (BHA) resistance of *Listeria monocytogenes*. Zbl. Bakt. Hyg. A. 258:42.

Ames, G.F. 1968. Lipids of *Salmonella typhimurium* and *Escherichia coli*: Structure and metabolism. J. Bacteriol. 95:833.

Asselineau, J. 1966. Lipid groups occurring in bacterial lipids. In: The Bacterial lipids. E. Lederer (ed.). Holden-Day Inc. California. U.S.A. p. 158.

Ayres, J.C., Mundt, J.O. and Sandine, W.E. 1980. Microbiology of foods. W.H. Freeman and Co. San Francisco, California. p. 132.

Banwart, G.J. 1979. "Basic Food Microbiology". AVI Publ., Westport, Conn.

Baranowski, J.D. and Nagel, C.W. 1983. Properties of alkyl hydroxycinnamates and effects on *Pseudomonas fluorescens*. Appl. Environ. Microbiol. 45:218.

Bargiota, E., Rico-munoz, E. and Davidson, P.M. 1987. Lethal effects of methyl and propyl parabens as related to *Staphylococcus aureus* lipid composition. Int. J. Food Microbiol. 4:257.

Binkerd, E.F. and Kolari, O.E. 1975. The history and use of nitrate and nitrite in the curing of meat. Food Cosmet. Toxicol. 13:655.

Bio-Rad. 1990. Protein and protease assays. P. 130 in "Chromatography, electrophoresis, immunochemistry, molecular biology, and HPLC." Chemical Division, Richmond, CA.

Bligh, E.G. and Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37:911.

Bolis, L. and Pethica, B.A. 1968. "Membrane Models and the Formation of Biological Membranes," p. 1, 6, 88, 138, 190, North-Holland Publishing Co., Amsterdam.

Boyd, R.F. and Hoerl, B. 1981. Cytology. p. 22. In "Basic Medical Microbiology," R.F. Boyd and B. Hoerl (eds.). Little, Brown and Company, Inc. U.S.A.

Branen, A.L., Davidson, P.M. and Katz, B. 1980. Antimicrobial properties of lipids and phenolic antioxidants. Food Technol. 34:42.

Brown, M.R.W. and Watkins, W.M. 1970. Low magnesium and phospholipid content of cell walls of *Pseudomonas aeruginosa* resistant to polymixin. Nature 227:1360.

Brown, M.R.W. and Wood, S.M. 1972. Relation between cation and lipid content of cell walls of *Pseudomonas aeruginosa, Proteus vulgaris,* and *Klebsiella aeruginosa* and their sensitivity to polymixin B and other antibacterial agents. J. Pharm. Pharmacol. 24:215.

Brundish, D.E. Shaw, N. and Braddiley, J. 1967. The structure and possible function of the glycolipid from *Streptococcus lactis* 13. Biochem. J. 105:885.

Busta, F.F. and Foegeding, P.M. 1983. Chemical food preservatives. Ch. 35. In "Disinfection, Sterilization, and Preservation." Block, S.S. (Ed.) p.656. Lea and Febiger, Philadelphia.

Canonica, F.P. and Pisano, M.A. 1987. Gas-liquid chromatographic analysis of fatty acid methyl esters of *Aeromonas hydrophila, Aeromonas sobria,* and *Aeromonas caviae*. J. Clin. Microbiol. 26:681.

Carbonnelle, B., Cottin, J., Parvery, F., Chambreuil, G., Kouyoumdjian, S., Lirzin, M. and vincent, F. 1978. Epidemie de listeriose dans l'oust de la France (1975-1976). Rev. Epidemiol. Sante Publique (Paris) 26:451

Carrol, K.K. Cutts, J.H. and Murray, E.G.D. 1968. The lipids of *Listeria monocytogenes*. Can J. Biochem. 46:899.

Chapman, D. 1968. Introduction. Recent physical studies of phospholipids and natural membranes. The nature of the interaction between protein and lipid during the formation of lipoprotein membranes. In "Biological Membranes, Physical Fact and Function," p. 103,125-202, 203-232, Academic Press, New York.

Chichester, D.F. and Tanner, F.W. 1972. Antimicrobial Food Additives. In "CRC Handbook of Food Additives." Furia, T.E. (Ed.) Vol 1. p. 115. CRC Press, Inc. Boca Raton, Fl.

Cho, K.Y. and Salton, M.R.J. 1966. Fatty acid composition of bacterial membrane and wall lipids. Biochim. Biophys. Acta 116:73.

Ciesielski, C.A., Hightower, A.W., Parsons, S.K. and C.V. Broome. 1988. Listeriosis in the United States: 1980-1982. Arch. Intern. Med. 148:1416-1419.

Code of Federal Regulations. 1988. Title 21. Food and drugs. Parts 100-199. Off. Fed. Reg., Natl. Arch. and Records Admin., Washington, D.C.

Conner, D.E., Brackett, R.E. and Beuchat, L.R. 1986. Effect of temperature, sodium chloride, and pH on growth of *Listeria monocytogenes* in cabbage juice. Appl. Environ. Microbiol. 52:59.

Costerton, J.W., Ingram, J.M. and Cheng, K.J. 1974. Structure and function of the cell envelope of Gram-negative bacteria. Bacteriol. Rev. 38:87.

Cronan, J.E. and Vagelos, R.R. 1972. Metabolism and function of membrane phospholipids of *Escherichia coli*. Biochim. Biophys. Acta 265:25.

Cronan, J.E. Jr. and Gelman, E.P. 1975. Physical properties of membrane lipids: biological relevance and regulation. Bacteriol. Rev. 39:232.

D'Agnolo, G., Rosenfeld, I.S., Awaya, J., Omura, S. and Vagelos, P.R. 1973. Inhibition of fatty acid synthesis by the antibiotic cerulenin. Specific inactivation of B-Ketoacyl-acyl carrier protein synthetase. Biochim. Biophys. Acta. 326:155.

Danielli, J.F. and Davson, H. 1935. A contribution to the theory of permeability of thin films. J. Cell. Comp. Physiol. 5:495.

Davidson, P.M. and Branen, A.L. 1980. Inhibition of two psychrotrophic *Pseudomonas* species by butylated hydroxyanisole. J. Food Sci. 45:1603.

Davidson, P.M. and Branen, A.L. 1981. Antimicrobial activity of non-halogenated phenolic compounds. J. Food Prot. 44:623.

Davidson, P.M., Brekke, C.J. and Branen, A.L. 1981. Antimicrobial activity of butylated hydroxyanisole, tertiary butylhydroquinone and potassium sorbate in combination. J. Food Sci. 46:314.

Davidson, P.M. 1983. Phenolic compounds. p. 37. In: "Antimicrobials in Foods". Branen, A.L. and Davidson, P.M. (Eds.). Marcel Dekker, New York.

Davidson, P.M. and Juneja, V.K. 1990. Antimicrobial agents. Ch. 4 in "Food Additives", Branen, A.L., Davidson, P.M. and Salminen, S. (ed.) Marcel Dekker, Inc. New York.

Degre, R. and Sylvestre, M. 1983. Effect of butylated hydroxyanisol on *Staphylococcus aureus* Wood 46. J. Food Prot. 46:206.

Dehaas, G.H., Bonsen, P.P.M. and Van Deenan, L.L.M. 1966. Studies on cardiolipin. III. Structural identity of ox-heart cardiolipin and synthetic diphosphatidyl glycerol. Biochim. Biophys. Acta 116:114.

Dickgieber, N. 1980. *Listeria monocytogenes* als Ursache von Krankenhausin-fektionen: Untersuchungen zum Uberleben der Erreger inder Aubenwelt. Infektion. 8:199.

Dje, Y., Payne, K.D. and Davidson, P.M. 1989. Inhibition of *Listeria monocytogenes* Scott A by propyl paraben in non-fat milk and chicken and hot dog suspensions. Ann. Mtg. Inst. Food Technol., Chicago, IL.

Dje, Y., Rico-Munoz, E. and Davidson, P.M. 1990. Effect of antimicrobials against *Listeria monocytogenes* on hot dogs and in brine. Inst. of Food Technol. 50th Ann. Mtg. Anaheim, CA.

Dunnick, J.K. and O'Leary, W.M. 1970. Correlation of bacterial lipid composition with antibiotic resistance. J. Bacteriol. 101:892.

Edwards, M.R. and Stevens, R.W. 1963. Fine structure of *Listeria monocytogenes*. J. Bacteriol. 86:414.

Eisenberg, A.D. and Corner, T.R. 1978. Effects of growth temperature on protoplast properties in *Bacillus megaterium*. Can. J. Microbiol. 24:386.

Eklund, T. 1980. Inhibition of growth and uptake processes in bacteria by some chemical food preservatives. J. Appl. Bacteriol. 48:423.

Eletr, S., Williams, M.A., Watkins, T. and Keith, A.D. 1974. Perturbations of the dynamics of lipid alkyl chains in membrane systems: Effect on the activity of membrane-bound enzymes. Biochim. Biophys. Acta 339:190.

Erickson, D.R. and Tompkin, R.B. 1977. Antimicrobial and antirancidity agent. U.S. Patent 4,044,160. August 23,1977.

Esfahani, M., Limbrick, A.R., Knutton, S., Oka, S. and Wakil, S.J. 1971. The molecular organization of lipids in the membrane of *Escherichia coli*: phase transitions. Proc. Natl. Acad. Sci. U.S.A. 68:3180.

Etemadi, A.H. and Convit, J. 1974. Mycolic acids from "noncultivable" mycobacteria. Infect. Immun. 10:236.

Fitz-James, P.C. 1960. Participation of the cytoplasmic membrane in the growth and spore formation of bacilli. J. Biophys. Biochem. Cytol. 8:507.

Fleming, D.W., Cochi, S.L., MacDonald, K.L., Brondum, J., Hayes, P.S., Plikaytis, B.D., Holmes, M.B., Audurier, A., Broome, C.V. and Reingold, A.L. 1985. Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. N. Engl. J. Med. 312:404-407.

