ANTIMICROBIAL PROPERTIES OF MONOLAURIN AND SELECTED ANTIOXIDANTS IN VITRO AND IN GROUND PORK

by

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This thesis is dedicated

to my parents

Mr. and Mrs. Tsung Yuan Cheng

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INTRODUCTION

Many GRAS (Generally Recognized As Safe) antimicrobial agents have been shown to be effective in inhibiting microbial growth in culture media but less effective in food systems, especially those containing lipids. While all these GRAS agents are effective in inhibiting microorganisms to various degrees, all have limitations when used alone. For example, fatty acids and their glyceride derivatives are effective against gram-positive organisms and fungi, but have little activity against gram-negative organisms; the phenolic antioxidants are active against a wide variety of organisms, but are limited by toxicological considerations. In order to maximize the effectiveness of these additives, it is necessary to adopt a "preservative system" to the challenge posed by the need for food preservation and to avoid the limitations of single chemical preservatives. The "preservative systems" advantageously utilized the multiple function of the food additives discussed and introduce them into food products in order to create an environment unfavorable to microorganisms.

A "preservative system" from currently approved food additives was initiated in 1978 by Kabara (1978). Since then intensive screening research works on this were followed by many food microbiologists. Kabara reported that for many applications a system of monoglyceride, food-grade phenolic, and chelator with a ratio of 1:1:1 was found to be satisfactory.

Under the nature and scope of this approach, the purposes of this study were to select the best combination (preservative system) from monolaurin (monoglyceride of lauric acid) and (food-grade phenolic antioxidants such as BHA, BHT, TBHQ, and PG) at pH 6.8, 5.6, and 4.5 to control the bacterial activity *in vitro* as well as to apply this preservative system to the ground pork to ascertain its practical value in a food system.

LITERATURE REVIEW

Antimicrobial Properties of Fatty Acids

Fatty acids were first mentioned on a 4,000 year-old clay tablet uncovered at Tello, Mesopotamia. With the property of reducing surface tension, they played a more prominant role as a surfactant than an antimicrobial reagent. With only a few exceptions, fatty acids are all straight-chains of carboxylic acids, ranging from three to eighteen carbons. The early literature relating their antimicrobial properties was reported by Bayliss (1936) who found that the optimum activity for even numbered carbon aliphatic fatty acids was C_{12} saturated fatty acids and C_{18} mono and di-unsaturated fatty acids. In subsequent years, the antifungal and bactericidal properties of fatty acids have been extensively investigated. Fatty acids like formic, acetic, diacetic, propionic, sorbic and caprylic acids are approved as antimicrobial food additives in many countries.

Hoffman et al. (1939) studied the fungistatic properties of the fatty acids. They proposed that at neutrality, the acids containing eight to 12 carbon atoms are the most effective for the inhibition of mold growth. A branched chain acid is less effective fungistatically than the corresponding straight chain acid.

The structural relationships of 30 straight-chain fatty acids and derivatives and their bactericidal properties were studied with 8 Gram negative and 12 Gram positive organisms by Kabara et al. (1972). They found that C_{12} (lauric acid) is the most inhibitory saturated fatty acid against Gram positive organisms. Alcohols and glyceryl esters were active only against Gram positive organisms. Esterification of the carboxyl group led to a compound which was less active. The free carboxyl group is necessary for the activity. However, the monolaurin was the only ester of a fatty acid having higher antimicrobial activity than it's free acid.

Freese et al. (1973) studied the mechanism of inhibition of lipophilic acids on Bacillus subtilis and Escherichia coli, and proposed that lipophilic acids prevent

bacterial growth by inhibiting the transport of amino acids, organic acids, and phosphate.

Galbraith and Miller (1973) studied the effect of long chain fatty acids on bacterial respiration and amino acid uptake. They found that lauric acid stimulated oxygen uptake by Bacillus megaterium at 0.5 mM and produced inhibition at 1.0 mM. Also 0.05 mM lauric acid stimulated glutamic acid uptake by B. megaterium but further increase in lauric acid concentration to 0.1 mM resulted in inhibition of glutamic acid uptake.

Woolford (1975) studied the effect of straight chain fatty acids against different groups of microorganisms isolated from silage. He reported that at pH 6.0, the lauric acid showed the minimum inhibitory concentration (MIC) of 2 mM against homofermentative lactic acid bacteria, 2 mM against heterofermentative lactic acid bacteria, 0.5 mM against Clostridium butyricum, 125 mM against Bacillus mycoides, 4 mM against yeast, 4 mM against mold, 1 mM against Staphylococcus aureus, and > 125 mM against Escherichia coli, respectively.

Kondo and Kanai (1976) worked on the lethal effect of long chain fatty acids on Mycobacterium bovis, and proposed that the killing effect was accompanied by inhibition of the membrane-bound acid phosphatase activity. They also suggested that the mycobactericidal action of long chain fatty acids is due to their detergent-like action on the cytoplasmic membrane, and that the determining factor for the fatty acid-sensitivity of bacteria is the property of the cell wall by which fatty acids are adsorbed so that the active site is brought into contact with the inner membrane.

Kabara et al. (1977) screened 40 natural or synthetic lipophilic compounds for antimicrobial activity. They found that Gram positive bacteria and yeasts but not Gram negative bacteria were affected by these agents. These facts suggest that the mechanism of bactericidal action of long chain fatty acids and derivatives

is due to a balance between hydrophilic and hydrophobic parts of the molecule; the fluidity of the cell membrane can be disturbed maximally by lipophilic compounds of particular chain lengths. Among the fatty acids and their corresponding monoglycerides tested, those with C_{12} were found to be most effective in inhibiting microorganisms.

Kato (1981) studied the antimicrobial activity of fatty acids (alkyl chain length 7, 9, 11, and 13) and their esters against a film yeast, Saccharomyces rouxii, in soy sauce, and found that capric acid and monolaurin had the highest inhibitory activity. However, although capric acid has a high antimicrobial activity against the yeast, it has a low water-solubility, undesirable odor and other properties which make it unsuitable as an antimicrobial agent. Two selected sugar esters (sucrose monocaprate and sucrose monolaurate) could not completely inhibit the growth of the test organism even after 3 weeks of contact.

Chipley et al. (1981) worked on inhibition of Aspergillus growth and extracellular aflatoxin accumulation by sorbic acid and derivatives of fatty acids. Mycelia grown in the presence of fatty acid derivatives contained less phosphorus, potassium, magnesium, phosphatidyl ethanolamine, phosphatidyl serine, cholesterol, and triglycerides. Sorbic acid at 1,000 ppm completely inhibited extracellular accumulation of aflatoxins B_1 and B_2 of Aspergillus flavus, and 750 ppm of monolaurin inhibited the extracellular accumulation of aflatoxins. They reported that fatty acid derivatives were more effective inhibitors of extracellular accumulation of aflatoxins and that sorbic acid was more effective as a general inhibitor of mycelial growth.

Antimicrobial Properties of Monolaurin

The antibacterial properties of monolaurin, a monoacyl ester of lauric acid to glycerol, were reported by Kabara et al. (1977) They found that monolaurin was

the most active ester among the esters of saturated fatty acids (C_{11}, C_{12}, and C_{13} fatty acids) in inhibiting Gram positive bacteria. In contrast to result obtained by Kabara et al. (1977), Robach et al. (1981) proposed that monolaurin was effective against the growth of Salmonella typhimurium and not effective against Staphylococcus aureus. They also found that monolaurin had more antimicrobial activity in laboratory media than in pork homogenate.

Kimsey and Adams (1981) found that the presence of monolaurin in the heating menstruum of Bacillus stearothermophilus 1518 spores increased the rates of spore inactivation at 113-121° C by 2-3 fold. They also observed that increasing the concentration of monolaurin from 0.4 mM to 3.6 mM increased the rate of inactivation, but concentrations higher than 3.6 mM did not appear to influence the effectiveness of monolaurin.

Notermans and Dufrenne (1981) reported that glyceryl monolaurate at 5 g per kg (5000 ppm) of meat slurry (pH 6.0 - 6.2) inhibited toxin production by Clostridium botulinum type A (strain 73 A), type B (strain OKRA) and type E (strain RIV 2). However, the addition of butylated hydroxy-anisole (BHA) to glyceryl monolaurate had no effect upon the concentration needed for inhibition of botulinum toxin production.

Monolaurin was tested against 16 fungi belonging to different groups and having different cell wall compositions, and in most cases monolaurin showed antifungal activity at a concentration of 0.5% (5000 ppm) (Lisker and Paster, 1981).

Baker et al. (1982) observed that addition of monolaurin (250 ppm) extended the shelf-life of mechanically deboned chicken meat, minced fish and chicken sausage by ca. 2 days when stored at 2° C. They also found that higher concentrations of monolaurin (1000 ppm) were not more beneficial than a concentration of 250 ppm.

Hierholzer (1982) studied the effects of monolaurin on human RNA and DNA enveloped viruses. He found that at concentrations of 1% (10,000 ppm) additive in the reaction mixture for 1 hour at 23° C, all viruses were reduced in infectivity by > 99.9%. The monolaurin reduced infectivity of all viruses tested by disintegrating the virus envelope.

Kabara (1984) studied the inhibition of Staphylococcus aureus 196E in an agar-meat system by monolaurin. He found that monolaurin at 5,000 ppm caused bactericidal effects on S. aureus 196E.

Antimicrobial Properties of Antioxidants

Ward and Ward (1967) studied the effect of butylated hydroxytoluene (BHT) upon Salmonella senftenberg. They observed that the inhibitory effects of BHT upon S. senftenberg appear to be slight. Inhibition beyond 24 hours would require the impractically high concentration of at least 1.0% (10,000 ppm) BHT.

Chang and Branen (1975) tested the antimicrobial effects of butylated hydroxyanisole (BHA) on Aspergillus parasiticus, Salmonella typhimurium, Staphylococcus aureus, and Escherichia coli. They found that 1,000 ppm of BHA totally prevented growth and aflatoxin production of A. parasiticus spores. Of the bacteria tested, S. aureus was the most sensitive to BHA. BHA at 150 - 200 ppm inactivated the initial 10^6/ml inoculum. However, 400 ppm of BHA was necessary to inhibit E. coli. Salmonella typhimurium was the least sensitive to BHA. After 4 hours of treatment at 400 ppm of BHA, the inoculum decreased by 99% but after 6 hours the organism started to grow.

Shih and Harris (1976) studied the antimicrobial activity of selected antioxidants and found propyl gallate (PG) had a lethal effect against Escherichia coli at 400 ppm level. However, BHA and combined PG-BHA were not as effective in killing E. coli. When tested against Staphylococcus aureus, BHA and combined

PG-BHA had strong antimicrobial activity at the 400 ppm level, but propyl gallate alone had little effect against Staphyloccus aureus.

Robach et al. (1977) studied the inhibition of Vibrio parahaemolyticus by butylated hydroxyanisole (BHA) in trypicase soy broth and in a homogenate of blue crab meat. They found that growth in trypicase soy broth was inhibited by 50 ppm of BHA while 400 ppm of BHA was required to inhibit growth in the crab meat homogenate. They assumed that the marked decrease in the inhibition of growth of Vibrio parahaemolyticus by BHA in the carb meat homogenate as compared to inhibition in trypicase soy broth may have been due to partial reduction of the antioxidant properties of BHA by the presence of oxidized crab meat lipids. Therefore, the antimicrobial activity of BHA may depend on the lipid content and degree of lipid oxidation in the food product.

Fung et al. (1977) worked on the effects of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) on growth and aflatoxin production of Aspergillus flavus. They found that BHA (0.005 - 0.020 g per plate) had an inhibitory effect on growth and toxigenesis of the test organisms, while BHT (0.005 -0.020 g per plate) had no visible inhibitory effects. Sporulation of the cultures had no direct relationship with toxigenesis in the presence of BHA.

Vardaman et al. (1978) tested the effect of butylated hydroxytoluene on Mycoplasma synoviae (Ms) in vitro and in the feed. Results of in vitro studies showed that 10 ppm of BHT in Mycoplasma medium prevented growth of all six M. synoviae isolates. BHT at 100, 200, and 400 ppm in the feed did not have any significant in vivo effect on the serological responses of M. synoviae.

Klindworth et al. (1979) worked on the inhibition of Clostridium perfringens by butylated hydroxyanisole. Three strains of C. perfringens were inhibited by 150 ppm BHA in fluid thioglycollate medium. BHA was equally effective as an autoclaved or filter-sterilized solution. At 100 and 200 ppm, BHA was found to be

bactericidal to C. perfringens cells in a dilution buffer. In the presence of a lipid and surfactant, the antimicrobial activity of BHA against C. perfringens was greatly reduced.

Robach and Pierson (1979) studied the inhibition of Clostridium botulinum type A and B by phenolic antioxidants. They reported that BHA inhibited growth of three proteolytic strains of C. botulinum in prereduced thiotone yeast-extract glucose medium (pH 7.0) at 37° C. There was outgrowth and toxin production by strain 10755A spores when 25 ppm of BHA was present in the medium, whereas growth was inhibited by 50 ppm of BHA. BHT and PG were less effective in inhibiting outgrowth of C. botulinum spores than was BHA. Spore outgrowth and toxin production were inhibited in the presence of 200 ppm of BHT in the thiotone yeast-extract glucose medium. PG exhibited the least inhibitory activity of the antioxidants tested. None of the levels (25 to 200 ppm) of PG tested delayed outgrowth or toxin formation of the culture for more than 24 hours.

Pierson et al. (1979) studied the inhibition of Salmonella typhimurium and Staphylococcus aureus by BHA and propyl ester of p-hydroxybenzoic acid. They reported that 200 ppm of BHA was bactericidal to Staphylococcus aureus, while up to 400 ppm of BHA was only restrictive to growth of Salmonella typhimurium. A gradual decline in viable cell numbers of S. aureus was noted with addition of 500 ppm of propylparaben while an initial reduction of S. typhimurium and subesquent growth occurred at a level of 300 ppm of propylparaben. In combination, BHA and propylparaben showed no additional inhibition against S. aureus in comparison to BHA alone. However, an additive effect of the two compounds was noticeable with S. typhimurium.

Surak and Singh (1980) studied butylated hydroxyanisole (BHA) induced changes in the synthesis of polar lipids. They found that when butylated hydroxyanisole (BHA) was added to cultures of Tetrahymena pyriformis at

concentrations up to 12.5 x 10^{-6} gram/ml, an inhibition in the synthesis of polar lipids was observed. Increasing concentrations of BHA decreased the percentage of Na-2-(^{14}C) - acetate incorporated into lysophosphatidylcholine, 2-aminoethylphosphonolipids, and unknown polar lipid L

Gailani and Fung (1984) studied the antimicrobial properties of BHA, BHT, TBHQ, and PG, alone or in combinations, on 16 Gram negative and 8 Gram positive bacteria in laboratory media. They found that antioxidants inhibited Gram-positive bacteria more than Gram-negative bacteria and the inhibitory effects were bactericidal rather than bacteriostatic.

