ANTIMICROBIAL PROPERTIES OF LIQUID SMOKE FRACTIONS

by

PAUL JESSE MILLY

(Under the Direction of Romeo T. Toledo)

ABSTRACT

Liquid smoke can be utilized for their antimicrobial properties against a variety of Gram positive and Gram negative bacteria, yeast and molds. Liquid smoke prolongs the lag phase of certain Gram positive and Gram negative bacteria and yeasts and decreases the rate of mold growth. Smoke extract F1 (overall MIC $\geq$ 1.5%) demonstrated the most versatile antimicrobial potential for industrial applications out of all the liquid smoke fractions tested. Liquid smoke condensates offer RTE meat processors a valuable option for complying with USDA/FSIS final rule (68 FR 34207) of employing a "post-pasteurization process." Extracts F1, F2, and F3 demonstrated an ability to inhibit the growth and destroy *Listeria innocua* M1 in food systems. Utilizing liquid smoke in processing RTE meat products offers the processor a means of guaranteeing product safety and complying with rules addressing environmental contamination from *Listeria monocytogenes*.

INDEX WORDS: *Listeria monocytogenes*, minimum inhibitory concentration, liquid smoke, *Listeria innocua*

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PAUL JESSE MILLY

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PAUL JESSE MILLY

Major Professor:	Romeo T. Toledo
Committee:	Jinru Chen William Kerr

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
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DEDICATION

I am dedicating this work to above all GOD. He has given me strength and will power required to pursue my goals in life. Without Him, I would be lost.

I would also like to dedicate all my accomplishments to my mother and father, Patricia Ann Melvin-Milly and Donald Francis Milly, respectively. Without their Love, friendship, advice, tolerance, patience, humor, strength and support, I would have nothing to offer this world. Everything that I am, I owe to them and their Love for each other and our family. I must never forget my two sisters, Janine Ann and Jenna Lyn, they will always be my reminders of how important family is. I Love you all.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Purpose of the Study

On June 6, 2003 USDA/FSIS published a final rule (68 FR 34207) amending previous regulations on ready-to-eat (RTE) meat and poultry products. The new rule mandates that RTE producers prevent product adulteration from the pathogenic environmental contaminant *Listeria monocytogenes.* Two of three new proposals for processing protocols to ensure the safety of RTE meat products involve the incorporation of antimicrobials alone or in conjunction with a "post-process pasteurization" treatment. For this reason, there is interest from both the ingredient suppliers and processors to find suitable antimicrobial agents. Depending upon the product and the target consumer market, chemical antimicrobial agents declared in the product label may have negative connotations. Thus, there is a need for different antimicrobial agents that processors can use to differentiate their product from others in the market. The purpose of this study was to explore the antimicrobial properties of several commercial liquid smoke fractions in a model system of microbiological media and in actual processed meat products. This research was directed towards the potential use of commercial liquid smoke condensate fractions as an antimicrobial in RTE meat products.

1.2 History of Smoking Foods

Incidental exposure to smoke from the pyrolysis of wood(s) has aided humans in the preservation of meats, fish, and hides for more than 1,000 years. In fact, smoke

tanned leather artifacts were found near Innsbruck, Austria in September of 1991. The leather artifacts were accompanying the 5300 year-old body of the suspected 'Ice Man.' The artifacts are believed to have been tanned from their exposure to open wood fires used to cook foods (Spindler, 1994).

Primitive conditioning methods used to extend the perishable nature of foods like meat and fish included salting, drying, and smoking (Barylco-Pikielna, 1977). The mechanisms of preservation associated with such methods must have had a profound effect on the way man began to develop ways of preparing large amounts of food for future consumption. These timeless mechanisms have carried over to modern methods of processing and preserving foods as well.

1.3 Traditional and Conventional Uses

Traditional methods of smoking foods involve fairly simple equipment based upon easily obtainable sensory properties. The traditional equipment used for smoking foods usually consisted of a smoker where pyrolysis of wood was induced. The smoker was loaded with wood that was burned and the resulting smoke was channeled in a direction so that direct contact with the food could be obtained. These traditional goals of smoking foods were to impart and develop desirable sensory (flavor, aroma, and appearance) properties a well as rendering the food product safe to eat (Fessmann, 1995). Traditional methods are preformed with either hot or cold smoking techniques. Hot smoking is executed at temperatures in the range of 55 to 80°C, and depending on the size and type of meat, requires a much shorter time of exposure to the smoke to obtain sought-after flavor and aroma. Hot smoking also leaves the food product in a state of being cooked due to the temperature of the smoke. Cold smoking is executed at

temperatures in the range of 15 to 25°C, and depending on the size and type of meat, requires a much longer time of exposure to the smoke to obtain desired flavor and aroma. In contrast with hot smoking, which can take only hours to properly cook something, cold smoking requires an extended amount of exposure time, and even then the product should be considered not properly cooked. Differences in hot and cold smoking methods can affect the sensory qualities of certain foods and have been well documented (Maga, 1988).

Conventional means of smoking foods have become more technologically advanced in the past few decades. Methods for smoking foods rely on new advances in smoke generation technology and application advantages. Cold smoking that utilizes smoke condensates have become the prominent means for applying smoke to foods (Fessmann, 1995; Sunen, 1988; Sunen, et al., 2001; Sunen, et al., 2003). Additional application technology allows for alternative means for smoking such as glimmer smoke, liquid smoke, friction smoke, wet smoke, and smoke chambers which can accommodate both batch and (semi) continuous flow systems in conjunction with computerized controls. Also, new smoke generation systems are mandated to employ equipment or scrubbers to aid in the cleaning of smoke, which strengthens its case for an environmentally friendly processing technology (Fessmann, 1995).

1.4 Advantages and Benefits

As mentioned before, new advances in smoke generation technology and application alternatives have increased the positive benefits of cold smoking foods. Advantages and benefits associated with smoking foods include environmentally-friendly application techniques, antioxidant potential, sensory control, and antimicrobial

properties. Smoke condensates are becoming more widely used compared to gaseous smoke because of better process control of drenching, dipping, or gaseous smoke regeneration to impart consistent quantitative and qualitative attributes of smoke flavor and color. Use of condensates in smoke applicators allows the processor to control the concentration of smoke being applied more readily than generating gaseous smoke *in situ* (Sunen, et al., 2001).

Antioxidant activity of smoke condensates has been extensively documented and results indicate potential to retard lipid oxidation in many meat products (Estrada-Munoz, et al., 1998; Maga, 1988).

Control of sensory properties of smoke has also been widely researched and documented. In fact, an entire lexicon of new descriptive language has been proposed to describe and evaluate commercial smoke condensate flavor profiles (Ojeda, 2002).

Antimicrobial properties of smoke condensates add to the advantages and benefits associated with the smoking of foods. Antimicrobial properties of smoke in foods have been documented comprehensively throughout the last fifty years, but most of this work has been on components of naturally deposited smoke (Estrada-Munoz, et al., 1998; Maga, 1988; Painter, 1998; Sunen, 1988; Sunen, et al., 2001; Sunen, et al., 2003). To add value to smoke, the industry has embarked on a program of fractionating smoke and determining difference in the functional (flavor, color, antimicrobial, protein cross-linking) attributes of the different smoke fractions. There has been no work published on antimicrobial properties of fractions of condensed wood smoke.

1.5 USDA/FSIS Regulations

New regulations set by the USDA, FSIS Pathogen Reduction: Hazard Analysis and Critical Control Point System (final rule 68 FR 34207) require that processors of ready-to-eat (RTE) meat be subjected to validating their process for the control of pathogenic bacteria starting October 2003. Currently, any RTE products that are found contaminated with *Salmonella* species, *E. coli* O157:H7, *L. monocytogenes*, or staphylococcal enterotoxin is deemed adulterated. Two of the three new alternative processing methods proposed by the FSIS for controlling *L. monocytogenes* involve the addition to/or treating the product with an antimicrobial alone or in conjunction with a post-thermal treatment. Liquid smoke offers a solution to finding the appropriate antimicrobial agent which fits the processor's concerns. Antimicrobial efficacy along with application flexibility, antioxidant capabilities, and quality enhancing properties such as flavor and appearance validates, if not encourages the implementation of liquid smoke products into one's RTE processing scheme.

Title 9 of the Coder of Federal Regulations sections 318.7 (c) (4) and 381.147 (f) (4) list smoke flavorings and artificial smoke flavorings as having expressed authorized intended use for meat and poultry products. When smoke flavorings are produced under good manufacturing conditions, FDA has approved their status to be considered Generally Regarded as Safe (GRAS) under the food additive provisions of the Federal Food, Drug and Cosmetic Act (FFDCA).

1.6 Wood Composition

Smoke is induced by the thermal degradation or pyrolysis of wood. Wood used for smoking can be categorized into two groups, commonly known as hardwoods and

softwoods. As one may suspect, there are compositional and organoleptic characteristic differences between the two smokes produced by pyrolysis of hardwoods and softwoods. For example, softwood smoke contains more resin acids and other organic-solvent soluble extractives when compared to that of hardwood. These subtle differences give rise to different compositional characteristics of smoke produced by wood pyrolysis. The most commonly used woods for smoking foods are hardwoods, like hickory, mesquite, oak and maple (Maga, 1988).

In general, wood is made up of three major components. As seen in Figure 1.1, structures of cellulose, hemicellulose, and lignin can be viewed. About 40 to 45 percent of the total dry weight of normal wood tissue is cellulose. Cellulose is a linear, organic, long-chain polymer glucan made up of anhydroglucopyranose or glucose units bonded by a β-(1→4) glycosidic bond (Horton, et al., 1996). Glucose units range from about 9,000 to 15,000 units in length depending upon species of wood. Figure 1.2

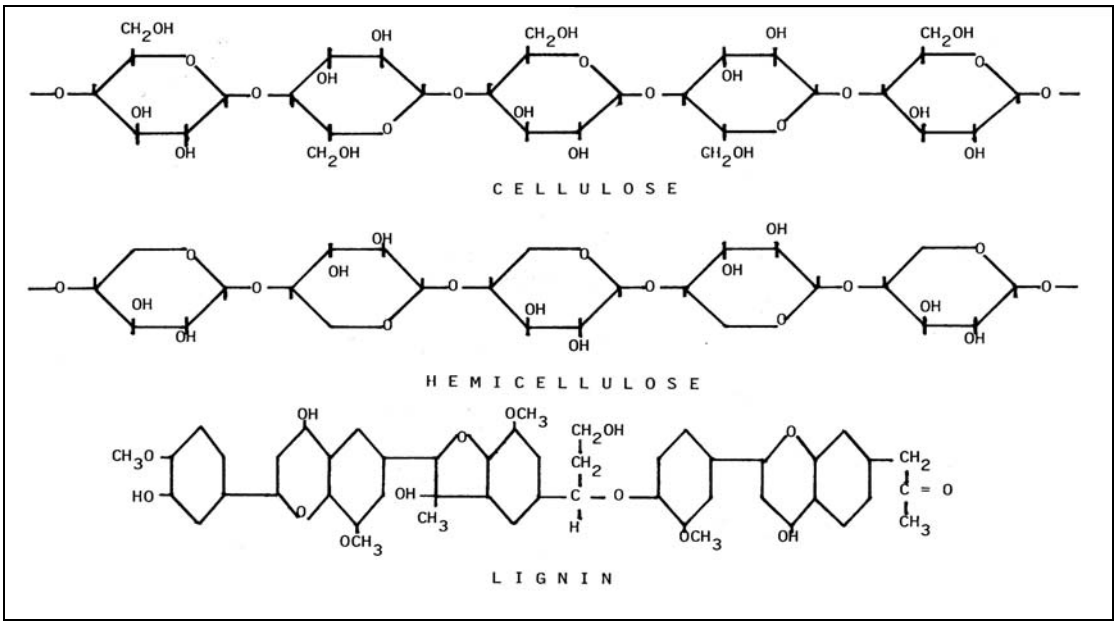

Figure 1.1- Structural illustration of the major components in wood (adapted from Maga, 1988).

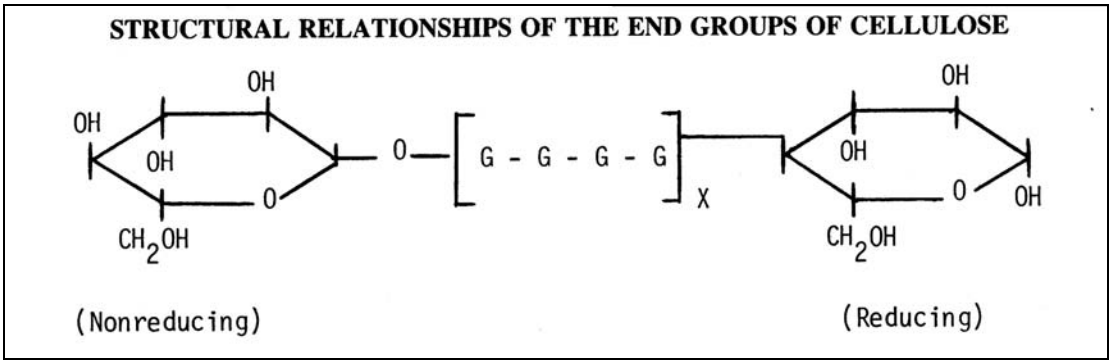

Figure 1.2- End group illustration of cellulose polymer (adapted from Maga, 1988).

illustrates how each polymer of cellulose begins with a reducing hemiacetyl group and is terminated with a nonreducing (or extra hydroxyl group) end group. Cotton is an example of fiber that is almost entirely made up of cellulose (Maga, 1988).

The second major component of wood, hemicellulose, comprises approximately 20 to 35 percent of total dry weight of normal wood. Hemicellulose can be branched and consists of more than one type of polysaccharide. Figure 1.3 illustrates some of the saccharides found in hemicellulose. It can be thought of as a mixture of glucose, galactose, mannose, xylose, rhamnose, arainose, 4-*O*-methylglucuronic, and galacturonic acid residues. Xylans, mannans, glucans, and galactans typically make up the chemical divisions of hemicellulose (Maga, 1988).

Lignin is the third major component of wood and can be found in amounts of 18 to 38 percent in mature wood. Hydroxycinnamyl alcohols *p*-coumaryl-, coniferyl-, and sinapyl alcohol structures can be seen in Figure 1.4. These alcohols, when dehydrogenated, form a copolycondensate that can be classified as phenolic-based compounds with a number of possible combinations. Ether bonds (phenolic ether and

dialkyl ether) and carbon-carbon (5,5[1], β-[5], β-β[1], and α-α[1]) allow linkages to be formed between the alcohols. Hardwoods usually consist of the major class of lignin known as guaiacyl-syringyl lignin (Maga, 1988).

Remaining wood composition can be generically grouped together into a term known as resin. Resin is more of a physical condition rather than a chemical classification. Resins usually consist of terpenes, lignans, stilbenes, flavonoids and aromatic compounds which are more commonly found in softwoods (Maga, 1988).