Fogg, A.H. and Lodge, R.M. 1945. The mode of antibacterial action of phenols in relation to drug fastness. Trans. Faraday Soc. 41:359.

Freese, E., Sheu, C.W. and Galliers, E. 1973. Function of lipophilic acids as antimicrobial food additives. Nature 241:321.

Fulco, A.J. 1967. The effect of temperature on the formation of Δ^5-unsaturated fatty acids by bacilli. Biochim. Biophys. Acta. 144:701.

Fulco, A.J. 1974. Metabolic alterations of fatty acids. Ann. Rev. Biochem. 43:215.

Fulko, J.A. and Fujii, D.K. 1980. Adaptive regulation of membrane lipid biosynthesis in *Bacilli* by environmental temperature. In "Membrane Fluidity-Biophysical Techniques and Cellular Regulation". M. Kates and Kuksis (eds.). The Humana Press, Inc. Clifton. N.J.

Furr, J.R. and Russell, A.D. 1972. Some factors influencing the activity of p-hydroxybenzoic acid against *Serratia marcescens*. Microbios 5:189.

Gelman, N.S., Lukoyanova, M.A. and Ostrovsky, D.N. 1966. The respiratory apparatus of bacteria. Nauke Publ., Moscow, Russia.

Ghosh, B.K. and Murray, R.G.E. 1967. Fine structure of *Listeria monocytogenes* in relation to protoplast formation. J. Bacteriol. 93:411.

Ghosh, B.K. and Carrol, K.K. 1968. Isolation, composition and structure of membrane of *Listeria monocytogenes*. J. Bacteriol. 95:688.

Ghosh, B.K., Lampen, J.O. and Remsen, C.C. 1969. Periplasmic structure of frozen-etched and negatively starved cells of *Bacillus licheniformis* as correlated with penicillinase formation. J. Bacteriol. 100:1002.

Ghosh, B.K. 1974. The mesosome-A clue to the evolution of the plasma membrane, sub-cell. Biochemistry 3:311.

Goldberg, I., Walker, J.R. and Bloch, K. 1973. Inhibition of lipid synthesis in *Escherichia coli* cells by the antibiotic cerulenin. Antimicrob. Agents Chemother. 3:549.

Hamilton, W.A. 1968. The mechanism of the bacteriostatic action of tetrachlorosalicylanilide: A membrane active antibacterial compound. J. Gen. Microbial. 50:441.

Hanna, K., Bengis-Garber, C., Kushner, D.J., Kogut, M. and Kates, M. 1984. The effect of salt concentration on the phospholipid and fatty acid composition of the moderate halophile, *Vibrio costicola*. Can. J. Microbiol. 30:669.

Harder, M.E., Ladenson, R.C., Schimmel, S.C. and Silbert, D.F. 1974. Mutants of *Escherichia coli* with temperature sensitive malonyl CoA-acyl carrier protein transacylase. J. Biol. Chem. 249:7468.

Hasegawa, Y., Kawada, N. and Nosoh, Y. 1980. Change in chemical composition of *Bacillus caldotenax* after shifting the growth temperature. Arch. Microbiol. 126:103.

Higgins, M.L. and Shockman, G.D. 1971. Procaryotic cell division with respect to wall and membrane. CRC Crit. Rev. Microbiol. 1:29.

Hill, M.J., James, A.M. and Maxted, W.R. 1963. Some physical investigations of the behavior of bacterial surfaces X. The occurrence of lipid in the streptococcal cell wall. Biochim. Biophys. Acta. 75:414.

Hiramatsu, T., Ohno, Y., Hara, H., Yano, I. and Masui, M. 1980. Effects of NaCl concentration on the envelope components in a moderately halophilic bacterium, *Pseudomonas halosaccharolytica*. pp. 189. In Morisita, H., Masui, M. (eds.). Saline environments. Proceedings of the Japanese conference on halophilic microbiology. Kyoto, Japan: Nakanishi Printing Co., Ltd.

Ho, J.L., Shands, K.N., Friedland, G., Eckind, P. and Fraser, D.W. 1986. An outbreak of type 4b *Listeria monocytogenes* infection involving patients from eight Boston hospitals. Arch. Intern. Med. 146:520-524.

Hughes, D.E. 1962. The bacterial cytoplasmic membrane. J. Gen. Microbiol. 29:39.

Hugo, W.B. and Stretton, R.J. 1966. The role of cellular lipid in the resistance of Gram-positive bacteria to penicillins. J. Gen. Microbiol. 42:133.

Hugo, W.B. and Franklin, I.E. 1968. Cellular lipid and the anti-staphylococcal activity of phenols. J. Gen. Microbiol. 52:365.

Hugo, W.B. and Davidson, J.R. 1973. Effect of cell lipid depletion in *Staphylococcus aureus* upon its resistance to antimicrobial agents. 2. A comparison of the response of normal and lipid depleted cells of *S. aureus* to antibacterial drugs. Microbios 8:63.

Hurst, A. and Stubbs, J.M. 1969. Electron microscopic study of membranes and walls of bacteria and changes occurring during growth initiation. J. Bacteriol. 97:1466.

Ingram, L.O. 1977. Changes in lipid composition of *Escherichia coli* resulting from growth with organic solvents and with food additives. Appl. Environ. Microbiol. 33:1233.

Jackson, M.B. and Cronan, J.E. 1978. An estimate of the minimum amount of fluid lipid required for the growth of *Escherichia coli*. Biochim. Biophys. Acta 512:472.

James, S.M., Fannin, S.L., Agree, B.A., Hall, B., Parker, E., Vogt, J., Run,G., Williams, J. and Lieb, L. 1985. Listeriosis outbreak associated with Mexican-style cheese. Calif. Morb. Mort. Weekly Rep. 34:357-359.

Janna, J., Jorma, H., Poul, H. and Esko, N. 1989. Effect of different levels of nitrite and nitrate on the survival of *Listeria monocytogenes* during the manufacture of fermented sausage. J. Food Prot. 52:158.

Jensen, L.B. 1954. Microbiology of meats. 3rd ed. The Garrard Press, Champaign, Il.

Jones, D. 1975. The taxonomic position of *Listeria*. In "Problems of Listeriosis". p. 4-17. Woodbine, M.(ed.), University Press, University of Nottingham, Leicester.

Jones, D. and Seeliger, H.P.R. 1986. International committee on systematic bacteriology. Subcommittee on the taxonomy of *Listeria* and related bacteria (Minutes of meeting, Nantes, France, Sept. 1985). Int. J. Syst. Bacteriol. 36:117.

Julak, J. and Mara, M. 1973. Effect of glucose of glycerine in cultivation media on the fatty acid composition of *Listeria monocytogenes*. J. Hyg. Epidemiol. Microbiol. Immunol. 17:327.

Kaback, H.R. and Hong, J.S. 1973. Membranes and transport. CRC Crit. Rev. Microbiol. 2:333.

Kaneda, T. 1963. Biosynthesis of branched chain fatty acids. I. Isolation and identification of fatty acids from *Bacillus subtilis* (ATCC 7059). J. Biol. Chem. 238:1222.

Kanemasa, Y., Yoshioka, T. and Hayashi, H. 1972. Alteration of the phospholipid composition of *Staphylococcus aureus* cultured in medium containing NaCl. Biochem. Biophys. Acta. 280:444.

Kanfer, J. and Kennedy, E.P. 1963. Metabolism and function of bacterial lipids. I. Metabolism of phospholipids in *Escherichia coli* B. J. Biol.Chem. 238:2919.

Kass, L.R. 1968. The antibacterial activity of 3-Decynoyl-N-acetylcysteamine. J. Biol. Chem. 243:3223.

Kates, M., Sastry, P.S. and Yengoyan, L.S. 1963. Isolation and characterization of a diether analog of phosphatidyl glycerophosphate from *Halobacterium cutirubrum*. Biochim. Biophys. Acta 70:705.

Kates, M. 1964. Bacterial lipids. Advan. Lipid Res. 2:17.

Kates, M. 1966. Biosynthesis of lipids in microorganisms. Ann. Rev. Microbiol., 20:13.

Kates, M. and Pugh, E. 1980. Role of phospholipid desaturases in control of membrane fluidity. p. 153. In Membrane Fluidity-Biophysical Techniques and Cellular Regulation. M. Kates and Kuksis (eds.). The Humana Press, Inc. Clifton. N.J.

Kawaguchi, A., Seyama, Y. Sasaki, K. Okuda, S. and Yamakawa, T. 1980. Thermal control of fatty acid synthetase from *Brevibacterium ammoniagenes*. p. 203. In Membrane Fluidity-Biophysical Techniques and Cellular Regulation. M. Kates and Kuksis (eds.). The Humana Press, Inc. Clifton. N.J.

Kawaguchi, A., Tomoda, H., Nozoe, S., Omura, S. and Okuda, S. 1982. Mechanism of action of cerulenin on fatty acid synthetase. Effect of cerulenin on iodoacetamide-induced malonyl-CoA decarboxylase activity. J. Biochem. 92:7.

Kellenberger, E. and Ryter, A. 1958. Cell wall and cytoplasmic membrane of *Escherichia coli*. J. Biophys. Biochem. Cytol. 4:323.

Khullar, G.K. and Goldfine, H. 1974. Phospholipids of *Clostridium butyricum*. V. Effects of growth temperature on fatty acid, alk-1-enyl ether group, and phospholipid composition. J. lipid research. 15:500.

Komaratat, P. and Kates, M. 1975. The lipid composition of a halotolerant species of *Staphylococcus epidermidis*. Biochem. Biophys. Acta. 398:464.

Krulwich, T.A., Clejan, S., Falk, L.H. and Guffanti, A.A. 1987. Incorporation of specific exogenous fatty acids into membrane lipids modulates protonophore resistance in *Bacillus subtilis*. J. Bacteriol. 169:4479.

