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The University of Arizona, 1988

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THE EFFECT OF LIQUID SMOKE ON
LISTERIA MONOCYTOGENES

by
Maria Cipolla Messina

A Thesis Submitted to the Faculty of the
DEPARTMENT OF NUTRITION AND FOOD SCIENCE
In Partial Fulfillment of the Requirements
For the Degree of
MASTER OF SCIENCE
WITH A MAJOR IN FOOD SCIENCE
In the Graduate College
THE UNIVERSITY OF ARIZONA

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4/6/88

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ABSTRACT

Four strains of Listeria monocytogenes (LCDC 81-861, ATCC 19115, M1 and M2) examined in pure culture behaved similarly when exposed to a concentration of 0.5% CharSol C-10 liquid smoke by reducing Listeria numbers to an undetectable level within 4 hours post treatment. However, at the lower concentration of 0.25% liquid smoke, differences in resistance to the antimicrobial properties of smoke components become evident among these strains indicating that a level of 0.5% liquid smoke is more effective in controlling this organism. CharSol C-10 liquid smoke was used as a full strength dip treatment for beef franks surface inoculated with six strains of L. monocytogenes (LCDC 81-861, ATCC 19115, M1, M2, M5, and C6) then vacuum packaged and stored at $4 \pm 1^{\circ}\text{C}$ for 72 hours. Beef franks dipped in CharSol C-10 liquid smoke exhibited a significant ($P < 0.001$) reduction in L. monocytogenes numbers after 72 hours of storage at both inoculum levels of 1×10^3 cells/ml and 1×10^5 cells/ml.

INTRODUCTION

Although no outbreaks of listeriosis have been recorded from consumption of meat and poultry products in the United States to date, outbreaks may have been overlooked in the past. Listeria monocytogenes is a Gram-positive bacterium which produces serious clinical manifestations in humans including meningitis and abortion with a mortality rate of approximately 30%. Documentation does exist concerning the ability of L. monocytogenes to survive and multiply in meat and meat products. Johnson, Doyle and Cassens (1986) inoculated ground beef with L. monocytogenes and after 14 days of storage at 4°C found no real decrease in cell numbers. Khan et al. (1972) observed the organisms's ability to multiply in meat drippings from lamb stored at 8°C. Karaioannoglou and Xenos (1980) inoculated meatballs with L. monocytogenes, cooked them, and tested for survival of the organism. They concluded that at an inoculation level of 1×10^4 to 1×10^5 organisms/g, L. monocytogenes can survive when the cooking temperature is low or when the cooling time is not adequate.

Tolerance to the preservatives sodium chloride and sodium nitrite (Shahamat, Seaman and Woodbine, 1980) and refrigeration indicates the potential that L. monocytogenes has of becoming a post-processing contaminant in refrigerated foods. L. monocytogenes is common to the environment of processing plants and can be isolated from peeler room exhaust hoods, floors and drains, collators and pans during frankfurter packaging production (R.B. Sleeth, 1987, personal communication).

On August 14, 1987, the USDA's Food Safety and Inspection Service (FSIS) announced its regulatory policy for L. monocytogenes in cooked and ready-to-eat meat and poultry products, in which it established a zero tolerance level of the pathogen (Anonymous, 1987e). The plants would be given a chance to improve sanitation before recalls would be made. The FSIS policy was invoked for the first time in November, 1987 when cooked corned beef samples from a Florida plant were found to be contaminated with L. monocytogenes. The product had been withheld from retail, therefore no recall was necessary.

The reason for a zero tolerance level of the organism is because an infective dose has not been determined in humans and even small initial numbers may attain high levels during refrigeration storage, especially with longer shelf-life products (Anonymous, 1987e). The infective dose of L. monocytogenes has been estimated at 1,000 to 100,000 microorganisms per gram of food for individuals not at high risk, but it may be as low as 100 organisms in susceptible individuals (Anonymous, 1987c). However, listeriosis has also been found among healthy persons indicating that there are additional risk factors that have not been uncovered or that some strains are more pathogenic than others.

Radecki et al. (1975) reported that when liquid smoke is present on the surface of processed meats, smoke components such as formaldehyde, acetic acid, creosote, and high-boiling phenols exhibit bactericidal properties. In addition, smoking of processed meats can produce desirable flavor and color in the products. The purpose of

this study was to investigate the antimicrobial effect of different liquid smoke products on L. monocytogenes in pure culture and to determine the most effective liquid smoke against L. monocytogenes after inoculation on frankfurter surfaces.

LITERATURE REVIEW

History of the Organism

The genus Listeria now is comprised of eight species, only four of the species are known to cause infections in humans and animals: L. monocytogenes, L. ivanovii, L. innocua, and L. seeligeri. The other species (L. denitrificans, L. murrayi, L. grayi, and L. welshimeri) have not been associated with naturally occurring infections. The vast majority of human listeric infections are caused by L. monocytogenes while only a few cases have been linked to L. ivanovii, L. innocua, and L. seeligeri (McLauchlin, 1987).

Murray et al. (1926) is credited with the first description of the organism now known as Listeria monocytogenes. They observed a spontaneous infection among laboratory rabbits and guinea-pigs caused by a Gram-positive bacillus which they named Bacterium monocytogenes because of the ability of the organism to cause a monocytosis in the blood of the infected animals. In 1927, Pirie isolated a similar organism from the liver of infected gerbils and named it Listerella hepatolytica (Gray and Killinger, 1966). The genus name was selected in honor of the English surgeon Lord Lister.

Nyfeldt, in 1929, was credited with the first report of L. monocytogenes' isolation from humans, the blood of a boy with infectious mononucleosis and then from 12 other patients with the same clinical manifestation. The cultures he isolated were all serotype 3, a serotype so rare it was not found again for 25 years (Gray and Killinger, 1966). Although monocytosis is now recognized as an uncommon manifestation of human listeriosis, the connection between L. monocytogenes and the monocyte is still an interesting phenomenon (Barza, 1985). The manifestations of listeriosis as well as the organism's predisposition for attacking the immunocompromised may be explained by the ability of L. monocytogenes to parasitize the mononuclear phagocyte (Barza, 1985).

Burn in 1933 observed L. monocytogenes as a cause of infection in the perinatal period and the following year described it as the causative agent in an adult meningitis case (Gray and Killinger, 1966). Also in 1933, Gill recognized that L. monocytogenes produced a characteristic meningoencephalitis in sheep he termed "circling disease". It was not until 1951 that Reiss et al. described granulomatosis infantiseptica, which is the most characteristic clinical manifestation of human listeriosis (Schlech, 1986).