Lin and Fung (1983) studied the effect of BHA, BHT, TBHQ, and PG on growth and toxigenesis of selected aspergilli in vitro and in salami. They concluded that BHT and PG (0.001, 0.005, 0.01, 0.02 g per plate) did not inhibit growth, sporulation, and toxigenesis of all cultures. Aflatoxin production by toxigenic aspergilli (B_1, B_2, G_1, and G_2) in presence of BHA, TBHQ, and a combination of BHA and TBHQ was reduced significantly ($P < 0.05$). In salami, BHA or TBHQ alone or in combination at 100 ppm decreased ($P < 0.05$) the aflatoxin production by aspergilli when compared to control samples. A combination of BHA and TBHQ showed synergistic inhibition in both studies.

Fung et al. (1985) concluded that antioxidants, BHA, BHT, TBHQ, and PG are potentially useful antimicrobial compounds in both laboratory media and in food system. They also suggested that antioxidants alone within legal limits cannot be used as antimicrobials in food. However, combinations of antioxidants with other antimicrobial agents may be beneficial.

The Effect of pH on Antimicrobial Activity

Kitajima and Kawamura (1932) observed that an increase from pH 6.5 to pH 7.5 increased the minimum inhibitory concentrations of the short-chain acids

(caproic, caprylic, capric) but decreased the minimal inhibitory concentrations of two medium-chain fatty acids (lauric, myristic). However the minimum inhibitory concentrations of the unsaturated fatty acids were unaffected by a change in pH value in the medium.

Chung and Goepfert (1970) observed that the growth of salmonellae occurred at pH values as low as 4.05 $\pm$ 0.05. The growth-limiting pH was dependent on several factors, and the most important factor was the acid molecule itself. They also found that the salmonellae could not be "trained" to grow at a lower pH by sequential transfer at near optimal pH values.

Stern et al. (1979) worked on the inhibition of Staphylococcus aureus growth by combinations of BHA, NaCl, and pH. They concluded that pH value of medium does affect the effectiveness of BHA in inhibition of microbial growth. Without adding BHT, S. aureus still can grow well in the media at pH 7, 6, and 5 after 48 hours. pH value of medium is the critical factor in determining the duration of lag phase among the growth cycle.

Kimsey and Adams (1981) reported that the influence of monolaurin on Bacillus stearothermophilus spores did not appear to be pH dependent over the range of pH 6 to 8.

Lahellec et al. (1981) worked on the growth effect of sorbate and selected antioxidants on toxigenic strains of Staphylococcus aureus. They found that potassium sorbate at 1, 3, and 5% levels in combination with BHA, BHT, PG (50 and 100 ppm) exerted greater bactericidal and bacteriostatic effects on S. aureus strains at pH 5 than at pH 7; at pH 6 the effect was more pronounced at 3 and 5% compared with 1% sorbate.

Notermans and Heuvelman (1983) worked on the combined effect of water activity, pH, and sub-optimal temperature on growth and enterotoxin production of Staphylococcus aureus. They concluded that growth of S. aureus was not observed

at A_w 0.85, at pH 4.3, or at 8° C. At 12° C no growth occurred at A_w 0.93 in combination with pH < 5.5. At A_w 0.96 no growth occurred at pH < 4.9.

Montville (1983) studied the interaction of pH and NaCl on culture density of Clostridium botulinum 62A. He observed that the growth rates of C. botulinum 62A declined with decreasing pH and increasing salt levels. Lysis rates, however, were affected only by pH. He concluded that growth occurred in media at pH 5.0, but only in the absence of added salt (2%), and the growth did not occur at pH 4.8.

Tuncan and Martin (1985) studied the effect of pH, temperature, and potassium sorbate on amino acid uptake in Salmonella typhimurium 7136. They found that low pH had an apparent synergistic effect on amimo acid uptake inhibition caused by sorbate. The inhibition of amino acid uptake by sorbate was much greater at pH 5.0 than at pH 6.0.

The Microbial Flora on Meats

Red meats, fish, and poultry meat contain appreciable but variable amounts of carbohydrates, amino acids, nucleotides, essential minerals and lipids in addition to protein. Hence they provide an ideal environment for the growth of microorganisms. During storage of meat, biochemical activties of these organisms are responsible for the changes in appearance, flavor, odor and texture, which eventually render the food unacceptable to the consumer.

Under aerobic conditions, spoilage of fish meat ($10^8/cm^2$) is dominated by Pseudomonas, Moraxella-Acinetobacter, and Alteromonas (Shewan, 1974). However, in the case of red meats, under low oxygen tension and high carbon dioxide tension (anaerobic condition), spoilage was by Gram positive bacteria mainly the lactic acid bacteria (Gill and Newton, 1978).

Kubokura (1983) studied the temperature requirements of bacterial isolates from raw meat (beef, pork, and chicken). They isolated 1056 strains of bacteria

from refrigerated and frozen raw meat after incubating agar plates at 7°, 25°, and 37° C. Of the strains isolated from plates incubated at 7° C, two (0.4%) were obligate psychrophilic bacteria, which grew only within a temperature range from 7° to 20° C. Among the strains isolated from plates incubated at 7° C and 25° C, 61.8% and 32.5%, respectively, could not grow at 35° C. All strain isolated at 7° C and 37° C could grow at 25° C, except for the two. Therefore, 25° C was presumed to be the best temperature to recover most bacteria in meats.

Mahoney and Campbell (1983) studied the total plate count, psychrotrophic count, coliform count, coagulase positive Staphylococcus count, and Salmonella count on meat samples (minced meat, sausage, cut pork, and primal cut). They found that in all samples studied the total plate counts obtained at 30° C and 4 - 6° C incubation temperature were similar, and suggested that psychrotrophs were the dominant flora of the meat samples. Of the 41 samples, 32% were reported to harbor low numbers of coagulase positive Staphylococcus. Salmonella was not recovered from any of the samples analysed in this study.

MATERIALS AND METHODS

I. Antimicrobial Properties of Monolaurin Combined with Selected Antioxidants in an in Vitro System

Organisms Tested

Sixteen bacterial species representing a variety of environment bacteria and potential pathogen were studied in an *in vitro* system to ascertain the effects of monolaurin and four selected antioxidants under different pH values. The organisms tested were obtained from the cultures collection of the Food Microbiology Labortory of Kansas State University.

A) Gram-negative bacteria

1. *Acinetobacter calcoaceticus*
2. *Bordetella bronchiseptica*
3. *Citrobacter freundii*
4. *Enterobacter aerogenes*
5. *Escherichia coli*
6. *Hafnia alvei*
7. *Klebsiella pneumoniae*
8. *Morganella morganii*
9. *Proteus mirabilis*
10. *Providencia stuartii*
11. *Pseudomonas fluorescens*
12. *Salmonella molade*
13. *Serratia marcescens*
14. *Shigella sonnei*

B) Gram-positive bacteria

1. *Staphylococcus aureus*
2. *Streptococcus faecalis*

All Gram negative cultures were identified by means of 26 different biochemical tests and Gram reaction. The biochemical tests used were as follows:

1. Indole test
2. Methyl red test
3. Voges-Proskauer test
4. Simmons' Citrate test
5. Hydrogen Sulfide test (TSI)
6. Urea test
6. KCN test
8. Motility test
9. Gelatin test (22° C)
10. Lysine Decarboxylase test

11. Arginine Dihydrolase	12. Ornithine Decarboxylase
13. Phenylalnine Deaminase	14. Malonate test
15. Gas from glucose	16. Lactose
17. Sucrose	18. D-Mannitol
19. Dulcitol	20. Salicin
21. Adonitol	22. i (meso) Inositol
23. D-Sorbitol	24. L-Arabinose
25. Raffinose	26. L-Rhamnose

The Gram positive cultures were identified by conventional procedures. The biochemical characteristics of individual bacteria studied were matched against information in Bergey's manual (Buchanan and Gibbons, 1974) and Difco's biochemical chart (Difco laboratories, Detroit, MI, 1984).

Media Used

Antimicrobial properties of antioxidants and monolaurin against test cultures were studied in both nutrient broth (Difco) and nutrient agar (Difco) to ascertain effectiveness of test compounds in a solid system as well as in a liquid system. Some researchers (Fung et al., 1977) considered a solid system to be more effective while others (Klindworth et al., 1979) considered a liquid system more satisfactory.

pH Values Tested

Three pH values (6.8, 5.6, 4.5) were tested in this challenge study. pH 6.8 is selected since it is the pH of nutrient broth and most bacteria grow best at this pH. pH 5.6 is selected because it is the pH value of fresh ground pork. pH 4.5 is selected because it is the critical pH that limits growth of most bacteria.

Antimicrobial Reagents Preparation

The stock solution of monolaurin, donated by J. Kabara, Michigan State University (Lauricidin Inc., Lot # 30301, Okemos, MI.), was prepared by dissolving 10 grams of monolaurin in 1 liter of 95% ethyl alcohol (w/v), and the final concentration of stock solution was 10,000 ppm.

The stock solutions of the four selected antioxidants BHA, BHT, PG (Sigma Chem. Co., St. Louis, MO) and TBHQ (Aldrich Chem. Co., Milwaukee, WI) were prepared by dissolving 10 grams of antioxidant in 1 liter of 95% of ethyl alcohol (w/v), making the final concentration of each stock solution 10,000 ppm.

Media Preparation

Proper amount of nutrient broth or nutrient agar in a flask was boiled in a water bath until completely dissolved. The media were cooled to 50° C before adjustment of pH by the addition of 8 N NaOH or 5 N HCl. Final pH value was measured by Beckman 43 pH meter.

Into aliquots of 10 ml pH-specified nutrient broth, predetermined quantities of monolaurin or selected antioxidants were introduced into individual tubes to make final concentrations of 100, 200, 300, 400, 500, 600, 700, 800, 900, and 1,000 ppm, respectively. Similarly into aliquots of 20 ml of pH-specified nutrient agar, predetermined quantities of monolaurin or selected antioxidants were introduced to individual test tubes to make final concentrations of 100, 200, 300, 400, 500, 600, 700, 800, 900, and 1,000 ppm, respectively. In studying the combination effect of monolaurin with selected antioxidant, all combinations were based on the ratio of 1 to 1. All tubes were sterilized after proper addition of compounds. Fung et al. (1977) indicated the sterilization did not affect activities of antioxidants.

This research involved 54 different combinations (Fig. 1).

Miniaturized Microbiological Testing Procedures for Nutrient Agar and Nutrient Broth

In studying the antimicrobial properties of monolaurin and antioxidants, all tests were conducted using the "miniaturized microbiological method" developed by Fung and colleagues (Fung and Kraft, 1968; Fung and Miller, 1970; Fung and Hartman, 1972 and 1975, and Fung, 1976). Tests were conducted in the wells of sterile microtiter plates (8 x 12 wells per plates) for the nutrient broth, and petri-dish (100 x 15 mm) for the nutrient agar. The sterile microtiter plates and sealers were obtained from Dyna-tech Lab group (Alexandria, VA). The sterile petri-dishes were obtained from Fisher Scientific (St. Louis, MO). For the nutrient broth, 0.2 ml of sterile media was added to each well. The plates were covered with sterile plate sealer, which prevented contamination and evaporation during storage and incubation of the plates. For the nutrient agar, sterilized media were poured into petri-dishes and incubated overnight at 37° C before use.

A master plate was prepared by aseptically transferring 4 drops (0.2 ml) of individual bacterial cultures into one of 96 wells of the sterile microtiter plate. An inoculation device having 20 pins, with their heads protuding outwards, was used for inoculation. The pins were spaced so that each one fits into a different one of the 20 wells in the microtiter plate. Four batches, each consists of 20 sub-units, with different concentrations of antimicrobial reagents were studied in one microtiter plate. The remaining 16 wells were used as a control group (without addition of antimicrobial reagents).

Sterilization of the inoculation device was implemented by dipping the pins into alcohol for 30 seconds, and then flaming, according to Fung and Hartman (1975). The sterile inoculation device was then loaded with bacterial cultures by dipping it into the master plate. Organisms were then transferred to media by introducing the loaded inoculator into the nutrient broth or to the surface of

nutrient agar. Each pin head transferred about 3×10^5 Colony Forming Units (CFU) of bacteria from a 24 hour old culture containing 5×10^8 CFU/ml (Fung and Miller, 1970).

After inoculation and incubation for 24 hours at 32° C, the plates were examined visually for turbidity in nutrient broth or the appearance of the colonies in nutrient agar. Results were recorded as positive or negative for growth or no growth of the cultures, respectively. Differentiation between bacteriostatic and bactericidal effects of monolaurin or antioxidants, in the nutrient broth, were made by sub-culturing liquid from wells that showed no growth, onto sterile nutrient agar. The agar plates were incubated at 32° C for 24 hours before checking for bacterial growth. Positive growth at this last stage indicated bacteriostatic activity. In this research, all tests were done in triplicate.

Statistical Analysis

In the in vitro system the results were recorded as the Minimum Inhibitory Concentration (MIC). The mean values from the triplicate data were reported instead of 3 individual values. To simplify the statistical work, 1,100 ppm was listed on the tables rather than > 1,000 ppm when bacteria showed growth at the concentration of 1,000 ppm.

Statistical analysis was done by analysis of variance with Least Significant Difference (LSD) test at probability of 0.05 used for determining differences between multiple means.

II. Antimicrobial Properties of Monolaurin and/or TBHQ in Ground Pork

Sample Preparation

Fresh pork sample, 20 lbs, was obtained from Department of Animal

Sciences and Industry, Kansas State University, Manhattan, Kansas. Fat tissues were trimmed from the cut, and lean meat and fat tissue were ground separately via coarse and then fine grinding consecutively. Hobart fat indicator instrument was adopted to determine the percentage of fat in the lean meat. The final percentage of fat was adjusted to 25% by addition of ground fat. The fat tissue and lean were mixed, reground, and divided into 50 gram portion per stomacher bag.

Predetermined quantities of monolaurin or TBHQ, which showed the best antimicrobial properties under the in vitro system at pH 5.6, were pipetted into 50 grams of meat samples to give the final concentrations of 200, 400, and 600 ppm. In meat, the addition of antioxidant is regulated by Food and Drug Administration in that the total content of antioxidant shall not be over 0.02 percent (200 ppm) of fat or oil content, including essential (volatile) oil content of food. Under this regulation, the addition of TBHQ is based on 25% fat content rather than the ground pork sample weight (50 grams) in this part of study. Monolaurin, a generally recognized as safe (GRAS) compound, is regulated under the good manufacture practices (GMP) which means monolaurin can be used in food as much as possible. In studying the combination effect of monolaurin with TBHQ, all combinations were based on the ratio of 1 to 1. Control groups contained no additives.

All the bags were labeled, rolled, and tied with rubber bands before storing at 4° C of refrigerator. Bags were taken out at 0, 1, 3, 5, and 7 days for microbiological analysis. All treatments were done in duplicate.

Microbiological Analysis

In meat samples, the organisms monitored are aerobic psychrotroph counts and fecal coliform counts.

A massaging device, the stomacher (Lab-Blender 400, London, U.K.), was

utilized to mix the sample with 0.1% peptone dilution buffer solution before performing viable cell counts (Sharpe and Jackson, 1972). The machine eliminated efforts involved in cleaning and sterilizing reusable blenders, and it releases significantly larger numbers of viable microorganisms than most other methods of homogenization.

After removal of sample bags from refrigeration, 200 ml of sterile buffer solution was added to the 50 grams of meat, and then the bag was treated for 2 minutes in the stomacher. The slurry was again diluted accordingly for viable cell count (APHA, 1985).