Lechevalier, M.P., Horan, A.C. and Lechevalier, H.A. 1971. Lipid composition in the classification of nocardiae and mycobacteria. J. Bacteriol. 105:313.

Lechevalier, M.P., Lechevalier, H.A. and Horan, A.C. 1973. Chemical characteristic and classification of nocardiae. Can. J. Microbiol. 19:965.

Lechevalier, M.P. 1977. Lipids in bacterial taxonomy-A taxonomists view. CRC Crit. Rev. Microbiol. 5:109.

Lehninger, A.L. 1975. "Biochemistry," 2nd edition. Worth Publishers, Inc., New York.

Lennon, D., Lewis, B., Mantell, C., Becroft, D., Dove, B., Farmer, K., Tonkin, S., Yeates, N., Stamp, R. and Mickleson, K. 1984. Epidemic perinatal listeriosis. Pediat. Dis. 3:30-34.

Lin, C.C.S. and Fung, D.Y.C. 1983. Effect of BHA, BHT, TBHQ, and PG on the growth and toxigenesis of selected aspergilli. J. Food Sci. 48(2):576.

Linnan, M.J., Mascola, L., Iou, X.D., Groulet, V., May, S., Salminen, C., Hird, D.W., Yonekura, M.L., Hayes, P., Weaver, R., Andurier, A., Plikaytis, B.D., Fannin, S.L., Kleks, A. and Broome, C.V. 1988. Epidemic listeriosis associated with Maxican-style cheese. New Engl. J. Med. 319:832.

Lopes, J.A. 1986. Evaluation of dairy and food plant sanitizers against *Salmonella typhimurium* and *Listeria monocytogenes*. J. Dairy Sci. 50:2791-2796.

Lovett, J.L. 1989. *Listeria monocytogenes*. p.288. In "Foodborne Bacterial Pathogens." Doyle, M.P. (ed.). Marcel Dekker, Inc. New York.

Lueck, E. 1980. "Antimicrobial Food Additives". Springer-Verlag, Berlin.

Malinverni, R., Glauser, M.P., Bille, J. and Rocourt, J. 1986. Unusual clinical features of an epidemic of listeriosis associated with a particular phage type. Eur. J. Clin. microbiol. 5:169-171.

Mara, M., Benes, B. and Patocka, F. 1971. *Listeria monocytogenes* lipids: their dependence on the cultivation conditions and strain virulence. Folia Microbiol. 16:520.

Mara, M. and Michalec, C. 1977. Chromatographic study of mycolic acid-like substances in lipids of *Listeria monocytogenes*. J. Chromatogr. 130:434.

Marr, A.G. and Ingraham, J.L. 1962. Effect of temperature on the composition of fatty acids in *Escherichia coli*. J. Bacteriol. 84:1260.

Marsden, J.L. 1980. The importance of sodium in processed meats. In "Proc. Meat Ind. Res. Conf"., p.77, Amer. Meat Inst., Washington, D.C.

Marth, E.H. 1988. Disease characteristics of *Listeria monocytogenes*. Food Technol. 42:165-168.

Masayori, I. 1979. Bacterial outer membranes-Biogenesis and functions. John Wiley and Sons, Inc. N.Y.

McElhaney, R.N. 1976. in *Extreme Environments: Mechanisms of Microbial Adaptation,* p. 255. Academic Press, London, New York, San Francisco.

McGarrity, J.T. and Armstrong, J.B. 1975. The effect of temperature on phospholipid and fatty acid composition in *E. coli* K-12. Biochem Biophys. Acta. 398:258.

McLauchlin, J., Audrier, A. and Taylor, A.G. 1986. Aspects of the epidemiology of human *Listeria monocytogenes* infections in Britain 1967-1984; The use of serotyping and phage-typing. J. Med. Microbiol. 22:367.

McLauchlin, J. 1987. *Listeria monocytogenes*, recent advances in the taxonomy and epidemiology of listeriosis in humans. J. Appl. Bacteriol. 63:1-11.

MIDI. no date. Identification of bacteria by gas chromatography of cellular fatty acids. MIDI, Newark, DE.

Miller, R.W. 1980. Homeostatic control of membrane lipid fluidity in *Fusarium*. p. 327. In "Membrane Fluidity-Biophysical Techniques and Cellular Regulation". M. Kates and Kuksis (eds.). The Humana Press, Inc. Clifton. N.J.

Miller, K.J. 1985. Effects of temperature and sodium chloride concentration on the phospholipid and fatty acid composition of a halotolerant *Planococcus* sp. J. Bacteriol. 162:263.

Moss, C.W. and Cherry, W.B. 1968. Characterization of the C_{15} branched-chain fatty acids of *Corynebacterium acnes* by gas chromatography. J. Bacteriol. 95:241.

Moss, W.C., Lambert, M.A. and Merwin, W.H. 1974. Comparison of rapid methods for analysis of bacterial fatty acids. Appl. Microbiol. 28:80.

Moustafa, A., El-Shenawy and Marth, E.M. 1988. Inhibition of *Listeria monocytogenes* by sorbic acid. J. Food Prot. 52:842.

Murray, E.G.D., Webb, R.A. and Swann, M.B.R. 1926. A disease of rabbits characterized by large mononuclear leucocytosis, caused by a hitherto undescribed *Bacterium monocytogenes*. J. Path. Bacteriol. 29:407-439.

Murray, E.G.D. 1955. A characterization of listeriosis in man and other animals. Can. Med. Assoc. J. 72:99.

Nozawa, Y. 1980. Modification of lipid composition and membrane fluidity. p. 399. In "Membrane Fluidity-Biophysical Techniques and Cellular Regulation". M. Kates and Kuksis (eds.). The Humana Press, Inc. Clifton. N.J.

Nyfeldt, A. 1929. Etologie de la mononucleose infectieuse. C.R. Soc. Biol. 101:590-591.

Ohno, Y., Yano, I. and Masui, M. 1979. Effect of NaCl concentration and temperature on the phospholipid and fatty acid composition of a moderately halophilic bacterium. *Psuudomonas halosaccharolytica*. J. Biochem. 80:217.

O'Leary, W.M. 1962. The fatty acids of bacteria. Bacteriol. Rev. 26:421.

O'Leary. W.M. 1967. in "The Chemistry and Metabolism of Microbial Lipids," p. 93. The World Publishing Co., Cleveland, Ohio.

Omura, S. 1976. The antibiotic cerulenin, a novel tool for biochemistry as an inhibitor for fatty acid synthesis. Bacteriol. Rev. 40:681.

Oram, J.D. and Reiter, B. 1968. The adsorption of phage to group N streptococci. The specificity of adsorption and the location of phage receptor substances in cell-wall and the plasma-membrane fractions. J. Gen. Virol. 3:103.

Otrel, S. 1968. Bakteriologische und epidemiologische untersuchungen wahrend einer listerios-epidemie. Deutsche Gesundheitswesen. 23:753-759.

Overath, P., Schairer, H.U. and Stoffel, W. 1970. Correlation of in vivo and in vitro phase transition s of membrane lipids in *Escherichia coli*.Proc. Nat. Acad. Sci. USA. 67:606.

Overton, E. 1895. In Sherbert, G.V. 1978. "The Biophysical Characterization of the Cell Surface," Academic Press, Inc., New York.

Papageorgiou, D.K. and Marth, E.H. 1989. Behaviour of *Listeria monocytogenes* at 4 and 22°C in whey and skim milk containing 6 or 12% sodium chloride. J. Food Prot. 52:625.

Payne, K.D., Rico-Munoz, E. and Davidson, P.M. 1989. The antimicrobial activity of phenolic compounds against *Listeria monocytogenes* and their effectiveness in a model milk system. J. Food Prot. 52:151.

Pechey, D.T., Yau, A.O.P. and James, A.M. 1974. Total and surface lipid of cells of *Pseudomonas aeruginosa* and their relationship to gentamycin resistance. Microbios 11:77.

Pirie, J.H.H. 1927. A new disease of wild rodents "Tiger River Disease." Publ. S. African Inst. Med. Res. 3:163-186.

Pirie, J.H.H. 1940. *Listeria*: change of name for a genus of bacteria. Nature. 145:264.

Post, L.S. 1982. The interrelationship of lipid composition and the bacterial activity of the antioxidant butylated hydroxyanisole. Ph.D. Thesis. The University of Tennessee. Knoxville.

Prescott, D.J. and Vagelos, P.R. 1972. Acyl carrier protein. Adv. Enzymol. 36:269.

Prindle, R.F. and Wright, E.S. 1977. Phenolic compounds. In "Disinfection, Sterilization, and Preservation", edited by S.S.Block. Lea and Febiger, Philadelphia, p.219.

Raccach, M. and Hemingsen, E.C. 1981. Antibacterial effect of *tertiary*-butylhydroquinone against two genera of gram positive cocci. J. Food Sci. 47:106.

Raetz, C.R. 1978. Enzymology, genetics and regulation of membrane phospholipid synthesis in *Escherichia coli*. Microbiol. Rev. 42:614.

Raines, L.J., Moss, C.W., Farshchi, D. and Pittman, B. 1968. Fatty acids of *Listeria monocytogenes*. J. Bacteriol. 96:2175.

Ralovich, B. 1984. Listeriosis Research - Present Situation and Perspective. Akademiai Kiado. Budapest.

Rico-Munoz, E. and Davidson, P.M. 1982. The effect of food components on the antimicrobial activity of phenolic antioxidants. 42nd Annual Meeting of the Institute of Food Technologists, Las Vegas, Nevada.

Rico-Munoz, E., Bargiota, E.E. and Davidson, P.M. 1987. Effect of selected phenolic compounds on the membrane-bound adenosine triphosphatase of *Staphylococcus aureus*. Food Microbiol. 4:239.

Rilfors, L., Wieslander, A. and Stahl, S. 1978. Lipid and protein composition of membranes of *Bacillus megaterium* variants in the temperature range 5 to 70°C. J. Bacteriol. 135:1043.