Morphology and Biochemical Characteristics

L. monocytogenes occurs in regular, short rods, 0.4 - 0.5 μ m in diameter and 0.5 - 2 μ m in length with rounded ends (Seeliger and Jones, 1986). Besides occurring singly and in pairs, short chains as well as diploforms that may be V- or Y- shaped are described (Seeliger

and Finger, 1985). The organism is nonacid-fast and does not produce spores or capsules. Young cultures are Gram-positive, but after 2 to 5 days the organisms tend to become Gram-variable. L. monocytogenes is facultatively anaerobic and exhibits a characteristic tumbling motility when cultured at 20-25°C. The colonies appear bluish gray by normal illumination and exhibit a characteristic blue-green sheen under obliquely transmitted light (Seeliger and Finger, 1985). On 5% sheep blood agar, the organism produces a narrow zone of beta hemolysis.

The temperature limits of growth for L. monocytogenes are between 1 and 45°C. The optimum growth temperature of the organism is between 30 and 35°C. The organism grows best at a pH between 6 and 9. Multiplication can also occur in nutrient broth supplemented with as much as 10% (w/v) NaCl (Seeliger and Jones, 1986).

L. monocytogenes is catalase-positive and oxidase-negative. L (+)-lactic acid is produced by the fermentative metabolism of glucose. The organism is methyl red positive, and Voges-Proskauer positive. Exogenous citrate is not utilized nor is indole produced. Esculin and sodium hippurate are hydrolyzed while urea, gelatin, casein, and milk are not hydrolyzed.

L. monocytogenes is pathogenic for humans and more than 50 animal species, particularly cattle and sheep. Practically all laboratory animals are susceptible, and this susceptibility can be significantly increased by administration of cortisone (Seeliger and Finger, 1985).

Thirteen serotypes have now been identified, but a large proportion of strains isolated from human and animal infections belong to only three serotypes: 1/2a, 1/2b, and 4b. The vast majority of isolations are found to be serotype 4b (McLauchlin, 1987).

This organism is widely distributed in nature and can be isolated from vegetation, raw milk, soil, sewage, silage, surface and stream waters, animal feed, slaughter-house waste and can be carried by healthy humans and animals.

Environmental Factors

Unlike most other foodborne pathogens, L. monocytogenes survives well at refrigerator temperatures. To the other extreme, some L. monocytogenes cells may survive the pasteurization of milk due to their intracellular location.

The evidence that listeriosis was transmitted by pasteurized milk in the 1983 Massachusetts and Connecticut outbreak raised questions about the current pasteurization regulations - i.e., 71.7°C for 15 sec. Even now there are many conflicting studies on the effectiveness of pasteurization against L. monocytogenes. More studies are needed to resolve the discrepancies that exist and to investigate the question of intraleukocytic protection from pasteurization by use of milk from naturally infected animals for pasteurization (Hird, 1987).

In a study by Dijkstra, naturally-infected suspensions of brain tissue, silage and feces, stored at 5°C, were routinely re-examined for the presence of L. monocytogenes for several years. The

average survival of the organism in brain tissue samples was 5 to 6 years, 5 years in silage suspensions, and 1 to 2 years in feces suspensions.

A parallel increase in the incidence of listeriosis occurred after the mid 1970s in humans and animals. In animals this increase may be associated with changes in agricultural practices such as the use of "big bale" type silage production (McLauchlin, 1987). It is also interesting to note that these increases coincide with increases in disease caused by intestinal pathogens in man, and also an increase in the use of untreated human sewage sludge and animal slurry on agricultural land (McLauchlin, 1987).

It has been reported (Blenden and Szatalowicz, 1967) that the level of iron in the environment has a strong effect on L. monocytogenes and the development of listeriosis. Iron compounds are stimulatory for in vitro growth of the organism and also will enhance the virulence of the organism.

The 1983 outbreak of listeriosis in which L. monocytogenes, serotype 4b, contaminated raw cabbage and coleslaw, prompted a study to be done by Conner, Brackett, and Beuchat to determine the influence of temperature, sodium chloride and pH on the growth of L. monocytogenes in cabbage juice up to 70 days of storage. Two strains of L. monocytogenes were used (Scott A, a human isolate, and LCDC 81-861, a pathogenic strain isolated from cabbage) both serotype 4b. To summarize their findings, the LCDC 81-861 strain was less sensitive to NaCl but more sensitive to refrigeration (5°C) than Scott A. Both

strains were also found to grow well below pH 5.6 which had previously been reported to be the minimum for growth, and survive at a pH of 4.8. Other researchers have shown L. monocytogenes to grow in 10% salt and survive for up to three weeks in 25% brine (R. B. Sleeth, 1987, personal communication).

Clinical Manifestations

Unlike many other foodborne illnesses which produce mainly gastrointestinal symptoms, human listeriosis has a variety of clinical manifestations. "Human listeriosis is characterized by the formation of miliary granulomas and focal necroses or by suppuration in the affected tissues" (Seeliger and Finger, 1985). The term "listerioma" is used to refer to the granulomas associated with listeriosis (Gray and Killinger, 1966). The clinical course listeriosis takes depends mainly on the site, number and location of the foci. It is also important to note that two or more manifestations of the disease may be present simultaneously or may follow each other (Seeliger and Finger, 1985).

A low grade listeric infection producing mild flu-like symptoms is a manifestation of the disease which is most likely to go unrecognized as listeriosis. Listeriosis during pregnancy, in many cases, begins as an acute febrile disease in the mother with chills and headaches and is sometimes accompanied by pharyngitis, diarrhea, pyelitis and backache a few days or weeks before abortion, stillbirth or delivery of an infant with a listeric infection (Gray and Killinger, 1966). Meningitis is only occasionally observed in

pregnant women. The most characteristic form of listeriosis in the newborn is "granulomatosis infantiseptica" (Seeliger, 1961), which was first described by Reiss and his co-workers in 1951. In stillborn infants Reiss described this manifestation as distinctive focal necrosis throughout the body. Microscopically it was seen as areas of focal necrosis infiltrated with mononuclear cells. The clinical symptoms of listeriosis in the newborn are somewhat variable and frequently go unrecognized. Respiratory distress and heart failure are the main symptoms, but the presence of listeriomias in the posterior pharyngeal wall are more characteristic.

The central nervous system forms of listeriosis are considered the most dangerous and include meningitis, meningoencephalitis and brain abscesses (Seeliger and Finger, 1985). *Listeria* meningitis and meningoencephalitis predominantly affect neonates and males over the age of 50 years. The fatality rate among untreated patients or those treated too late is approximately 70 percent.

A septicemic form accompanied by pharyngitis and a mononucleosis-like syndrome is seen mainly in adults. A transition to the meningitic form may occur but usually the prognosis for the "anginose-septic form with mononucleosis" is good although convalescence may require several months. Another septicemic form known as the granulomatosis septica and typhoid-pneumonic form may resemble typhoid fever accompanied by pneumonia or endocarditis. A

transition to listeriosis of the central nervous system may also occur with this septicemic form (Seeliger and Finger, 1985).