For the aerobic psychrotroph counts, the plates (Standard Plate Count Agar, Difco) were put into plastic bags and incubated at 7° C for 10 days. Enumeration of fecal coliforms was made by the procedure of Klein and Fung (1976). Pour plates of Violet Red Bile Agar (Difco) were prepared and allowed to solidify, then another layer of the medium was poured on the top to prevent spreaders before incubation at 45° C for 24 hours. Duplication of each dilution were done in all treatments.

Statistical Analysis

In ground pork, results were recorded as the number of bacteria counts per gram along with the storage period. The mean values from the duplicate data were reported instead of 2 individual values.

Statistical analysis was done by analysis of variance with Least Significant Difference (LSD) test at probability of 0.05 used for determining difference between multiple means.

RESULTS

Antimicrobial Properties of Monolaurin with Selected Antioxidants in an in Vitro System

Effect on Acinetobacter calcoaceticus

For Acinetobacter calcoaceticus, the poorest antimicrobial effect was BHT in nutrient broth or nutrient agar at pH 6.8 and BHT in nutrient agar at pH 5.6; a large number of treatments were effective against this organism at lower concentrations. For example, monolaurin with BHA in nurtrient agar at pH 5.6, BHA in rutrient broth at pH 6.8, and monolaurin in nutrient agar at pH 4.5 (Table 1, Appendix p. 41).

In comparing the effects of media on growth of this organism, at all concentrations tested this organism was inhibited more effectively in nutrient agar than nutrient broth ($P < 0.05$) (Table 20, Appendix p. 80).

Within the 3 pH values studied, both pH 6.8 and 5.6 showed no difference ($P > 0.05$) and less effective than pH 4.5 in inhibiting this organism ($P < 0.05$) (Table 21, Appendix p. 81).

Among the 9 reagents tested on this organism, BHT showed the least effect of inhibition, and BHA had the best inhibiting effect (Table 22, Appendix p. 83).

Effect on Bordetella bronchiseptica

For Bordetella bronchiseptica, the poorest antimicrobial effects were from BHT in nutrient broth at pH 6.8, monolaurin with BHT in nutrient agar at pH 6.8, BHT in nutrient agar at pH 5.6, and monolaurin in nutrient agar at pH 5.6 but not different than 4 other treatments; a number of treatments were equally effective against this organism at lower concentrations. For example, PG in nutrient broth at pH 4.5, monolaurin in nutrient agar at pH 4.5, and monolaurin with TBHQ in

nutrient agar at pH 4.5 (Table 2, Appendix p. 43).

In comparing the effects of media on growth of this organism at all concentrations tested, nutrient broth and nutrient agar were not different ($P > 0.05$) (Table 20, Appendix p. 79).

Within the 3 pH values studied, pH 6.8 showed the least effect, pH 5.6 was in between, and pH 4.5 had the best performance in inhibiting this organism ($P < 0.05$) (Table 21, Appendix p. 81).

Among the 9 reagents tested on this organism, BHT showed less inhibition than all treatments except monolaurin plus BHT; TBHQ, PG, BHA, monolaurin with PG, and monolaurin with TBHQ had the best inhibiting effects (Table 22, Appendix p. 83).

Effect on Citrobacter freundii

For Citrobacter freundii, the least effective antimicrobial agents were combinations of monolaurin with BHT in nutrient agar at pH 5.6, monolaurin in nutrient broth at pH 5.6, monolaurin in nutrient agar at pH 5.6, and BHT in nutrient agar at pH 5.6 but not different than 11 other treats; a lot of treatments were effective against this organism. For example, TBHQ in nutrient broth at pH 4.5 and PG in nutrient broth at pH 4.5 (Table 3, Appendix p. 45).

In comparing the effects of media on growth of this organism at all concentrations tested, this organism was inhibited more effectively in nutrient broth than in nutrient agar ($P < 0.05$) (Table 20, Appendix p. 79).

Within the 3 pH values studied, both pH 5.6 and 6.8 were similar ($P > 0.05$), but were less effective than pH 4.5 in inhibiting the organism ($P < 0.05$) (Table 21, Appendix p. 81).

Among the 9 reagents tested on this organism, BHT, monolaurin, monolaurin with BHT showed the least inhibition; TBHQ, PG, and BHA had the best inhibiting

effects (Table 22, Appendix p. 83).

Effect on Enterobacter aerogenes

For Enterobacter aerogenes, a large number of treatments were equally and less effective. For example, monolaurin with BHT in nutrient agar at pH 5.6, BHT in nutrient broth at pH 6.8, and BHT in nutrient agar at pH 4.5; the best antimicrobial effects were PG in nutrient broth at pH 4.5, TBHQ in nutrient broth at pH 4.5, and BHA in nutrient broth at pH 4.5 but not different than 13 other treatments (Table 4, Appendix p. 47).

In comparing the effects of media on growth of this organism, at all concentrations tested this organism was inhibited more effectively in nutrient broth than in nutrient agar ($P < 0.05$) (Table 20, Appendix p. 79).

Within the 3 pH values studied, both pH 5.6 and 6.8 were not different ($P > 0.05$), but were less effective than pH 4.5 in inhibiting this organism ($P < 0.05$) (Table 21, Appendix p. 81).

Among the 9 reagents tested on this organism, monolaurin with BHT, BHT, monolaurin with BHA, and monolaurin showed the least effect of inhibition; TBHQ had the best inhibition (Table 22, Appendix p. 83).

Effect on Escherichia coli

For Escherichia coli, a number of individual treatments were less effective. The best antimicrobial effect was the combination of monolaurin with PG in nutrient broth at pH 4.5 but not different than 10 other treatments (Table 5, Appendix p. 49).

In comparing the effects of media on growth of this organism, at all concentrations tested, this organism was inhibited more effectively in nutrient broth than in nutrient agar ($P < 0.05$) (Table 20, Appendix p. 79).

Within the 3 pH values studied, both pH 5.6 and 6.8 were not different ($P > 0.05$), but were less effective than pH 4.5 in inhibiting the organism ($P < 0.05$) (Table 21, Appendix p. 81).

Among the 9 reagents tested on this organism, monolaurin, BHT, and monolaurin with BHT showed the least inhibition; PG and BHA had the best inhibiting effects (Table 22, Appendix p. 83).

Effect on Hafnia alvei

For Hafnia alvei, a number of treatments were less effective. TBHQ in nutrient agar at pH 6.8 tended to show the the best antimicrobial effect but not different than 11 other treatments (Table 6, Appendix p. 51).

This organism was inhibited more effectively in nutrient broth than in nutrient agar ($P < 0.05$) (Table 20, Appendix p. 79).

Within the 3 pH values studied, pH 5.6 showed the least effect, pH 6.8 was intermediate, and pH 4.5 had the best inhibition ($P < 0.05$) (Table 21, Appendix p. 81).

Among the 9 reagents tested on this organism, BHT, monolaurin, and monolaurin with BHT showed the least inhibition; TBHQ and BHA showed the best inhibiting effects (Table 22, Appendix p. 83).

Effect on Klebsiella pneumoniae

For Klebsiella pneumoniae, many treatments were equally and less effective. The best antimicrobial effect was the combination of BHA in nutrient broth at pH 4.5 but not different than 9 other treatments (Table 7, Appendix p. 53).

This organism was inhibited more effectively in nutrient broth than in nutrient agar ($P < 0.05$) (Table 20, Appendix p. 79).

Within the 3 pH values studied, both pH 5.6 and 6.8 showed no significant difference from each other ($P > 0.05$), but were less effective than pH 4.5 in inhibiting the organism ($P < 0.05$) (Table 21, Appendix p. 81).

Among the 9 reagents tested on this organism, monolaurin and BHT showed the least inhibition; TBHQ had the best (Table 22, Appendix p. 83).

Effect on Morganella morganii

For morganella morganii, the least antimicrobial effect was from monolaurin or BHT in nutrient agar at pH 5.6 but not different than 9 other treatments. Many treatments were effective against this organism at lower concentrations (Table 8, Appendix p. 55).

In comparing the effects of media on growth of this organism, at all concentrations tested nutrient broth and nutrient agar were not different ($P > 0.05$) (Table 20, Appendix p. 79).

For the 3 pH values studied, pH 5.6 showed the least effect, pH 6.8 was intermediate, and pH 4.5 resulted in the best inhibition of this organism ($P < 0.05$) (Table 21, Appendix p. 81).

Among the 9 reagents tested on this organism, BHT, monolaurin, and monolaurin with BHT were least effective; BHA and PG had the best inhibition (Table 22, Appendix p. 83).

Effect on Proteus mirabilis

For Proteus mirabilis, the least antimicrobial effect was from monolaurin in nutrient agar at pH 5.6, BHT in nutrient agar at pH 5.6, and monolaurin with BHA in nutrient agar at pH 5.6 but not different than 15 other treatments. The best antimicrobial effect was from the combination of monolaurin with PG in nutrient broth at pH 4.5 but not different than 16 other treatments (Table 9, Appendix p.

57).

In comparing the effects of media on growth of this organism, at all concentrations tested this organism was inhibited more effectively in nutrient broth than in nutrient agar ($P < 0.05$) (Table 20, Appendix p. 79).

For the 3 pH values studied, both pH 5.6 and 6.8 were not different ($P > 0.05$), but less effective than pH 4.5 in inhibiting the organism ($P < 0.05$) (Table 21, Appendix p. 81).

Among the 9 reagents tested on this organism, minimum inhibitory concentration was not very different, although TBHQ had a lower inhibitory concentration than BHT and monolaurin (Table 22, Appendix p. 83).

Effect on Providencia stuartii

For Providencia stuartii, a number of treatments were less effective against this organism. For example, monolaurin in nutrient agar at pH 5.6, monolaurin combined with BHT in nutrient agar at pH 6.8, and BHT in nutrient agar at pH 5.6. Many treatments were equally effective against this organism at lower concentrations (Table 10, Appendix p. 59).

In comparing the effects of media on this organism, nutrient broth and nutrient agar were not different ($P > 0.05$) (Table 20, Appendix p. 79).

For the 3 pH values studied, both pH 6.8 and 5.6 were not different ($P > 0.05$), but were less effective than pH 4.5 in inhibiting the organism ($P < 0.05$) (Table 21, Appendix p. 81).

Among the 9 reagents tested on this organism, monolaurin, BHT, and monolaurin with BHT showed the least effects of inhibition; BHA or TBHQ had the best inhibiting effects (Table 22, Appendix p. 83).

Effect on Pseudomonas fluorescens

For Pseudomonas fluorescens, the combination of monolaurin with BHA in nutrient agar at pH 5.6 tended to be least effective but not different than 9 other treatments. A lot of treatments were effective against this organism at lower concentrations (Table 11, Appendix p. 61).

In comparing the effects of media on growth of this organism, at all concentrations tested this organism was inhibited more effectively in nutrient broth than in nutrient agar ($P < 0.05$) (Table 20, Appendix p. 79).

For the 3 pH values studied, pH 5.6 showed the least effect, pH 6.8 was intermediate, and pH 4.5 had the best performance in inhibiting this organism ($P < 0.05$) (Table 21, Appendix p. 81).

Among the 9 reagents tested on this organism, considerable overlapping of significance was found (Table 22, Appendix p. 83).

Effect on Salmonella molade

For Salmonella molade, the poorest antimicrobial effect was from the combination of BHT in nutrient agar at pH 4.5 but not different than 8 other treatments. No treatments had a clear advantage for the best antimicrobial effect (Table 12, Appendix p. 63).

This organism was inhibited more effectively in nutrient broth than in nutrient agar ($P < 0.05$) (Table 20, Appendix p. 79).

For the 3 pH values studied, both pH 5.6 and 6.8 were not different ($P > 0.05$), but had less effect than pH 4.5 in inhibiting this organism ($P < 0.05$) (Table 21, Appendix p. 81).

Among the 9 reagents tested on this organism, BHT showed the least effect of inhibition, and BHA had the best inhibiting effects (Table 22, Appendix p. 83).

Effect on Serratia marcescens

For Serratia marcescens, Many treatments were equally and less effective against this organism. For example, monolaurin in nutrient agar at pH 5.6, BHT in nutrient broth at pH 6.8, and monolaurin with BHT in nutrient agar at pH 6.8; the best antimicrobial effect was the combination of BHA in nutrient agar at pH 4.5 but not different than 16 other treatments (Table 13, Appendix p. 65).

In comparing the effects of media on growth of this organism, at all concentrations tested this organism was inhibited more effectively in nurtrient broth than in nutrient agar ($P < 0.05$) (Table 20, Appendix p. 79).

Within the 3 pH values studied, both pH 5.6 and 6.8 were not different ($P > 0.05$), but were less effective than pH 4.5 in inhibiting this organism ($P < 0.05$) (Table 21, Appendix p. 81).

Among the 9 reagents tested on this organism, monolaurin and BHT showed the least inhibition, and TBHQ had the best inhibiting effect (Table 22, Appendix p. 83).

Effect on Shigella sonnei

For Shigella sonnei, the least antimicrobial effect was from BHT in nutrient agar at pH 5.6 but not different than 6 other treatments. A large number of treatments were effective against this organism. For example, monolaurin with PG in nutrient broth at pH 4.5, BHT in nutrient broth at pH 4.5, BHA in nutrient broth at pH 4.5, monolaurin with TBHQ in nutrient broth, BHA in nutrient agar at pH 4.5, and PG in nutrient broth at pH 4.5 (Table 14, Appendix p. 67).

In comparing the effects of media on growth of this organism, nutrient broth and nutrient agar were not different ($P > 0.05$) (Table 20, Appendix p. 79).

For the 3 pH values studied, both pH 5.6 and 6.8 were not different ($P > 0.05$), but were less effective than pH 4.5 in inhibiting the organism ($P < 0.05$) (Table 21, Appendix p. 81).

Among the 9 reagents tested on this organism, BHT showed the least effects of inhibition; BHA had the best inhibiting effects (Table 22, Appendix p. 83).

Effect on Staphylococcus aureus

For Staphylococcus aureus, the least antimicrobial effect was from BHT in nutrient agar at pH 6.8 and pH 5.6, BHT in nutrient broth at pH 6.8 and pH 5.6; A large number of treatments were effective against this organism at lower concentrations (Table 15, Appendix p. 69).

In comparing the effects of media on growth of this organism, at all concentrations tested nutrient broth and nutrient agar were not different ($P > 0.05$) (Table 20, Appendix p. 79).

For the 3 pH values studied, both pH 6.8 and 5.6 were not different ($P > 0.05$), but were less effective than pH 4.5 in inhibiting the organism ($P < 0.05$) (Table 21, Appendix p. 81).

Among the 9 reagents tested on this organism, BHT showed the least effect of inhibition, and TBHQ tended to have the best inhibiting effect (Table 22, Appendix p. 83).

Effect on Streptococcus faecalis

For Streptococcus faecalis, the least antimicrobial effect was from combinations of BHT in nutrient agar at pH 6.8, BHT in nutrient broth at pH 6.8, and BHT in nutrient agar at pH 5.6. A number of treatments showed the best antimicrobial effects (Table 16, Appendix p. 71).

In comparing the effects of media on growth of this organism, at all concentrations tested this organism was inhibited more effectively in nutrient agar than in nutrient broth ($P < 0.05$) (Table 20, Appendix p. 79).