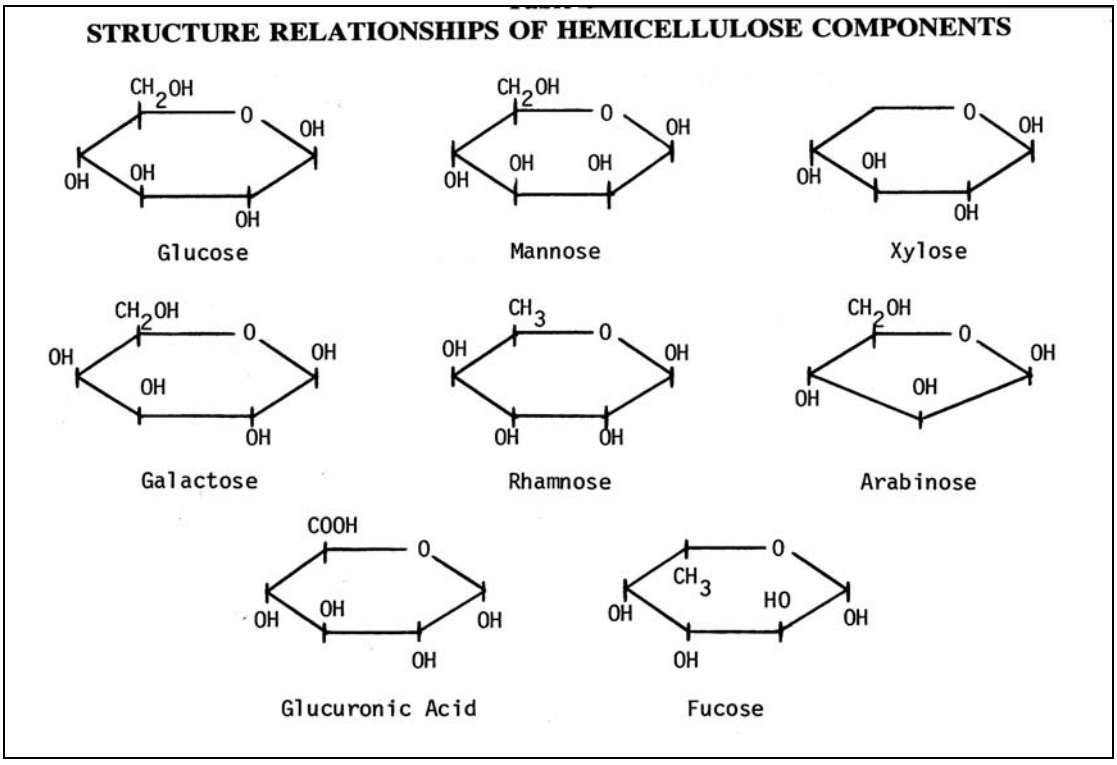

Figure 1.3- Structural illustrations of hemicellulose components (adapted from Maga, 1988).

Volatile oils, terpenes and related compounds, fatty acids, carbohydrates polyhydric alcohols, nitrogen compounds, phenolic compounds and inorganic constituents are also found in wood at varying concentrations depending upon the species

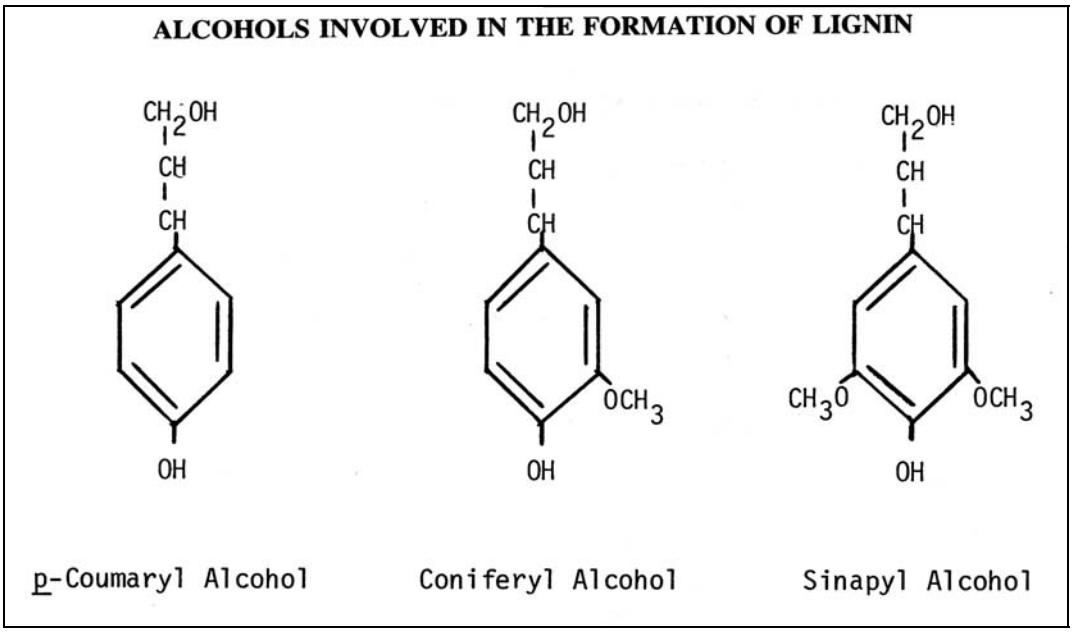

Figure 1.4- Structural illustrations of the alcoholic precursors to the formation of lignin (adapted from Maga, 1988).

of wood, climate, and time of harvest. Because of the relevance associated with phenolic compounds and their documented antimicrobial properties, a list of common phenolic compounds found in wood is listed in Table 1.1.

1.7 Wood Pyrolysis and Products

The amount of water vapor existing during wood pyrolysis, the oxygen concentration, wood composition, and most importantly, temperature all influence the products of thermal reactions in wood. Table 1.2 allows one to map the degradation of the major wood components in relation to temperature increase. As temperatures rise to approximately 170°C, the wood experiences drying and loss of water. Acetic acid is usually formed at these low temperatures. When the temperature reaches a range of about 200 to 260°C, hemicellulose is the first of the three major components to decompose. At this temperature range, both acetic and formic acid begin to from. Generally speaking, decomposition of hemicellulose produces furans, furan

Table 1.1- Common phenolic-based compounds in wood (adapted form Maga, 1988).

PHENOLIC-BASED COMPOUNDS IDENTIFIED IN WOOD

Phthalic acid	Chrysin	Tannins
Piperonylic acid	Morin	Maclurin
Gallic acid	Fisetin	Cotoin
Ellagic acid	Robinetin	Anibine
p-Hydroxybenzoic acid	Quercetin	Euxanthone
Vanillic acid	Kaempferol	Brazilin
Syringic acid	Pinocembrin	Haematin
Ferulic acid	Naringenin	Lapachol
Vanillin	Taxifolin	Desoxysantalin
Syringaldehyde	Aromadendrin	Chrysarobin
Coniferin	Dihydromorin	Tectoquinone
Syringin	Dihydrorobinetin	
Matairlesinol	Pinistrobin	
Lariciresinol	Pinobanksin	
Pinoresinol	Stropopinin	
Conidendrin	Cryptostrobin	
Secoisolariciresinol	Strobobanksin	
Livoviol	Tectochysin	
Oxomatairesinol	Afzelin	
Hydroxymatairesinol	Ayanin	
Allo-hydroxymatairesinol	Santal	
Pinosylvin	Prunetin	
4-Hydroxystilbene	Muningin	
4-Methoxystilbene	Podospicatin	
2,4,3′,5′-Tetrahydroxystilbene	Pterocarpin	
Okanin	Homopterocarpin	
Isookanin	Catechin	
Neoplathymenin	Leucofisentinidin	

Table 1.2- Degradation of wood components and thermal ranges (adapted from Maga, 1988).

INFLUENCE OF TEMPERATURE ON THE THERMAL DESTRUCTION OF WOOD

Temperature (C°)	Reaction
Up to 170	Loss of water, drying
200—260	Decomposition of hemicellulose
260—310	Decomposition of cellulose
310—500	Decomposition of lignin
Above 500	Secondary reactions including oxidation, polymerization, condensation, and pyrolysis

derivatives,and a chain of aliphatic carboxylic acids. Figure 1.5 illustrates the products of the degradation of hemicellulose. Because hardwoods contain pentose-based hemicellulose, compared to hexosan-based softwoods, pyrolysis of hardwoods produce more acids than softwoods. Cellulose is the next component to experience decomposition at temperature ranges of 260 to 310°C. Cellulose can undergo two different thermally induced pathways of decomposition. One pathway is prevalent at lower temperatures, while the other pathway is more common at temperatures higher than 300°C. The lower temperature decomposition is characterized as having three distinct steps; the commencement of pyrolysis, propagation, and end product formation. This low temperature reaction produces char, carbon monoxide, carbon dioxide, and water. At temperatures above 300°C, decomposition entails cleavage of molecules by fission, transglycosylation, and reactions producing an array of tarry anhydro sugars and volatile compounds. Figure 1.6 demonstrates how intermediate anhydro sugars are formed from

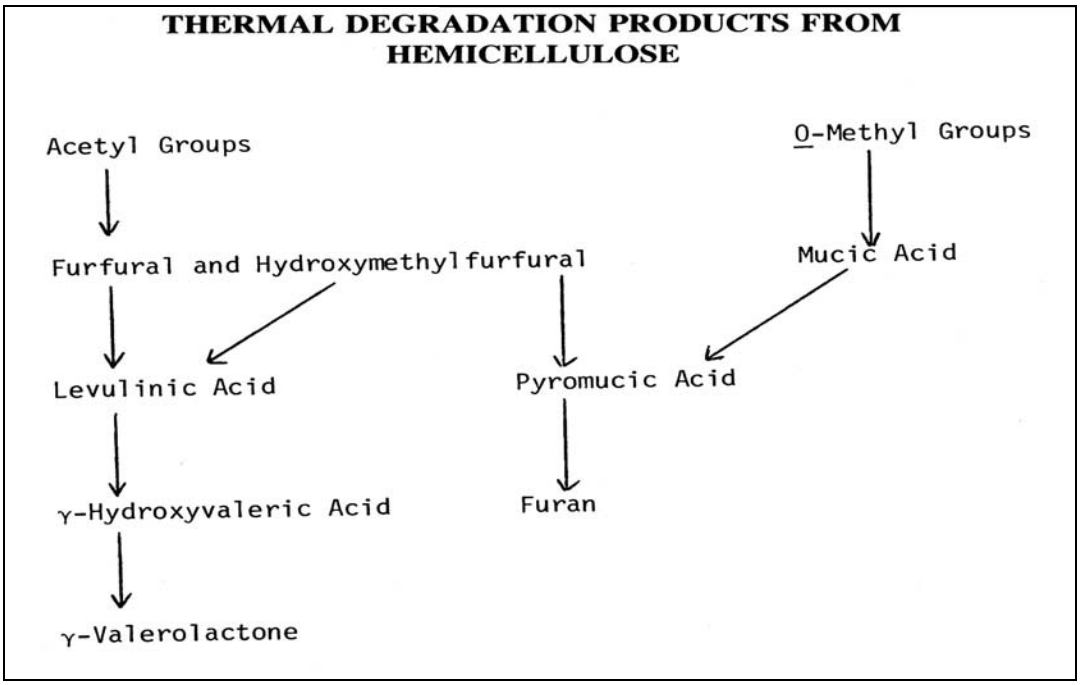

Figure 1.5- Product formation from the degradation of hemicellulose (adapted from Maga, 1988).

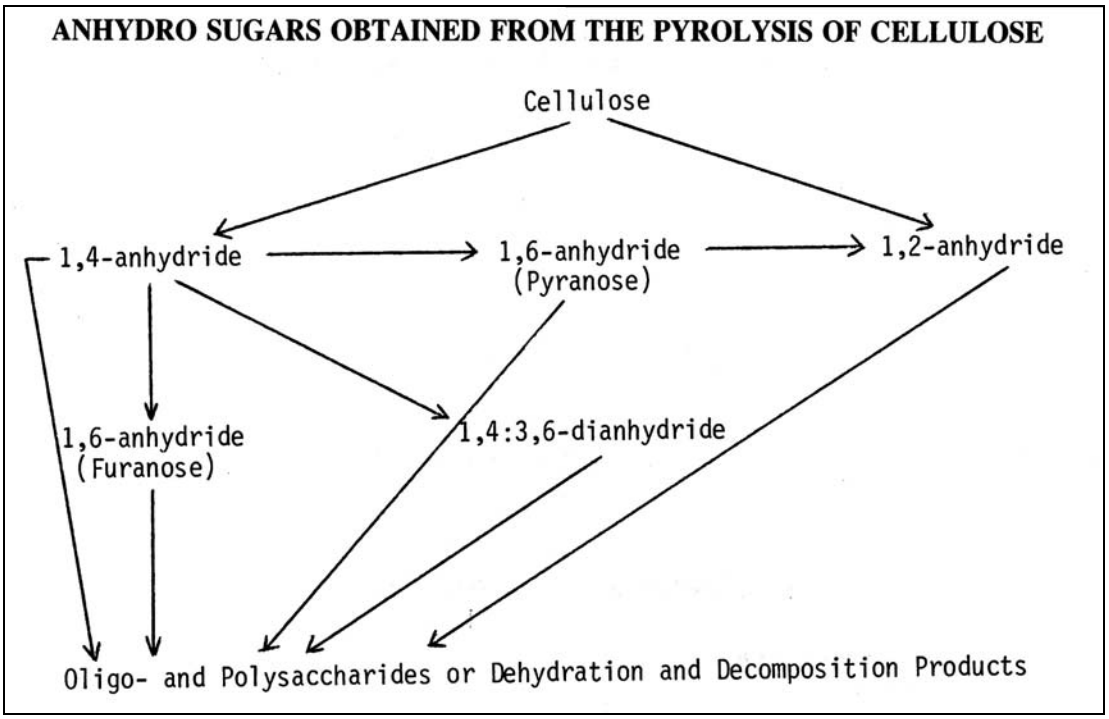

Figure 1.6- Formation of anhydro sugars from the pyrolysis of cellulose (adapted from Maga, 1988).

pyrolysis of cellulose. The main anhydro sugar formed is known as levoglucosan, and constitutes approximately 50% of the anhydro sugars formed. The dehydration of levoglucosan, monosaccharides, and oligosaccharides, followed by fission of these sugars results in the formation of abundant carbonyl compounds. Further heating can produce a number of different compounds. For example, 86 different compounds from cellulose pyrolysis have been isolated and grouped together to make up 12 phenolics, 16 aliphatic and cyclic hydrocarbons, 12 aromatics, 25 ketones, 8 aliphatic and cyclic alcohols, aldehydes, esters, and 13 furans (Maga, 1988). Table 1.3 and Figure 1.7 summarize the products formed from cellulose pyrolysis and the flow diagram of cellulose pyrolysis, respectively.

Table 1.3- Products of cellulose pyrolysis (adapted from Maga, 1988).

PYROLYSIS PRODUCTS (600°C) OF CELLULOSE

Compound	Relative %
Acetaldehyde	2.3
Furan	1.6
Acetone/propionaldehyde	1.5
Propenal	3.2
Methanol	2.1
2,3-Butanedione	2.0
1-Hydroxy-2-propanone	2.1
Glyoxal	2.2
Acetic acid	6.7
2-Furaldehyde	1.1
Formic acid	0.9
5-Methyl-2-furaldehyde	0.7
2-Furfuryl alcohol	0.5
Carbon dioxide	12.0
Water	18.0
Char	15.0
Tar	28.0

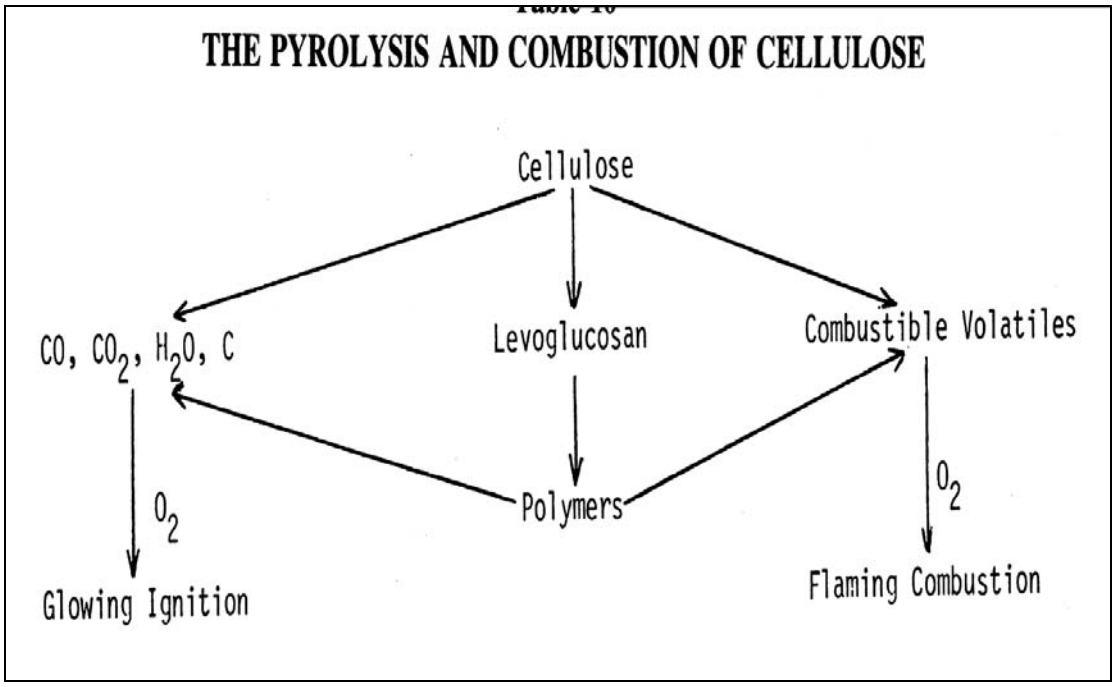

Figure 1.7- Pyrolysis of cellulose (adapted from Maga, 1988).