Other forms of listeric infection include external and internal abscesses, cutaneous lesions produced in persons having close contact with animals infected with L. monocytogenes, conjunctivitis which is sometimes seen during other clinical manifestations but not commonly observed, and urethritis and the cervicoglandular form which also are rarely seen. Low grade listeric infections and conjunctivitis are forms of human listeriosis observed in some laboratory technicians working with L. monocytogenes (Gray and Killinger, 1966). Mental retardation in children and psychosis in children as well as adults has also been associated with listeric infections. In addition, approximately 5 percent of the general population is an asymptomatic carrier of L. monocytogenes (Barza, 1985).

Treatment

The mortality rate associated with human listeriosis is approximately 30 percent. The prognosis depends on early antibiotic treatment and untreated individuals usually do not survive more than 4 days. Without early antibiotic therapy, recovery is sometimes followed by permanent damage, such as hydrocephalus, ptosis and strabismus (Seeliger and Finger, 1985). Effective antibiotics include the usage of tetracyclines in adults, erythromycin in children, and ampicillin in newborns. Prompt treatment of children and adults with ampicillin can cure both the extraneural and neural forms of

listeriosis without permanent damage (Seeliger and Finger, 1985). Ampicillin combined with an aminoglycoside has been suggested as the most effective treatment to combat relapses (Seeliger and Finger, 1985).

High Risk Individuals

Listeriosis is not a disease that primarily targets healthy individuals in the general population. High risk groups include pregnant women, neonates, and the elderly. Immunocompromised persons, such as alcoholics, those suffering from neoplastic diseases, chemotherapy patients, individuals receiving cortisone or corticosteroids, AIDS patients, diabetics, those undergoing dialysis, and leukemia patients all have a predisposition for contracting some form of listeric infection. Also at high risk are individuals on antacid medication, cimetidine or antihistamines, all of which neutralize gastric acidity (Seeliger, 1961; Gray and Killinger, 1966; and Schlech, 1984).

Foodborne Outbreaks

Burn, in 1936, (Seeliger and Finger, 1985) first suggested that L. monocytogenes may be transmitted to humans through food, however, Potel, in 1953, is credited with the first description of foodborne listeriosis in humans (Hird, 1987). He isolated L. monocytogenes from a cow with Listeria mastitis and from stillborn twins of a woman who had ingested raw milk from this animal prior to delivery.

Foods in which L. monocytogenes has been isolated from include: milk (Fleming et al, 1985), cheeses (Ryser and Marth, 1987), raw vegetables (Schlech et al., 1983 and Ho et al., 1986), meat products (Ralovich, 1984b), fermented sausages (Ralovich, 1984a), poultry (Ralovich, 1984a), ice cream, shrimp, crab meat, langostinos, lobster tails and fish (Anonymous, 1987d). L. monocytogenes survives longer in soil, silage, feces and milk longer than most other foodborne pathogens (Silliker, 1986). Since the organism can be airborne, a possible mode of transmission is dust particles (Seeliger, 1961) carrying L. monocytogenes which can contaminate food surfaces. Flies and ticks are also capable of transmitting the organism. In addition, there exists a possible increase in virulence of the organism when growth occurs at refrigerator temperatures (Farber, 1986).

Keeping in mind that listeriosis has only been a reportable disease since 1986, in the United States, there are only a small number of documented foodborne outbreaks of listeriosis that have occurred in the United States and foreign countries. The first of these outbreaks occurred in Halle, Germany from 1949 to 1957. The vehicle of transmission was suspected to be unpasteurized milk (Seeliger, 1961). Moreover, a renewed interest in human listeriosis has occurred in the last 10 years as a result of more recent outbreaks.

The first of these outbreaks happened during September and October 1979, involving 20 patients from eight Boston hospitals (Ho et

al, 1986). All 20 isolates were L. monocytogenes serotype 4b. Twelve of the 20 patients had received antacids or cimetidine prior to the onset of listeriosis. Both medications reduce gastric acidity which is an important defense against listeric infections. Three foods were preferred by these patients: tunafish, chicken salad, and cheese. However, the only common feature was the serving of these foods with raw celery, tomatoes and lettuce. The raw vegetables may have been contaminated with L. monocytogenes, which was then able to survive ingestion because of gastric acid neutralization. Listeric infections in the patients manifested themselves as enteritis, bacteremia, and meningitis resulting in three deaths. Although raw vegetables are believed to be the source of this outbreak, pasteurized milk has not been excluded.

From January to November, 1980, 29 cases of listeriosis were diagnosed in Auckland, New Zealand (Lennon et al., 1984). The predominant serotype of L. monocytogenes in these cases was 1b. Twenty two of these cases were perinatal infections resulting in 5 fetal deaths. Shellfish and raw fish consumption may have served as vehicles of transmission, but the cause of the epidemic was not discovered. It has been suggested that environmental pollution may play a key role (Seeliger and Finger, 1985). L. monocytogenes can be isolated from sewage effluent, and this could have caused contamination of fish or shellfish (Lennon et al., 1984).

Between March and September 1981, a large foodborne outbreak occurred in the Maritime Provinces of Canada (Schlech, 1984). Thirty

four perinatal cases (47% fatality rate) and 7 adult cases were identified. The vehicle of transmission was coleslaw which, when obtained from the refrigerator of one patient, had the same serotype of L. monocytogenes (4b) and phage type that had been isolated from the patient's blood. Of the 7 nonpregnant adult patients, 6 had meningitis and 1 aspiration pneumonia, but none had any underlying immunosuppressive condition suggesting the importance of undefined susceptibility factors. The coleslaw was prepared from cabbage obtained from a farm known to have had cases of ovine listeriosis. The cabbage was grown in fields fertilized with both raw and composted manure from the sheep flock. The cabbage, after harvesting, was not shipped out until the following winter and spring. "The prolonged cold storage of the cabbage could have enriched for L. monocytogenes as well as caused a die-off of the normal cabbage microflora" (Palumbo, 1986). Also, L. monocytogenes serotype 4b was isolated from two unopened packages of coleslaw purchased in two different retail markets.

In Massachusetts, during the summer of 1983, 49 cases of human listeriosis resulting in 14 deaths were attributed to the consumption of whole or 2% pasteurized milk. The listeric infections were manifested by either septicemia or meningitis. Seven newborns and 42 immunocompromised adults were involved in the outbreak (Fleming et al., 1985). Inspection of the milk-processing facility failed to uncover any evidence of improper handling or pasteurization of the milk. However, the implicated milk came from farms where listeriosis

in dairy cows was known to have occurred at the time of the outbreak. In addition, milk in the bulk tanks of one of the farms supplying the processing plant contained L. monocytogenes serotype 4b, the same serotype as that found in 32 of 40 case isolates available for testing (Farber, 1986). It has been suggested (Fleming et al., 1985) that a few organisms may have survived pasteurization by being protected in their intracellular location and noted that the pasteurization plant did not use clarification (the removal of leukocytes by a centrifugal filtering process). Postpasteurization contamination has been considered but seems unlikely, because no potential source in the plant could be found and skim milk processed with the same equipment each day was not affected.