For the 3 pH values studied, pH 6.8 showed the least effect, pH 5.6 was intermediate, and pH 4.5 had the best performance in inhibiting the organism ($P < 0.05$) (Table 21, Appendix p. 81)

Among the 9 reagents tested on this organism, BHT showed the least inhibition, and TBHQ had the best inhibiting effect (Table 22, Appendix p. 83)

The Mean Values of Minimum Inhibitory Concentrations

Table 17 (Appendix p. 73) indicated the mean values of minimum inhibitory concentration of monolaurin and/or selected antioxidants on 16 bacterial cultures. The least Antimicrobial effect out of 54 combinations was BHT in nutrient agar at pH 5.6. Statistical results (Table 17) indicated that there was no inhibition as the concentration was increased to 1,000 ppm under this combination.

TBHQ in nutrient broth at pH 4.5 was the treatment which showed the best antimicrobial effect. Under this combination, the mean value of minimum inhibitory concentration was 202 ppm which is close to the practical usage level 200 ppm (100 ppm if single antioxidant).

The Maximum Values of Minimum Inhibitory Concentrations

Table 18 (Appendix p. 75) listed the maximum values of minimum inhibitory concentration among 16 bacteria cultures. Many treatments showed the highest maximum values of minimum inhibitory concentration, > 1,000 ppm, out of 54 combinations. In these combinations as high as 1,000 ppm showed no inhibitory effect.

The lowest maximum value of minimum inhibitory concentrations was 400 ppm shown by PG and TBHQ in nutrient broth at pH 4.5. This indicated that all 16 bacteria cultures were effectively inhibited and showed no growth with this treatment.

The Minimum Values of Minimum Inhibitory Concentrations

Table 19 (Appendix p. 77) indicated the minimum values of minimum inhibitory concentration among 16 bacteria cultures. BHT in nutrient agar at pH 5.6 was the combination which showed the highest minimum values of minimum inhibitory concentration, 866 ppm, out of 54 combinations. This indicated that bacteria were inhibited by no lower than 866 ppm under this combination.

The lowest minimum value of minimum inhibitory concentration was, 100 ppm, a value shown by many treatments. This indicated that bacteria were inhibited effectively as the dosage as low as 100 ppm.

Antimicrobial Properties of Monolaurin and/or TBHQ in Ground Pork

In a food system using ground pork with antioxidants and monolaurin, the effect on growth of psychrotroph and fecal coliforms was monitored. Of the 4 antioxidants (BHA, BHT, TBHQ, PG) tested in vitro, TBHQ was determined to be the most inhibitory to all cultures at pH 5.6 and was selected for future study in ground pork system. The pH value of the meat is also 5.6.

Effect on Psychrotrophs

Table 23 (Appendix p. 88) listed the results of antimicrobial properties of monolaurin and/or TBHQ on psychrotrophs at fixed intervals. In the control group, 8.9×10^3/g of psychrotroph were present in the meat sample at 0 time. After 24 hours of storage in 4° C, the psychrotroph counts increased less than 2 log cycles to not over 2.6×10^5/g. At the third day, the meat recovered high psychrotroph counts of over 10^7/g. TBHQ at 200, 400 ppm and monolaurin at 200 ppm showed similar counts at 1, 3, 5, and 7 days with the psychrotroph count of about 10^5/g, 10^6/g, 10^8/g, and 10^8/g, respectively. TBHQ at 600 ppm and monolaurin with TBHQ

at 200 ppm had similar bacterial growth patterns at 1, 3, 5, and 7 days with psychrotroph counts of about 10^4/g, 10^5/g, 10^8/g, and 10^8/g, respectively. Monolaurin at 400 and 600 ppm, and monolaurin with TBHQ at 400 ppm had similar bacterial growth patterns at 1, 3, 5, and 7 days with the psychrotroph counts of about 10^4/g, 10^5/g, 10^7/g, and 10^8/g, respectively. Monolaurin with TBHQ at 600 ppm had bacterial counts of about 10^4/g, 10^4/g, 10^6/g, and 10^7/g at 1, 3, 5, and 7 day intervals, which indicated that this combination effectively controlled psychrotrophs.

Effect on Fecal Coliform

Table 24 (Appendix p. 89) listed the results of antimicrobial properties of monolaurin and/or TBHQ on fecal coliforms at 1, 3, 5, and 7 day intervals. In the control group (without addition of antimicrobial reagents) as well as samples with TBHQ at 200, 400, 600, and monolaurin at 200 ppm, no fecal coliform growth was until the fifth day. Monolaurin at 400 and 600 ppm were effective in suppressing fecal coliform growth until the seventh day; monolaurin with TBHQ at 200, 400, and 600 ppm totally inhibited fecal coliform growth throughout the entire storage period.

DISCUSSIONS

Antimicrobial Properties of Monolaurin with Selected Antioxidants in an in Vitro System

The composite mean values of combined minimum inhibitory concentration data combined indicate that bacteria grow better in a solid (nutrient agar) than in a liquid medium (nutrient broth) ($P < 0.05$) (Table 20). This is different from the observation of Gailani and Fung (1984), who found that inhibition was greater in nutrient agar (solid medium) than in brain heart infusion (liquid medium). It could be attributed to the difference in nutrient contents between nutrient broth and brain heart infusion broth. Brain heart infusion is so nutritious that only bacteria (streptococci, pneumococci, and meningococci) considered difficult to cultivate are grown in it. Comparatively, the nutrient broth is nutrient-inferior and recommended for general laboratory use for the cultivation of the microorganisms that are not exacting in nutrient requirements (Difco manual).

Table 21 indicates the mean values of minimum inhibitory concentration (MIC) of 3 different pH values. At pH values tested, there is no significant difference ($P > 0.05$) between pH 5.6 and 6.8 in inhibiting bacterial activity. A similar result was reported by Kimsy and Adams (1981), who found that the influence of monolaurin on Bacillus stearothermophilus spores did not appear to be pH dependent over the range of pH 6 to 8. As expected, at pH 4.5, bacterial activity was significantly decreased.

Table 22 lists the mean values of minimum inhibitory concentration (MIC) of 9 individual antimicrobial reagents. In agreement with Lin and Fung (1983), Shih and Harris (1976), these mean values indicate that antioxidants, TBHQ, BHA, and PG, alone have significant antimicrobial activity, but BHT does not. Within the combinations between monolaurin and selected antioxidants, monolaurin increases ($P < 0.05$) its antimicrobial activity, however the antioxidants decrease ($P < 0.05$) their antimicrobial activity. Monolaurin alone shows the antimicrobial property at

700 ppm which is an acceptable level under the limitation of Good Manufacture Practice (GMP). According to this study, BHT is the least effective antioxidant against bacteria.

Antimicrobial Properties of Monolaurin and/or TBHQ in Ground Pork

In ground pork, TBHQ is less effective than monolaurin as a bacterial inhibitor. The reduction in activity could be attributed to: 1) the fact that allowable amount of TBHQ in the meat sample is based upon fat content rather than meat weight; 2) the inherent nature of TBHQ as an antioxidant against oxidation of lipid lessens its antimicrobial activity (Klindworth et.al., 1979); 3) TBHQ has a nonpolar character, therefore it might migrate and solubilize in lipid of a medium, and become unavailable to act on microorganisms (Branen et al., 1980).

Table 23 and 24 show that monolaurin alone or in combination with TBHQ can be used in meat to create environments which are inhibitory to microorganisms. In agreement with Lisker and Paster (1981) and Kabara (1984), synergistic effect were observed when combinations of monolaurin with other preservatives (BHA or sorbic acid) was used. In this study, a combination of monolaurin and TBHQ gave a greater inhibitory effect than either of the substances alone. Comparatively, concentration factor is less important than reagent factor against bacterial growth. A similar result was reported by Kimsey and Adams (1981).

CONCLUSIONS

Antimicrobial Properties of Monolaurin with Selected Antioxidants in Vitro

1. Nutrient broth (average MIC 497.5 ppm) created a somewhat more inhibitory environment to microorganisms than nutrient agar (average MIC 578.9 ppm).

2. Bacterial activity was significantly inhibited at pH 4.5, but not at pH 5.6 and 6.8. There were no consistent differences between pH 5.6 and 6.8.

3. TBHQ frequently showed the best antimicrobial activity of 9 reagent combinations tested.

4. TBHQ in nutrient broth at pH 4.5 is frequently the best combination of 54 treatments studied.

5. No synergistic effects were observed when combinations of monolaurin and selected antioxidants were tested in vitro.

Antimicrobial Properties of Monolaurin and/or TBHQ in Ground Pork

1. When compared to TBHQ, monolaurin was more active against bacterial growth.

2. The combination of monolaurin with TBHQ gave a greater inhibitory effect than either of the substances alone.

3. Comparatively, concentration factor is less important than reagent factor.

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APPENDIX

Figure 1. 54 Treatments Studied in an in Vitro System

In Vitro System

Treatment

Nutrient Agar			Nutrient Broth		
pH			pH		
6.8	5.6	4.5	6.8	5.6	4.5
BHA BHT TBHQ PG LAU LA LT LQ LG			BHA BHT TBHQ PG LAU LA LT LQ LG		

Each treatment was tested against 14 gram negative bacteria and 2 gram positive bacteria.

2 x 3 x 9 = 54 Treatments

Abbreviation

BHA: Butylated Hydrosyanisole

BHT: Butylated Hydroxytoluene

TBHQ: Tert-butyl Hydroquinone

PG: Propyl Gallate

LAU: Monolaurin

LA: Monolaurin and Butylated Hydroxyanisole

LT: Monolaurin and Butylated Hydroxytoluene

LQ: Monolaurin and Tert-butyl Hydroquinone

LG: Monolaurin and Propyl Gallate

Table 1: Minimum Inhibitory Concentration (MIC) of Monolaurin and/or Selected Antioxidants on Acinetobacter calcoaceticus

Legend

Ranking: 54 treatments were ranked according to the order of antimicrobial efficiency. The higher rank a treatment is, the less antimicrobial effect the treatment has.

Grouping: 54 treatments were grouped in terms of statistical analysis, which having the same letter/letters are no significant difference at a P value of 0.05.

Mean: In the in vitro system, the mean values of Minimum Inhibitory Concentration (MIC) resulted from the triplicate data of each treatment were recorded instead of 3 sets of individual value.

Repeat: It represents the times of experimental data being used to calculate the mean values.

Reagent: See page----(Figure 1)

Medium: In the in vitro system, both nutrient broth (NB) and nutrient agar (NA) were used as growth medium.

Organism: Acinetobacter calcoaceticus

Rank	Grouping							Mean(ppm)	Repeat	pH	Reagent	Medium
54				A				1033.3	3	6.8	BHT	NB
53		B		A				933.3	3	6.8	BHT	NA
53		B		A				933.3	3	5.6	BHT	NA
51		B		C				700.0	3	5.6	LAU	NB
50		B		C		D		633.3	3	5.6	LT	NB
49		E		C		D		600.0	3	5.6	BHT	NB
48		E	F	C		D		533.3	3	6.8	LA	NB
48		E	F	C		D		533.3	3	6.8	LT	NB
46	G	E	F	C		D		500.0	3	6.8	LT	NA
45	G	E	F	C		D	H	466.7	3	6.8	LAU	NA
45	G	E	F	C		D	H	466.7	3	6.8	LAU	NB
43	G	E	F	C	I	D	H	433.3	3	6.8	LG	NB
43	G	E	F	C	I	D	H	433.3	3	6.8	LA	NA
43	G	E	F	C	I	D	H	433.3	3	5.6	LQ	NA
43	G	E	F	C	I	D	H	433.3	3	5.6	LA	NB
39	G	E	F	J	I	D	H	366.7	3	4.5	LT	NB
39	G	E	F	J	I	D	H	333.3	3	5.6	LQ	NB
39	G	E	F	J	I	D	H	333.3	3	4.5	LA	NB
39	G	E	F	J	I	D	H	333.3	3	5.6	LG	NB
39	G	E	F	J	I	D	H	333.3	3	4.5	LQ	NB
34	G	E	F	J	I		H	300.0	3	6.8	LQ	NB
34	G	E	F	J	I		H	300.0	3	6.8	TBHQ	NB
32	G		F	J	I		H	233.3	3	5.6	LAU	NA
32	G		F	J	I		H	233.3	3	5.6	PG	NA
32	G		F	J	I		H	233.3	3	4.5	LG	NB
32	G		F	J	I		H	233.3	3	6.8	PG	NB
28	G			J	I		H	200.0	3	6.8	PG	NA
28	G			J	I		H	200.0	3	5.6	PG	NB
28	G			J	I		H	200.0	3	4.5	PG	NB
28	G			J	I		H	200.0	3	5.6	LT	NA
24				J	I		H	166.7	3	4.5	BHT	NB
24				J	I		H	166.7	3	6.8	LG	NA
24				J	I		H	166.7	3	4.5	TBHQ	NB
21				J	I			133.3	3	5.6	TBHQ	NA
21				J	I			133.3	3	6.8	LQ	NA
21				J	I			133.3	3	4.5	LG	NA
21				J	I			133.3	3	6.8	BHA	NA
21				J	I			133.3	3	5.6	LG	NA
21				J	I			133.3	3	4.5	LAU	NB
21				J	I			133.3	3	6.8	TBHQ	NA
14				J				100.0	3	4.5	BHA	NB
14				J				100.0	3	4.5	LA	NA
14				J				100.0	3	5.6	TBHQ	NB
14				J				100.0	3	4.5	LT	NA
14				J				100.0	3	4.5	LQ	NA
14				J				100.0	3	4.5	PG	NA
14				J				100.0	3	4.5	BHA	NA
14				J				100.0	3	4.5	TBHQ	NA
14				J				100.0	3	4.5	BHT	NA
14				J				100.0	3	5.6	BHA	NA
14				J				100.0	3	5.6	BHA	NB
14				J				100.0	3	4.5	LAU	NA
14				J				100.0	3	6.8	BHA	NB
14				J				100.0	3	5.6	LA	NA

Table 2: Mimimum Inhibited Concentration (MIC) of Monolaurin and/or Selected Antioxiants on Bordetella bronchiseptica

Legend identical to Table 1.