Robert, M.H. and Rahn, O. 1946. The amount of enzyme inactivation at bacteriostatic and bactericidal concentrations of disinfectants. J. Bacteriol. 52:639.

Rogers, H.J. and Perkins, H.R. 1968. "Cell Walls and Membranes," E. and F.M. Spon Ltd., London.

Russell, N.J. and Sandercock, S.P. 1980. The regulation of bacterial membrane fluidity by modification of phospholipid fatty acyl chain length. p. 181. In "Membrane Fluidity-Biophysical Techniques and Cellular Regulation". M. Kates and Kuksis (eds.). The Humana Press, Inc. Clifton. N.J.

Salton, M.R.J. 1964. Physiochemical properties and chemical composition of walls. In the bacterial cell wall. Elsevier Publishing Company. N.Y.

Salton, M.R.J. 1967. Structure and function of bacterial cell membranes. Ann. Rev. Microbiol. 21:417.

Salton, M.R.J. 1971. The bacterial membrane. Ch.1. In "Biomembranes." Vol.1. Manson, L.A. (Ed.) Plenum press, New York.

Sandermann, H. 1978. Regulation of membrane enzymes by lipids. Biochem. Biophys. Acta 515:209.

Schlech, W.F., Lavigne, P.M., Bortolussi, R.A., Allen, A.C., Haldane, E.V., Wort, A.J., Hightower, A.W., Johnson, S.E., King, S.H., Nicholls, E.S. and Broome, C.V. 1983. Epidemic listeriosis: Evidence for transmission by food. N.Engl. J. Med. 308:203-206.

Seeliger, H.P.R. 1961. "Listeriosis." Hafner Pub. Co., New York.

Seeliger, H.P.R. and Jones, D. 1986. *Listeria*. p.1235. In "Bergey's Manual of Systematic Bacteriology." vol. 2. Sneath, P.H.A. (ed.) William and Wilkins, Baltimore, MD.

Seeliger, H.P.R. and Finger, H. 1976. Listeriosis. In "Infectious Diseases of the Fetus and Newborn Infants," ed. J.S. Remington and J.O. Klein, p. 333. W.B. Sounders Co., Philadelphia.

Shahamat, M., Seaman, A. and Woodbine, M. 1980. Survival of *Listeria monocytogenes* in high salt concentrations. Zentralbl. Bakteriol. Hyg., I. Abt. Orig. A 246:506.

Shaw, N. 1970. Bacterial glycolipids. Bacteriol. Rev. 34:365.

Shaw, N. 1974. Lipid composition as a guide to the classification of bacteria. Adv. Appl. Microbiol. 17:63.

Shaw, N. and Braddiley, J. 1968. Structure and distribution of glycosyl diglycerides in bacteria. Nature 217:142.

Sherbet, G.V. 1978. "The Biophysical Characterization of the Cell Surface,"Academic Press, Inc, New York.

Sherwin, E.R. and Thompson, J.W. 1967. *tertiary*-Butylhydroquinone-an antioxidant for fats and oils and fat-containing foods. Food Technol. 21(6):106.

Sherwin, E.R. 1990. Antioxidants. Ch. 5 in "Food Additives", Branen, A.L., Davidson, P.M. and Salminen, S. (ed.) Marcel Dekker, Inc. New York.

Silliker, J.H. 1987. New bacteria in the news: A special symposium. *Listeria monocytogenes*. Food Technol. 40(8): 24.

Silvius, J.R., Mak, N. and McElhaney, R.N. 1980. Why do prokaryotes regulate membrane fluidity? p. 213. In "Membrane Fluidity-Biophysical Techniques and Cellular Regulation." M. Kates and Kuksis (eds.). The Humana Press, Inc. Clifton. N.J.

Sinensky, M. 1974. Homeoviscous adaptation: a homeostatic process that regulates the viscosity of membrane lipids in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. 71:522.

Singer, S.J. and Nicolson, G.L. 1972. The fluid mosaic model of the structure of cell membranes. Science 175:720.

Smith, P.F. and Mayberry, W.R. 1968. Identification of major glycolipid from *Mycoplasma* sp., strain J as 3,4,6-triacyl-B-D-glucopyranose. Biochemistry 7:2706.

Souza, K.A., Kostiw, L.L. and Tyson, B.J. 1974. Alteration in normal fatty acid composition in a temperature sensitive mutant of a thermophilic bacillus. Arch. Microbiol. 97:89.

Steel, R.G.D. and Torrie, J.H. 1960. Analysis of Variance II, Multiway classifications. Ch. 3. In "Principles and Procedures of Statistics." McGraw-Hill Book Co., Inc. New York, NY.

Strominger, J.L., Park, J.T. and Thompson, R.E. 1959. Composition of the cell wall of *Streptococcus aureus*: Its relation to the mechanism of action of penicillin. J. Biol. Chem. 234:3263.

Suganuma, A. 1966. Further studies on the plasma membrane of *Staphylococcus aureus*. J. Cell Biol. 30:208.

Sullivan, K.H., Jain, M.K. and Koch, A.L. 1974. Activation of the B-galactoside transport system in *Escherichia coli* by n-alkanols. Modification of lipid-protein interaction by a change in bilayer fluidity. Biochem. Biophys. Acta 352:287.

Tadayon, R.A. Carroll, K.K. and Murray, R.G.E. 1969. Factors affecting the yield and biological activity of lipid extracts of *Listeria monocytogenes*. Can J, Microbiol. 15:421.

Thompson, Q.A. 1980. "The Regulation of Membrane Lipid Metabolism," CRC Press, Inc., Boca Raton, FL.

Tomlins, R.I., Watkins, T.R. and Gray, R.J.H. 1982. Membrane lipid alterations and thermal stress in *Salmonella typhimurium 7136*.Appl. and Environ. Microbiol. 44:1110.

Troller, J.A. 1980. Influence of water activity on microorganisms in foods. Food Technol. 34:76.

Uchida, K. and Mogi, K. 1973. Cellular fatty acid spectra of *Sporolactobacillus* and some other *Bacillus-Lactobacillus* intermediates as a guide to their taxonomy. J. Gen. Appl. Microbiol. 19:129.

Vaczi, L. 1973. The Biological Role of Bacterial Lipids. Akademiai Kiado, Budapest.

Vaczi, L., Redai, I. and Rethy, A. 1967. Changes in the fatty acid composition of *Staphyloccus aureus* under various cultural conditions. Acta Microbiol. Acad. Sci. Hung. 14:293.

Van Deenen, L.L.M. 1972. Lipid composition, permeability and structure of biological membranes. J. Biochem. 28:19.

Van Iterson, W. 1965. Symposium on the fine structure and replication of bacteria and their parts. II. Bacterial cytoplasm, Bacteriol. Rev. 29:299.

Vas, K. 1953. Mechanism of antimicrobial action. Interference with the cytoplasmic membrane. Agrokem. Talajtan 2:1-16 [Chem. Abstr. (1954) 48:794d].

Vorbeck, M.L. and Marinetti, G.V. 1965. Intracellular distribution and characterization of the lipids of *Streptococcus faecalis* (ATCC 9790). Biochemistry 4:296.

Watkins, J. and Sleath, K.P. 1981. Isolation and enumeration of *Listeria monocytogenes* from sewage, sewage sludge, and river water. J. Appl. Bacteriol. 50:1.

Weerkamp, A. and Heinen, W. 1972. Effect of temperature on the fatty acid composition of the extreme thermophiles, *Bacillus caldolyticus* and *Bacillus caldotenax*. J. Bacteriol. 109:443.

Welker, N.M. 1976. In "Extreme Environments: Mechanisms of Microbial Adaptation". p. 229. Academic Press, London, New York, San Francisco.

Welshimer, H.J. 1960. Survival of *Listeria monocytogenes* in soil. J. Bacteriol. 80:316.

Welshimer, H.J. 1981. The genus *Listeria* and related organisms. In "The Prokaryotes. A Handbook on Habitats, Isolation, and Identification of Bacteria." Starr, M.P.(Ed.) Vol. 2, p. 1680. Springer-Verlag, Berlin and New York.

White, D.A., Albright, F.R., Lennarz, W.J. and Schnaitman, C.A. 1971. Distribution of phospholipid-synthesizing enzymes in the wall and membrane subfractions of the envelope of *Escherichia coli*. Biochim. Biophys. Acta. 249:636.

Wiedel, W. and Primosigh, J. 1958. Biochemical parallels between lysis by virulent phage and lysis by penicillin. J. Gen. Microbiol. 18:513.

Wilkins, P.O., Bourgeois, R. and Murray, R.G.E. 1972. Psychrotrophic properties of *Listeria monocytogenes*. Can. J. Microbiol. 18:543.

Wille, W., Eisenstadt, E. and Willecke, K. 1975. Inhibition of de novo fatty acid synthesis by the antibiotic cerulenin in *Bacillus subtilis:* effects on citrate-Mg++ transport and synthesis of macromolecules. Antimicrob. Agents Chemother. 8:231.

World Health Organization Working group. 1988. Foodborne listeriosis. Bull. WHO. 66(4):421.

APPENDIX

Table A-1. Retention Times of Fatty Acids Found in *Listeria monocytogenes* (Strains Scott A and LM4A), Grown on Tryptose Phosphate Agar at 35°C and 7°C.

Retention Time Minutes	Fatty Acid	Retention Time Minutes	Fatty Acid
5.1	11:0	19.4	16:0
5.9	2-OH 10:0	21.1	i-17:0
6.0	UNK 1	21.3	17:0Δ
6.6	12:0	21.6	17:0
6.9	UNK 2	22.0	2-OH 16:0
7.2	13:0	22.5	UNK 7
8.0	UNK 3	23.5	18:2
9.3	2-OH 12:0	23.7	18:1
10.0	UNK 4	23.9	18:1
11.7	3-OH 12:0	24.1	UNK 8
13.1	UNK 5	24.4	18:0
14.2	14:0	25.3	UNK 9
15.9	i-15:0	26.4	19:0Δ
16.1	a-15:0	26.8	19:0
16.9	15:0	28.1	UNK 10
18.3	UNK 6	29.1	20:0
18.5	2-OH 14:0	29.5	UNK 11
18.7	3-OH 14:0	30.9	UNK 12
18.8	i-16:0	33.4	UNK13
19.1	16:1	UNK 14	UNK 14

[a]Chromatographic conditions: column temperature:150°C, held for 4 minutes, temperature programmed from 150°C to 250°C at 4°C/min; injection temperature 250°C; detector: flame ionization at 280°C; carrier gas flow rate linear velocity, 20 cm/sec of helium.