The outbreak that has probably brought the most attention to foodborne transmission of listeriosis occurred in the spring of 1985 in Southern California due to the consumption of Jalisco brand Mexican-style cheese. There were approximately 300 cases with nearly 100 deaths. Approximately two-thirds of the cases occurred in perinatal pairs with most of the deaths occurring among infants who were stillborn or died shortly after birth (Ryser and Marth, 1987). The presence of L. monocytogenes serotypes 4b in the cheese was confirmed by the Centers for Disease Control, Atlanta, GA, and resulted in a nationwide recall of the product. Production records reveal that raw milk might have been added to pasteurized milk used for cheese manufacture, but the epidemic serotype of L. monocytogenes was not isolated from raw milk used by the cheese plant. However, L.

monocytogenes was ubiquitous in the factory environment, indicating the possibility of post-processing contamination. Whether L. monocytogenes was present in the milk used to make the cheese or post-pasteurization occurred, there were a number of favorable conditions present for multiplication of L. monocytogenes. These conditions included cold-enrichment (refrigeration), lack of competition (no starter culture was used), the substrate itself (a soft cheese with high moisture content), and pH (approximately 6.6), all factors which would promote survival and multiplication of this organism (Hird, 1987).

In addition, some smaller and more recent outbreaks have occurred. L. monocytogenes was isolated from Liederkranz cheese, in the United States in 1985, producing an unknown number of cases of listeriosis. During February and March of 1986, the organism was isolated from Brie cheese imported from France. Several cases were reported in both England and Canada, however, no cases of listeriosis were connected with consumption of the cheese in the United States. At least three cases of listeriosis were attributable to soft Mexican-style cheese consumption in Arizona, in 1986.

A foodborne outbreak involving rice soup with cream has also been reported as well as two outbreaks in the Soviet Union involving pork (Ralovich, 1984b). Furthermore, approximately 1,000 sporadic cases of human listeriosis occur in the United States each year and an unknown number of these may be caused by consumption of contaminated foods (Anonymous, 1987e).

Preventive Measures

Only when the source of listeric infection is known can the transmission of the agent to man be controlled or entirely prevented. To attain this goal, human and veterinary public health services need to inform each other of proved occurrences of listeriosis (Seeliger, 1961).

Farm workers, animal breeders and veterinarians should take proper precautions when attending to sick animals. Workers in slaughter houses and abattoirs as well as the personnel of certain soap manufacturers using fat of animal carcasses for soap production should be informed of the dangers.

High risk individuals should avoid consumption of raw milk and its products, contact with animals, and contact with other humans infected with listeriosis. Transmission of listeriosis has been known to occur in obstetrical wards from one infant to another as well as to hospital personnel such as doctors and nurses who came in contact with listeric patients.

Prophylaxis depends mainly on general sanitary measures. Of particular importance is the enforcement of controlling the distribution of raw animal products destined for human use and consumption (Seeliger, 1961).

Research is now in progress which will alter the microflora of food stuffs as a preventative measure. Even though L. monocytogenes may not exist at a detectable level initially, extended storage at refrigerator temperatures will allow multiplication of the organism to high numbers in many foods. Currently, organisms are

being found that may be used as starter cultures or fermentors which produce antagonist metabolites (antibiotics) to control the growth of L. monocytogenes in dairy products, fermented sausage and other processed meats (Anonymous, 1987a).

Detection Methods

Currently, there are a number of detection and enumeration methods utilized in the isolation of L. monocytogenes from biological as well as food samples. Misdiagnosis of foodborne listeric infections in the past is partly due to the difficulty associated with having a rapid and reliable method of detection. Some of the procedures used to identify L. monocytogenes now include: cold enrichment, direct plating, a selective enrichment procedure (Doyle and Schoeni, 1986 and 1987), the FDA procedure, the USDA-FSIS procedure, gene probes, ELISA, and fluorescent antibody.

Cold enrichment is one of the oldest methods employed in the detection of L. monocytogenes. Ryser and Marth (1987) have frequently used this procedure for isolation of the organism from cheeses. Samples are first diluted in Tryptose broth and then surface plated on McBride Listeria Agar (MLA). Plates are incubated for 48 hours at 35 C, in a 5% O₂, 10% CO₂, 85% N₂ atmosphere. Typical L. monocytogenes colonies were described as smooth, bluish gray, slightly raised, translucent, watery consistency, 0.5 - 1.5 mm in diameter, and weakly beta-hemolytic. These colonies were counted and biochemical tests were done for confirmation. If the organism was not detected with the original plating, the initial dilutions stored at 3 °C were plated on

MLA after 2, 4, 6 and 8 weeks. Ryser and Marth (1987) were able to detect levels of 10 organism/g or ml of sample with this procedure. Although low levels of detection are possible with this method, it is a slow procedure sometimes requiring months to complete.

A more rapid method involves directly surface plating dilutions of sample onto selective media such as MLA without enrichment. Plates should be incubated for 48 hr and an increased CO atmosphere is helpful. However, this procedure is not recommended for samples with very low levels of L. monocytogenes, and it does not allow for recovery of injured cells.

A selective enrichment procedure (SEP) developed by Doyle and Schoeni (1986 and 1987) can be used for analyzing biological and fecal specimens as well as food samples. The sample is homogenized in a Stomacher with 100 ml of enrichment broth containing tryptose, defibrinated sheep blood, glucose, K_2HPO_4 , polymixin B, acriflavine HCl and nalidixic acid. The sample is then incubated with agitation at 37 °C, under the microaerobic conditions for 24 hr then surface plated onto MLA plates. After a 48 hr incubation period the MLA plates are examined for typical L. monocytogenes colonies.

With the FDA procedure for isolation of L. monocytogenes, samples are added to 225 ml of enrichment broth composed of: trypticase soy broth, yeast extract, acriflavine HCl, nalidixic acid and cycloheximide. The enrichment broth and sample are then homogenized in a Stomacher and incubated at 30°C. At 24 and 48 hr of incubation, enrichment cultures are both: (a) surface plated (0.1 ml)

directly onto duplicate plates of MLA; and (b) alkali treated (1 ml into 9 ml of 0.5% KOH) and the surface plated (0.1 ml) onto duplicate plates of MLA.