Organism: Bordetella bronchiseptica

Rank	Grouping							Mean(ppm)	Repeat	pH	Reagent	Medium
54				A				1100.0	3	6.8	BHT	NB
54				A				1100.0	3	6.8	LT	NA
54				A				1066.7	3	5.6	BHT	NA
54				A				1033.3	3	5.6	LAU	NA
50		B		A				966.7	3	6.8	LT	NB
49		B		A		C		933.3	3	6.8	LAU	NA
49		B		A		C		933.3	3	5.6	LT	NA
49		B		A		C		933.3	3	6.8	BHT	NA
46		B		D		C		766.7	3	5.6	BHT	NB
46		B		D		C		766.7	3	6.8	LA	NA
46		B		D		C		766.7	3	6.8	LAU	NB
43				D		C		733.3	3	6.8	LA	NB
42		E		D				600.0	3	5.6	LT	NB
41		E		D		F		566.7	3	5.6	LA	NB
41		E		D		F		566.7	3	5.6	LAU	NB
39		E		G		F		500.0	3	5.6	LA	NA
39		E		G		F		500.0	3	5.6	LQ	NA
39		E		G		F		500.0	3	5.6	LQ	NB
39		E		G		F		500.0	3	5.6	LG	NA
35		E	H	G		F		466.7	3	6.8	LG	NB
34	I	E	H	G		F		433.3	3	6.8	BHA	NA
34	I	E	H	G		F		433.3	3	6.8	LQ	NB
32	I	E	H	G		F	J	400.0	3	6.8	BHA	NB
32	I	E	H	G		F	J	400.0	3	6.8	TBHQ	NB
30	I	K	H	G		F	J	366.7	3	6.8	PG	NA
29	I	K	H	G		L	J	333.3	3	5.6	TBHQ	NA
29	I	K	H	G		L	J	333.3	3	5.6	PG	NA
29	I	K	H	G		L	J	333.3	3	5.6	LG	NB
29	I	K	H	G		L	J	333.3	3	5.6	PG	NB
29	I	K	H	G		L	J	333.3	3	6.8	PG	NB
24	I	K	H	G	M	L	J	300.0	3	5.6	TBHQ	NB
24	I	K	H	G	M	L	J	300.0	3	6.8	LG	NA
24	I	K	H	G	M	L	J	300.0	3	5.6	BHA	NB
21	I	K	H		M	L	J	266.7	3	4.5	BHT	NB
21	I	K	H		M	L	J	266.7	3	4.5	LAU	NB
19	I	K			M	L	J	233.3	3	5.6	BHA	NA
18		K			M	L	J	200.0	3	4.5	LT	NB
18		K			M	L	J	200.0	3	4.5	BHT	NA
16		K			M	L		166.7	3	6.8	LQ	NA
16		K			M	L		166.7	3	6.8	TBHQ	NA
16		K			M	L		166.7	3	4.5	LQ	NB
16		K			M	L		166.7	3	4.5	LA	NB
12					M	L		133.3	3	4.5	TBHQ	NB
12					M	L		133.3	3	4.5	LG	NB
12					M	L		133.3	3	4.5	BHA	NB
12					M	L		133.3	3	4.5	LT	NA
8					M			100.0	3	4.5	BHA	NA
8					M			100.0	3	4.5	TBHQ	NA
8					M			100.0	3	4.5	PG	NA
8					M			100.0	3	4.5	LG	NA
8					M			100.0	3	4.5	LA	NA
8					M			100.0	3	4.5	LQ	NA
8					M			100.0	3	4.5	LAU	NA
8					M			100.0	3	4.5	PG	NB

Table 3: Minimum Inhibitory Concentration (MIC) of Monolaurin and/or Selected Antioxidants on Citrobacter freundii

Legend identical to Table 1.

Organism: Citrobacter freundii

Rank	Grouping						Mean(ppm)	Repeat	pH	Reagent	Medium
54				A			1100.0	3	5.6	LT	NA
54				A			1100.0	3	5.6	LAU	NA
54				A			1100.0	3	5.6	LAU	NB
54				A			1100.0	3	5.6	BHT	NA
50		B		A			1066.7	3	6.8	BHT	NB
50		B		A			1066.7	3	6.8	LT	NA
50		B		A			1066.7	3	4.5	BHT	NA
47		B		A	C		1000.0	3	6.8	LAU	NA
46		B	D	A	C		966.7	3	5.6	LA	NA
46		B	D	A	C		966.7	3	4.5	LAU	NA
46		B	D	A	C		966.7	3	6.8	BHT	NA
46		B	D	A	C		966.7	3	5.6	BHT	NB
46		B	D	A	C		966.7	3	5.6	LQ	NA
46		B	D	A	C		966.7	3	6.8	LT	NB
40	E	B	D	A	C		933.3	3	4.5	LT	NA
39	E	B	D	F	C		900.0	3	6.8	LA	NA
38	E	G	D	F	C		866.7	3	5.6	LT	NB
37	E	G	D	F	C	H	833.3	3	5.6	LG	NA
36	E	G	D	F		H	800.0	3	6.8	LG	NA
35	E	G		F		H	766.7	3	6.8	LAU	NB
34		G		F	I	H	733.3	3	6.8	LA	NB
34		G		F	I	H	733.3	3	5.6	PG	NA
32		G		J	I	H	700.0	3	6.8	LQ	NA
32		G		J	I	H	700.0	3	6.8	BHA	NA
32		G		J	I	H	700.0	3	5.6	TBHQ	NA
29				J	I	H	666.7	3	5.6	LA	NB
28		K		J	I		566.7	3	5.6	LQ	NB
27		K		J	L		533.3	3	5.6	LG	NB
27		K		J	L		533.3	3	4.5	LAU	NB
27		K		J	L		533.3	3	6.8	LQ	NB
27		K		J	L		533.3	3	6.8	BHA	NB
27		K		J	L		533.3	3	6.8	LG	NB
22		K		M	L		466.7	3	5.6	BHA	NA
22		K		M	L		433.3	3	6.8	TBHQ	NB
22		K		M	L		433.3	3	4.5	LQ	NA
19		K	N	M	L		400.0	3	6.8	TBHQ	NA
18		O	N	M	L		366.7	3	4.5	LA	NA
18		O	N	M	L		366.7	3	5.6	TBHQ	NB
18		O	N	M	L		366.7	3	6.8	PG	NB
18		O	N	M	L		366.7	3	6.8	PG	NA
18		O	N	M	L		366.7	3	4.5	LT	NB
13		O	N	M	P		333.3	3	5.6	PG	NB
13		O	N	M	P		333.3	3	4.5	BHT	NB
11	Q	O	N	M	P		300.0	3	4.5	TBHQ	NA
11	Q	O	N	M	P		300.0	3	5.6	BHA	NB
9	Q	O	N		P		233.3	3	4.5	LQ	NB
9	Q	O	N		P		233.3	3	4.5	PG	NA
9	Q	O	N		P		233.3	3	4.5	LA	NB
6	Q	O			P		200.0	3	4.5	LG	NA
6	Q	O			P		200.0	3	4.5	BHA	NA
4	Q				P		166.7	3	4.5	BHA	NB
3	Q						133.3	3	4.5	LG	NB
3	Q						133.3	3	4.5	TBHQ	NB
3	Q						133.3	3	4.5	PG	NB

Table 4: Minimum Inhibitory Concentration (MIC) or Monolaurin and/or Selected Antioxidants on Enterobacter aerogenes

Legend identical to Table 1.

Organism: Enterobacter aerogenes

Rank	Grouping							Mean(ppm)	Repeat	pH	Reagent	Medium
54				A				1100.0	3	5.6	LT	NA
54				A				1100.0	3	6.8	BHT	NB
54				A				1100.0	3	4.5	BHT	NA
54				A				1100.0	3	5.6	LAU	NB
54				A				1100.0	3	4.5	LA	NA
54				A				1100.0	3	5.6	BHT	NA
54				A				1100.0	3	6.8	LG	NA
54				A				1100.0	3	5.6	LA	NA
54				A				1100.0	3	6.8	LAU	NA
54				A				1100.0	3	6.8	LA	NA
54				A				1100.0	3	6.8	LT	NA
54				A				1100.0	3	5.6	LAU	NA
54				A				1100.0	3	4.5	LT	NA
54				A				1100.0	3	5.6	LG	NA
54				A				1066.7	3	5.6	BHA	NA
54				A				1066.7	3	4.5	LAU	NA
38		B		A				1033.3	3	6.8	BHT	NA
37		B		A		C		1000.0	3	6.8	LT	NB
37		B		A		C		1000.0	3	5.6	PG	NA
35		B	D	A		C		966.7	3	6.8	BHA	NB
35		B	D	A		C		966.7	3	5.6	BHT	NB
35		B	D	A		C		966.7	3	6.8	LA	NB
35		B	D	A		C		966.7	3	6.8	BHA	NA
31	E	B	D	A		C		933.3	3	6.8	LQ	NA
31	E	B	D	A		C		933.3	3	5.6	TBHQ	NA
29	E	B	D	A		C	F	900.0	3	5.6	LQ	NA
29	E	B	D	A		C	F	900.0	3	5.6	LA	NB
29	E	B	D	A		C	F	900.0	3	5.6	LT	NB
26	E	B	D	A	G	C	F	866.7	3	6.8	LAU	NB
25	E	B	D	H	G	C	F	766.7	3	4.5	PG	NA
25	E	B	D	H	G	C	F	766.7	3	5.6	BHA	NB
25	E	B	D	H	G	C	F	766.7	3	6.8	PG	NA
22	E		D	H	G	C	F	733.3	3	5.6	LG	NB
22	E		D	H	G	C	F	733.3	3	4.5	LG	NA
22	E		D	H	G	C	F	733.3	3	4.5	LQ	NA
19	E		D	H	G		F	700.0	3	5.6	LQ	NB
19	E		D	H	G		F	700.0	3	6.8	LG	NB
19	E		D	H	G		F	700.0	3	4.5	LT	NB
16	E	I		H	G		F	666.7	3	6.8	LQ	NB
16	E	I		H	G		F	666.7	3	4.5	LA	NB
16	E	I		H	G		F	666.7	3	4.5	TBHQ	NA
13		I		H	G		F	633.3	3	6.8	PG	NB
12		I		H	G			600.0	3	4.5	BHT	NB
11		I		H				566.7	3	4.5	BHA	NA
11		I		H				566.7	3	6.8	TBHQ	NB
11		I		H				533.3	3	4.5	LAU	NB
11		I		H				533.3	3	6.8	TBHQ	NA
11		I		H				533.3	3	5.6	PG	NB
6		I		H				500.0	3	5.6	TBHQ	NB
6		I		H				500.0	3	4.5	LG	NB
6		I		H				500.0	3	4.5	LQ	NB
3		I						400.0	3	4.5	BHA	NB
3		I						400.0	3	4.5	TBHQ	NB
3		I						400.0	3	4.5	PG	NB

Table 5: Minimum Inhibitory Concentration (MIC) of Monolaurin and/or Selected Antioxidants on Escherichia coli

Legend identical to Table 1.

Organism: Escherichia coli

RANK	GROUPING						MEAN(ppm)	REPEAT	pH	REAGENT	MEDIUM
54				A			1100.0	3	5.6	LAU	NB
54				A			1100.0	3	6.8	BHT	NB
54				A			1100.0	3	6.8	LT	NA
54				A			1100.0	3	5.6	LAU	NA
54				A			1100.0	3	5.6	BHT	NA
49		B		A			1066.7	3	5.6	LT	NA
49		B		A			1066.7	3	4.5	LAU	NA
47		B		A	C		1033.3	3	6.8	BHT	NA
46		B	D	A	C		1000.0	3	6.8	LAU	NA
46		B	D	A	C		1000.0	3	6.8	LT	NB
44	E	B	D	A	C		966.7	3	5.6	BHT	NB
44	E	B	D	A	C		933.3	3	4.5	LT	NA
44	E	B	D	A	C		933.3	3	5.6	LQ	NA
41	E	B	D	A	C	F	866.7	3	5.6	LT	NB
41	E	B	D	A	C	F	866.7	3	6.8	LAU	NB
39	E	B	D	G	C	F	833.3	3	6.8	LA	NA
39	E	B	D	G	C	F	833.3	3	5.6	LG	NA
37	E		D	G	C	F	800.0	3	4.5	BHT	NB
37	E		D	G	C	F	800.0	3	6.8	LQ	NA
35	E	H	D	G		F	766.7	3	5.6	LA	NA
35	E	H	D	G		F	766.7	3	5.6	LA	NB
35	E	H	D	G		F	766.7	3	4.5	BHT	NA
32	E	H		G	I	F	733.3	3	4.5	LAU	NB
31		H	J	G	I	F	666.7	3	6.8	LA	NB
31		H	J	G	I	F	666.7	3	4.5	LT	NB
31		H	J	G	I	F	666.7	3	5.6	LQ	NB
28	K	H	J	G	I	F	633.3	3	6.8	LQ	NB
28	K	H	J	G	I	F	633.3	3	6.8	LG	NA
28	K	H	J	G	I	F	633.3	3	5.6	TBHQ	NA
25	K	H	J	G	I	L	600.0	3	5.6	LG	NB
25	K	H	J	G	I	L	600.0	3	5.6	BHA	NA
23	K	H	J	M	I	L	533.3	3	4.5	LQ	NA
23	K	H	J	M	I	L	533.3	3	6.8	TBHQ	NA
23	K	H	J	M	I	L	533.3	3	6.8	LG	NB
20	K	N	J	M	I	L	500.0	3	4.5	LG	NA
20	K	N	J	M	I	L	500.0	3	5.6	PG	NA
20	K	N	J	M	I	L	500.0	3	6.8	TBHQ	NB
20	K	N	J	M	I	L	500.0	3	6.8	BHA	NA
16	K	N	J	M	O	L	433.3	3	5.6	TBHQ	NB
15	K	N		M	O	L	400.0	3	6.8	PG	NB
15	K	N		M	O	L	400.0	3	6.8	PG	NA
15	K	N		M	O	L	400.0	3	4.5	TBHQ	NA
15	K	N		M	O	L	400.0	3	6.8	BHA	NB
11		N	P	M	O	L	366.7	3	4.5	LA	NB
10		N	P	M	O		333.3	3	4.5	LQ	NB
10		N	P	M	O		333.3	3	4.5	PG	NA
8		N	P		O		266.7	3	5.6	BHA	NB
8		N	P		O		266.7	3	5.6	PG	NB
6			P		O		233.3	3	4.5	LA	NA
5			P		O		200.0	3	4.5	BHA	NB
5			P		O		200.0	3	4.5	PG	NB
5			P		O		200.0	3	4.5	TBHQ	NB
3			P		O		200.0	3	4.5	BHA	NA
1			P				133.3	3	4.5	LG	NB

Table 6: Minimum Inhibitory Concentration (MIC) of Monolaurin and/or Selected Antioxidants on Hafnia alvei

Legend identical to Table 1.