Table A-2. Mean Relative Percentage of Fatty Acids Found in *Listeria monocytogenes* Strain Scott A at five Sodium Chloride Concentrations, Grown on Tryptose Phosphate Agar at 35°C.

	Sodium Chloride (w/v)					
	0%	1%	2%	3%	4%	5%
Fatty	Mean Relative Percentage					
2-OH	0.19[a]	0.21[a]	0.33[a]	0.44[a]	0.72[a]	0.89[a]
UNK[f]1	0.59[c]	0.65[c]	0.92[bc]	1.17[b]	1.89[a]	2.22[a]
12:0	0.22[c]	0.23[c]	0.34[bc]	0.38[b]	0.72[ab]	0.78[a]
13:0	0.38[d]	0.42[d]	0.61[c]	0.99[bc]	1.52[b]	1.68[a]
14:0	0.89[e]	0.97[e]	1.31[d]	1.51[c]	1.67[b]	1.87[a]
Br-15:0[g]	62.43[a]	58.97[b]	54.95[c]	54.59[c]	42.16[d]	50.18[d]
15:0	0.18[b]	0.22[ab]	0.28[a]	0.28[a]	-	-
2-OH	1.86[c]	2.30[c]	2.66[b]	3.01[a]	2.55[bc]	2.77[b]
3-OH	0.16[a]	0.14[a]	0.34[a]	-	-	-
16:0	2.53[d]	2.76[c]	3.54[bc]	4.64[b]	5.96[a]	4.62[b]
Br-17:0[h]	25.27[b]	25.68[b]	23.70[b]	28.83[a]	25.81[b]	28.02[a]
18:2	0.17[c]	0.21[bc]	0.81[bc]	0.97[a]	0.90[b]	-
18:1	1.22[b]	1.08[b]	4.06[a]	1.38[b]	4.41[a]	4.72[a]
18:0	1.31[b]	0.30[c]	0.56[c]	0.97[c]	3.15[a]	1.43[b]
19:0Δ	0.23[b]	0.95[ab]	-	-	1.64[a]	-
UNK 10	0.28[b]	1.37[b]	-	-	6.88[a]	-
20:0	0.18[b]	0.22[b]	3.59[a]	-	-	0.82[b]
UNK[i]	1.64[a]	3.28[a]	2.00[a]	1.20[a]	-	-
TOTAL	99.73	99.96	100.00	100.36	99.98	100.00

[a-e]Means in rows with different superscripts are significantly different ($p<0.05$).
[f]UNK = Unknown Fatty Acid.
[g]i-15:0 and a-15:0.
[h]i-17:0 and 17:0Δ.
[i]Unknown fatty acids in which total was <1% for most treatments.
[j]Means represent two replications.

Table A-3. Mean Relative Percentage of Fatty Acids Found in *Listeria monocytogenes* Strain LM4A at five Sodium Chloride Concentrations, Grown on Tryptose Phosphate Agar at 35°C.

	Sodium Chloride (w/v)					
	0%	1%	2%	3%	4%	5%
Fatty Acid	Mean Relative Percentage					
2-OH 10:0	0.28[a]	0.34[a]	0.40[a]	0.44[a]	0.79[a]	0.80[a]
UNK[f]1	0.82[b]	0.76[b]	0.96[b]	0.96[b]	2.09[a]	2.02[a]
12:0	0.27[b]	0.32[b]	0.32[b]	0.38[b]	0.74[a]	0.65[a]
13:0	0.50[d]	0.58[cd]	0.72[bc]	0.89[b]	1.46[a]	1.36[a]
UNK 3	0.71[bc]	0.94[ab]	1.11[a]	1.04[ab]	0.82[abc]	0.53[c]
14:0	0.88[c]	1.18[bc]	1.43[ab]	1.36[b]	1.53[ab]	1.73[a]
Br-15:0[g]	56.43[a]	57.16[a]	54.91[b]	49.89[c]	43.24[d]	38.37[e]
15:0	0.25[b]	0.25[b]	0.33[b]	0.30[b]	0.46[ab]	0.60[a]
2-OH 14:0	2.97[c]	3.27[bc]	4.18[a]	4.45[a]	3.80[b]	3.04[c]
16:0	3.24[d]	4.08[c]	4.27[c]	3.70[cd]	5.33[b]	6.26[a]
Br-17:0[h]	30.29[a]	26.69[b]	27.65[b]	29.78[ab]	31.70[a]	30.16[a]
2-OH 16:0	-	-	-	-	-	0.89
UNK 7	-	-	-	-	1.07[a]	2.36[a]
18:2	-	-	-	1.82[a]	-	0.58[b]
18:1	0.89[c]	1.89[b]	0.91[c]	-	1.21[bc]	3.82[a]
18:0	0.63[c]	0.50[c]	0.51[c]	0.59[c]	1.39[b]	3.21[a]
UNK 9	-	1.00[b]	-	-	2.97[a]	1.08[b]
20:0	0.19[b]	-	-	3.07[a]	0.39[b]	0.90[b]
UNK[i]	1.65[ab]	1.02[ab]	2.28[a]	1.33[ab]	0.99[b]	1.45[ab]
TOTAL	100.00	99.98	99.98	100.00	99.98	99.82

[a-c]Means in rows with different superscripts are significantly different ($p<0.05$).
[f]UNK = Unknown Fatty Acid.
[g]i-15:0 and a-15:0.
[h]i-17:0 and 17:0Δ.
[i]Unknown fatty acids in which total was <1% for most treatments.
[j]Means represent two replications.

Table A-4. Mean Relative Percentage of Fatty Acids Found in *Listeria monocytogenes* Strain Scott A at five Sodium Chloride Concentrations, Grown on Tryptose Phosphate Agar at 7°C.

	Sodium Chloride (w/v)					
	0%	1%	2%	3%	4%	5%
Fatty Acid	Mean Relative Percentage					
11:0	-	0.56[a]	0.86[a]	0.90[a]	-	0.97[a]
UNK[c]1	2.17[c]	2.94[c]	4.78[b]	5.15[b]	8.18[a]	4.56[b]
12:0	0.50[c]	0.75[c]	1.67[b]	1.29[b]	2.19[a]	1.36[b]
13:0	-	0.13[a]	0.33[a]	-	-	-
2-OH 12:0	0.52[a]	-	0.42[a]	-	-	-
UNK 4	1.79[a]	2.44[a]	2.78[a]	3.38[a]	3.32[a]	3.92[a]
3-OH 12:0	-	0.21	-	-	-	-
UNK 5	1.06[b]	1.09[b]	1.14[b]	1.56[b]	-	2.24[a]
14:0	1.88[b]	1.20[b]	1.36[b]	1.41[b]	3.14[a]	1.44[b]
Br-15:0[f]	75.68[a]	70.27[b]	65.86[c]	71.78[b]	71.07[b]	63.20[d]
15:0	0.15[a]	-	0.34[a]	-	-	-
2-OH 14:0	0.82[b]	1.24[a]	1.27[a]	1.28[a]	-	1.21[a]
3-OH 14:0	0.36	-	-	-	-	-
i-16:0	-	0.36[a]	0.28[a]	-	-	-
16:0	2.71[c]	3.11[bc]	4.29[b]	2.94[c]	5.81[a]	3.96[b]
Br-17:0[g]	9.13[a]	11.86[a]	10.51[a]	9.35[a]	6.28[b]	7.41[b]
18:2	0.46[b]	0.58[b]	0.80[ab]	-	-	1.04[a]
18:1	0.40[b]	0.54[b]	0.41[b]	-	-	1.32[a]
18:0	0.66[a]	0.59[a]	0.84[a]	-	-	-
19:0Δ	-	-	-	-	-	2.93
19:0	-	-	-	-	-	0.74
UNK 10	-	-	-	-	-	3.24
20:0	-	0.30[a]	-	-	-	0.45[a]
UNK[h]	1.59[a]	2.29[a]	2.32[a]	0.95[a]	-	-
TOTAL	99.88	100.46	100.26	99.99	99.99	99.99

[a-d]Means in rows with different superscripts are significantly different ($p<0.05$).
[c]UNK = Unknown Fatty Acid.
[f]i-15:0 and a-15:0.
[g]i-17:0 and 17:0Δ.
[h]Unknown fatty acids in which total was <1% for most treatments.
[i]Means represent two replications.

Table A-5. Mean Relative Percentage of Fatty Acids Found in *Listeria monocytogenes* Strain LM4A at five Sodium Chloride Concentrations, Grown on Tryptose Phosphate Agar at 7°C.