Doyle and Schoeni (1987) compared three isolation procedures of L. monocytogenes from soft surface-ripened cheese and found that in 41 isolations made, the most were made (21) by the cold enrichment, with 16 and 13 isolations made by the FDA and selective enrichment procedures respectively. The authors concluded that the more often the enrichment culture is sampled, the more likely L. monocytogenes will be isolated. This may explain why the least number of Listeria isolations were made by the SEP method, because this procedure involved only a single sampling, whereas the FDA procedure required 4 samplings and the cold enrichment procedure used 6 samplings. However, in another study done by Doyle and Schoeni (1986) in which they compared SEP with direct plating on MLA and cold enrichment for isolation of L. monocytogenes from fecal and biological specimens, they found that the SEP procedure isolated L. monocytogenes from approximately 2 and 5 times as many specimens as the cold enrichment and direct plating procedures, respectively. These findings exemplify the importance of evaluating a detection method by considering the type of sample to be tested (different types of food, biological and fecal specimens).

A complex procedure, but one used widely in the food industry is the USDA-FSIS method (McClain) of isolation and identification of L. monocytogenes. With this method, detection of the organism is

possible in 3 to 4 days. Examination of meat samples is done by taking a 25 g sample and mixing it in a Stomacher with 225 ml of University of Vermont enrichment broth. The homogenate is incubated overnight at 30°C for primary enrichment. In the secondary enrichment step 0.1 ml of the incubated primary enrichment broth is transferred to a tube with 10 ml of secondary enrichment broth (primary enrichment broth with increased acriflavin), incubated overnight at 30°C and then streaked on LPM agar for 24-48 hr. Bluish-gray colonies were identified as L. monocytogenes when examined under a 45° transillumination microscope arrangement. Confirmation was made with Gram staining, catalase reaction, and additional biochemical testing.

In a recent paper accepted for publication, Smith and Archer (Anonymous, 1987b) evaluated 33 different culture media and found that most, including those used by the FDA and USDA, failed to support growth of heat-injured L. monocytogenes. The paper indicates that the presence of phenylethanol in the FDA and USDA media prevents repair and subsequent colony formation of heat-injured cells. Smith and Archer state that before accurate surveys can be done to determine the incidence of *Listeria* in foods and food products receiving a thermal treatment, a medium must be developed that allows the recovery of injured cells.

Research efforts have also been concentrated on developing gene probes for the detection of L. monocytogenes. GENE-TRAK Systems (Framingham, MA) has recently developed a gene probe called GENE-TRAK *Listeria* Assay that is now commercially available. Another rapid

method of detection was described by Farber and Speirs (1986). They developed an ELISA technique for detection of L. monocytogenes in raw milk. Also, fluorescent antibody techniques have been modified for usage in dairy products giving presumptive results within 26 hr. This method would be useful in monitoring outbreaks of listeriosis where analysis of large samples in a short time would be necessary. Obviously much more research is needed to develop reliable, simple, rapid methods of isolation of L. monocytogenes from foods.

MATERIALS AND METHODS

Experiment I.

Preparation of Inoculum. L. monocytogenes LCDC 81-861, an isolate from raw cabbage and a serotype 4b strain, originally isolated by Dr. L. Beuchat, University of Georgia, was obtained from Campbell Institute for Research and Technology, Camden, NJ, courtesy of Dr. Donald L. Zink. This particular strain was chosen because it is a demonstrated pathogen (Conner, Brackett and Beuchat, 1986). The culture was maintained on tryptic soy agar (Difco Laboratories, Detroit, MI) slants at 5°C. The organism was grown in tryptic soy broth (Difco) for 24 hr at 37°C. The bacterial suspension was diluted using 0.85% saline: 0.1 M sodium phosphate buffer (3:1) to adjust the culture to a McFarland 1.0 nephelometer standard (Finegold, Martin and Scott, 1978) which makes the cell density approximately 3×10^8 cells/ml. Subsequent dilutions were made from this adjusted culture to approximate 1×10^6 cells/ml.

Preparation of Liquid Smoke. Four different smoke concentrates (CharSol C-10, Aro-Smoke P-50, Char-Oil Hickory, CharSol PN-9) and one spray dried smoke (CharDex Hickory) were obtained from Red Arrow Products, Manitowoc, WI (see Appendix A). Two different concentrations of smoke (0.5% and 0.25%) were prepared in a 0.85% saline: 0.1 M sodium phosphate buffer (3:1). The pH of the mixture was 5.64.

The diluted liquid smoke preparations (99 ml) were inoculated with 1 ml of the bacterial suspension containing 7.3×10^6 cells/ml

in the 0.5% concentration of smoke and 3.2×10^6 cells/ml in the 0.25% preparation and held at room temperature. The viable cell counts were determined at 0, 4, and 24 hr for the 0.5% liquid smoke preparation and extended to 96 hr for the 0.25% liquid smoke preparation. At each sampling time, a dilution series was made in 0.1% Phytone peptone (BBL, Cockeysville, Maryland) and 0.1 ml of each dilution was surface plated onto duplicate tryptic soy agar plates. Plates were incubated in a 5% CO₂ incubator at 37°C for 24 hr and then counted for viable organisms.

Experiment II.

Preparation of Inoculum. Three different strains of L. monocytogenes were used in this experiment: L. monocytogenes, a serotype 4b derived from ATCC 19115; M1; M2 (both were isolated from raw ground beef). The same procedures for maintenance, growth and adjustment of the bacterial suspensions were followed as described for Experiment I.

Preparation of Liquid Smoke. Two different concentrations of CharSol C-10 (0.5% and 0.25%) were prepared in a 0.85% saline: 0.1 M sodium phosphate buffer (3:1). The pH of the mixture was 5.61.

The diluted liquid smoke preparations (99 ml) were inoculated with 1 ml of the bacterial suspension containing 8.5×10^5 cells/ml, 7.8×10^5 cells/ml, and 6.1×10^5 for strains ATCC 19115, M1 and M2, respectively, in the 0.5% concentration of smoke and 9.5×10^5 cells/ml, 1.0×10^6 cells/ml, and 1.2×10^6 cells/ml, respectively, for these three strains in the 0.25% preparation and held at room

temperature. The viable cell counts were determined at 0 and 4 hr for the 0.5% liquid smoke preparation and extended to include 48 hr for the 0.25% liquid smoke preparation. Enumeration of surviving colonies was done by using the method described in Experiment I.

Experiments III and IV.