Organism: Hafnia alvei

Rank	Grouping							Mean(ppm)	Repeat	pH	Reagent	Medium
54				A				1100.0	3	5.6	LAU	NB
54				A				1100.0	3	6.8	BHT	NB
54				A				1100.0	3	5.6	LAU	NA
54				A				1100.0	3	6.8	LG	NA
54				A				1100.0	3	5.6	BHT	NA
49		B		A				1066.7	3	6.8	LT	NA
48		B		A		C		1033.3	3	6.8	LT	NB
47		B	D	A		C		1000.0	3	5.6	BHT	NB
47		B	D	A		C		1000.0	3	4.5	BHT	NA
47		B	D	A		C		1000.0	3	5.6	LT	NA
47		B	D	A		C		966.7	3	6.8	BHT	NA
47		B	D	A		C		966.7	3	5.6	PG	NA
42	E	B	D	A		C		933.3	3	4.5	LT	NA
42	E	B	D	A		C		933.3	3	6.8	LAU	NA
42	E	B	D	A		C		933.3	3	4.5	LAU	NA
39	E	B	D	A		C	F	900.0	3	6.8	LA	NA
38	E	B	D	A	G	C	F	866.7	3	5.6	LG	NA
38	E	B	D	A	G	C	F	866.7	3	5.6	TBHQ	NA
36	E	B	D	H	G	C	F	833.3	3	5.6	LT	NB
35	E	I	D	H	G	C	F	800.0	3	5.6	LA	NA
35	E	I	D	H	G	C	F	800.0	3	5.6	LQ	NA
33	E	I	D	H	G	J	F	766.7	3	6.8	LG	NB
33	E	I	D	H	G	J	F	766.7	3	6.8	LA	NB
31	E	I	K	H	G	J	F	700.0	3	5.6	LA	NB
30	L	I	K	H	G	J	F	666.7	3	5.6	LG	NB
30	L	I	K	H	G	J	F	666.7	3	6.8	LAU	NB
30	L	I	K	H	G	J	F	666.7	3	6.8	PG	NA
30	L	I	K	H	G	J	F	666.7	3	4.5	LAU	NB
26	L	I	K	H	G	J	M	633.3	3	5.6	LQ	NB
25	L	I	K	H	N	J	M	600.0	3	5.6	BHA	NA
25	L	I	K	H	N	J	M	600.0	3	4.5	LG	NA
25	L	I	K	H	N	J	M	600.0	3	6.8	BHA	NA
22	L	I	K		N	J	M	566.7	3	4.5	LQ	NA
22	L	I	K		N	J	M	566.7	3	6.8	PG	NB
20	L		K	O	N	J	M	533.3	3	6.8	LQ	NA
20	L		K	O	N	J	M	533.3	3	4.5	LT	NB
20	L		K	O	N	J	M	533.3	3	4.5	PG	NA
17	L	P	K	O	N		M	500.0	3	5.6	TBHQ	NB
17	L	P	K	O	N		M	500.0	3	6.8	BHA	NB
17	L	P	K	O	N		M	500.0	3	4.5	LA	NA
17	L	P	K	O	N		M	500.0	3	4.5	LA	NB
17	L	P	K	O	N		M	500.0	3	5.6	BHA	NB
12	L	P	K	O	N	Q	M	466.7	3	5.6	PG	NB
12	L	P	K	O	N	Q	M	466.7	3	6.8	LQ	NB
12	L	P	K	O	N	Q	M	466.7	3	4.5	LG	NB
9	L	P		O	N	Q	M	433.3	3	4.5	BHT	NB
8		P		O	N	Q	M	400.0	3	4.5	TBHQ	NA
8		P		O	N	Q	M	400.0	3	4.5	LQ	NB
6		P		O	N	Q		366.7	3	4.5	BHA	NA
6		P		O	N	Q		366.7	3	4.5	PG	NB
6		P		O	N	Q		366.7	3	6.8	TBHQ	NB
3		P		O		Q		300.0	3	4.5	BHA	NB
2		P				Q		266.7	3	4.5	TBHQ	NB
1						Q		233.3	3	6.8	TBHQ	NA

Table 7: Minimum Inhibitory Concentratron (MIC) of Monolaurin and/or Selected Antioxidants on Klebsiella pneumoniae

Legend identical to Table 1.

Organism: Klebsiella pneumonia

RANK	GROUPING							MEAN(ppm)	REPEAT	pH	REAGENT	MEDIUM
54				A				1100.0	3	5.6	LT	NA
54				A				1100.0	3	6.8	LAU	NA
54				A				1100.0	3	4.5	BHT	NA
54				A				1100.0	3	5.6	LAU	NB
54				A				1100.0	3	5.6	LA	NA
54				A				1100.0	3	5.6	BHT	NA
54				A				1100.0	3	6.8	LG	NA
54				A				1100.0	3	6.8	LT	NA
54				A				1100.0	3	5.6	LQ	NA
54				A				1100.0	3	6.8	LA	NA
54				A				1100.0	3	5.6	LG	NA
54				A				1100.0	3	5.6	LAU	NA
54				A				1100.0	3	4.5	LT	NA
41		B		A				1066.7	3	5.6	PG	NA
41		B		A				1066.7	3	6.8	BHT	NA
41		B		A				1066.7	3	4.5	LAU	NB
41		B		A				1066.7	3	4.5	LAU	NA
37		B		A	C			1033.3	3	5.6	BHT	NB
37		B		A	C			1033.3	3	6.8	LT	NB
35		B	D	A	C			1000.0	3	6.8	BHT	NB
35		B	D	A	C			1000.0	3	4.5	LG	NA
35		B	D	A	C			966.7	3	6.8	BHA	NA
32	E	B	D	A	C			933.3	3	6.8	LAU	NB
32	E	B	D	A	C			933.3	3	6.8	LA	NB
32	E	B	D	A	C			933.3	3	5.6	LA	NB
29	E	B	D	A	C	F		900.0	3	5.6	LT	NB
28	E	B	D	A	C	F	G	866.7	3	6.8	LQ	NA
27	E	B	D	H	C	F	G	833.3	3	5.6	TBHQ	NA
26	E	I	D	H	C	F	G	800.0	3	6.8	BHA	NB
25	E	I	D	H	J	F	G	766.7	3	5.6	BHA	NA
25	E	I	D	H	J	F	G	766.7	3	6.8	LG	NB
25	E	I	D	H	J	F	G	766.7	3	4.5	BHT	NB
22	E	I	K	H	J	F	G	700.0	3	5.6	LG	NB
22	E	I	K	H	J	F	G	700.0	3	6.8	LQ	NB
22	E	I	K	H	J	F	G	700.0	3	4.5	PG	NA
19	L	I	K	H	J	F	G	666.7	3	6.8	PG	NA
18	L	I	K	H	J	M	G	633.3	3	4.5	TBHQ	NA
17	L	I	K	H	J	M	N	600.0	3	5.6	LQ	NB
17	L	I	K	H	J	M	N	600.0	3	4.5	LA	NA
17	L	I	K	H	J	M	N	600.0	3	6.8	PG	NB
14	L	I	K		J	M	N	566.7	3	5.6	BHA	NB
14	L	I	K		J	M	N	566.7	3	4.5	LA	NB
12	L		K		J	M	N	533.3	3	6.8	TBHQ	NA
12	L		K		J	M	N	533.3	3	4.5	LT	NB
10	L		K	O		M	N	500.0	3	4.5	LQ	NB
10	L		K	O		M	N	500.0	3	5.6	TBHQ	NB
10	L		K	O		M	N	500.0	3	4.5	LG	NB
10	L		K	O		M	N	466.7	3	6.8	TBHQ	NB
6	L			O		M	N	433.3	3	5.6	PG	NB
6	L			O		M	N	433.3	3	4.5	BHA	NA
4				O		M	N	400.0	3	4.5	LQ	NA
3				O			N	366.7	3	4.5	PG	NB
3				O			N	366.7	3	4.5	TBHQ	NB
1				O				266.7	3	4.5	BHA	NB

Table 8: Minimum Inhibitory Concentration (MIC) of Monolaurin and/or Selected Antioxidants on Morganella morganii

Legend identical to Table 1.

Organism: Morganella morganii

Rank	Grouping						Mean(ppm)	Repeat	pH	Reagent	Medium
54				A			1100.0	3	5.6	LAU	NA
54				A			1100.0	3	5.6	BHT	NA
52		B		A			1066.7	3	6.8	LT	NA
52		B		A			1033.3	3	6.8	LT	NB
52		B		A			1000.0	3	6.8	BHT	NB
52		B		A			1000.0	3	5.6	LAU	NB
48		B		A	C		966.7	3	5.6	LT	NA
48		B		A	C		966.7	3	6.8	LAU	NA
48		B		A	C		966.7	3	6.8	BHT	NA
48		B		A	C		933.3	3	5.6	BHT	NB
44		B			C		866.7	3	6.8	LAU	NB
43				D	C		766.7	3	5.6	LT	NB
42		E		D			600.0	3	6.8	LA	NB
42		E		D			600.0	3	5.6	LQ	NA
40		E		F			533.3	3	5.6	LQ	NB
40		E		F			533.3	3	5.6	LA	NB
38		E		F	G		500.0	3	5.6	LA	NA
37		E	H	F	G		466.7	3	5.6	LG	NB
37		E	H	F	G		466.7	3	5.6	LG	NA
37		E	H	F	G		466.7	3	5.6	PG	NA
34	I	E	H	F	G		433.3	3	5.6	TBHQ	NB
34	I	E	H	F	G		433.3	3	6.8	LA	NA
34	I	E	H	F	G		433.3	3	6.8	TBHQ	NB
34	I	E	H	F	G		433.3	3	5.6	TBHQ	NA
30	I	E	H	F	G	J	400.0	3	6.8	LG	NA
30	I	E	H	F	G	J	400.0	3	4.5	BHT	NA
28	I	K	H	F	G	J	333.3	3	6.8	LQ	NB
28	I	K	H	F	G	J	333.3	3	6.8	PG	NB
26	I	K	H	L	G	J	300.0	3	5.6	BHA	NB
26	I	K	H	L	G	J	300.0	3	6.8	LG	NB
24	I	K	H	L		J	266.7	3	6.8	BHA	NB
23	I	K		L		J	233.3	3	4.5	LA	NB
23	I	K		L		J	233.3	3	6.8	BHA	NA
23	I	K		L		J	233.3	3	5.6	BHA	NA
23	I	K		L		J	233.3	3	5.6	PG	NB
23	I	K		L		J	233.3	3	4.5	LT	NA
18		K		L		J	200.0	3	4.5	TBHQ	NA
18		K		L		J	200.0	3	4.5	LQ	NA
18		K		L		J	200.0	3	4.5	LAU	NB
15		K		L			166.7	3	6.8	PG	NA
15		K		L			133.3	3	4.5	PG	NB
15		K		L			133.3	3	6.8	LQ	NA
15		K		L			133.3	3	4.5	LQ	NB
15		K		L			133.3	3	4.5	LAU	NA
15		K		L			133.3	3	6.8	TBHQ	NA
15		K		L			133.3	3	4.5	LA	NA
15		K		L			133.3	3	4.5	TBHQ	NB
15		K		L			133.3	3	4.5	LT	NB
6				L			100.0	3	4.5	LG	NB
6				L			100.0	3	4.5	LG	NA
6				L			100.0	3	4.5	BHT	NB
6				L			100.0	3	4.5	BHA	NB
6				L			100.0	3	4.5	PG	NA
6				L			100.0	3	4.5	BHA	NA

Table 9: Minimum Inhibitory Concentration (MIC) of Monolaurin and/or Selected Antioxidants on *Proteus mirabilis*

Legend identical to Table 1.

Organism: Proteus mirabilis

Rank	Grouping						Mean(ppm)	Repeat	pH	Reagent	Medium
54				A			1100.0	3	5.6	LA	NA
54				A			1100.0	3	5.6	LAU	NA
54				A			1100.0	3	5.6	BHT	NA
51		B		A			1066.7	3	6.8	LT	NA
50		B		A	C		1033.3	3	5.6	PG	NA
50		B		A	C		1033.3	3	6.8	LT	NA
50		B		A	C		1033.3	3	6.8	LA	NA
50		B		A	C		1033.3	3	6.8	LG	NA
46		B	D	A	C		1000.0	3	6.8	LAU	NA
45	E	B	D	A	C		966.7	3	6.8	BHT	NA
45	E	B	D	A	C		966.7	3	5.6	TBHQ	NA
45	E	B	D	A	C		966.7	3	5.6	LQ	NA
45	E	B	D	A	C		966.7	3	5.6	LG	NA
45	E	B	D	A	C		933.3	3	6.8	BHA	NA
45	E	B	D	A	C		933.3	3	5.6	LAU	NB
39	E	B	D	A	C	F	900.0	3	6.8	BHT	NB
39	E	B	D	A	C	F	900.0	3	6.8	PG	NA
39	E	B	D	A	C	F	900.0	3	5.6	BHA	NA
36	E	B	D	G	C	F	833.3	3	5.6	BHA	NB
35	E	H	D	G	C	F	800.0	3	5.6	LT	NB
35	E	H	D	G	C	F	800.0	3	6.8	PG	NB
33	E	H	D	G		F	766.7	3	6.8	LQ	NA
33	E	H	D	G		F	766.7	3	6.8	LAU	NB
33	E	H	D	G		F	766.7	3	6.8	LT	NB
33	E	H	D	G		F	766.7	3	6.8	LA	NB
33	E	H	D	G		F	766.7	3	5.6	BHT	NB
33	E	H	D	G		F	766.7	3	5.6	LQ	NB
27	E	H		G	I	F	733.3	3	5.6	LA	NB
27	E	H		G	I	F	733.3	3	6.8	BHA	NB
25		H		G	I	F	666.7	3	6.8	TBHQ	NB
25		H		G	I	F	666.7	3	6.8	LG	NB
23		H	J	G	I		633.3	3	5.6	LG	NB
23		H	J	G	I		633.3	3	6.8	LQ	NB
20		H	J	K	I		566.7	3	5.6	PG	NB
20		H	J	K	I		566.7	3	5.6	TBHQ	NB
19		L	J	K	I		500.0	3	6.8	TBHQ	NA
18		L	J	K	M		400.0	3	4.5	BHT	NA
17		L	N	K	M		333.3	3	4.5	BHA	NB
17		L	N	K	M		333.3	3	4.5	LAU	NA
17		L	N	K	M		333.3	3	4.5	BHT	NB
17		L	N	K	M		333.3	3	4.5	LT	NB
13		L	N		M		300.0	3	4.5	LT	NA
13		L	N		M		300.0	3	4.5	LAU	NB
13		L	N		M		266.7	3	4.5	LQ	NB
13		L	N		M		266.7	3	4.5	LQ	NA
13		L	N		M		266.7	3	4.5	LA	NB
13		L	N		M		266.7	3	4.5	LA	NA
7			N		M		233.3	3	4.5	TBHQ	NA
7			N		M		233.3	3	4.5	LG	NA
7			N		M		200.0	3	4.5	BHA	NA
7			N		M		200.0	3	4.5	PG	NB
7			N		M		200.0	3	4.5	TBHQ	NB
7			N		M		166.7	3	4.5	PG	NA
1			N				133.3	3	4.5	LG	NB

Table 10: Minimum Inhibitory Concentration (MIC) of Monolaurin and/or Selected Antioxidants on Providencia stuartii

Legend identical to Table 1.