Lignin is the last of the three major wood components to undergo decomposition at temperature ranges of about 310 to 500°C. This step in the pyrolysis of wood is quite important from a flavor standpoint. Combustion of lignin is associated with the generation of phenols and phenolic esters (guaiacol and syringol). Also, an array of compounds that occupy methyl, ethyl, propyl, vinyl, allyl, and propnyl sidechains arise. As ether linkages, pyran rings, and heterocyclic furans in lignin undertake fission, guaiacol is generated which in turn become degraded into phenols and cresols. Figure 1.8 depicts the products generated from the pyrolysis of lignin (Maga, 1988).

Overall, the most significant classes of chemical compounds found in liquid smoke are phenols, carbonyls, acids, furans, alcohols and esters, lactones, and polycyclic aromatic hydrocarbons (PAH's) (Hamm, 1977).

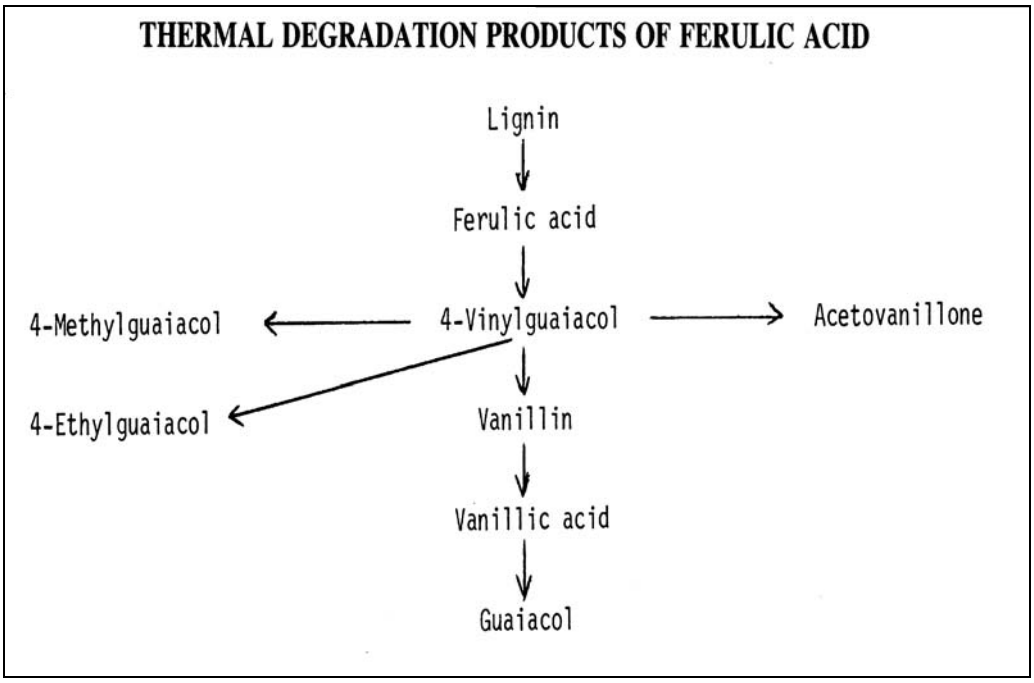

Figure 1.8- Product generation from the pyrolysis of lignin (adapted from Maga, 1988).

1.8 Liquid Smoke Generation

There are several methods for generating smoke to be applied to foods. Some methods include smoldering, friction, wet smoke or condensate method, fluidization, modified fluidization (two stage), carbonization, electrostatic smoking, and a few other technologies. One of these miscellaneous technologies is known as liquid smoke generation.

Some of the advantages of liquid smoke use in food processing include: (1) flavor can be integrated uniformly into the product rather than only on the surface, (2) flavor intensity can be manipulated, (3) processors have more control on intended flavor profile, (4) fractionation can be done more readily and harmful components of the condensate (PAH's) can be removed, (5) diversified application to products not customarily smoked,

(6) consumer friendly with respect to home use, (7) environmentally friendly, (8) wide options on application of the smoke such as: dipping, spraying, and integrated mixing.

Liquid smoke can be formed by dissolving a smoke condensate into water, oil, or some organic solvents. The smoke condensate can also be absorbed onto solids like spices, sugars, and proteins to create dry or powdered forms. Condensates that are dissolved in water may result in polymerized products, tarry products, and PAH's settling out over time, changing the smoke solution color to a light yellow. However, if certain solvents are present such as ethanol or glycol, the polymerized products, tarry products, and PAH's will not precipitate out of solution. Table 1.4 summarizes the composition of commercial liquid smokes.

Table 1.4- Typical composition of commercial liquid smoke condensates (adapted from Maga, 1988).

COMPOSITION OF COMMERCIAL LIQUID SMOKES

Fraction	%
Water	11—92
Phenols	0.2—2.9
Acids	2.8—9.5
Carbonyls	2.6—4.6
Tar	1—17

1.9 Antioxidant Properties of Wood Smoke

Research shows that wood smoke condensates possess antioxidant properties (Maga, 1988). The capability of smoke condensates to hinder lipid oxidation in meat products has also been reported. Attempts to isolate the active compounds in liquid smoke that possess antioxidant properties (Toth and Potthast, 1984) resulted in the observation that when liquid smoke is fractionated into its acidic, neutral, and basic components, it is the neutral portion that exhibits the highest antioxidant activity. Since a majority of the phenolics in liquid smoke is contained in the neutral portion, these results indicate that phenolics are the source of the antioxidant activity in smoke.

Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are some of the U.S.'s most popular synthetic antioxidants and they are also phenolic in nature. Moreover, phenolic compounds which have very high boiling points are also found in liquid smokes and they seem to posses the highest antioxidant activity. Some of these phenolic compounds include: 2,6-dimethoxyphenol, 2,6-dimethoxy-4-methylphenol, and 2,6-dimethoxy-4-ethylphenol. Table 1.5 gives a list of proven antioxidant compounds found in liquid smoke.

Table 1.5- Antioxidant compounds found in liquid smoke (adapted from Maga, 1988).

SMOKE-RELATED PHENOLIC COMPOUNDS WITH PROVEN ANTIOXIDATIVE PROPERTIES
Pyrocatechol
Hydroquinone
Guaiacol
Eugenol
Isoeugenol
Vanillin
Salicylaldehyde
2-Hydroxybenzoic acid
4-Hydroxybenzoic acid

1.10 Antimicrobial Properties of Smoke

Several publications document the antimicrobial activity of smoke condensates (Estrada-Munoz, et al., 1998; Maga, 1988; Painter, 1998; Sunen, 1988; Sunen, et al., 2001; Sunen, et al., 2003). Research proved the difficulty of identifying the mechanism and compounds responsible for microbial inhibition (Maga, 1988). Different species of organisms behave differently and can show signs of varying susceptibility within differing strains of the same organism when treated with smoke condensates. Thus, effectiveness of antimicrobial capability of liquid smoke must be assessed using different species and strains of microorganisms. Moreover, some research suggests that smoke condensates have no antimicrobial potential at all. This again suggests that relative effectiveness may be due to differences in composition of the smoke condensates. Other research proves that determining synergistic effects amongst smoke condensates can be equally difficult. Therefore, the efficacy of smoke condensates with regard to antimicrobial potential depends on the concentration of phenols, carbonyls, and organic acids and the test microorganism.

The amount of phenols present in commercial liquid smoke condensates has been reported to be approximately 0.2 to 2.9 percent (Maga, 1988). Naturally occurring phenols in foods are classified as simple phenols, flavonoids, hydroxycinnamic acid and derivatives, and phenolic acids. Cytoplasmic membranes are disturbed by phenolic compounds and cause the intracellular fluids in microorganism to leak (Davidson, 1997). Wood smoke condensates have been deemed one of the few practical sources of natural phenolic compounds useful in the preservation of foods. They mainly contribute to the smoke flavor profiles, antioxidant activity, and antimicrobial properties.

Reports show that carbonyls have been reported to materialize in amounts of approximately 2.6 to 4.6 percent in commercial liquid smoke condensates (Maga, 1988). The role of carbonyls as an antimicrobial has limited documentation. However, their efficacy when in the form of smoke condensates can be inferred based on the 133 different aldehydes and ketones present in smoke. Of these carbonyls, formaldehyde and acrolein have proven toxicity against microorganisms. Carbonyls inhibit microbial growth by penetrating the cell wall and inactivating enzymes located in the cytoplasm and the cytoplasmic membrane.

Carbonyls also utilize three means (Type A, B, and C) to inhibit growth of microorganisms through passive inhibition by nutrient deprivation. Type A inhibition is thought to "sequester" low molecular weight nutrients in the growth medium, ultimately lowering the effective concentration available for the microorganism. Additional carbonyl compounds chelate with essential, multivalent metal cations to form stable complexes. Some of the carbonyls include: α-keto-carboxylic acids, 3-hydroxyketones, 1,3-diones, and enediols. Type B inhibition works by inactivating or immobilizing exocellular enzymes secreted by saprogenic bacteria and molds. These enzymes are needed to break down polymerized substrates like proteins and glycans. Carbonyls inhibit the depolymerisation of these polymers through inactivating secreted enzymes or isolating them away from the substrates. This ultimately deprives the microorganism of the essential amino acids and sugars needed for normal metabolism. Type C inhibition involves the direct modification of the polymeric substrates that the depolymerases are attempting to break down. The carbonyls modify the polymers so as to leave them in a less available or vulnerable state for the enzymes to act upon (Painter, 1998).

Organic acids constitute up to 2.8 to 9.5 percent of commercial smoke condensates. Of the reported organic acids found in smoke condensates, acetic acid, propionic acid, and benzoic acid are credited with possessing the most antimicrobial potential (Davidson, 1997). The antimicrobial potential of organic acids is accredited to the influence on overall pH and the undissociated form of the acid. The cell membrane lipid bilayer can be easily penetrated by organic acids in their undissociated forms. Because the pH inside the cell is higher than the exterior, the acid is highly dissociated inside the cell. Once dissociated inside the cell, the cell will deplete all of its ATP reserve energy transporting the dissociated protons out of the cell. This leaves the cell unable to perform essential metabolic pathways needed to sustain life (Davidson, 1997).

Phenols, carbonyls, and organic acids significantly contribute to smoke condensates' antimicrobial potential.

1.11 Microorganisms Used and Characteristics

The present study utilized several microorganisms that are commonly associated with food spoilage and safety concerns in the food industry. Gram positive and Gram negative bacteria, yeast and mold were exposed to several commercial smoke condensates and the minimum inhibitory concentration (MIC) of the smoke for the respective microorganisms were determined. The Gram negative organisms used in this experiment were *Escherichia coli* 8677*, Pseudomonas putida, Salmonella* Muenster*, Salmonella* Seftenburg*, and Salmonella* Typhimurium. The Gram positive organisms were *Lactobacillus plantarum* and *Listeria innocua* M1. The yeast and mold used in this experiment were *Saccharomyces cerevisiae* and *Aspergillus niger*, respectively.

A Gram positive cell is defined as a prokaryotic cell whose cell wall is made up of 90 percent peptidoglycan (or murein). In contrast, a Gram negative cell is defined as a prokaryotic cell whose cell wall consists of only 10 percent peptidoglycan, and is surrounded by an outer membrane made up of lipoprotein, lipopolysaccharide, and other intricate macromolecules. A prokaryotic cell is defined as a cell that lacks a membrane-encapsulated nucleus and is characterized as usually having a single circular DNA molecule as its chromosomes (Brock, et al., 1997a).

In contrast to prokaryotic cells, fungi (yeast, mold, and mushrooms) are classified as eukaryotes. Eukaryotic classified cells contain a membrane-enclosed nucleus along with other major organelles like vacuoles and mitochondria. Fungi cell walls are mainly made up of chitin. Chitin is a common building block in fungal cell walls. Usually, fungal cell walls are made up of 80 to 90 percent polysaccharide, mixed in with proteins, polyphosphates, lipids, and inorganic ions. Chemoorganotrophs are organisms that utilize organic compounds for energy sources, and all fungi fall under this classification. Molds are considered filamentous fungi and yeasts are classified as unicellular fungi. Overall, yeast cells are much larger than that of bacteria. Often the most effective way of differentiating between fungi species is based upon morphological properties and sexual reproduction cycles (Brock, et al., 1997b).

1.12 *Escherichia coli*

Escherichia coli 8677 is one of the five Gram-negative microorganisms evaluated in this experiment. *E. coli* 8677 is a nonpathogenic form of *E. coli*, and is used as an indicator organism for testing effective means of reducing, eliminating, and or removing *E. coli*. *E. coli spp*. inhabit the intestinal tract of humans and warm blooded animals, and

are a part of the standard facultative anaerobic microflora found within the intestines. *Escherichia* species belong to the family *Enterobacteriaceae.* They are characterized as being enteric bacteria, which falls into the phylogenetic phylum of Proteobacteria. This group is classified as having several unique characteristics such as: Gram negative straight rods, motile by body-covering flagella (or nonmotile), nonsporulating, facultative aerobes, acid producing from glucose, catalase-positive, oxidase-negative, optimal growth at 37°C, and sodium is neither required nor stimulatory (Doyle, et al., 1997). Differences among the surface antigens allow for serological classification between species of *E. coli.* The surface antigens are as such: the O (somatic), H (flagella), and K (capsule) antigens. Certain strains of *E. coli* can cause diarrheal illnesses and is grouped together depending on virulence characteristics, clinical set of symptoms, method of pathogenicity, and specific O:H serogroups. The most notable of the categories is that of the enterohemorrhagic *E. coli* strains (EHEC). *E. coli* O157:H7 is the most predominant cause of EHEC-related disease in the United States. Other categories of *E. coli* strains include: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), diffuse-adhering *E. coli* (DAEC), and enteroaggregative *E. coli* (EAggEC). A food contaminated with *E. coli* entails possible risk of being contaminated with other enteric pathogenic bacteria (Doyle, et al., 1997). Common foods contaminated with *E. coli spp*. are meat and poultry (beef, chicken, and pork), fruits and vegetables, dairy products, as well as water supplies used in plants and farms (Jackson, et al., 1997; Jay, 1998c).

1.13 *Pseudomonas putida*

P. putida is one of the five Gram negative microorganisms evaluated in this experiment. *P. putida* is a nonpathogenic species of the group *Pseudomonas*. Some species of *Pseudomonas* can be considered pathogenic; however, *P. putida* is not. This group is classified as having several unique characteristics such as: Gram negative straight or curved rods but not vibrioid, no spores, polar flagella, no sheaths or buds, respiratory metabolism, never fermentative (although is capable of aerobically generating small amounts of acid from glucose), utilizes low molecular weight organic compounds (not polymers), some are chemolithotrophic (using only H_2 or CO as exclusive electron donor), optimal growth at 25°C, always oxidase positive (except for enteric forms), and always catalase positive (Brock, et al., 1997e). *P. putida* concerns commonly arise from spoilage problems associated with the organism. Common foods contaminated with *P. putida* species are fresh meat, poultry, seafood, fruits and vegetables, milk and water (Jackson, et al., 1997; Jay, 1998d).