Six different strains of L. monocytogenes were used in these experiments: L. monocytogenes, a serotype 4b derived from ATCC 19115; LCDC 81-861, a serotype 4b; M1; M2; M5 (all isolated from raw ground beef); C6 (isolated from raw chicken). Strains M1, M2, M5, and C6 are isolates that were obtained from Campbell Institute for Research and Technology and have not been serotyped. These strains were grown separately on TSA slants for 24 hr at 37°C, then mixed together to allow for more variability by transferring 1 loopful of each strain from the TSA slant into a flask containing 100 ml tryptic soy broth which was then incubated for 24 hr at 37°C. An appropriate volume of the bacterial suspension in TSB (about 1 ml) was transferred to a test tube containing 9 ml of 0.85% saline: 0.1 M sodium phosphate buffer (3:1) pH 7.2 to adjust the culture to a McFarland 1.0 nephelometer standard. Further dilutions were made from this tube in 0.85% saline: 0.1 M sodium phosphate buffer to approximate 1×10^3 cells/ml for Experiment III and 1×10^5 cells/ml for Experiment IV in a total volume of 3 liters. Direct plating was done on TSA plates that indicated the inoculum used had 8.00×10^2 cells/ml in Trial I and 1.13×10^3 cells/ml in Trials II and III for Experiment III and 1.33×10^5 cells/ml in both Trials I and II for Experiment IV.

Inoculation of Beef Franks. Thirty-two beef franks (CFR 319.180 standard of identity for beef franks) were immersed (8 franks at a time) in the inoculum solution, and removed 2 franks at a time, which took 2-4 min. Upon removal from the inoculating solution, the franks were air dried on absorbing paper. One-half of the inoculated franks (16) were dipped in full strength CharSol C-10, then air dried. The remaining franks (16) were immersed in non-inoculated saline-sodium phosphate buffer to serve as a control. Each of the above groups were divided into two samples (8 franks each) in order to test the viable colony forming units, after dipping, at zero time (actually 15 min. had elapsed between air drying and initial sampling) and after 72 hr of storage in vacuum (22 in Hg vac.) at $4 \pm 1^{\circ}\text{C}$. P.51B barrier bags (Viskase, Chicago, IL) were utilized in vacuum packaging the franks. The zero time samples were tested without packaging. Three trials were conducted in Experiment III and two trials in Experiment IV.

The isolation and identification of L. monocytogenes was a modification of the USDA-FSIS procedure (McClain). Each sample (227 g) was blended in 227 ml Listeria enrichment broth (Gibco) and 50 grams of the blended mixture were weighed and suspended in 200 ml Listeria enrichment broth to give a 1:10 dilution. Further dilutions of 1:100, 1:1000, and 1:10000 were made. These tubes were incubated in a 5% CO₂ incubator at 30°C for 24 hr. After 24 hr, 0.1 ml from each tube in each dilution was surface plated onto McBride's Listeria Agar (Difco) and incubated in a 5% CO₂ incubator at 30°C for 48 hr.

Enumeration of L. monocytogenes was done using the 3 tube Most Probable Number procedure (FDA, 1978). The colonies were examined through a transillumination microscope angled at 45°. Plates containing bluish gray colonies which were also found to be Gram-positive and catalase positive were identified as L. monotocytogenes positive plates. No further tests were performed to confirm L. monocytogenes since our purpose in this investigation was not to isolate naturally occurring L. monocytogenes colonies but to be able to distinguish between L. monocytogenes and lactic acid bacteria. Gram staining, catalase reaction and the characteristic bluish gray color of the colonies on selective media (MLA) were sufficient to make this distinction.

RESULTS AND DISCUSSION

Experiment I.

Figure 1 depicts the survival curve for L. monocytogenes LCDC 81-861 in 0.5% liquid smoke preparations. The number of viable cells detected in the control after 4 hrs showed an initial decline in Listeria level that remained unchanged after 24 hrs. The time required for the significant reduction ($P < 0.005$) of microbial numbers below detection level was variable among the different types of liquid smoke used. CharSol C-10, Aro-Smoke P-50, and CharDex Hickory exhibited similar antimicrobial effects by eliminating the viable cell population within 4 hrs. CharSol PN-9 and CharOil Hickory also demonstrated similar but weaker antimicrobial properties by eliminating Listeria numbers within 24 hrs.

Figure 2 represents survival of L. monocytogenes LCDC 81-861 in 0.25% liquid smoke preparations. The control samples again exhibited an initial reduction in the Listeria level within 4 hrs while bacterial numbers then remained largely unchanged after 96 hrs. Within 4 hrs, CharSol C-10 and Aro-Smoke P-50 again demonstrate their effectiveness as strong antimicrobial agents by reducing ($P < 0.005$) the viable cell population to an undetectable level. CharDex Hickory liquid smoke was less effective when its concentration was lowered to 0.25%. At this concentration of CharDex Hickory, 24 hrs were required

Figure 1. Survival curve for *L. monotycoenes* LCDC 81-61 in 0.85% saline: 0.1 M sodium phosphate buffer (pH 5.64) using 0.5% liquid smoke preparations.

Figure 2. Survival curve for *L. monocytogenes* LCDC 81-861 in 0.85% saline: 0.1 M sodium phosphate buffer (pH 5.64) using 0.25% liquid smoke preparations.

for reduction of microbial numbers below detection, whereas at 0.5% concentration, only 4 hours were necessary for the elimination of viable cells. CharSol PN-9 and CharOil Hickory also showed a reduction in their antimicrobial activity when their concentrations were lowered to 0.25% by requiring 48 hrs and 96 hrs, respectively to lower the Listeria level below detection.

Experiment II

Table 1 shows the survival of L. monocytogenes strains ATCC 19115, M1, and M2 in 0.5% CharSol C-10 liquid smoke expressed as cells/ml. The average of duplicate plate counts was then taken and Table 2 expresses survival data as the negative $\log_{10}$ of the survival ratio N_t/N_o , where N is the number of cells at 0 hr and N_o is the number of cells surviving after a given period of time. A less than 1- $\log_{10}$ reduction in viable cells was observed in the controls for all 3 strains after 4 hrs. However, treatment with CharSol C-10 caused a greater ($P < 0.001$) than 5- $\log_{10}$ reduction in cell numbers in the ATCC 19115 and M1 strains, and a 6- $\log_{10}$ reduction in colony forming units in the M2 strain resulting in undetectable levels of the organisms after 4 hrs.

Table 3 represents the survival of L. monocytogenes strains ATCC 19115, M1 and M2 in 0.25% CharSol-10 liquid smoke preparations expressed as cells/ml. Table 4 expresses survival data as the negative $\log_{10}$ of the survival ratio N_t/N_o . The controls for L. monocytogenes strains ATCC 19115, M1, and M2 showed a less than 1- $\log_{10}$ decline in viability after 24 hrs. The control for the L. monocytogenes ATCC 19115 strain which was held up to 48 hrs then

Table 1. SURVIVAL OF L. MONOCYTOGENES AFTER TREATMENT
WITH 0.5% CHAR SOL C-10 LIQUID SMOKE ^a

Strain	Treatment	Time - Hours	
		0	4
		Organisms/ml	
1 ^a	Control	7.00 x 10 ⁵	3.30 x 10 ⁵
		1.00 x 10 ⁶	2.00 x 10 ⁵
	C-10	9.10 x 10 ⁴	0.00
		1.53 x 10 ⁵	0.00
2 ^a	Control	6.80 x 10 ⁵	4.00 x 10 ⁵
		8.70 x 10 ⁵	5.00 x 10 ⁵
	C-10	6.30 x 10 ⁵	0.00
		6.90 x 10 ⁵	0.00
3 ^a	Control	6.30 x 10 ⁵	4.70 x 10 ⁵
		5.90 x 10 ⁵	3.30 x 10 ⁵
	C-10	9.40 x 10 ⁵	0.00
		1.04 x 10 ⁶	0.00

^aStrains of L. monocytogenes: 1. Serotype 4b derived from ATCC 19115;
2. M1 isolated from ground beef; 3. M2 isolated from ground beef.