Orgaanism: Providencia stuartii

Rank	Grouping							Mean(ppm)	Repeat	pH	Reagent	Medium
54				A				1100.0	3	5.6	LAU	NA
54				A				1100.0	3	6.8	LT	NA
54				A				1100.0	3	5.6	BHT	NA
50		B		A				1066.7	3	6.8	BHT	NB
49		B		A	C			1000.0	3	6.8	BHT	NA
49		B		A	C			1000.0	3	6.8	LT	NB
49		B		A	C			966.7	3	6.8	LAU	NA
49		B		A	C			966.7	3	6.8	LAU	NB
45		B	D	A	C			900.0	3	5.6	LT	NA
44		B	D	E	C			800.0	3	5.6	LAU	NB
44		B	D	E	C			800.0	3	6.8	LA	NB
44		B	D	E	C			800.0	3	5.6	BHT	NB
41		F	D	E	C			766.7	3	5.6	LT	NB
40	G	F	D	E	C			733.3	3	5.6	LA	NB
39	G	F	D	E	H			666.7	3	5.6	LQ	NB
39	G	F	D	E	H			666.7	3	6.8	LG	NA
37	G	F	D	E	H	I		633.3	3	6.8	LA	NA
37	G	F	D	E	H	I		633.3	3	5.6	LG	NA
37	G	F	D	E	H	I		633.3	3	5.6	LG	NB
34	G	F	J	E	H	I		566.7	3	5.6	LA	NA
34	G	F	J	E	H	I		566.7	3	5.6	LQ	NA
32	G	F	J	K	H	I		500.0	3	5.6	PG	NA
32	G	F	J	K	H	I		500.0	3	6.8	BHA	NB
30	G	L	J	K	H	I		466.7	3	6.8	LQ	NB
29	M	L	J	K	H	I		433.3	3	6.8	LG	NB
28	M	L	J	K	H	I	N	400.0	3	6.8	PG	NA
28	M	L	J	K	H	I	N	400.0	3	4.5	LA	NB
26	M	L	J	K	O	I	N	366.7	3	6.8	PG	NB
26	M	L	J	K	O	I	N	366.7	3	5.6	BHA	NB
24	M	L	J	K	O		N	333.3	3	6.8	TBHQ	NB
24	M	L	J	K	O		N	333.3	3	4.5	LT	NB
24	M	L	J	K	O		N	300.0	3	5.6	TBHQ	NA
24	M	L	J	K	O		N	300.0	3	5.6	BHA	NA
24	M	L	J	K	O		N	300.0	3	5.6	TBHQ	NB
19	M	L		K	O		N	266.7	3	6.8	BHA	NA
19	M	L		K	O		N	266.7	3	4.5	LG	NA
19	M	L		K	O		N	266.7	3	4.5	LQ	NB
19	M	L		K	O		N	266.7	3	4.5	LQ	NA
19	M	L		K	O		N	233.3	3	5.6	PG	NB
19	M	L		K	O		N	233.3	3	4.5	LG	NB
13	M	L			O		N	200.0	3	4.5	LT	NA
13	M	L			O		N	200.0	3	6.8	TBHQ	NA
11	M				O		N	166.7	3	4.5	LAU	NB
11	M				O		N	166.7	3	4.5	BHT	NA
11	M				O		N	166.7	3	6.8	LQ	NA
11	M				O		N	166.7	3	4.5	TBHQ	NB
11	M				O		N	166.7	3	4.5	BHT	NB
11	M				O		N	166.7	3	4.5	LA	NA
11	M				O		N	166.7	3	4.5	PG	NB
5					O		N	133.3	3	4.5	BHA	NB
5					O		N	133.3	3	4.5	LAU	NA
3					O			100.0	3	4.5	TBHQ	NA
3					O			100.0	3	4.5	PG	NA
3					O			100.0	3	4.5	BHA	NA

Table 11: Minimum Inhibitory Concentration (MIC) of Monolaurin and/or Selected Antioxidants on Pseudomonas fluorescens

Legend identical to Table 1.

Organism: Pseudomonas fluorescens

Rank	Grouping							Mean(ppm)	Repeat	pH	Reagent	Medium
54				A				1066.7	3	5.6	LA	NA
53			B		A			1000.0	3	6.8	LA	NA
52			B		A	C		933.3	3	5.6	LAU	NB
52			B		A	C		933.3	3	5.6	BHT	NA
52			B		A	C		866.7	3	6.8	BHT	NA
52			B		A	C		866.7	3	5.6	LT	NA
52			B		A	C		866.7	3	5.6	LAU	NA
47			B	D	A	C		833.3	3	5.6	LQ	NA
47			B	D	A	C		833.3	3	5.6	TBHQ	NA
47			B	D	A	C		833.3	3	6.8	LT	NA
44			B	D	E	C		766.7	3	6.8	LAU	NA
44			B	D	E	C		766.7	3	6.8	BHT	NB
44			B	D	E	C		766.7	3	6.8	LT	NB
44			B	D	E	C		733.3	3	5.6	LG	NA
44			B	D	E	C		733.3	3	5.6	BHA	NA
39			F	D	E	C		666.7	3	5.6	BHT	NB
39			F	D	E	C		666.7	3	6.8	LG	NA
39			F	D	E	C		666.7	3	5.6	PG	NA
36			F	D	E	G		566.7	3	6.8	LA	NB
36			F	D	E	G		566.7	3	6.8	BHA	NA
34			F	H	E	G		533.3	3	5.6	LQ	NB
33	I		F	H	E	G		500.0	3	6.8	LQ	NA
32	I		F	H	J	G		433.3	3	6.8	LQ	NB
32	I		F	H	J	G		433.3	3	5.6	LA	NB
32	I		F	H	J	G		433.3	3	5.6	LT	NB
32	I		F	H	J	G		433.3	3	5.6	LG	NB
32	I		F	H	J	G		433.3	3	6.8	LG	NB
27	I		F	H	J	G	K	400.0	3	6.8	PG	NA
26	I		L	H	J	G	K	366.7	3	6.8	BHA	NB
26	I		L	H	J	G	K	366.7	3	5.6	BHA	NB
24	I		L	H	J	G	K	333.3	3	6.8	TBHQ	NB
24	I		L	H	J	G	K	333.3	3	6.8	LAU	NB
24	I		L	H	J	G	K	333.3	3	5.6	TBHQ	NB
24	I		L	H	J	G	K	333.3	3	4.5	BHT	NB
20	I		L	H	J		K	266.7	3	4.5	BHT	NA
20	I		L	H	J		K	266.7	3	6.8	PG	NB
20	I		L	H	J		K	266.7	3	6.8	TBHQ	NA
17	I		L		J		K	233.3	3	4.5	TBHQ	NA
17	I		L		J		K	233.3	3	4.5	LAU	NA
17	I		L		J		K	233.3	3	4.5	LQ	NB
14			L		J		K	200.0	3	4.5	BHA	NA
14			L		J		K	200.0	3	4.5	LQ	NA
14			L		J		K	200.0	3	4.5	LT	NA
14			L		J		K	200.0	3	4.5	LT	NB
14			L		J		K	166.7	3	5.6	PG	NB
14			L		J		K	166.7	3	4.5	LG	NA
14			L		J		K	166.7	3	4.5	BHA	NB
14			L		J		K	166.7	3	4.5	LAU	NB
14			L		J		K	166.7	3	4.5	TBHQ	NB
14			L		J		K	166.7	3	4.5	PG	NB
14			L		J		K	166.7	3	4.5	LA	NB
14			L		J		K	166.7	3	4.5	PG	NA
2			L				K	133.3	3	4.5	LA	NA
1			L					100.0	3	4.5	LG	NB

Table 12: Minimum Inhibited Concentration (MIC) of Monolaurin and/or Selected Antioxidants on Salmonella molade

Legend identical to Table 1.

Organism: Salmonella molade

Rank	Grouping								Mean(ppm)	Repeat	pH	Reagent	Medium
54				A					1066.7	3	4.5	BHT	NA
53		B		A					1000.0	3	6.8	BHT	NB
52		B		A		C			900.0	3	4.5	LT	NA
51		B	D	A		C			866.7	3	6.8	LT	NB
51		B	D	A		C			866.7	3	4.5	LAU	NA
51		B	D	A		C			866.7	3	5.6	LAU	NB
51		B	D	A		C			866.7	3	5.6	LAU	NA
51		B	D	A		C			866.7	3	5.6	BHT	NA
46	E	B	D	A		C			833.3	3	5.6	BHT	NB
45	E	B	D	F		C			766.7	3	6.8	LAU	NB
44	E	G	D	F		C			733.3	3	6.8	BHT	NA
44	E	G	D	F		C			733.3	3	6.8	LAU	NA
44	E	G	D	F		C			733.3	3	5.6	LT	NA
41	E	G	D	F		C	H		700.0	3	6.8	LT	NA
40	E	G	D	F	I	C	H		666.7	3	5.6	LG	NA
39	E	G	D	F	I	J	H		633.3	3	5.6	PG	NA
39	E	G	D	F	I	J	H		633.3	3	6.8	LQ	NA
39	E	G	D	F	I	J	H		633.3	3	6.8	LA	NB
39	E	G	D	F	I	J	H		633.3	3	6.8	LG	NA
35	E	G	K	F	I	J	H		600.0	3	5.6	LT	NB
34	L	G	K	F	I	J	H		566.7	3	5.6	LQ	NA
34	L	G	K	F	I	J	H		566.7	3	6.8	LQ	NB
34	L	G	K	F	I	J	H		533.3	3	5.6	TBHQ	NA
31	L	G	K	M	I	J	H		500.0	3	5.6	LG	NB
31	L	G	K	M	I	J	H		500.0	3	4.5	LAU	NB
29	L	N	K	M	I	J	H		466.7	3	5.6	LQ	NB
29	L	N	K	M	I	J	H		466.7	3	6.8	LA	NA
27	L	N	K	M	I	J	O		433.3	3	5.6	PG	NB
27	L	N	K	M	I	J	O		433.3	3	5.6	LA	NB
27	L	N	K	M	I	J	O		433.3	3	6.8	TBHQ	NB
27	L	N	K	M	I	J	O		433.3	3	6.8	LG	NB
23	L	N	K	M	P	J	O		400.0	3	6.8	PG	NA
23	L	N	K	M	P	J	O		400.0	3	4.5	LQ	NA
21	L	N	K	M	P	Q	O		366.7	3	5.6	LA	NA
21	L	N	K	M	P	Q	O		366.7	3	5.6	BHA	NB
21	L	N	K	M	P	Q	O		366.7	3	4.5	LT	NB
21	L	N	K	M	P	Q	O		366.7	3	5.6	TBHQ	NB
21	L	N	K	M	P	Q	O		366.7	3	6.8	TBHQ	NA
21	L	N	K	M	P	Q	O		366.7	3	6.8	PG	NB
15	L	N	R	M	P	Q	O		333.3	3	6.8	BHA	NB
15	L	N	R	M	P	Q	O		333.3	3	4.5	BHT	NB
13		N	R	M	P	Q	O		266.7	3	4.5	TBHQ	NA
13		N	R	M	P	Q	O		266.7	3	4.5	LQ	NB
13		N	R	M	P	Q	O		266.7	3	6.8	BHA	NA
13		N	R	M	P	Q	O		266.7	3	4.5	PG	NA
9		N	R		P	Q	O		233.3	3	5.6	BHA	NA
9		N	R		P	Q	O		233.3	3	4.5	LA	NB
7			R		P	Q	O		200.0	3	4.5	BHA	NB
7			R		P	Q	O		200.0	3	4.5	TBHQ	NB
5			R		P	Q			166.7	3	4.5	LG	NB
5			R		P	Q			166.7	3	4.5	LA	NA
3			R			Q			133.3	3	4.5	LG	NA
3			R			Q			133.3	3	4.5	BHA	NA
1			R						100.0	3	4.5	PG	NB

Table 13: Minimum Inhibitory Concentration (MIC) of Monolaurin and/or Selected Antioxidnats on Serratia marcescens

Legend identical to Table 1.

Organism: Serretia marcescens

Rank	Grouping								Mean(ppm)	Repeat	pH	Reagent	Medium
54					A				1100.0	3	5.6	LAU	NA
54					A				1100.0	3	6.8	BHT	NB
54					A				1100.0	3	6.8	LT	NA
54					A				1100.0	3	6.8	LAU	NA
54					A				1100.0	3	5.6	BHT	NA
49		B			A				1066.7	3	5.6	LT	NA
48		B			A		C		1033.3	3	5.6	LAU	NB
48		B			A		C		1033.3	3	6.8	LG	NA
46		B		D	A		C		1000.0	3	6.8	BHT	NA
46		B		D	A		C		1000.0	3	6.8	LT	NB
46		B		D	A		C		1000.0	3	5.6	LG	NA
43	E	B		D	A		C		966.7	3	5.6	PG	NA
43	E	B		D	A		C		966.7	3	5.6	LA	NA
41	E	B		D	A		C	F	933.3	3	5.6	BHT	NB
40	E	B		D	A	G	C	F	900.0	3	6.8	LA	NB
40	E	B		D	A	G	C	F	900.0	3	6.8	BHA	NA
40	E	B		D	A	G	C	F	900.0	3	6.8	LAU	NB
40	E	B		D	A	G	C	F	900.0	3	5.6	LT	NB
36	E	B		D	H	A	G	C	F	866.7	3	4.5	BHT	NA
36	E	B		D	H	A	G	C	F	866.7	3	5.6	BHA	NA
36	E	B		D	H	A	G	C	F	866.7	3	5.6	TBHQ	NA
33	E	B	I	D	H	A	G	C	F	833.3	3	6.8	LG	NB
32	E	B	I	D	H	J	G	C	F	800.0	3	6.8	PG	NA
32	E	B	I	D	H	J	G	C	F	800.0	3	5.6	LA	NB
30	E	K	I	D	H	J	G	C	F	766.7	3	6.8	PG	NB
30	E	K	I	D	H	J	G	C	F	766.7	3	6.8	LA	NA
30	E	K	I	D	H	J	G	C	F	766.7	3	5.6	LQ	NB
30	E	K	I	D	H	J	G	C	F	766.7	3	5.6	LG	NB
26	E	K	I	D	H	J	G	L	F	733.3	3	5.6	BHA	NB
26	E	K	I	D	H	J	G	L	F	733.3	3	6.8	LQ	NA
26	E	K	I	D	H	J	G	L	F	733.3	3	4.5	LAU	NA
23	E	K	I		H	J	G	L	F	700.0	3	4.5	LAU	NB
23	E	K	I		H	J	G	L	F	700.0	3	5.6	LQ	NA
23	E	K	I		H	J	G	L	F	700.0	3	4.5	LT	NB
20		K	I	M	H	J	G	L	F	666.7	3	6.8	BHA	NB
19	N	K	I	M	H	J	G	L		633.3	3	6.8	LQ	NB
19	N	K	I	M	H	J	G	L		633.3	3	4.5	LA	NB
17	N	K	I	M	H	J		L	O	600.0	3	4.5	PG	NA
16	N	K	I	M		J		L	O	566.7	3	4.5	LT	NA
16	N	K	I	M		J		L	O	566.7	3	4.5	LG	NA
14	N	K		M		J		L	O	533.3	3	4.5	BHT	NB
13	N	K		M				L	O	500.0	3	5.6	PG	NB
13	N	K		M				L	O	500.0	3	6.8	TBHQ	NB
13	N	K		M				L	O	500.0	3	5.6	TBHQ	NB
13	N	K		M				L	O	500.0	3	4.5	LQ	NB
13	N	K		M				L	O	500.0	3	6.8	TBHQ	NA
8	N			M				L	O	466.7	3	4.5	LG	NB
8	N			M				L	O	466.7	3	4.5	BHA	NB
6	N			M					O	400.0	3	4.5	PG	NB
5	N								O	366.7	3	4.5	LG	NA
5	N								O	366.7	3	4.5	TBHQ	NA
5	N								O	366.7	3	4.5	LA	NA
5	N								O	366.7	3	4.5	TBHQ	NB
1									O	333.3	3	4.5	BHA	NA

Table 14: Minimum Inhibitory Concentration (MIC) of Monolaurin and/or Selected Antioxidants on Shigella sonnei

Legend identical to Table 1.