	Sodium Chloride (w/v)					
	0%	1%	2%	3%	4%	5%
Fatty Acid	Mean Relative Percentage					
11:0	-	-	0.46[a]	0.54[a]	0.69[a]	0.52[a]
UNK[e]1	2.64[b]	3.09[ab]	2.97[ab]	2.67[b]	3.48[a]	2.64[b]
12:0	0.73[b]	-	0.86[a]	0.65[b]	0.69[b]	0.46[b]
UNK 2	-	-	0.77[a]	0.82[a]	1.12[a]	-
13:0	-	-	-	0.13[a]	0.45[a]	-
2-OH 12:0	0.87[a]	-	-	0.44[b]	-	-
UNK 4	2.23[a]	1.87[a]	1.80[a]	2.08[a]	2.47[a]	1.96[a]
UNK 5	1.79[b]	1.44[b]	1.52[ab]	2.32[a]	2.54[a]	2.65[a]
14:0	1.59[b]	1.83[a]	1.71[a]	1.35[b]	1.59[b]	1.60[b]
Br-15:0[f]	79.82[a]	63.15[d]	64.72[d]	75.18[b]	72.26[c]	72.87[c]
2-OH 14:0	1.02[b]	2.60[a]	1.43[ab]	1.83[a]	2.10[a]	2.20[a]
16:0	1.93[c]	6.07[a]	6.13[a]	2.35[bc]	2.80[b]	2.82[b]
Br-17:0[g]	6.35[a]	10.01[a]	10.02[a]	9.04[a]	9.04[a]	9.96[a]
18:2	0.51[b]	1.62[a]	0.81[ab]	-	-	1.01[a]
18:1	0.50[b]	2.22[a]	2.81[a]	0.58[b]	0.78[b]	0.72[b]
18:0	-	2.63[a]	2.44[a]	-	-	0.59[b]
UNK 11	-	1.87[a]	1.52[a]	-	-	-
UNK 12	-	1.62	-	-	-	-
TOTAL	99.98	100.02	99.97	99.98	100.01	100.00

[a-d]Means in rows with different superscripts are significantly different ($p<0.05$).
[c]UNK = Unknown Fatty Acid.
[f]i-15:0 and a-15:0.
[g]i-17:0 and 17:0Δ.
[h]Means represent two replications.

Table A-6. Mean Relative Percentage of Fatty Acids Found in *Listeria monocytogenes* Strain Scott A at five Methyl Paraben Concentrations (ppm), Grown on Tryptose Phosphate Agar at 35°C.

	Methyl Paraben (ppm)					
	0	100	200	300	400	500
Fatty Acid	Mean Relative Percentage					
11:0	-	0.47[a]	0.60[a]	0.92[a]	0.66[a]	0.43[a]
2-OH 10:0	-	0.23	-	-	-	-
UNK[c]1	0.11[c]	2.67[b]	3.01[b]	4.56[a]	3.31[b]	2.38[b]
12:0	0.25[c]	0.74[b]	0.74[b]	1.06[a]	0.83[b]	0.66[b]
13:0	0.03[b]	0.13[a]	0.21[a]	-	0.27[a]	-
2-OH 12:0	-	0.26[a]	-	-	0.15[a]	-
3-OH 12:0	-	0.33[a]	0.31[a]	-	0.31[a]	-
14:0	0.67[c]	0.91[ab]	0.97[ab]	-	1.05[a]	0.84[bc]
Br-15:0[f]	49.84[b]	57.55[a]	50.05[b]	39.11[c]	49.31[b]	51.13[b]
15:0	0.17[a]	0.21[a]	-	-	-	-
2-OH 14:0	1.89[ab]	1.94[a]	2.04[a]	1.57[b]	1.84[ab]	1.97[a]
3-OH 14:0	0.13[b]	-	-	-	-	1.54[a]
i-16:0	-	0.18[a]	1.84[a]	-	-	-
16:0	2.77[bc]	3.06[b]	3.93[a]	3.08[b]	2.63[c]	2.74[c]
Br-17:0[g]	33.00[a]	27.47[b]	25.12[bc]	16.10[d]	23.64[c]	26.85[b]
17:0	-	-	-	-	-	0.19
2-OH 16:0	0.07[a]	-	0.11[a]	-	0.18[a]	-
18:1	0.85[ab]	0.91[ab]	1.81[a]	-	0.69[b]	-
18:0	0.69[b]	0.79[b]	1.02[a]	-	0.59[b]	-
UNK 9	-	-	-	-	3.69	-
19:0Δ	0.12[c]	-	1.29[b]	-	-	2.47[a]
19:0	-	-	0.75[b]	2.62[a]	0.56[b]	2.53[a]
UNK 10	0.71	-	-	-	-	-
20:0	0.24[d]	0.06[d]	1.03[c]	27.22[a]	4.16[b]	1.93[c]
UNK 11	-	-	-	-	-	3.90
UNK 12	1.95[a]	-	1.41[a]	-	1.32[a]	-
UNK 13	2.16[b]	0.44[c]	2.83[a]	-	1.29[b]	-
UNK 14	2.96[a]	-	-	3.09[a]	1.40[b]	-
UNK[h]	1.57[a]	1.62[a]	0.87[b]	0.67[b]	2.07[a]	0.44[b]
Total	100.18	99.97	99.94	100.00	99.95	100.00

[a-d]Means in rows with different superscripts are significantly different ($p<0.05$).
[c]UNK = Unknown Fatty Acid.
[f]i-15:0 and a-15:0.
[g]i-17:0 and 17:0Δ.
[h]Unknown fatty acids in which total was <1% for most treatments.
[1]Means represent two replications.

Table A-7. Mean Relative Percentage of Fatty Acids Found in *Listeria monocytogenes* Strain Scott A at five Methyl Paraben Concentrations (ppm), Grown on Tryptose Phosphate Agar at 7°C.

	Methyl Paraben (ppm)					
	0	100	200	300	400	500
Fatty Acid	Mean Relative Percentage					
11:0	-	-	0.92[b]	0.88[b]	2.10[a]	-
UNK[e]1	2.17[c]	3.44[c]	7.06[b]	5.18[b]	10.18[a]	12.79[a]
12:0	0.50[b]	0.94[b]	-	1.28[a]	1.80[a]	-
2-OH 12:0	0.52[a]	-	-	0.39[a]	-	-
UNK 4	2.16[a]	1.43[a]	1.18[a]	1.49[a]	1.10[a]	-
UNK 5	1.06[a]	1.45[a]	-	0.87[a]	-	-
14:0	1.88[b]	1.61[b]	1.45[b]	2.61[a]	2.30[a]	-
Br-15:0[f]	75.68[a]	68.49[b]	68.85[b]	71.28[b]	67.10[b]	68.77[b]
15:0	0.15	-	-	-	-	-
2-OH 14:0	0.82[b]	0.98[b]	3.03[a]	0.85[b]	-	-
3-OH 14:0	0.36	-	-	-	-	-
16:0	2.71[d]	11.43[a]	6.90[c]	4.53[c]	5.29[c]	9.39[b]
Br-17:0[g]	9.13[a]	7.65[a]	7.95[a]	8.73[a]	9.20[a]	9.04[a]
18:2	0.46[a]	0.58[a]	-	0.61[a]	-	-
18:1	0.40[b]	0.76[b]	1.29[a]	-	-	-
18:0	0.66[b]	1.24[ab]	1.36[a]	0.58[b]	0.92[b]	-
UNK[h]	1.21[a]	-	-	0.68[a]	-	-
TOTAL	99.87	100.00	99.99	99.96	99.99	99.99

[a-d]Means in rows with different superscripts are significantly different ($p<0.05$).
[e]UNK = Unknown Fatty Acid.
[f]i-15:0 and a-15:0.
[g]i-17:0 and 17:0Δ.
[h]Unknown fatty acids in which total was <1% for most treatments.
[1]Means represent two replications.

Table A-8. Mean Relative Percentage of Fatty Acids Found in *Listeria monocytogenes* Strain LM4A at five Methyl Paraben Concentrations (ppm), Grown on Tryptose Phosphate Agar at 35°C.

	Methyl Paraben (ppm)					
	0	100	200	300	400	500
Fatty Acid	Mean Relative Percentage					
11:0	-	0.57[a]	0.94[a]	0.32[a]	0.64[a]	0.52[a]
UNK[c]1	0.10[c]	2.72[b]	4.60[a]	3.18[b]	3.40[b]	2.59[b]
12:0	0.17[b]	0.67[b]	1.17[a]	0.87[ab]	0.95[ab]	0.70[b]
UNK 2	-	-	-	-	1.19	-
13:0	-	-	-	-	0.20[a]	0.10[a]
2-OH 12:0	0.45	-	-	-	-	-
14:0	0.52[b]	0.85[ab]	0.76[ab]	0.74[ab]	0.65[b]	0.90[a]
Br-15:0[f]	40.66[d]	55.52[a]	40.04[d]	47.60[c]	51.87[b]	52.74[b]
2-OH 14:0	2.15[b]	2.91[a]	2.85[a]	2.47[a]	2.62[a]	2.57[a]
i-16:0	-	-	1.61[a]	-	-	1.55[a]
16:0	2.27[c]	2.86[b]	2.69[bc]	2.85[b]	2.84[b]	3.73[a]
Br-17:0[g]	32.71[a]	31.53[a]	23.00[c]	27.54[b]	30.25[a]	31.43[a]
17:0	0.17	-	-	-	-	-
2-OH 16:0	-	-	-	5.26	-	-
18:2	-	-	-	-	-	0.51
18:1	0.38[b]	-	1.10[a]	1.98[a]	-	1.10[a]
UNK 8	0.83[a]	-	1.55[a]	-	-	-
18:0	0.25[b]	-	0.59[ab]	0.57[ab]	-	0.91[a]
UNK 9	1.39	-	-	-	-	-
19:0Δ	2.20[a]	-	2.73[a]	-	-	0.15[b]
UNK 10	3.68	-	-	-	-	-
20:0	0.40[c]	-	4.15[b]	6.18[a]	4.76[b]	-
UNK 11	5.65[a]	1.27[b]	-	-	-	-
UNK 12	4.87[a]	0.46[b]	-	-	-	-
UNK 13	0.43[b]	-	5.62[a]	-	-	-
UNK 14	-	-	6.01	-	-	-
UNK[h]	0.31[a]	0.61[a]	0.58[a]	0.44[a]	0.60[a]	0.48[a]
TOTAL	99.59	99.97	99.99	100.00	99.97	99.98

[a-d]Means in rows with different superscripts are significantly different ($p<0.05$).
[c]UNK = Unknown Fatty Acid.
[f]i-15:0 and a-15:0.
[g]i-17:0 and 17:0Δ.
[h]Unknown fatty acids in which total was <1% for most treatments.
[i]Means represent two replications.