TABLE 2. EFFECT ON SURVIVAL OF L. MONOCYTOGENES AFTER
TREATMENT WITH 0.5% CHAR SOL C-10 LIQUID SMOKE ^a

Strain	Treatment	Time - Hours	
		0	4
1 ^b	Control	0.00	-0.49
	C-10	0.00	-5.09
2 ^b	Control	0.00	-0.23
	C-10	0.00	-5.83
3 ^b	Control	0.00	-0.18
	C-10	0.00	-6.00

^aExpressed as the negative $\log_{10}$ of the survival ratio N_t/N_o .

^b Strains of L. monocytogenes: 1. Serotype 4b derived from ATCC 19115; 2. M1 isolated from ground beef; 3. M2 isolated from ground beef.

TABLE 3. SURVIVAL OF L. MONOCYTOGENES AFTER TREATMENT
WITH 0.25% CHAR SOL C-10 LIQUID SMOKE ^a

		Time - Hours			
Strain	Treatment	0	4	24	48
		Organisms/ml			
1 ^a	Control	9.90 x 10 ⁵	1.51 x 10 ⁵	1.50 x 10 ⁵	4.40 x 10 ⁴
		9.10 x 10 ⁵	1.52 x 10 ⁵	1.48 x 10 ⁵	4.40 x 10 ⁴
	C-10	1.03 x 10 ⁶	3.60 x 10 ²	4.00 x 10 ¹	0.00
		9.70 x 10 ⁵	4.30 x 10 ²	0.00	0.00
2 ^a	Control	1.23 x 10 ⁶	8.90 x 10 ⁵	8.50 x 10 ⁵	
		7.90 x 10 ⁵	7.30 x 10 ⁵	7.10 x 10 ⁵	
	C-10	7.20 x 10 ⁵	1.35 x 10 ⁴	0.00	
		7.80 x 10 ⁵	1.32 x 10 ⁴	0.00	
3 ^a	Control	1.30 x 10 ⁶	7.30 x 10 ⁵	5.30 x 10 ⁵	
		1.09 x 10 ⁶	6.00 x 10 ⁵	6.50 x 10 ⁵	
	C-10	9.90 x 10 ⁵	6.80 x 10 ³	0.00	
		1.12 x 10 ⁶	7.70 x 10 ³	0.00	

^aStrains of L. monocytogenes: 1. Serotype 4b derived from ATCC 19115; 2. M1 isolated from ground beef; 3. M2 isolated from ground beef.

TABLE 4. EFFECT ON SURVIVAL OF L. MONOCYTOGENES AFTER
TREATMENT WITH 0.25% CHAR SOL C-10 LIQUID SMOKE ^a

Strain	Treatment	Time - Hours			
		0	4	24	48
1 ^b	Control	0.00	-0.80	-0.81	-1.34
	C-10	0.00	-3.40	-4.60	-6.00
2 ^b	Control	0.00	-0.11	-0.12	
	C-10	0.00	-1.75	-5.88	
3 ^b	Control	0.00	-0.25	-0.31	
	C-10	0.00	-2.16	-6.03	

^aExpressed as the negative $\log_{10}$ of the survival ratio N_t/N_o .

^b Strains of L. monocytogenes: 1. Serotype 4b derived from ATCC 19115; 2. M1 strain isolated from ground beef; 3. M2 strain isolated from ground beef.

showed a slightly greater than 1-log reduction in cell numbers. In the 0.25% concentration of CharSol C-10, 24 hrs were required to reduce ($P < 0.001$) Listeria numbers for strains M1 and M2 to an undetectable Table 1 level and 48 hrs for no viable colonies to be formed with strain ATCC 19115, whereas only 4 hrs were required in the 0.5% concentration of CharSol C-10 preparation to reduce cell numbers of all strains to below detection of the organisms. Although strain ATCC 19115 exhibited a greater decline in cell numbers at 4 hrs (3-log_{10}) than strains M1 and M2 (2-log_{10}), at 24 hrs M2 showed a 6-log_{10} reduction in viable cells count, M1 exhibited a greater than 5-log_{10} reduction followed by ATCC 19115 with a 4-log_{10} decline in cell numbers.

Experiment III

In untreated beef franks (no liquid smoke) that had been vacuum packaged and stored at $4 \pm 1^{\circ}\text{C}$, the numbers of L. monocytogenes remaining after dipping did not change after 72 hrs (Table 5) indicating favorable storage conditions for the survival for this organism. On the other hand, the Listeria population decreased more than 99.9% in the liquid smoke treated franks vacuum packaged and stored at $4 \pm 1^{\circ}\text{C}$ for 72 hrs. Furthermore, the CharSol C-10 liquid smoke used in treating the franks in Trials I and II reduced the viable cell counts by more than 60% (at zero time) and in Trial III reduced Listeria numbers below a detectable level.

TABLE 5. SURVIVAL OF L. MONOCYTOGENES^a INOCULATED
 ONTO FRANKFURTER SURFACES AFTER DIPPING IN CHAR SOL C-10
 LIQUID SMOKE^b

Trial No.	Treatment	Time-Hours	
		0	72
I ^c	Control	2.36	2.36
	Smoke	1.56	0.00
II ^d	Control	2.36	2.36
	Smoke	1.96	0.00
III ^d	Control	2.36	2.36
	Smoke	0.00	0.00

^a Strains of L. monocytogenes: 1. LCDC 81-861, a serotype 4b; 2. A serotype 4b derived from ATCC 19115; 3. M1 isolated from ground beef; 4. M2 isolated from ground beef; 5. M5 isolated from ground beef; 6. C6 isolated from chicken.

^b Number of organisms/g expressed as log₁₀ values.

^c The log₁₀ of the number of cells per ml of the inoculum solution was 2.90.

^d The log₁₀ of the number of cells per ml of the inoculum solution was 3.05.