1.14 *Salmonella* Muenster, *Salmonella* Seftenburg, and *Salmonella* Typhimurium

Almost all *Salmonella* species are pathogenic to humans and or warm blooded animals. *Salmonella* species continue to be the leading cause of foodborne bacterial illnesses (D'Aoust, 1997). *Salmonella* species belong to the family *Enterobacteriaceae,* and are characterized as being facultative anaerobes and Gram negative rods. They are characterized as being enteric bacteria, which falls into the phylogenetic phylum of Proteobacteria. This group is classified as having several characteristics such as: chemoorganotrophic with available respiratory or fermentative pathways for metabolizing nutrients, optimal growth at 37°C, acid and gas production from

catabolizing D-glucose and carbohydrates, utilize citrate as solitary carbon source, catalase positive, and oxidase negative. *Salmonella* species also display psychrotrophic growth characteristics (D'Aoust, 1997).

Salmonella Muenster is a problem with milk and cheese processing. *Salmonella* Seftenburg has been associated with chocolate products. *Salmonella* Typhimurium is usually associated with meat, poultry, and eggs. *Salmonella* Typhimurium is the most common cause of salmonellosis in humans. Common foods contaminated with *Salmonella spp*. are fresh meat, poultry, eggs, milk, and milk products (Brock, et al., 1997d; Jackson, et al., 1997; Jay, 1998d).

1.15 *Lactobacillus plantarum*

L. plantarum is one of the two Gram positive organisms utilized in this experiment. *L. plantarum* belongs to the family *Lactobacillaceae* and are considered part of the group of lactic acid bacteria. This group is classified as having several characteristics such as: often nonmotile, nonsporulating, generate lactic acid as exclusive product from fermentative metabolism, can only obtain energy by substrate level phosphorylation, aerotolerant anaerobes, heterofermentative, typically restricted to environments in which sugars are present, usually catalase negative (some strains positive), and require amino acids, vitamins, purines, and pyrimidines (Brock, et al., 1997e).

Lactobacilli grow well in acidic habitats and can thrive in a pH range of about 4 to 5. They are often used in the dairy industry to aid in the production of yogurt and acidified milks (Brock, et al., 1997e). Other species have been used in the fermentations

of sauerkraut, silage, and pickles. They can also be used for fermented meats such as sausage (Jackson, et al., 1997; Jay, 1998a; Ricke and Keeton, 1997).

1.16 *Listeria innocua* M1

L. innocua M1 is the second Gram positive bacterium utilized in this research. The genus *Listeria* is positioned among the *Clostridium* subbranch, and is grouped together along with *Lactobacillus*, *Brochothrix*, *Staphylococcus*, and *Streptococcus*. *L. innocua* is the most common species of *Listeria* to be detected in the food industry. Although it is not a pathogenic form of *Listeria*, the organism is a good indicator of poor sanitation conditions, which in turn increases the risk of food contamination from *L. monocytogenese.* This organism is classified as having several characteristics such as: ubiquitous in nature, motile, H_2S negative, oxidase negative, nonsporeforming, catalase positive, produce lactic acid from glucose and other fermentable sugars, resistant to low pH and high NaCl environments, microaerobic and psychrotrophic (Jay, 1998b; Rocourt and Cossart, 1997).

Because of *Listeria*'s ubiquitous nature, and its virulent methods of sickening people with immune deficiencies, the organism has forced research in areas to develop quick detection methods as well as sanitation and HACCP plans addressing the organism solely (Rocourt and Cossart, 1997).

1.17 *Saccharomyces cerevisiae*

S. cerevisiae is a unicellular fungi belonging to the family *Saccharomycetaceae*. Yeast cells are typically oval, cylindrical, or spherical. *S. cerevisiae*'s main means of reproducing is through multilateral budding. However, *S. cerevisiae* has been shown to form filamentous growth complexes when subjected to certain growth conditions (Brock,

et al., 1997b). Other traits of the yeast include an inability to metabolize lactose, optimal growth at 37°C, spherical spore former, and used for baking, wine, and beer fermentations. *S. cerevisiae* is not considered a pathogenic organism, nor is associated with food quality deterioration (Jay, 1998e).

1.18 *Aspergillus niger*

A. niger is a filamentous fungi, or mold, belonging to the family *Trichocomaceae.* This mold generates large amounts of citric acid from aerobic fermentation, particularly when subjected to an iron (Fe) deficient medium (Brock, et al., 1997c). *A. niger* grows optimally at 37°C and forms asexual spores called conidia. These spores are airborne and can be easily spread through the environment and are considered a potential containment in most food processing facilities. *A. niger* has been linked to the spoilage of fruits, vegetables, and grains. The type of spoilage associated with the mold is classified as black rot in fruits and black mold rot in vegetables (Brackett, 1997).

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CHAPTER 2

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATIONS OF LIQUID SMOKE FRACTIONS[1]

[1] Milly, P.J. and R.T. Toledo. To be submitted to J. Food Sci.

Abstract

The minimum inhibitory concentrations (MIC) of nine liquid smoke fractions against pathogenic and food spoilage organisms were determined using broth or agar dilution methods. Smoke fractions F1 to F9 have pH in increasing order as follows: (F1=F3=F5=F6)<F4<F7<(F2=F8=F9); and carbonyls in increasing order as follows: F4<(F6=F7=F9)<(F2=F3=F5=F8)<F1. F1 (highest carbonyl content and lowest pH) was most effective against all microorganisms. MIC of F1 was 0.75% against *Lactobacillus plantarum*; 1.5% against *Listeria innocua* M1, *Salmonella* and *E. coli spp.*, *Saccharomyces cerevisiae* and *Aspergillus niger*; and 2% against *Pseudomonas putida*. F4 (highest phenol content and low carbonyls) was not as effective as F1 with MIC > 10% against *Lactobacillus plantarum*. The least effective smoke fraction was F9 with MIC > 9% against most organisms tested, had high pH, low phenols, and low carbonyls. Growth curves of individual bacteria and yeast below the MIC exhibited prolonged lag phase that increased with increasing smoke concentrations.

Introduction

Advantages and benefits associated with the use of liquid smoke in foods include ease and consistency of application to optimize antioxidant potential, sensory, and antimicrobial properties. Liquid smoke preparations can be easily controlled and evaluated for composition and consistency of application. Using condensates for smoke application allows the processor to dictate the concentration of smoke being applied more readily than using gaseous smoke (Sunen, et al., 2001). The applied smoke can be evaluated for flavor acceptability in the product to determine the most suitable concentration. The antioxidant potential of smoke condensates has also been extensively documented and its potential to retard lipid oxidation in many meat products is an added benefit in meat products (Estrada-Munoz, et al., 1998; Maga, 1988). The primary objective of smoking foods using a liquid form is to induce both a sought-after flavor and preservative affect. In addition there may be microbiological effects of the applied smoke condensates.

The techniques for determining minimum inhibitory concentration (MIC) of antimicrobial agents have been published (Davidson and Parish, 1989). Smoking has been proven to exhibit antimicrobial effects on both fungi and bacteria in foods (Maga, 1988).

The purpose of this study was to determine the MIC of nine commercial liquid smokes and to evaluate changes in the growth phases of five individual organisms (*Salmonella* Seftenburg, *Escherichia coli* 8677, *Listeria innocua* M1, *Aspergillus niger* and *Saccharomyces cerevisiae*) in the presence of the smoke fractions at sub-MIC concentrations. Changes in the growth curve or growth pattern of the organisms may

suggest potential antimicrobial properties of the smoke extracts. Similar studies concerning smoke condensates and their antimicrobial potential have been preformed (Estrada-Munoz, et al., 1998; Maga, 1988; Painter, 1998; Sunen, 1988; Sunen, et al., 2001; Sunen, et al., 2003). The difference between these previous studies and the present is the composition of the smoke fractions used.

Materials and Methods

Test microorganisms

The Gram negative organisms used in this experiment were *Salmonella* Muenster*, Salmonella* Seftenburg*, Salmonella* Typhimurium*, Escherichia coli* 8677*,* and *Pseudomonas putida.* A cocktail consisting of Gram negative organisms: *Salmonella* Muenster*, Salmonella* Seftenburg*, Salmonella* Typhimurium*, and Escherichia coli* 8677 was tested as a group. *P. putida* was tested separately because its optimal growing temperature was lower than the other Gram negative bacteria in the test. The Gram positive organisms were *Lactobacillus plantarum* and *Listeria innocua* M1. The yeast and fungi were *Saccharomyces cerevisiae* and *Aspergillus niger*, respectively.

All stock cultures were preserved on Microbank^{TM} beads (Pro-Lab Diagnostics, Austin, Tex., U.S.A) at -15°C. Each Gram negative culture, excluding *P. putida,* was grown in 9 ml of tryptic soy broth (TSB, Difco Laboratories, Division of Becton Dickinson and Co., Sparks, Md., U.S.A.) at 37°C for 24 h. *P. putida* was also grown in TSB tryptic soy broth medium but was incubated at 25°C for 24 h. *L. plantarum* was grown anaerobically in Man Rogosa Sharpe (MRS, Difco Laboratories, Division of Becton Dickinson and Co., Sparks, Md., U.S.A.) broth at 37°C for 24 h. *S. cerevisiae*

was grown in malt extract broth (MEB, Difco Laboratories) at 37°C for 24 h. *A. niger* spores were grown and collected on citric acid acidified (pH = 4.5) potato dextrose agar (PDA, Difco Laboratories) at 37°C for 48 h. All cultures except *A. niger* were activated from stock in the recommended medium for their culture and incubated at the optimum growth temperature. The activated cultures were then inoculated into fresh culture medium and incubated for 18 h to obtain the inoculum for the tests.

Each test culture used as inoculum except *A. niger* was standardized to permit inoculation of 10^3 CFU/ml in the test medium with smoke. This was done by pour plating the 18 h old cultures to determine CFU/mL and diluting with peptone water (0.1%, pH 7.2) to the desired 10^3 CFU/ml. *L. innocua* M1 cultures were 24 h old at the time of inoculation into the test medium with smoke.

A. niger spores were collected from a growing culture on acidified PDA by adding 5 ml of peptone water (0.1%, pH 7.2) to surface of the dish. The spores were then dislodged from the solid medium using a glass hockey stick. The spore suspension in peptone water was then collected and stored at 4°C until needed

Smoke extracts

All liquid smoke fractions were provided by a commercial purveyor of liquid smoke (Mastertaste, Inc., Brentwood, TN). Nine commercial liquid smokes were tested in this experiment (F1, F2, F3, F4, F5, F6, F7, F8, and F9). The properties of the smoke fractions are listed in Table 2.1. Components of the liquid smoke fractions were quantified as follows: acidity quantified as acetic acid; carbonyls quantified as 2-butanone; and phenols quantified as 2,6-dimethoxy phenol. The factions listed in

ascending pH are (F1=F3=F5=F6)<F4<F7<(F2=F8=F9). The phenol content was low and similar for all fractions except F4 which contained about 5 times the phenol level in all the other fractions. The carbonyl content in increasing order was F4<(F6=F7=F9)<(F2=F3=F5=F8)<F1). The liquid smoke fractions were used directly as received from the purveyor. All liquid smoke fractions were added to the test medium before inoculation to obtain the desired concentration (v/v) after mixing with 9 ml of the medium and 1 ml of the inoculum.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration was defined as the concentration of smoke that prevented growth in 100% of inoculated cultures. The MIC of each smoke is specific for the organism tested. Therefore the MIC for the Gram negative cocktail may differ from that of the Gram positive organism(s). MIC for both bacteria and yeast was determined by the broth dilution method of Davidson and Parish (1989). Inoculum was 10^3 CFU/tube in this experiment.

The Gram negative cocktail and two Gram positive bacteria were inoculated into culture medium containing each of the nine liquid smoke fractions. Concentrations of the liquid smoke fractions (v/v) were 0.5%, 0.75%, 1.0%, 1.5%, and 2.0% to 10.0%, in 1% increments in the medium inoculated with the bacteria. The first sequence was a screening to determine which component of smoke had the most influence on the MIC. Smoke fractions 1, 2, 3 and 8, were tested first and after establishing that MIC was less than 2% , succeeding tests with these fractions were conducted at levels < 2%.

To determine the MIC of smoke against yeast and mold, smoke was added to the test medium (MEB and acidified PDA) at concentrations ranging from 0.5% to 5.0% in 0.5% increments. Liquid smoke was added to the melted acidified PDA before pouring into petri dishes.

A second test was conducted using sub-MIC concentration of smoke extracts 1, 2, 3, and 8 to determine the growth patterns of five individual organisms (*S.* Seftenburg, *E. coli* 8677, *L. innocua* M1, *A. niger* and *S. cerevisiae*) in the presence of sub-lethal concentrations of smoke. All inoculated test tubes were allowed to incubate at 37°C during examination.

P. putida was subjected to only four of the nine liquid smoke fractions (F1, F2, F3, and F8). Inoculated test tubes were allowed to incubate at 25°C for up to 96 h. *A. niger* was subjected to only four of the nine liquid smoke fractions (F1, F2, F3, and F8). Inoculated test tubes were allowed to incubate at 37°C for up to 96 h.

No turbidity after incubation was indicative of growth inhibition. Test tubes with no smoke extracts that were inoculated with the Gram negative cocktail, *P. putida*, the two Gram positive organisms, and *S. cerevisiae* provided positive controls. The experiments were executed in triplicate and repeated three times.

Standard growth curves for *E. coli* 8677, *S.* Seftenburg, *L. innocua* M1 and *S. cerevisiae* were constructed using a Million Ray Spectronic 20+ at 530 nm. All four organism's growth curves were conducted while incubating at 37°C. The extent of inhibition induced by the smoke extracts were observed as the length of the lag phase, rate of growth in the logarithmic phase and the maximum cell mass at the stationary

phase. Averages of three growth curves per organism with each smoke extract were determined.

The MIC for *A. niger* was determined using an agar dilution method (Davidson and Parish, 1989). Petri dishes containing various smoke concentrations were inoculated and allowed to incubate at 37°C for 96 hours. Petri dishes with no smoke extracts that were inoculated with *A. niger* provided positive controls. Absence of mold growth or spore formation was indicative of growth inhibition. The experiment was conducted in triplicate and repeated three times.

A. niger spores were then subjected to one half of the determined MIC for each smoke. The diameter of colonies was measured over several days used as an indicator of growth rate. Diameters of three colonies each in separate plates were measured and the experiment was replicated three times.

Results and Discussion

Minimum inhibitory concentration

The MIC values of smoke extracts associated with the Gram negative cocktail, *L. plantarum*, *L. innocua* M1, and *A. niger* are displayed in Figures 2.1, 2.2, 2.3, and 2.4, respectively. The efficacy of each smoke to inhibit growth was different for each test organism. F1 was most effective in inhibiting growth of all organisms except *P. putida* and *S. cerevisiae*. MIC values for F1 were < 2.0% for all microorganisms tested. F1 was most effective against all Gram negative bacteria (MIC < 1.5%). F1 and F2 were most effective in inhibiting *P. putida* (MIC < 2.0%), and F3 and F8 were least effective (MIC > 2.0%). F1 was most effective against *L. innocua* M1 (MIC < 1.5%). F1 displayed the

greatest inhibitory affect against *L. plantarum* (MIC < 0.75%) and *A. niger* (MIC < 1.5%). F1, F2, F3, and F8 shared the same inhibitory effects against *S. cerevisiae* (all MIC < 1.5%).

Results for the MIC values of smoke extracts against the Gram negative bacteria cocktail are displayed in Figure 2.1. The Gram negative cocktail (*S.* Muenster*, S.* Seftenburg*, S.* Typhimurium*, E. coli* 8677) was most susceptible to inhibition by F1 with a MIC value of 1.5% smoke. F1 has the lowest pH, the highest carbonyl content and was low in phenols. The second most effective growth inhibitor of the Gram negative cocktail was shared by F3, F5, and F6, each with a MIC value of 2.0%. These fractions share similar pH and phenol level as F1 but their carbonyl content is lower than F1. MIC values of F2 and F8 against the Gram negative organisms exceeded the 2% concentration used in the test. These fractions have the highest pH, low in phenols, and high in carbonyl content. F4, F7 and F9 inhibited growth with MIC values of 3.0%, 5.0%, and 9%, respectively. F4 had higher pH than F1, F3, F5 and F6 but the phenol content was the highest and the carbonyl content the lowest among all the fractions. The Gram negative organisms were most resistant to F7 and F9. F9 had the highest pH and similar carbonyls as F7, but F7 had slightly lower pH than F9. It appears that the slightly lower pH of F7 (5.1-6) compared to F9 (6.1 to 7.0) made a big difference in the inhibitory capacity for Gram negative organisms, all other attributes being equal, to raise the MIC from 5.0 to 9.0 % (v/v).