Table A-9. Mean Relative Percentage of Fatty Acids Found in *Listeria monocytogenes* Strain LM4A at five Methyl Paraben Concentrations (ppm), Grown on Tryptose Phosphate Agar at 7°C.

	Methyl Paraben (ppm)					
	0	100	200	300	400	500
Fatty Acid	Mean Relative Percentage					
11:0	-	0.27[a]	0.43[a]	0.95[a]	0.52[a]	0.51[a]
UNK[c]1	2.64[b]	2.78[b]	5.26[a]	5.67[a]	2.90[b]	5.74[a]
12:0	0.73[b]	-	-	-	0.87[a]	0.81[a]
2-OH 12:0	0.87[a]	-	0.93[a]	-	0.30[a]	-
UNK 4	2.24[b]	2.24[b]	-	2.26[b]	3.87[a]	2.65[b]
UNK 5	1.79[b]	2.66[b]	-	2.86[b]	3.54[a]	2.28[b]
14:0	1.59[c]	1.51[c]	1.79[bc]	1.89[ab]	1.76[bc]	2.09[a]
Br-15:0[f]	79.82[a]	75.49[bc]	75.22[bc]	75.81[bc]	77.98[b]	75.14[c]
2-OH 14:0	1.02[ab]	0.66[b]	1.62[a]	0.56[b]	1.22[ab]	-
3-OH 14:0	-	-	3.55[a]	-	-	3.29[a]
16:0	1.93[d]	2.43[c]	3.75[b]	3.93[b]	2.62[bc]	4.77[a]
Br-17:0[g]	6.35[a]	5.07[b]	5.18[b]	3.88[bc]	2.87[c]	3.01[c]
18:2	0.50[b]	0.93[a]	0.40[b]	0.82[a]	0.74[ab]	-
18:1	0.50[a]	0.32[a]	0.45[a]	0.71[a]	0.33[a]	-
18:0	-	0.38[a]	-	0.57[a]	-	-
19:0Δ	-	0.51	-	-	-	-
19:0	-	0.47	-	-	-	-
20:0	-	0.25[b]	1.43[a]	-	0.46[b]	-
TOTAL	99.98	95.97	100.01	99.91	99.98	100.29

[a-d]Means in rows with different superscripts are significantly different ($p<0.05$).
[c]UNK = Unknown Fatty Acid.
[f]i-15:0 and a-15:0.
[g]i-17:0 and 17:0Δ.
[i]Means represent two replications.

Table A-10. Mean Relative Percentage of Fatty Acids Found in *Listeria monocytogenes* Strain Scott A at five Propyl Paraben Concentrations (ppm), Grown on Tryptose Phosphate Agar at 35°C.

	Propyl Paraben (ppm)					
	0	100	200	300	400	500
Fatty Acid	Mean Relative Percentage					
11:0	-	0.39[b]	0.59[b]	0.59[b]	3.25[a]	3.77[a]
UNK[f]1	0.11[d]	2.36[c]	3.05[c]	3.04[c]	17.64[b]	22.18[a]
12:0	0.25[c]	0.57[c]	0.81[c]	0.78[c]	4.60[b]	5.30[a]
13:0	0.03[a]	0.14[a]	-	-	0.77[a]	-
2-OH 12:0	-	0.12[b]	-	-	2.99[a]	-
14:0	0.67[c]	0.93[bc]	1.10[b]	1.12[b]	4.00[a]	3.10[a]
Br-15:0[g]	49.84[b]	54.03[a]	48.81[b]	45.87[c]	7.95[d]	3.14[c]
15:0	0.17[a]	0.15[a]	-	-	-	-
2-OH 14:0	1.89[a]	2.24[a]	2.20[a]	2.32[a]	-	-
i-16:0	-	0.53[b]	0.62[b]	1.49[b]	1.73[b]	2.61[a]
16:0	2.77[c]	3.71[c]	6.31[b]	6.09[b]	19.81[a]	20.45[a]
Br-17:0[h]	33.00[a]	29.40[a]	26.74[a]	32.30[a]	2.43[b]	-
2-OH 16:0	0.07[b]	-	-	-	1.73[a]	-
18:2	0.19	-	-	-	-	-
18:1	0.85[c]	2.07[b]	4.13[b]	3.91[b]	14.24[a]	12.46[a]
18:0	0.69[c]	0.85[c]	3.92[b]	1.54[bc]	14.10[a]	15.27[a]
UNK 9	-	0.29	-	-	-	-
19:0Δ	0.12	-	-	-	-	-
UNK 10	0.71	-	-	-	-	-
20:0	0.24[a]	0.44[a]	-	0.19[a]	-	-
UNK 11	1.95[c]	-	0.72[c]	0.35[c]	4.75[b]	11.71[a]
UNK 12	5.11[a]	-	0.99[b]	0.39[b]	-	-
UNK[i]	1.57[a]	1.61[a]	-	-	-	-
Total	100.23	99.83	99.99	99.98	99.99	99.99

[a-c]Means in rows with different superscripts are significantly different ($p<0.05$).
[f]UNK = Unknown Fatty Acid.
[g]i-15:0 and a-15:0.
[h]i-17:0 and 17:0Δ.
[i]Unknown fatty acids in which total was <1% for most treatments.
[j]Means represent two replications.

Table A-11. Mean Relative Percentage of Fatty Acids Found in *Listeria monocytogenes* Strain Scott A at five Propyl Paraben Concentrations (ppm), Grown on Tryptose Phosphate Agar at 7°C.

	Propyl Paraben (ppm)					
	0	100	200	300	400	500
Fatty Acid	Mean Relative Percentage					
UNK[f]1	2.17[d]	5.81[d]	10.64[c]	21.13[a]	16.43[b]	16.15[b]
12:0	0.50	-	-	-	-	-
UNK 2	0.50[d]	1.59[cd]	2.67[c]	5.48[a]	4.05[b]	4.15[b]
2-OH 12:0	0.52	-	-	-	-	-
UNK 4	2.16[a]	1.69[a]	2.74[a]	-	-	-
UNK 5	1.06[a]	0.92[a]	1.63[a]	-	-	-
14:0	1.89[c]	2.40[b]	3.75[b]	2.74[b]	2.30[b]	4.26[a]
Br-15:0[g]	75.68[a]	64.13[b]	55.70[c]	16.88[d]	3.18[c]	-
15:0	0.15	-	-	-	-	-
2-OH 14:0	0.82[a]	0.49[a]	-	-	-	-
3-OH 14:0	0.36[b]	0.51[b]	2.04[a]	3.67[a]	0.84[b]	-
16:0	2.71[d]	6.71[c]	8.39[c]	12.42[b]	13.43[b]	19.24[a]
Br-17:0[h]	9.13[a]	9.87[a]	7.44[a]	3.24[b]	1.82[b]	-
UNK 7	-	-	-	-	2.93[a]	4.33[a]
18:2	0.46[d]	1.37[c]	2.64[b]	11.12[a]	3.11[b]	1.71[bc]
18:1	0.40[d]	1.17[d]	-	17.61[a]	5.04[c]	8.15[b]
18:0	0.66[c]	3.22[d]	2.35[d]	5.69[c]	8.94[b]	13.09[a]
UNK 9	-	-	-	-	6.47[a]	6.98[a]
20:0	-	0.09[c]	-	-	3.68[b]	7.37[a]
UNK 11	-	-	-	-	2.87[a]	1.74[a]
UNK 12	-	-	-	-	7.30[a]	5.48[a]
UNK 13	-	-	-	-	9.17[a]	2.11[b]
UNK 14	0.61[b]	-	-	-	8.42[a]	5.24[a]
UNK[i]	0.60	-	-	-	-	-
TOTAL	99.88	99.97	99.99	99.98	99.98	100.00

[a-c]Means in rows with different superscripts are significantly different ($p<0.05$).
[f]UNK = Unknown Fatty Acid.
[g]i-15:0 and a-15:0.
[h]i-17:0 and 17:0Δ
[i]Unknown fatty acids in which total was <1% for most treatments.
[j]Means represent two replications.

Table A-12. Mean Relative Percentage of Fatty Acids Found in *Listeria monocytogenes* Strain LM4A at five Propyl Paraben Concentrations (ppm), Grown on Tryptose Phosphate Agar at 35°C.

	Propyl Paraben (ppm)					
	0	100	200	300	400	500
Fatty Acid	Mean Relative Percentage					
11:0	-	0.67^{c}	0.15^{c}	0.96^{bc}	1.33^{ab}	2.44^{a}
UNK[f]1	0.10^{d}	3.15^{c}	2.24^{c}	4.66^{bc}	7.06^{b}	13.41^{a}
12:0	0.17^{d}	0.76^{c}	0.58^{c}	1.20^{b}	1.73^{b}	3.60^{a}
13:0	-	0.07^{a}	-	0.23^{a}	0.19^{a}	-
2-OH 12:0	0.45^{a}	-	-	-	1.31^{a}	-
14:0	0.52^{b}	0.97^{b}	0.86^{b}	1.70^{a}	2.88^{a}	-
Br-15:0[g]	40.66^{b}	53.05^{a}	39.21^{b}	22.09^{c}	2.54^{d}	-
15:0	0.15^{a}	0.22^{a}	-	-	0.78^{a}	-
2-OH 14:0	2.15^{a}	2.73^{a}	2.09^{a}	1.44^{b}	-	-
3-OH 14:0	0.14^{b}	0.63^{ab}	-	0.97^{a}	0.77^{ab}	-
i-16:0	-	-	-	-	0.47	-
16:0	2.27^{d}	3.90^{d}	2.95^{d}	7.91^{c}	18.65^{a}	9.81^{b}
br-17:0[h]	32.71^{a}	26.42^{b}	20.27^{c}	14.08^{d}	0.85^{e}	-
17:0	0.17^{b}	-	12.18^{a}	-	-	-
2-OH 16:0	-	-	-	-	1.12	-
UNK 7	-	-	-	-	3.39	-
18:1	0.38^{d}	2.47^{c}	0.68^{d}	6.36^{c}	20.11^{a}	11.88^{b}
UNK 8	0.83^{a}	-	-	2.21^{a}	-	-
18:2		-	-	0.76^{b}	1.21^{b}	5.25^{a}
18:0	0.25^{c}	0.50^{c}	0.77^{c}	6.14^{b}	14.60^{a}	7.16^{b}
UNK 9	1.39	-	-	-	-	-
19:0Δ	2.20^{b}	0.46^{c}	-	1.42^{bc}	11.84^{a}	13.04^{a}
19:0		-	-	-	1.07^{b}	33.40^{a}
UNK 10	3.68	-	-	-	-	-
20:0	0.40^{b}	0.74^{b}	17.64^{a}	2.13^{b}	2.60^{b}	-
UNK 11	-	-	-	15.72	-	-
UNK 12	5.65^{a}	1.19^{b}	-	3.00^{b}	-	-
UNK 13	4.87^{a}	-	-	4.56^{a}	1.54^{b}	-
UNK 14	0.43^{b}	1.63^{ab}	-	2.47^{a}	3.92^{a}	-
UNK[i]	0.31^{a}	0.82^{a}	-	-	-	-
TOTAL	99.88	100.38	99.62	100.01	99.96	99.99