Experiment IV

Trial I showed that in untreated beef franks the numbers of L. monocytogenes surviving after dipping declined by approximately 15% after 72 hrs of storage (Table 6) but in Trial II cell numbers remained virtually unchanged. As in Experiment III, the initial Listeria numbers in smoke treated franks were less than in untreated Table 5 franks in both Trials I and II. Although in Trial I, Listeria numbers remained the same in smoke treated franks after 72 hrs, Trial II showed that the Listeria population in smoke treated franks was reduced to an undetectable level after 72 hrs of refrigerated storage. Experiments III and IV clearly illustrate that dipping in CharSol C-10 liquid smoke significantly ($P < 0.001$) reduces the level of Listeria on beef frank surfaces at both inoculation levels.

TABLE 6. EFFECT OF SURVIVAL ON L. MONOCYTOGENES^a INOCULATED
 ONTO FRANKFURTER SURFACES AFTER DIPPING IN CHAR SOL C-10
 LIQUID SMOKE^b

Trial No.	Treatment	Time-Hours	
		0	72
I	Control	3.96	3.38
	Smoke	2.36	2.36
II	Control	3.36	3.38
	Smoke	2.36	0.00

^aStrains of L. monocytogenes: 1. LCDC 81-861, a serotype 4b; 2. A serotype 4b derived from ATCC 19115; 3. M1 isolated from ground beef; 4. M2 isolated from ground beef; 5. M5 isolated from ground beef; 6. C6 isolated from chicken.

^bNumber of organisms/g expressed as log₁₀ values.

^cThe log₁₀ value of the number of cells per ml of the inoculum solution was 5.12.

CONCLUSION

The continuous decline in the viability of L. monocytogenes, suspended in saline buffer to which 0.25 or 0.5% liquid smoke was added provides strong evidence of the antimicrobial effectiveness of liquid smoke against this bacteria. The four strains of L. monocytogenes (LCDC 81-861, ATCC 19115, M1, and M2) examined in pure culture behaved similarly when exposed to a concentration of 0.5% CharSol C-10 by reducing Listeria numbers to an undetectable level within 4 hours post-treatment. However, at the lower concentration of 0.25% liquid smoke, differences in resistance to the antimicrobial properties of smoke components become evident among these strains indicating that a level of 0.5% liquid smoke is more effective in controlling this organism. In support of this observation, Donnelly, Ziegler and Acton (1982) recommended a level of 0.5% liquid smoke as the optimum to give desirable color and flavor to fermented sausage without altering consumer acceptance. This suggests that liquid smoke can be used as an aid to control the hazard of L. monocytogenes in processed meat products without reducing their acceptability.

Some of the liquid smoke products like CharSol-10 and Aro-Smoke P-50 possess higher antimicrobial activity than the others tested in this study. This is probably due to a higher polar phenol content (Hollenbeck, 1987, personal communication) of these products which would intensify their antimicrobial properties. Since these phenols are water soluble, it is also reasonable to assume that the rate of transfer of these components into the bacterium would be

greater than for those which are oil soluble. As a result, rate of kill of the microbes would be increased.

All liquid smoke products used were effective over time in eliminating L. monocytogenes in pure culture. In addition, CharSol-C-10 demonstrated its effectiveness in controlling L. monocytogenes upon dipping franks in full strength liquid smoke if contamination occurs post peeling.

APPENDIX A**LIQUID SMOKE SPECIFICATIONS**

LIQUID SMOKE SPECIFICATIONS

CharSol C-10

The Product: CharSol is a natural smoke flavoring produced by controlled pyrolysis of hardwoods.

Chemical Properties: Total Acidity (as acetic acid) 10.5 - 12.0%

Smoke Flavor Compounds 13.0 - 16.0 mg/ml

Carbonyls 12.0 - 13.0%

Density 8.9 lb. 1 gal.

Physical Properties: Clear, brown liquid with characteristic hardwood smoke aroma.

Suggested Uses: Use as a substitute for vaporous smoking without violating air quality standards. Excellent as the smoke flavor in meat and poultry products, sauces, cheese, seafood, snacks, and pet foods. Methods of application include direct addition, and surface applications such as spraying, dipping, or vaporization. Recommended usage levels are 2-6 oz/100 lbs of product.

Aro-Smoke P-50

The Product: Aro-Smoke is a natural hickory flavor concentrate in an emulsifier, which is totally soluble in brine or pickling solutions.

Chemical Properties: Total Acidity (as acetic acid) 4.0% max.

Smoke Flavor Compounds 40.0 - 45.0 mg/g

Viscosity (at 70°F) 1000 cp. Max.

Density 9.0 lb/gal

Physical Properties: Clear, brown, viscous liquid with pungent hickory smoke aroma.

Suggested Uses: Use for cured meats by dissolving in brine prior to injection at a level to insure 1-3 oz per hundred weight in the finished cured product. Use in sausage and other formed products by direct addition at a level of 0.5 - 1.5 oz per hundred weight. May also be used in snack foods, dehydrated soups, etc.,* where the addition of excessive water is a disadvantage.

CharOil Hickory

The Product: Char Oil is a vegetable oil extract of a natural hickory smoke flavor solution.

Chemical Properties: Smoke Flavor Compounds 7.3 - 8.1 mg/ml

AOM Stability, Hours 30 plus

Moisture 2% Max.

Density 7.7 lb/gal

Physical Properties: Golden brown oil with a mellow hickory smoke aroma.

Suggested Uses: With its ham-like flavor, CharOil can be used as an effective flavoring in canned meats and fish, sausage, snack foods, meat analogues, bean products and salad oils. Recommended for direct addition application with usage levels of 2-5 oz/100 lb product.

CharSol PN-9

The Product: CharSol PN-9 is a partially neutralized natural smoke flavoring with a food grade emulsifier added.

Chemical Properties: Smoke Flavor Compounds 9-12 mg/ml

pH 5.0 - 5.5

Density 8.9 lg/gal.

Physical Properties: Clear, brown liquid with characteristic hardwood smoke aroma.

Suggested Uses: Use for internal addition to meat emulsions or for meat or food products that cannot tolerate the higher acid concentrations in standard smoke solutions. Recommended usage levels are 2-6 oz/100 lbs of product.

CharDex Hickory

The Product: CharDex Hickory is a spray-dried natural smoke flavoring produced by combining malto-dextrin with CharSol Hickory.

Chemical Properties: Moisture 7.0 - 10.0%

Total Acidity (as acetic acid) 6.0 - 8.5%

Smoke Flavor Compounds 16.0 - 20.0 mg/g

Bulk Density .50 g/cc

Physical Properties: Golden brown powder. Passes through 40 mesh screen.

Suggested Uses: The bland nature of the carrier makes CharDex an excellent replacement for aqueous smoke in formulation that cannot tolerate added moisture such as sausage. Other uses include pet foods, dry seasoning blends, gravies, soups and nut meats. Recommended usage level are 1-5 oz/100 lb of product.

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