The MIC values of F1 and F2 against *P. putida* are similar at 2.0%. F3 and F8 exhibited MIC value > 2.0%. The effect of pH was not as strong on *P. putida* as it was for the Gram negative cocktail. The major difference between F1 and F3 is in the level of

carbonyls (F1>F3), all other components are similar. F8 has high pH but also contains high levels of carbonyls. Because the values are reported in ranges, it is difficult to contribute antimicrobial properties to any one factor.

The MIC values of smoke extracts against the Gram positive organism *L. plantarum* are displayed in Figure 2.2. F1 had the lowest MIC value of 0.75%. F2 and F3 had MIC values of 1.5 and 2.0%, respectively, against *L. plantarum*. The pH effect seems to be the reverse with *L. plantarum* since F3 had a lower pH than F2 and all other components are similar. Smoke extracts F5, F6, and F7 had 100% MIC values of 4.0%, 5.0%, and 7.0%, respectively. F4 and F9 had MIC > 10.0%. F9 had the highest pH and was moderate (about 1/3 of F1) in level of carbonyls. F4 was about median in pH and high in phenols but low in carbonyls. F8 MIC value was greater than 2.0%. Carbonyls seem to be the driving factor behind the antimicrobial efficacy against *L. plantarum.*

Results for the MIC values of smoke extracts associated with the Gram positive organism *L. innocua* M1 are displayed in Figure 2.3. F1 was the most effective in inhibiting growth with an MIC value of 1.5%. F2, F4, F5, F6, and F8 all had MIC values of 2.0%. F7 and F9 had MIC values of 4.0% and 6.0%, respectively. F3 MIC value was greater than 2.0%.

The MIC value of F1, F2, F3 and F8 against *S. cerevisiae* was 1.5%. When preparing the medium, F1 and F3 precipitated out of solution when mixed in malt extract broth (MEB) at a concentration greater than or equal to 1.5%.

MIC values of smoke fractions against the mold *A. niger* are displayed in Figure 2.4. F1 had an MIC value of 1.5%. F2 and F3 had MIC values of 2.5%. F8 had an MIC value greater than 5.0%.

Growth curves at sub-MIC levels

The growth curve of four select organisms (*E. coli* 8677, *S.* Seftenburg, *L. innocua* M1*, and S. cerevisiae*) can be seen in Figures 2.5, 2.7, 2.9, and 2.11, respectively. A general trend of prolonged lag phases was evident in all growth curves that were subjected to liquid smoke concentrations below the predetermined MIC level.

In Figure 2.5, the lag phase of growth of *E. coli* 8677 was prolonged by all the smoke fractions, ranging from 6 h for F1 and F2 to 12 h for F3. Growth in the presence of all smoke fractions had similar slope in the exponential growth phase. Although F1 and F2 only prolonged the lag phase by 2 h over that of control, the maximum cell concentration in the stationary phase was not as high as control or F8. In Figure 2.6, *E. coli* 8677 growth medium was treated with F1 at varying levels. Lag phase was increased with increasing smoke concentration. Cell mass at the stationary phase also decreased with increasing smoke concentration.

In Figure 2.7, *S.* Seftenburg also exhibited prolonged lag phase, but F1 and F2 almost had the lag phase as control. F3 and F8 exhibited longer lag phase than F1, F2, and control. Maximum cell mass in the stationary phase is lower in the smoke treated medium compared to control. In Figure 2.8, *S.* Seftenburg was only subjected to F1 at varying levels. There was little difference in the length of the lag phase in the smoke treated medium compared to control but decreasing maximum cell mass values at the stationary phase is evident with increasing smoke concentration.

In Figure 2.9, *L. innocua* M1 showed similar effects of smoke extracts prolonging the lag phase of growth. F1 and F3 prolonged the lag phase the most while F2 and F8 were close to the control. In Figure 2.10, *L. innocua* M1was exposed only to F1 at

varying levels. Increasing the smoke concentration seems to increase the lag phase of the organism.

In Figure 2.11, *S. cerevisiae* also was affected by the smoke by prolonging the lag phase of growth. F2 and F8 just slightly prolonged the lag phase but F1 and F3 prolonged the lag phase the longest. With all smoke concentrations being the same for all extracts, F1 prolonged the lag phase the longest. In Figure 2.12, *S. cerevisiae* was treated with F1 at varying levels. Treatments at 0.5% extended the lag phase to almost double that of control and no growth was observed up to 80 h of incubation when smoke was added at 0.75%.

The effect of smoke on growth of *A. niger* at half the MIC is shown as the increase in diameter of the colony with time of incubation in Figure 2.13. F1 inhibited *A. niger* to the slowest growth rate compared to F2 and F3. F8 inhibited growth only to day 3 but the diameter after 7 days was not much different from control.

Conclusions

In conclusion, liquid smoke fractions possess antimicrobial properties against a variety of Gram positive and Gram negative bacteria, yeast and molds. Above the MIC, growth does not occur and at concentrations of half the MIC, growth occurred but with a prolonged lag phase. The most effective smoke fraction with the highest antimicrobial activity is one with low pH, and high carbonyl content. Phenols do not seem to have as much of an antimicrobial effect compared to the low pH and high carbonyl content of the smoke fractions. The smoke fraction with the highest carbonyl content and the lowest pH appear to have broad spectrum antimicrobial activity against Gram positive bacteria,

Gram negative bacteria, yeasts and molds as well as against pathogens *Salmonella*, *Listeria*, and *E. coli*.

Table 2.1 – Properties of smoke fractions

Smoke Extract	Acidity (%)	pH	Phenol content (mg/ml)	Carbonyl content (mg/ml)
F1	4.5 - 5.9	2 – 3.0	0 - 5	151 - 200.9
F2	0 - 1.4	6.1 - 7.0	0 - 5	101 - 150.9
F3	6.0 - 7.4	2 – 3.0	0 - 5	101 - 150.9
F4	3.0 - 4.4	4.1 - 5.0	20.1 -25.0	0 - 50.9
F5	6.0 - 7.4	2 – 3.0	0 - 5	101 - 150.9
F6	6.0 - 7.4	2 – 3.0	0 - 5	51 - 100.9
F7	1.5 - 2.9	5.1 - 6.0	0 - 5	51 - 100.9
F8	0 - 1.4	6.1 - 7.0	0 - 5	101 - 150.9
F9	0 - 1.4	6.1 - 7.0	0 - 5	51 - 100.9

Source: MasterTaste Inc.

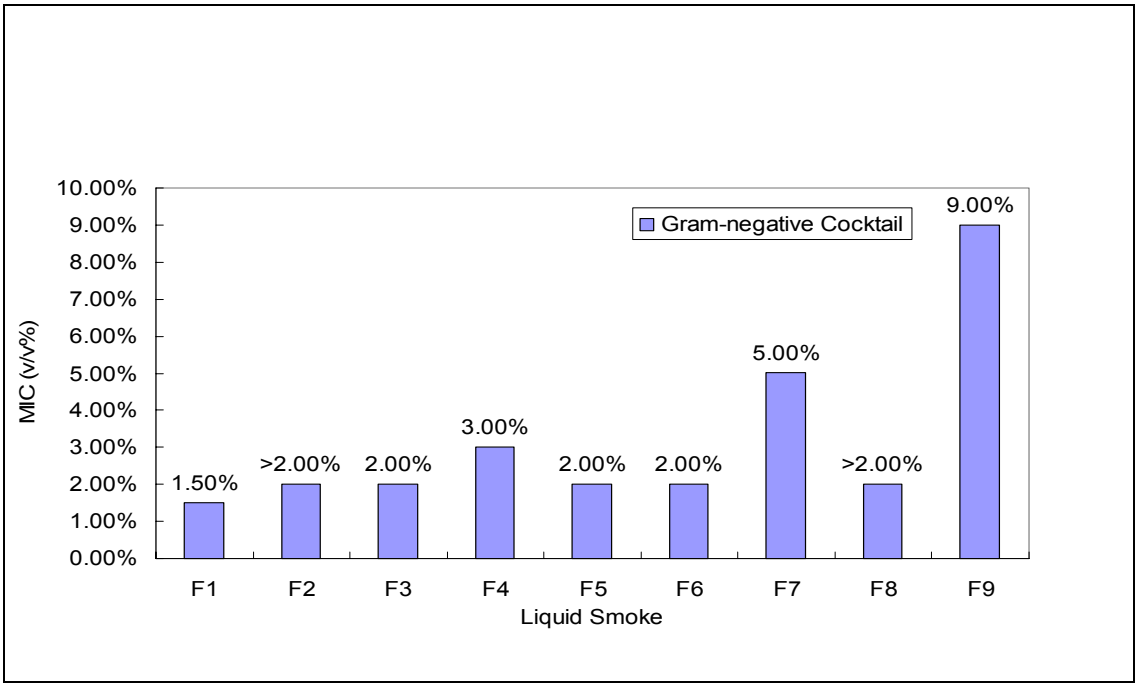

Figure 2.1 - 100% MIC values for gram-negative cocktail.

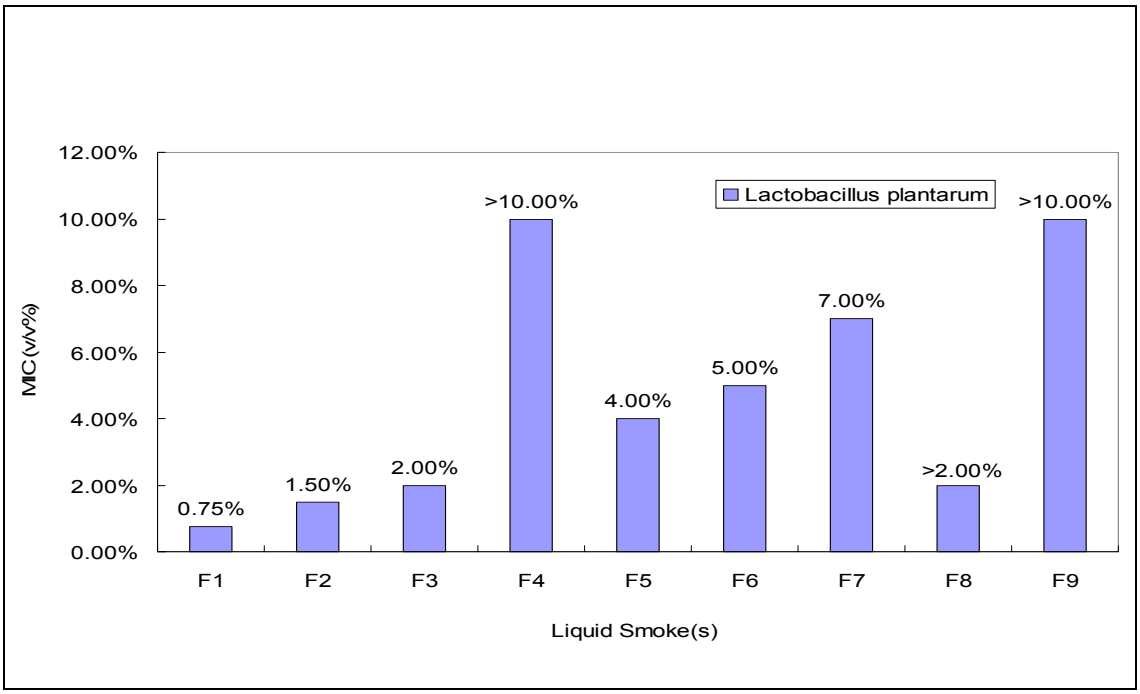

Figure 2.2 - 100% MIC values for *Lactobacillus plantarum*.

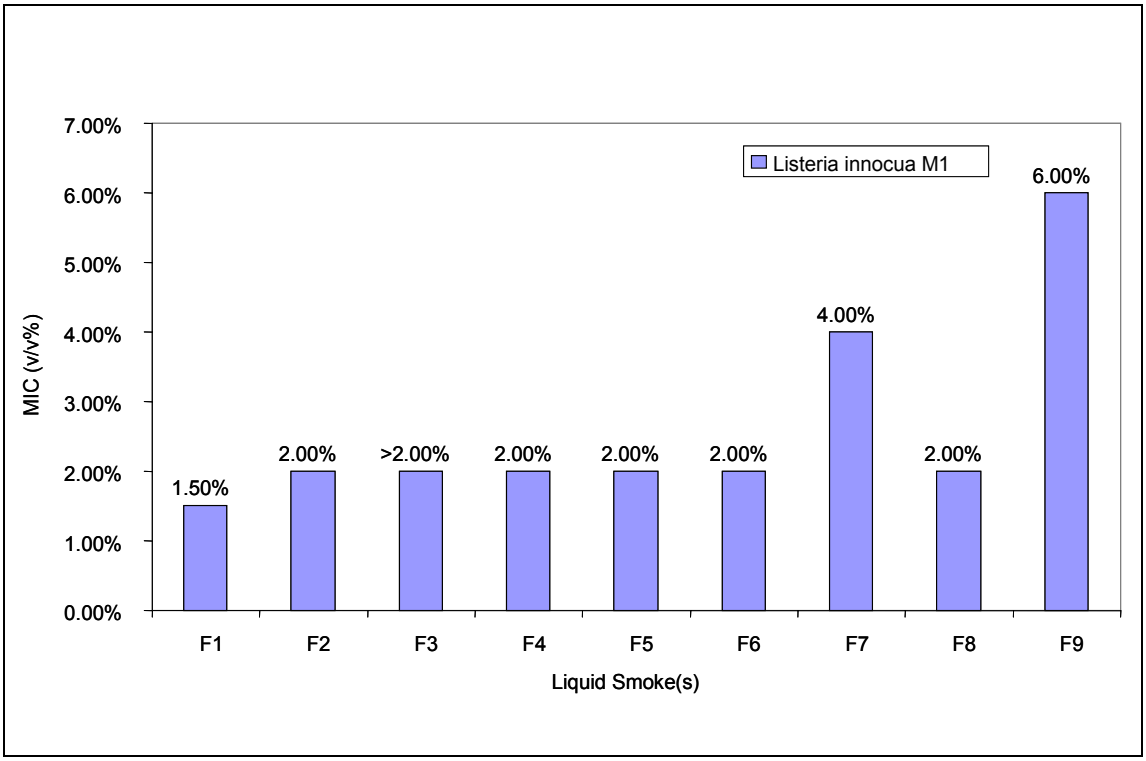

Figure 2.3 - 100% MIC values for *Listeria innocua* M1.