[a-e]Means in rows with different superscripts are significantly different ($p<0.05$).
[f]UNK = Unknown Fatty Acid.
[g]i-15:0 and a-15:0.
[h]i-17:0 and 17:0Δ.
[i]Unknown fatty acids in which total was <1% for most treatments.
[j]Means represent two replications.

Table A-13. Mean Relative Percentage of Fatty Acids Found in *Listeria monocytogenes* Strain LM4A at five Propyl Paraben Concentrations (ppm), Grown on Tryptose Phosphate Agar at 7°C.

	Propyl Paraben (ppm)					
	0	100	200	300	400	500
Fatty Acid	Mean Relative Percentage					
UNK[f]1	2.64[d]	6.59[c]	6.73[c]	29.80[b]	34.12[a]	29.56[b]
12:0	0.73[c]	1.86[b]	1.76[bc]	7.08[a]	8.23[a]	8.07[a]
UNK 2	-	0.63[b]	0.53[b]	2.49[a]	3.22[a]	2.19[a]
UNK 3	-	0.47[b]	0.95[b]	-	3.05[a]	3.64[a]
2-OH 12:0	0.87[a]	-	0.62[a]	-	-	-
UNK 4	2.24[a]	3.22[a]	2.75[a]	-	1.11[b]	1.06[b]
UNK 5	1.79[a]	2.84[a]	2.89[a]	-	-	-
14:0	1.59[d]	2.74[cd]	3.60[bc]	6.46[a]	3.43[bc]	4.19[b]
Br-15:0[g]	79.82[a]	51.09[b]	40.15[c]	8.79[d]	3.19[e]	1.73[f]
UNK 6	-	-	1.07	-	-	-
3-OH 14:0	1.02[b]	1.55[ab]	1.98[a]	-	-	2.11[a]
16:0	1.93[d]	9.31[c]	13.30[bc]		20.27[a]	15.70[b]
Br-17:0[h]	6.35[a]	1.87[b]	2.15[b]	-	-	-
UNK 7	-	1.46[a]	1.66[a]	-	-	-
18:2	0.50[b]	1.25[b]	2.30[a]	-	2.34[a]	1.77[ab]
18:1	0.50[c]	2.39[bc]	5.89[b]	5.14[b]	4.42[b]	20.97[a]
18:0	-	4.13[c]	7.84[bc]	11.36[a]	11.92[a]	9.00[ab]
20:0	-	1.64	-	-	-	-
UNK 11	-	1.83	-	-	-	-
UNK 12	-	2.78[b]	2.49[b]	10.04[a]	4.68[b]	-
UNK 13	-	1.40[b]	1.34[b]	2.49[a]	-	-
UNK[i]	-	0.91	-	-	-	-
TOTAL	99.98	99.96	100.00	99.99	99.98	99.99

[a-c]Means in rows with different superscripts are significantly different ($p<0.05$).
[f]UNK = Unknown Fatty Acid.
[g]i-15:0 and a-15:0.
[h]i-17:0 and 17:0Δ.
[i]Unknown fatty acids in which total was <1% for most treatments.
[j]Means represent two replications.

Table A-14. Mean Relative Percentage of Fatty Acids Found in *Listeria monocytogenes* Strain Scott A, Grown on Tryptose Phosphate Agar at five Temperatures.

	Temperature (°C)				
	7	15	24	30	35
Fatty Acid	Mean Relative Percentage				
UNK[f]1	2.17[c]	3.21[a]	1.75[d]	2.74[b]	0.11[c]
12:0	0.50[ab]	0.78[a]	0.33[ab]	0.60[ab]	0.25[b]
13:0	-	-	-	-	0.03
2-OH 12:0	0.52	-	-	-	-
UNK 4	1.79[a]	1.05[b]	0.74[bc]	-	0.36[bc]
UNK 5	1.06[a]	1.01[a]	0.77[a]	0.94[a]	0.26[a]
14:0	1.88[a]	1.46[ab]	0.97[bc]	1.03[bc]	0.67[c]
Br-15:0[g]	75.68[a]	66.17[b]	65.04[b]	57.66[c]	49.84[d]
15:0	0.15[a]	-	-	-	0.17[a]
2-OH 14:0	0.82[d]	1.63[c]	2.02[b]	3.66[a]	1.89[b]
3-OH 14:0	0.36[a]	-	-	-	0.13[a]
16:0	2.71[a]	1.72[b]	2.47[a]	2.54[a]	2.77[a]
UNK 7	0.60[b]	-	1.60[ab]	2.57[a]	-
Br-17:0[h]	9.13[c]	10.73[c]	20.67[b]	20.19[b]	33.00[a]
17:0	-	-	-	0.54	-
2-OH 16:0	-	-	-	-	0.07
18:2	0.46[a]	-	0.54[a]	-	0.19[b]
18:1	0.40[b]	-	0.42[b]	-	0.85[a]
18:0	0.66[a]	-	0.43[b]	-	0.69[a]
UNK 9	-	1.58	-	-	-
19:0Δ	-	1.71[a]	-	-	0.12[b]
20:0	-	3.03[a]	0.76[b]	3.70[a]	0.24[b]
UNK 11	-	2.66[a]	0.94[c]	1.74[b]	1.95[b]
UNK 12	.61[b]	-	-	-	2.16[a]
UNK 13	-	3.24[a]	0.67[c]	2.07[b]	2.96[ab]
UNK[i]	0.38[b]	-	0.31[b]	-	0.96[a]
TOTAL	99.88	99.98	100.43	99.98	99.67

[a-c]Means in rows with different superscripts are significantly different ($p<0.05$).
[f]UNK = Unknown Fatty Acid.
[g]i-15:0 and a-15:0.
[h]i-17:0 and 17:0Δ
[i]Unknown fatty acids in which total was <1% for most treatments.
[j]Means represent two replications.

Table A-15. Mean Relative Percentage of Fatty Acids Found in *Listeria monocytogenes* Strain LM4A, Grown on Tryptose Phosphate Agar at five Temperatures.

	Temperature (°C)				
	7	15	24	30	35
Fatty Acid	Mean Relative Percentage				
UNK[f]1	2.64[ab]	2.67[ab]	0.63[b]	3.80[a]	0.10[b]
12:0	0.73[a]	-	-	0.75[a]	0.17[b]
2-OH 12:0	0.87[a]	-	-	-	0.45[a]
UNK 4	1.75[a]	0.95[b]	-	-	-
UNK 5	1.79[ab]	2.24[a]	1.43[b]	1.01[bc]	0.31[c]
14:0	1.59[a]	1.97[a]	1.21[ab]	1.23[ab]	0.52[b]
Br-15:0[g]	79.82[a]	77.48[a]	57.01[b]	52.27[c]	40.66[d]
15:0	-	-	-	-	0.15
2-OH 14:0	1.02[c]	2.29[a]	3.32[a]	2.96[b]	2.15[b]
3-OH 14:0	-	-	-	-	0.14
16:1	-	-	-	-	0.09
16:0	1.93[b]	1.99[b]	2.54[ab]	3.56[a]	2.27[ab]
Br-17:0[h]	6.35[c]	10.41[d]	19.61[c]	24.65[b]	32.71[a]
17:0	-	-	-	2.50[a]	0.17[b]
18:2	0.50[a]	-	0.60[a]	-	-
18:1	0.50[a]	-	0.64[a]	-	0.38[a]
18:0	-	-	-	-	0.25
UNK 9	-	-	-	-	1.39
19:0Δ	-	-	1.20[a]	-	2.20[a]
UNK 10	-	-	-	-	3.68
20:0	-	-	2.26[b]	4.10[a]	0.40[c]
UNK 11	-	-	3.00[b]	-	5.65[a]
UNK 12	-	-	3.71[a]	-	4.87[a]
UNK 13	-	-	2.81[a]	3.16[a]	0.43[b]
UNK[i]	0.45[a]	-	-	-	0.83[a]
TOTAL	99.94	100.00	99.97	99.99	99.97

[a-c]Means in rows with different superscripts are significantly different ($p<0.05$).
[f]UNK = Unknown Fatty Acid.
[g]i-15:0 and a-15:0.
[h]i-17:0 and 17:0Δ
[i]Unknown fatty acids in which total was <1% for most treatments.
[j]Means represent two replications.

VITA

The author, Vijay Kumar Juneja, was born in India. Upon graduation from Central School, New Delhi, India, he entered Rajasthan University, India, for Pre-medical studies. In July 1974, he entered G.B. Pant University of Agriculture and technology, Pantnagar, India and graduated in Veterinary Medicine in 1978. He worked as a Veterinarian till June 1985 in private and government organizations in India.

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