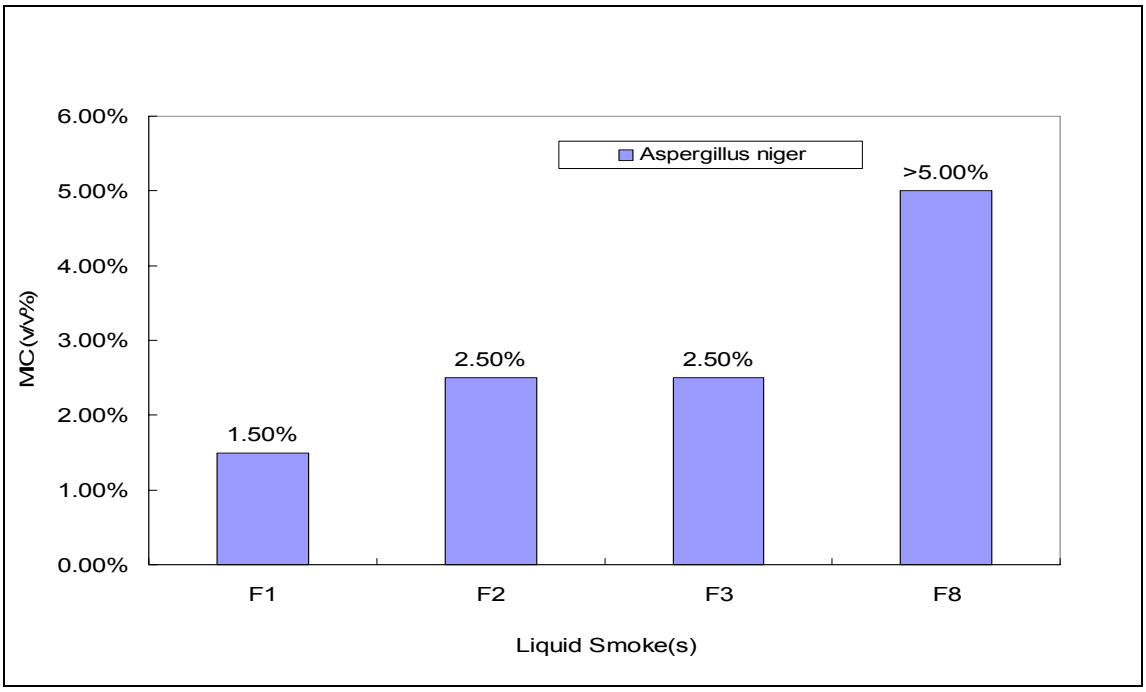

Figure 2.4 - 100% MIC values for *Aspergillus niger*.

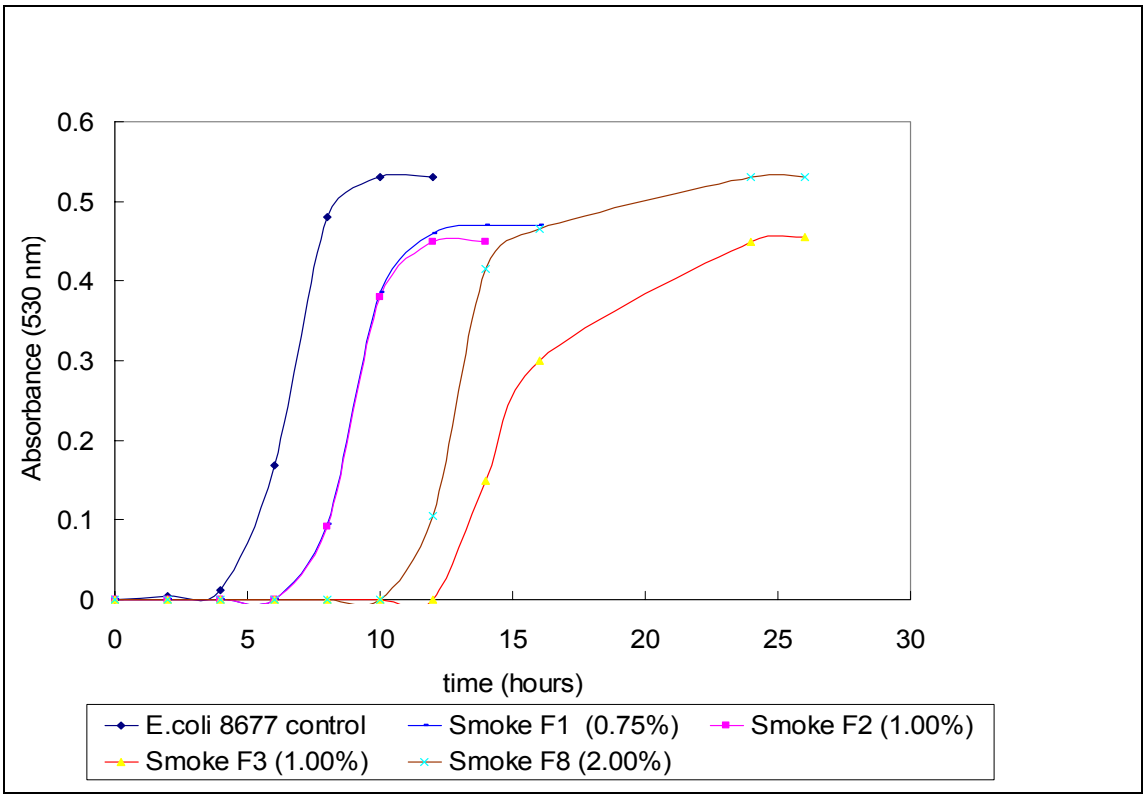

Figure 2.5 - *E. coli* 8677 growth curves below the MIC value.

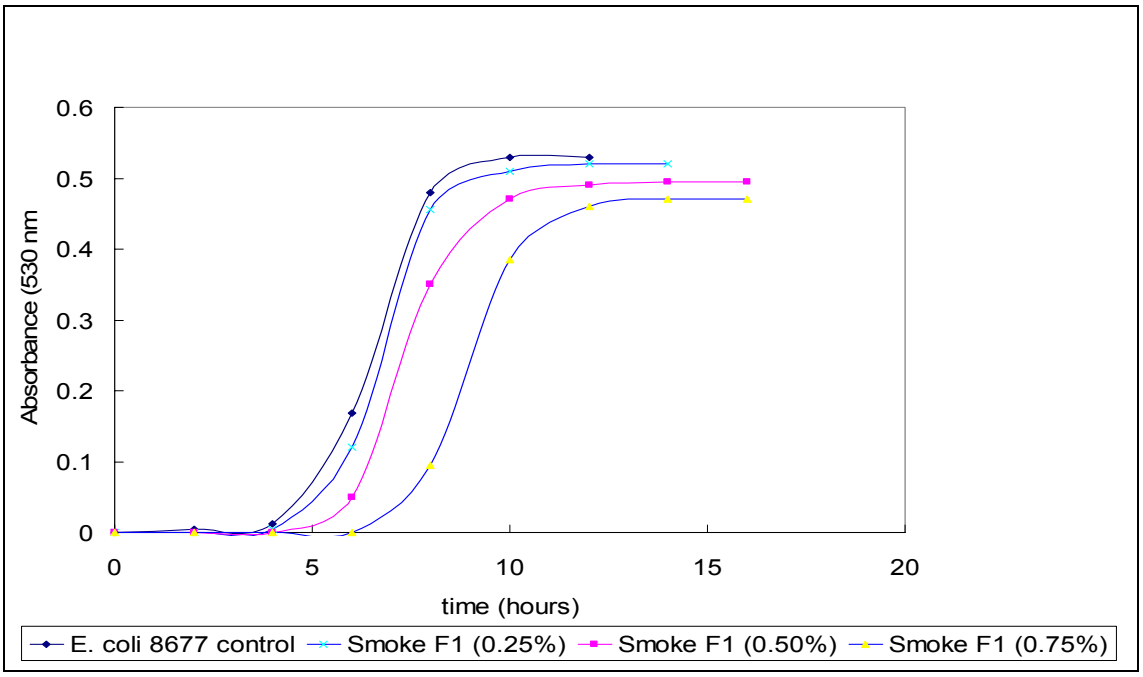

Figure 2.6 - *E. coli* 8677 growth curves against varying concentrations of smoke F1.

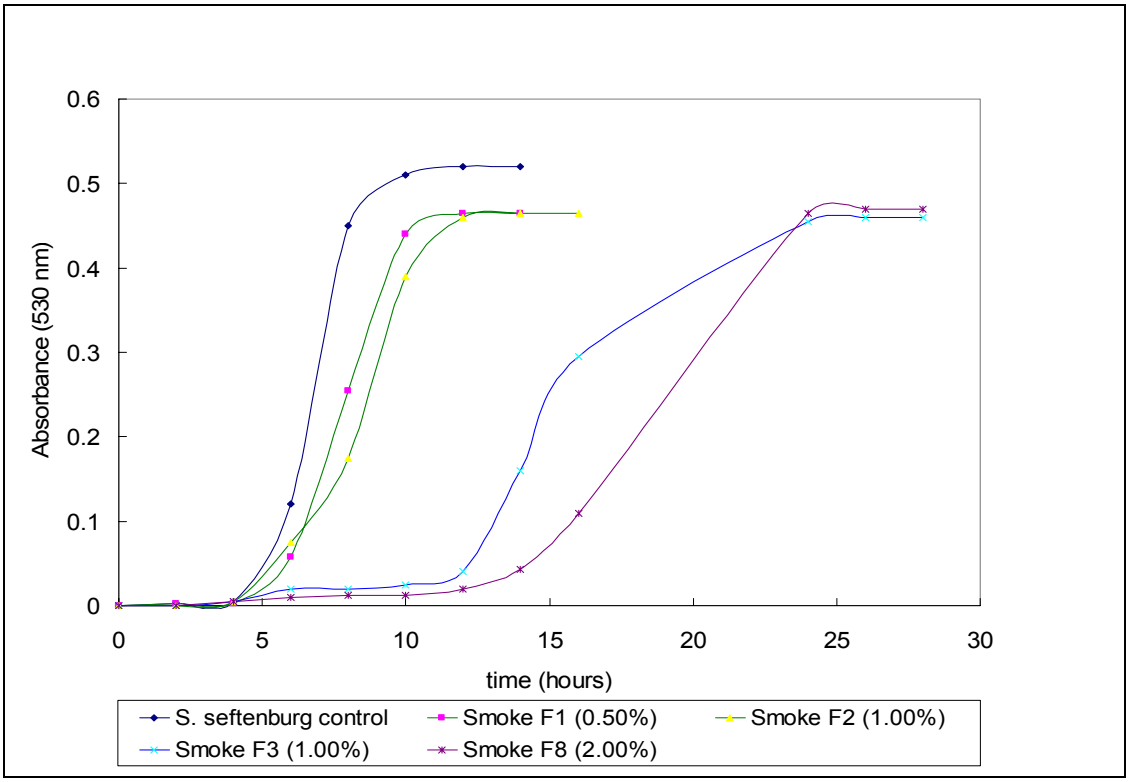

Figure 2.7 - *S. seftenburg* growth curves below the MIC value.

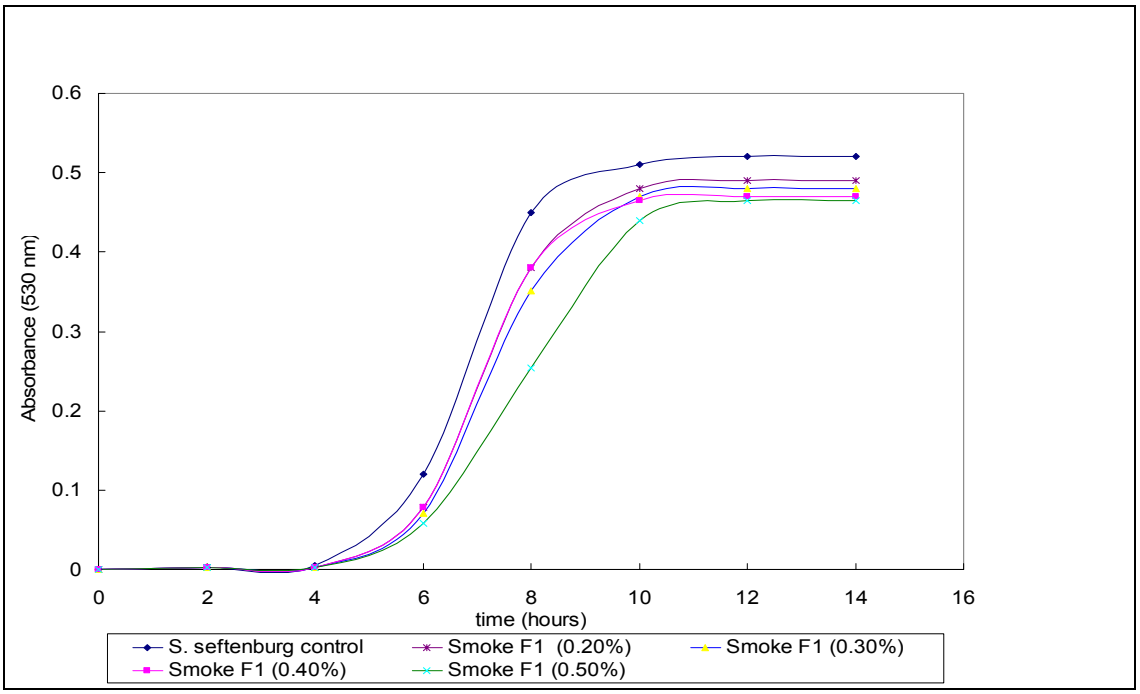

Figure 2.8 - *S. seftenburg* growth curves against varying concentrations of smoke F1.

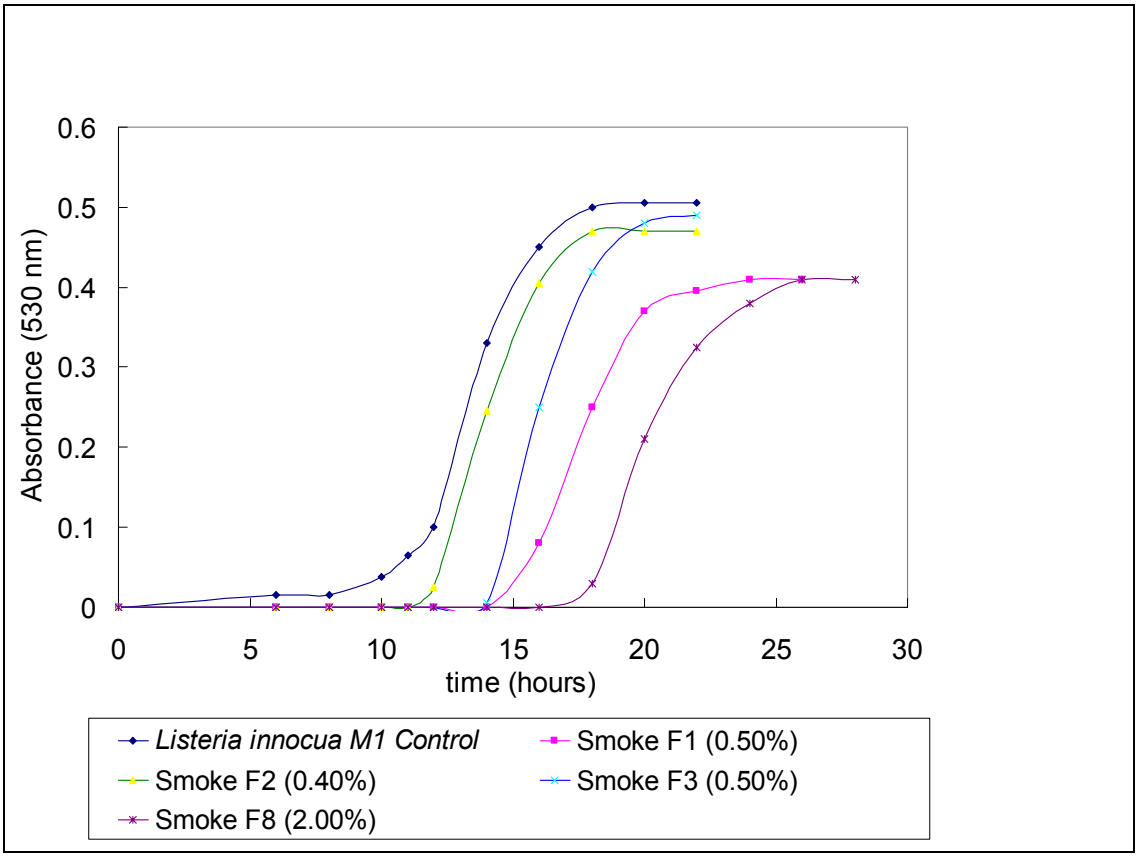

Figure 2.9 - *L. innocua* M1 growth curves below the MIC value.

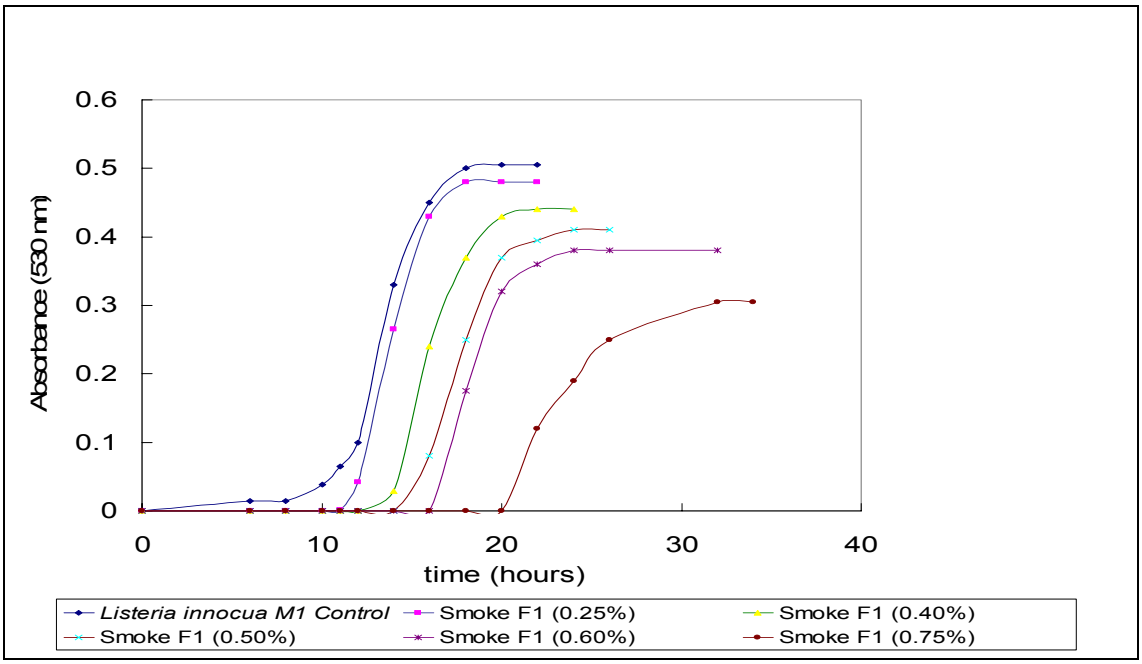

Figure 2.10 - *L. innocua* M1 growth curves against varying concentrations of smoke F1.

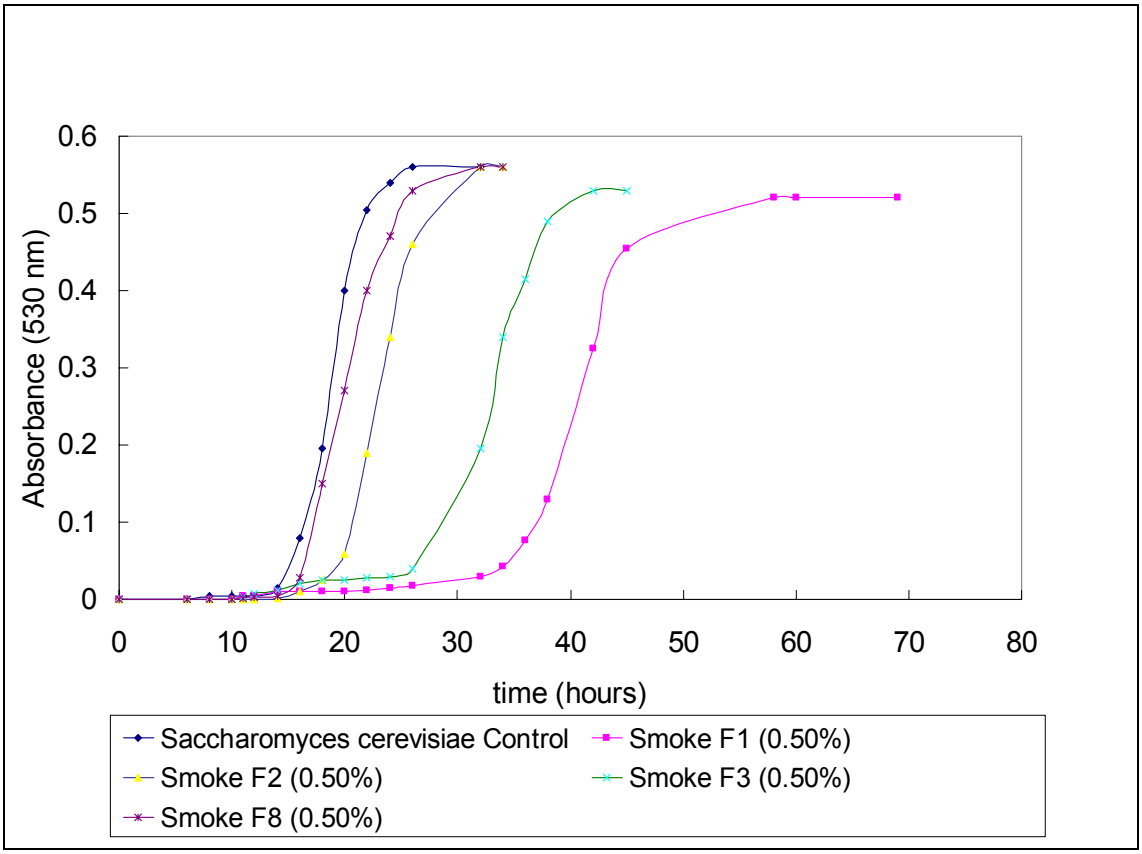

Figure 2.11 - *S. cerevisiae* growth curves below the MIC value.

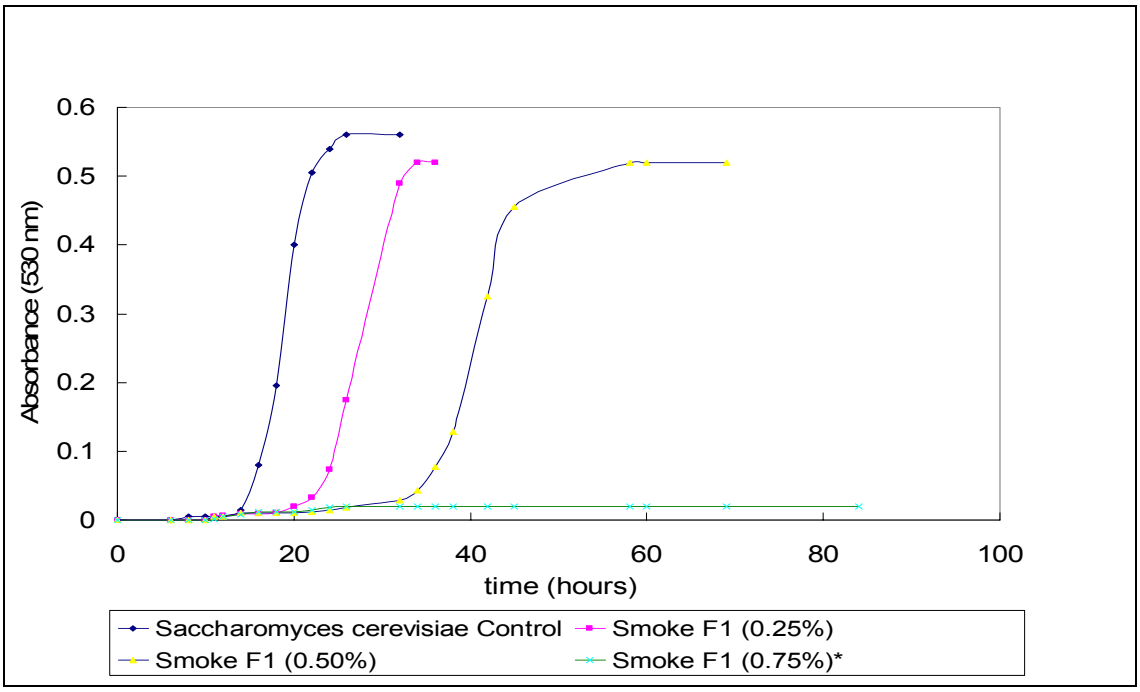

Figure 2.12 - *S. cerevisiae* growth curves against varying concentrations of smoke F1.

***S. cerevisiae did not assume any growth after 80 h**

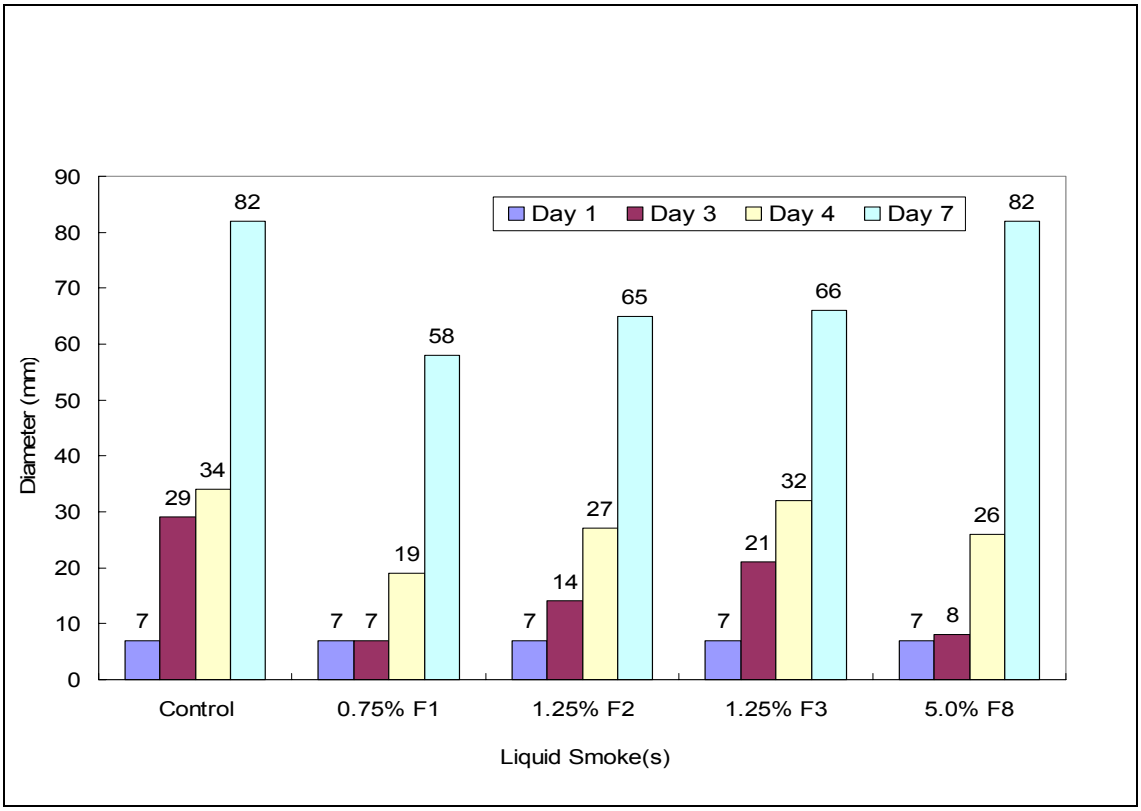

Figure 2.13 - *A. niger* rate of diameter growth.

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CHAPTER 3

VALIDATION OF LIQUID SMOKE TREATED READY-TO-EAT (RTE) MEAT PRODUCTS FOR CONTROL OF *Listeria innocua* M1[1]

[1] Milly, P.J. and R.T. Toledo. To be submitted to J. of Food Sci.

Abstract

Four liquid smoke fractions (F1, F2, F3, and F4) were applied on ready-to-eat (RTE) meat products to control growth of inoculated *Listeria innocua* M1. Turkey rolls (high and low end) and roast beef products were dipped in liquid smoke, the surface inoculated with *L. innocua* M1 (10^2 CFU/50µL), vacuum packaged, and stored at 4°C. Section 36.512 of USDA's enrichment procedure for isolation of *L. monocytogenes* was employed in conjunction with a Micro ID™ system for *Listeria* identification. Positive identification of *Listeria* at time = 0 was observed in all products. All products treated with smoke fractions F1, F2, and F3 were negative for *Listeria* at two and four weeks of incubation at 4°C. All products treated with F4 were positive for *Listeria* at time = 0, two, and four weeks.

Introduction

On June 6, 2003 USDA/FSIS published a final rule (68 FR 34207) amending previous regulations on ready-to-eat (RTE) meat and poultry products. The new rule mandates that RTE producers prevent product adulteration from a pathogenic environmental contaminant *Listeria monocytogenes.* Two of three new proposals for processing protocols to ensure safety of RTE meat products involve the incorporation of antimicrobials alone or in conjunction with a "post-process pasteurization" treatment. For this reason, there is interest from both the ingredient suppliers and processors to find suitable antimicrobial agents. Depending upon the product and the target consumer market, chemical antimicrobial agents declared in the product label may have negative connotations. Thus, there is a need for different antimicrobial agents that processors can use to differentiate their product from others in the market. Previous tests in a model system executed by the authors are the basis for this study in actual food products. The purpose of this study was to explore the antimicrobial properties of four commercial liquid smoke fractions in actual processed meat products. This research is directed towards the potential use of commercial liquid smoke fractions as an antimicrobial in RTE meat products.

The prevalence of *L. monocytogenes* in the US meat and food supply places food processors under constant pressure to address the issue of zero tolerance for this ubiquitous organism (Jay, 1996; Shank, et al., 1996). Several publications document the antimicrobial activity of smoke condensates in model systems (Estrada-Munoz, et al., 1998; Maga, 1988; Painter, 1998; Sunen, 1988; Sunen, et al., 2001; Sunen, et al., 2003). Limited research has been preformed on actual food systems utilizing liquid smoke as an

antimicrobial agent against *Listeria spp*. and other pathogenic microorganisms (Guillen and Errecalde, 2002; Mourey and Canillac, 2002).

Materials and Methods

Ready-To-Eat (RTE) Meat Products

RTE high and low end turkey rolls and roast beef cuts from a commercial manufacturer were utilized in this experiment. The turkey products ranged from 3.5 to 4.5 kg and were cooked in hot water in shrinkable cook-in bags to an internal temperature of 71 °C followed by cooling in water to an internal temperature of 7 °C. Roast beef were marinated sirloin tips smokehouse-cooked to an internal temperature of 69 °C and cooled in a chill room to an internal temperature of 7 °C. Each piece of the roast beef was about 1 kg and was in case-ready shrink film packages. All products were shipped to the University of Georgia overnight in Styrofoam boxes with polyfoam refrigerant packs. RTE meat products are designed for deli and lunch meat distribution operations. They are considered ready-to-eat once sliced to the consumer's preference. High end turkey rolls are characterized as whole parts of turkey breast formed to have no less than 40 percent binders and broth added. Low end turkey rolls are characterized as minced turkey breast parts that can have up to 60 percent binders and broth added before forming and cooking. Roast beef cuts are whole meats, and are considered a high value product. Each piece of roast beef was used as a unit while the whole turkey roll was cut into 4 sections with each section considered as a unit in these experiments.

Smoke Extracts

All liquid smoke fractions were obtained from Mastertaste, Inc., Brentwood, TN. Four commercial liquid smoke fractions F1, F2, F3, and F4, were tested in this experiment. Initial experiments were conducted to determine how much weight of the smoke was deposited on the meat by dipping. In previous work in a model system, the minimum inhibitory concentration (MIC) of these liquid smoke fractions was about 2%. We hypothesized that by measuring the surface area of the meat and calculating a volume of a layer 2 mm deep, we can determine an amount of smoke that must adhere to the meat to obtain a concentration of about 2% in this layer. Preliminary experiments confirmed that it would not be possible to attain the target concentration if the liquid smoke preparations were diluted. Thus, meats were dipped in the undiluted liquid smoke as received from the manufacturer. RTE meat products were dipped into the liquid smoke and remained submerged for no less than 60 seconds. After removal from the smoke solution the meats were allowed to air dry over a screen at room temperature for no less than five minutes before they were inoculated and used in the experiments.

Inoculum

L. innocua M1, a strain of *Listeria* resistant to the antibiotics streptomycin and rifampicin, was originally from Dr. P.M. Foegeding, North Carolina State University, Raleigh, North Carolina. This strain of bacteria is more heat resistant than *L. monocytogenese* and is considered a surrogate or indicator organism for inactivation of *L. monocytogenese* (Fairchild and Foegeding, 1993).

***L. innocua* M1 inoculation and recovery**

Two 25 cm^2 areas on the surface of each piece of smoke treated RTE meats were marked for application of the inoculum. The areas were marked with food grade ink using a sterile template (Reynolds, et al, 2001). The marked areas were inoculated with 50 µL of an actively growing (18 h) culture of *L. innocua* M1 to obtain a total inoculum of 10^2 CFU/25cm^2. Each piece was placed inside a Cryovac barrier bag and vacuum sealed. The packaged products were then incubated at 4°C.

Viable *L. innocua* M1 was evaluated at time = 0, 2, and 4 weeks at 4°C. The marked area was aseptically cut out using a sterile scalpel and transferred to a Stomacher bag. 100 mL of 0.1% sterile peptone was added and the mixture was subjected to the Stomacher for 2 min. Aliquots were taken and directly plated or dilutions were made enumerated. Enumeration of viable *L. innocua* M1 cells was done by direct spiral plating methods (Autoplate 4000 Spiral Biotech. Bethesda, Md., U.S.A.) using tryptic soy agar (TSA, Difco Laboratories, Division of Becton Dickinson and Co., Sparks, Md., U.S.A.) supplemented with 250 mg/L of streptomycin and 50 mg/L rifampicin.

Number of CFU reported is an average of CFU from the two designated areas on each product and two samples of purge. There were three batches of each RTE product (high end turkey roll, low end turkey roll, and roast beef cut) with 15 tests conducted on each batch. Three samples from each batch served as positive controls (no smoke treatment), one each for each sampling time. Each smoke fraction was applied to three pieces of meat from each batch and viable *L. innocua* M1 CFU were enumerated at time = 0, 2, and 4 weeks.

In addition to the CFU enumeration, enrichment procedures were carried out on the marked areas and the purge at time = two and four weeks. Section 36.512 of USDA's enrichment procedure for isolation of *L. monocytogenes* was employed (Donnelly, 2001). This procedure calls for a 225 g sample; however, we used all of the remaining undiluted extract (after removal of an aliquot for CFU enumeration) for the enrichment. Once presumptive *Listeria* was identified using enrichment procedures, *Listeria* isolates were identified using the Micro-ID™ *Listeria* system (Remel, Lenexa, Kans., U.S.A.). Results were provided as a positive (+) or negative (-) identification for *L. innocua*.

Results and Discussion

Recovery of *L. innocua* M1 at 0 week storage

Viable cell counts of the inoculated organism recovered from the meat pieces immediately after inoculation are shown in Tables 3.1 to 3.3. The bactericidal action of the smoke on *Listeria* was not immediate. After vacuum packaging the inoculated pieces and sampling right after, counts were at the same level as the inoculum. There was no purge in the packages at this sampling time, therefore there were no counts reported for the purge.

Recovery of *L. innocua* M1 after 2 and 4 weeks storage

F1, F2, and F3 exhibited antimicrobial activity on the surface of all three RTE meat products against *L. innocua* M1 at two and four weeks of storage in vacuum sealed packages at 4°C (Tables 3.4 to 3.9).

Table 3.4 shows recovery as $\log_{10}$ CFU/25 cm^2 $\pm$ S.D. and positive or negative identification for *L. innocua* on the surface of high end turkey rolls and in the purge at

two weeks of storage in vacuum sealed packages at 4°C. Control meat packages have viable cells in the purge of 6.99 $\pm$ 0.48 $\log_{10}$ CFU/mL and the inoculated meat surface contained 4.33 $\pm$ 0.44 ($\log_{10}$ CFU/25 cm^2). Smoke fraction F1, F2, and F3 inactivated *L. innocua* M1 on the meat and there was no transfer of viable cells in the purge so no viable cells were recovered from the purge. However meat pieces treated with F4 proved positive for *L. innocua*, although no CFU enumeration was recovered from the meat or in the purge. F4 did not inactivate *L. innocua* M1 completely fast enough to prevent some viable cells from transferring from the meat surface to the purge. However, very low numbers in the purge of the F4 treated meat pieces prevented recovery of CFU but a positive test for *L. innocua* indicate a few viable cells or injured cells which may have been activated by enrichment. Enrichment did not result in the recovery of *L. innocua* M1 from the inoculated meat surfaces of meat pieces treated with F4.

Table 3.5 shows *L. innocua* M1 CFU on low end turkey rolls and results of the confirmation test for the presence of *L. innocua* in packaged meat pieces stored two weeks of storage in vacuum sealed packages at 4°C. The control contained 7.90 $\pm$ 0.65 ($\log_{10}$ CFU/mL) in the purge and 3.60 $\pm$ 0.32 ($\log_{10}$ CFU/25 cm^2) on the inoculated meat surface. *L. innocua* M1 grew in the packaged meat to exhibit about a one log increase on the meat surface and a 7 log increase in the purge. The organism grew much faster in the purge than on the meat surface. F1, F2, and F3 inactivated the organism and resulting in undetectable CFU from both the purge and meat surface. Both meat and purge also tested negative for *L. innocua* M1. F4 did not inactivate the organism on the meat surface but inhibited its growth as evidenced by a $\log_{10}$ CFU count less than that at 0

week storage. No viable *L. innocua* M1 were recovered by enrichment in the purge of meats treated with F4.

Table 3.6 represents recovery of *L. innocua* M1 on roast beef surface and purge at two weeks of storage in vacuum sealed packages at 4°C. The control meats contained 5.54 ± 0.45 ($\log_{10}$ CFU/mL) in the purge and 3.81 ± 0.31 ($\log_{10}$ CFU/25 cm^2) from the surface of the meat. Extracts F1, F2, and F3 provided undetectable numbers from direct enumeration and a negative identification for *L. innocua* from purge and meat surface. Extract F4 provided a negative identification for *L. innocua*, with undetectable numbers from the purge for direct spiral plating techniques. Extract F4 yielded an undetectable number of viable cell numbers from the surface of the meat, however, a positive identification for *L. innocua* was observed. Extract 4 failed to destroy the intended microorganism.

The type of meat appears to differ in the way they support the growth of *L. innocua* M1. Comparing counts in the control meat pieces for the high end (Table 3.4), low end (Table 3.5) and roast beef (Table 3.6), it is apparent that the high end turkey rolls supported growth of the organism better than the low end turkey and the roast beef. Increase in CFU in the purge however was similar for the three different types of meats.

Recovery of *L. innocua* M1 in meats at 4 weeks storage

Tables 3.7 to 3.9 show recovery of CFU's from meat surface and purge after 4 weeks of storage. Counts on the control meat surface and purge were slightly higher after 4 weeks than at two weeks for all meat types. No recovery of CFU by direct plating and no recovery of viable *L. innocua* M1 was achieved after enrichment of either purge or

meat surface in all meats treated with F1, F2, and F3 smoke fractions. It appears that the inactivation of *L. innocua* M1 was complete in these treated meats a few days after packaging. Meats treated with F4 on the other hand were positive for *L. innocua* M1 in all meat types in either the purge or on the meat.

Conclusions

Liquid smoke fractions offer RTE meat processors a valuable option for complying with USDA/FSIS final rule (68 FR 34207) of employing a "post-pasteurization process." Of the four smoke fractions tested, fractions F1, F2, and F3 have proved effectiveness in destroying *L. innocua* M1 in an actual food system. One extract inhibited growth but allowed the persistence of injured cells which tested positive for *L. innocua* after enrichment. Liquid smoke applied before final packaging of RTE meat products offers the processor a means of guaranteeing product safety and complying with the new rules addressing environmental contamination from *L. monocytogenes*.

Table 3.1 – Viable cells (log_{10} CFU/25 cm^2 ± S.D.) of *L. innocua* M1 at week = 0 in vacuum packaged high end turkey roll.

High End Turkey Roll at 0 week		
Treatment	**Purge (CFU/mL) [1]**	**Log $_{10}$(CFU/25cm^2)**
Control	-	2.07 ± 0.11
F1	-	2.06 ± 0.11
F2	-	2.08 ± 0.11
F3	-	2.12 ± 0.16
F4	-	2.10 ± 0.12

Results are the mean value from the two marked areas on each piece of meat.
1 not determined. No purge at 0 day storage

Table 3.2 - Viable cells (log_{10} CFU/25 cm^2 ± S.D.) of *L. innocua* M1 at week = 0, at 4°C for low end turkey roll.

Low End Turkey Roll ; time = 0 week		
Treatment	**Purge (CFU/mL)[1]**	**Log_{10}(CFU/25cm^2)**
Control	-	2.06 ± 0.13
F1	-	2.06 ± 0.16
F2	-	2.04 ± 0.12
F3	-	2.18 ± 0.15
F4	-	2.19 ± 0.08

Results are the mean value from the two marked areas on each piece of meat.
1 not determined. No purge at 0 day storage

Table 3.3 - Viable cells (log_{10} CFU/25 cm^2 $\pm$ S.D.) of *L. innocua* M1 at week = 0, at 4°C for roast beef cut.

Roast Beef Cut : time = 0 week		
Treatment	**Purge (CFU/mL)[1]**	**Log_{10} (CFU/25cm^2)**
Control	-	2.13 $\pm$ 0.18
F1	-	2.24 $\pm$ 0.15
F2	-	2.13 $\pm$ 0.17
F3	-	2.08 $\pm$ 0.12
F4	-	2.22 $\pm$ 0.15

Results are the mean value from the two marked areas on each piece of meat.
1 not determined. No purge at 0 day storage

Table 3.4 - Viable cells (log_{10} CFU/25 cm^2 $\pm$ S.D.) of *L. innocua* M1 at time = 2 weeks, at 4°C on high end turkey roll, and recovery of injured cells after enrichment.

High End Turkey Roll (Dipped into 100%); time = 2 weeks				
Treatment	**Purge (CFU/mL)**	**Identification (+) or (-)**	**(Log_{10} CFU/25cm^2)**	**Identification (+) or (-)**
Control	6.99 $\pm$ 0.48	(+)	4.33 $\pm$ 0.44	(+)
F1	ND	(-)	ND	(-)
F2	ND	(-)	ND	(-)
F3	ND	(-)	ND	(-)
F4	ND	(+)	ND	(-)

Results are the mean value of two designated areas.
ND, no detection by cell count procedure (limit 10 CFU/mL).

Table 3.5 - Viable cells (log_{10} CFU/25 cm^2 $\pm$ S.D.) of *L. innocua* M1 at time = 2 weeks, at 4°C on low end turkey roll, and recovery of injured cells after enrichment.

Low End Turkey Roll (Dipped into 100%); time = 2 weeks				
Treatment	**Purge (CFU/mL)**	**Identification (+) or (-)**	**(Log_{10} CFU/25cm^2)**	**Identification (+) or (-)**
Control	7.90 $\pm$ 0.65	(+)	3.60 $\pm$ 0.32	(+)
F1	ND	(-)	ND	(-)
F2	ND	(-)	ND	(-)
F3	ND	(-)	ND	(-)
F4	ND	(-)	1.65 $\pm$ 0.18	(+)

Results are the mean value of two designated areas.
ND, no detection by cell count procedure (limit 10 CFU/mL).

Table 3.6 - Viable cells (log_{10} CFU/25 cm^2 $\pm$ S.D.) of *L. innocua* M1 at time = 2 weeks, at 4°C on roast beef cut, and recovery of injured cells after enrichment.

Roast Beef Cut (Dipped into 100%); time = 2 weeks				
Treatment	**Purge (CFU/mL)**	**Identification (+) or (-)**	**(Log_{10} CFU/25cm^2)**	**Identification (+) or (-)**
Control	5.54 $\pm$ 0.45	(+)	3.81 $\pm$ 0.31	(+)
F1	ND	(-)	ND	(-)
F2	ND	(-)	ND	(-)
F3	ND	(-)	ND	(-)
F4	ND	(-)	ND	(+)

Results are the mean value of two designated areas.
ND, no detection by cell count procedure (limit 10 CFU/mL).

Table 3.7 - Viable cells (log_{10} CFU/25 cm^2 $\pm$ S.D.) of *L. innocua* M1 at time = 4 weeks, at 4°C on high end turkey roll, and recovery of injured cells after enrichment.

High End Turkey Roll (Dipped into 100%); time = 4 weeks				
Treatment	**Purge (CFU/mL)**	**Identification (+) or (-)**	**(Log_{10} CFU/25cm^2)**	**Identification (+) or (-)**
Control	7.96 $\pm$ 0.68	(+)	4.49 $\pm$ 0.45	(+)
F1	ND	(-)	ND	(-)
F2	ND	(-)	ND	(-)
F3	ND	(-)	ND	(-)
F4	ND	(+)	ND	(-)

Results are the mean value of two designated areas.
ND, no detection by cell count procedure (limit 10 CFU/mL).

Table 3.8 - Viable cells (log_{10} CFU/25 cm^2 $\pm$ S.D.) of *L. innocua* M1 at time = 4 weeks, at 4°C on low end turkey roll, and recovery of injured cells after enrichment.

Low End Turkey Roll (Dipped into 100%); time = 4 weeks				
Treatment	**Purge (CFU/mL)**	**Identification (+) or (-)**	**(Log_{10} CFU/25cm^2)**	**Identification (+) or (-)**
Control	7.80 $\pm$ 0.66	(+)	4.71 $\pm$ 0.48	(+)
F1	ND	(-)	ND	(-)
F2	ND	(-)	ND	(-)
F3	ND	(-)	ND	(-)
F4	ND	(+)	ND	(-)

Results are the mean value of two designated areas.
ND, no detection by cell count procedure (limit 10 CFU/mL).

Table 3.9 - Viable cells (log_{10} CFU/25 cm^2 $\pm$ S.D.) of *L. innocua* M1 at time = 4 weeks, at 4°C on roast beef cut, and recovery of injured cells after enrichment.

Roast Beef Cut (Dipped into 100%); time = 4 weeks				
Treatment	**Purge (CFU/mL)**	**Identification (+) or (-)**	**(Log_{10} CFU/25cm^2)**	**Identification (+) or (-)**
Control	6.26 $\pm$ 0.45	(+)	4.57 $\pm$ 0.45	(+)
F1	ND	(-)	ND	(-)
F2	ND	(-)	ND	(-)
F3	ND	(-)	ND	(-)
F4	ND	(-)	ND	(+)

Results are the mean value of two designated areas.
ND, no detection by cell count procedure (limit 10 CFU/mL).

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CHAPTER 4

SUMMARY AND CONCLUSIONS

Certain liquid smoke fractions can be utilized for their antimicrobial properties against a variety of Gram positive and Gram negative bacteria, yeast and molds. Liquid smoke has been shown to prolong the lag phase of certain gram-positive and gram-negative bacteria and yeast and decrease the rate of mold growth. They can also be used as an effective means of prolonging the shelf life of high-quality smoke flavored ready-to-eat meats and ensure their safety.

To be effective in food systems, the liquid smoke must exhibit a low minimum inhibitory concentration (MIC) so that this level can be achieved in the food product without adversely affecting the organoleptic quality of the product. The new smoke fractions with low smoke flavor intensity and low staining properties that are now commercially available appear to meet the requirement for an effective antimicrobial agent in meat products. These new fractions have MIC of 1.5% against a broad spectrum of microorganisms including *Listeria* and *Salmonella spp*.

The antimicrobial properties of smoke condensates have traditionally been accredited to the phenolic components. The MIC of the various smoke fractions indicate that high carbonyl levels and low pH, high titratable acidity combine to make a smoke fraction highly effective as a microbial growth inhibitor.

Liquid smoke condensates offer RTE meat processors a valuable option for complying with USDA/FSIS final rule (68 FR 34207) of employing a "post-pasteurization process." Applying the liquid smoke as a dip on pre-cooked turkey and beef products, followed by vacuum packaging and storage at 4°C, inactivated *L. innocua* M1 completely such that no viable cells could be recovered with enrichment. Three different liquid smoke fractions exhibited this effect. One fraction inhibited growth but

permitted injured but viable cells to remain. These injured cells were recovered by enrichment.

Utilizing liquid smoke in processing RTE meat products offers the processor a means of guaranteeing product safety and complying with new rules addressing environmental contamination from *L. monocytogenes*.