Organism: Shigella sonnei

RANK	GROUPING						MEAN(ppm)	REPEAT	pH	REAGENT	MEDIUM
54				A			1100.0	3	5.6	BHT	NA
53		B		A			1000.0	3	6.8	BHT	NA
53		B		A			1000.0	3	5.6	LAU	NB
51		B		A	C		966.7	3	6.8	BHT	NB
50		B	D	A	C		933.3	3	6.8	LT	NB
50		B	D	A	C		866.7	3	5.6	LAU	NA
48	E	B	D	A	C		833.3	3	6.8	LT	NA
47	E	B	D		C		800.0	3	5.6	LT	NA
47	E	B	D		C		800.0	3	5.6	BHT	NB
45	E	B	D	F	C		733.3	3	6.8	LA	NB
44	E	G	D	F	C		700.0	3	6.8	LAU	NA
44	E	G	D	F	C		700.0	3	6.8	LAU	NB
42	E	G	D	F	H		666.7	3	5.6	LT	NB
41	E	G	I	F	H		566.7	3	5.6	LA	NB
41	E	G	I	F	H		566.7	3	5.6	LQ	NB
41	E	G	I	F	H		566.7	3	6.8	LA	NA
38	J	G	I	F	H		500.0	3	5.6	PG	NA
38	J	G	I	F	H		500.0	3	5.6	LG	NA
38	J	G	I	F	H		500.0	3	6.8	LQ	NB
35	J	G	I	F	H	K	466.7	3	4.5	BHT	NA
35	J	G	I	F	H	K	466.7	3	6.8	LG	NA
35	J	G	I	F	H	K	466.7	3	6.8	LG	NB
32	J	G	I		H	K	433.3	3	5.6	LQ	NA
31	J		I	L	H	K	400.0	3	5.6	LA	NA
30	J	M	I	L		K	366.7	3	5.6	TBHQ	NA
30	J	M	I	L		K	366.7	3	5.6	LG	NB
30	J	M	I	L		K	366.7	3	4.5	LT	NA
30	J	M	I	L		K	333.3	3	6.8	PG	NB
30	J	M	I	L		K	333.3	3	6.8	LQ	NA
30	J	M	I	L		K	300.0	3	5.6	TBHQ	NB
30	J	M	I	L		K	300.0	3	6.8	BHA	NA
23	J	M		L		K	266.7	3	5.6	BHA	NB
23	J	M		L		K	266.7	3	6.8	TBHQ	NB
23	J	M		L		K	266.7	3	6.8	PG	NA
23	J	M		L		K	266.7	3	6.8	BHA	NB
23	J	M		L		K	233.3	3	4.5	LA	NB
23	J	M		L		K	233.3	3	5.6	PG	NB
23	J	M		L		K	233.3	3	5.6	BHA	NA
23	J	M		L		K	233.3	3	6.8	TBHQ	NA
15		M		L		K	200.0	3	4.5	LAU	NB
14		M		L			133.3	3	4.5	TBHQ	NA
14		M		L			133.3	3	4.5	PG	NA
14		M		L			133.3	3	4.5	LAU	NA
14		M		L			133.3	3	4.5	LG	NA
14		M		L			133.3	3	4.5	LQ	NA
14		M		L			133.3	3	4.5	TBHQ	NB
14		M		L			133.3	3	4.5	LA	NA
14		M		L			133.3	3	4.5	LT	NB
6		M					100.0	3	4.5	LG	NB
6		M					100.0	3	4.5	BHT	NB
6		M					100.0	3	4.5	BHA	NB
6		M					100.0	3	4.5	LQ	NB
6		M					100.0	3	4.5	BHA	NA
6		M					100.0	3	4.5	PG	NB

Table 15: Minimum Inhibitory Concentration (MIC) of Monolaurin and/or Selected Antioxidants on Staphylococcus aureus

Legend identical to Table 1.

Organism: Staphylococcus aureus

Rank	Grouping			Mean(ppm)	Repeat	pH	Reagent	Medium
54		A		1000.0	3	6.8	BHT	NA
54		A		1000.0	3	5.6	BHT	NA
54		A		933.3	3	6.8	BHT	NB
54		A		833.3	3	5.6	BHT	NB
50		B		566.7	3	5.6	LAU	NB
49	C	B		433.3	3	5.6	LQ	NB
49	C	B		433.3	3	5.6	PG	NA
49	C	B		433.3	3	6.8	LT	NB
46	C	B	D	366.7	3	6.8	LA	NA
45	C	E	D	333.3	3	6.8	LAU	NB
44	C	F E	D	300.0	3	6.8	LA	NB
44	C	F E	D	300.0	3	6.8	LT	NA
44	C	F E	D	300.0	3	4.5	BHT	NA
44	C	F E	D	266.7	3	5.6	LQ	NA
44	C	F E	D	266.7	3	6.8	LAU	NA
44	C	F E	D	233.3	3	6.8	LG	NB
44	C	F E	D	233.3	3	5.6	PG	NB
37		F E	D	200.0	3	5.6	LA	NB
37		F E	D	200.0	3	6.8	BHA	NB
37		F E	D	200.0	3	6.8	TBHQ	NB
37		F E	D	200.0	3	6.8	PG	NA
37		F E	D	200.0	3	5.6	LG	NB
37		F E	D	200.0	3	5.6	BHA	NB
37		F E	D	166.7	3	6.8	LQ	NB
37		F E	D	166.7	3	4.5	PG	NA
37		F E	D	166.7	3	6.8	LG	NA
37		F E	D	166.7	3	6.8	PG	NB
37		F E	D	166.7	3	6.8	BHA	NA
26		F E		133.3	3	6.8	LQ	NA
26		F E		133.3	3	4.5	BHT	NB
26		F E		133.3	3	5.6	LT	NB
26		F E		133.3	3	4.5	LT	NB
26		F E		133.3	3	4.5	LG	NB
26		F E		133.3	3	4.5	LQ	NB
20		F		100.0	3	4.5	BHA	NA
20		F		100.0	3	4.5	BHA	NB
20		F		100.0	3	5.6	LAU	NA
20		F		100.0	3	5.6	TBHQ	NA
20		F		100.0	3	4.5	LAU	NB
20		F		100.0	3	4.5	LA	NA
20		F		100.0	3	5.6	LG	NA
20		F		100.0	3	4.5	LQ	NA
20		F		100.0	3	4.5	LG	NA
20		F		100.0	3	4.5	LT	NA
20		F		100.0	3	4.5	LAU	NA
20		F		100.0	3	5.6	LT	NA
20		F		100.0	3	4.5	PG	NB
20		F		100.0	3	4.5	TBHQ	NA
20		F		100.0	3	4.5	TBHQ	NB
20		F		100.0	3	5.6	BHA	NA
20		F		100.0	3	5.6	TBHQ	NB
20		F		100.0	3	4.5	LA	NB
20		F		100.0	3	6.8	TBHQ	NA
20		F		100.0	3	5.6	LA	NA

Table 16: Minimum Inhibitory Concentration (MIC) of Monolaurin and/or Selected Antioxidants on Streptococcus faecalis

Legend identical to Table 1.

Organism: Streptococcus faecalis

Rank	Grouping			Mean(ppm)	Repeat	pH	Reagent	Medium
54		A		1100.0	3	6.8	BHT	NA
54		A		1100.0	3	6.8	BHT	NB
54		A		1100.0	3	5.6	BHT	NA
51	B	A		1066.7	3	5.6	BHT	NB
50	B	C		900.0	3	5.6	PG	NA
49	D	C		733.3	3	6.8	PG	NA
49	D	E		666.7	3	5.6	LAU	NB
49	D	E		600.0	3	6.8	LT	NA
49	D	E		566.7	3	6.8	PG	NB
45	F	E		533.3	3	6.8	LT	NB
45	F	E		533.3	3	5.6	PG	NB
43	F	E	G	500.0	3	4.5	BHT	NB
42	F	H	G	366.7	3	6.8	LAU	NB
42	F	H	G	366.7	3	6.8	LA	NB
42	F	H	G	366.7	3	4.5	BHT	NA
42	F	H	G	366.7	3	6.8	LG	NB
38	I	H	G	333.3	3	6.8	BHA	NB
38	I	H	G	333.3	3	6.8	LAU	NA
38	I	H	G	333.3	3	5.6	LT	NB
35	I	H	J	300.0	3	6.8	LG	NA
35	I	H	J	300.0	3	5.6	LQ	NB
33	I K	H	J	266.7	3	5.6	BHA	NB
33	I K	H	J	266.7	3	5.6	LG	NB
33	I K	H	J	233.3	3	4.5	PG	NA
33	I K	H	J	233.3	3	5.6	LA	NB
33	I K	H	J	233.3	3	6.8	BHA	NA
33	I K	H	J	200.0	3	6.8	LQ	NB
33	I K	H	J	200.0	3	6.8	LA	NA
33	I K	H	J	200.0	3	5.6	BHA	NA
25	I K		J	166.7	3	4.5	PG	NB
25	I K		J	166.7	3	4.5	LG	NB
25	I K		J	166.7	3	4.5	LQ	NB
22	K		J	133.3	3	4.5	LT	NB
22	K		J	133.3	3	4.5	BHA	NA
22	K		J	133.3	3	5.6	LG	NA
22	K		J	133.3	3	6.8	LQ	NA
22	K		J	133.3	3	6.8	TBHQ	NB
22	K		J	133.3	3	4.5	LA	NB
16	K			100.0	3	5.6	LAU	NA
16	K			100.0	3	4.5	BHA	NB
16	K			100.0	3	4.5	LA	NA
16	K			100.0	3	4.5	LAU	NB
16	K			100.0	3	5.6	LQ	NA
16	K			100.0	3	4.5	LT	NA
16	K			100.0	3	4.5	LQ	NA
16	K			100.0	3	5.6	LT	NA
16	K			100.0	3	4.5	LG	NA
16	K			100.0	3	4.5	TBHQ	NA
16	K			100.0	3	4.5	TBHQ	NB
16	K			100.0	3	5.6	TBHQ	NA
16	K			100.0	3	5.6	TBHQ	NB
16	K			100.0	3	4.5	LAU	NA
16	K			100.0	3	6.8	TBHQ	NA
16	K			100.0	3	5.6	LA	NA

Table 17: The Mean Values of Minimum Inhibitory Concentration (MIC) of Monolaurin and/or selected Antioxidants on 16 Bacteria Tested

Legend identical to Table 1.

Mean of MIC

Rank	Grouping					Mean(ppm)	Repeat	pH	Reagent	Medium
54				A		1056.3	3	5.6	BHT	NA
53			B		A	1020.8	3	6.8	BHT	NB
52			B		A	C	972.9	3	6.8	BHT	NA
51			B		D	C	914.6	3	6.8	LT	NA
50			B		D	C	910.4	3	5.6	LAU	NB
49					D	C	872.9	3	5.6	LAU	NA
49					D	C	870.8	3	5.6	BHT	NB
49					D	C	866.7	3	6.8	LT	NB
46			E		D		835.4	3	6.8	LAU	NA
46			E		D		816.7	3	5.6	LT	NA
44			E		F		718.8	3	6.8	LA	NA
44			E		F		708.3	3	6.8	LAU	NB
42			G		F		687.5	3	5.6	LT	NB
42			G		F		687.5	3	6.8	LA	NB
40			G		F	H	683.3	3	5.6	PG	NA
40			G		F	H	666.7	3	5.6	LQ	NA
40			G		F	H	660.4	3	5.6	LG	NA
37			G		F	H	660.4	3	6.8	LG	NA
36			G		F	H	656.3	3	5.6	LA	NA
35			G	I	F	H	602.1	3	4.5	BHT	NA
35			G	I	F	H	602.1	3	5.6	LA	NB
33			G	I	J	H	564.6	3	5.6	LQ	NB
32				I	J	H	558.3	3	5.6	TBHQ	NA
31			K	I	J		522.9	3	6.8	LG	NB
31			K	I	J		512.5	3	4.5	LT	NA
31			K	I	J		510.4	3	6.8	BHA	NA
31			K	I	J		510.4	3	5.6	LG	NB
31			K	I	J		504.2	3	4.5	LAU	NA
26			K	I	J	L	481.3	3	6.8	PG	NA
26			K	I	J	L	479.2	3	6.8	LQ	NA
26			K	I	J	L	477.1	3	5.6	BHA	NA
26			K	I	J	L	477.1	3	6.8	LQ	NB
22			K		J	L	460.4	3	6.8	BHA	NB
21			K	M	J	L	443.8	3	6.8	PG	NB
20			K	M	N	L	406.3	3	5.6	BHA	NB
19		O	K	M	N	L	397.9	3	4.5	LAU	NB
19		O	K	M	N	L	395.8	3	6.8	TBHQ	NB
17		O		M	N	L	368.8	3	4.5	BHT	NB
17		O		M	N	L	364.6	3	4.5	LT	NB
15		O	P	M	N	L	356.3	3	5.6	PG	NB
15		O	P	M	N	L	356.3	3	5.6	TBHQ	NB
13		O	P	M	N	Q	327.1	3	4.5	LA	NB
13		O	P	M	N	Q	316.7	3	4.5	LG	NA
11		O	P		N	Q	308.3	3	6.8	TBHQ	NA
11		O	P		N	Q	306.3	3	4.5	LQ	NA
11		O	P		N	Q	293.8	3	4.5	PG	NA
11		O	P		N	Q	285.4	3	4.5	LA	NA
11		O	P		N	Q	283.3	3	4.5	LQ	NB
6		O	P			Q	270.8	3	4.5	TBHQ	NA
5			P			Q	231.3	3	4.5	LG	NB
4						Q	210.4	3	4.5	BHA	NA
4						Q	206.3	3	4.5	PG	NB
4						Q	204.2	3	4.5	BHA	NB
4						Q	202.1	3	4.5	TBHQ	NB

Table 18: The Maximum Values of Minimum Inhibitory Concentration (MIC) of Monolaurin and/or Selected Antioxidants on 16 Bacteria tested

Legend identical to Table 1.

Maximum of MIC

Rank	Grouping				Mean(ppm)	Repeat	pH	Reagent	Medium
54				A	1100.0	3	5.6	LT	NA
54				A	1100.0	3	6.8	BHT	NB
54				A	1100.0	3	4.5	BHT	NA
54				A	1100.0	3	5.6	BHA	NA
54				A	1100.0	3	4.5	LA	NA
54				A	1100.0	3	5.6	BHT	NA
54				A	1100.0	3	6.8	LG	NA
54				A	1100.0	3	6.8	BHT	NA
54				A	1100.0	3	5.6	LA	NA
54				A	1100.0	3	6.8	LA	NA
54				A	1100.0	3	5.6	LAU	NB
54				A	1100.0	3	6.8	LAU	NA
54				A	1100.0	3	4.5	LT	NA
54				A	1100.0	3	5.6	LG	NA
54				A	1100.0	3	5.6	LQ	NA
54				A	1100.0	3	5.6	LAU	NA
54				A	1100.0	3	6.8	LT	NA
37		B		A	1066.7	3	4.5	LAU	NA
37		B		A	1066.7	3	5.6	PG	NA
37		B		A	1066.7	3	4.5	LAU	NB
37		B		A	1066.7	3	6.8	LT	NB
37		B		A	1066.7	3	5.6	BHT	NB
32		B		A	C	1033.3	3	6.8	LAU	NB
32		B		A	C	1033.3	3	6.8	BHA	NB
32		B		A	C	1033.3	3	5.6	LA	NB
29		B	D	A	C	1000.0	3	4.5	LG	NA
29		B	D	A	C	966.7	3	6.8	BHA	NA
29		B	D	A	C	966.7	3	6.8	LA	NB
29		B	D	A	C	966.7	3	5.6	TBHQ	NA
29		B	D	A	C	966.7	3	6.8	LQ	NA
29		B	D	A	C	966.7	3	4.5	LQ	NA
23	E	B	D	A	C	933.3	3	5.6	LT	NB
22	E	B	D	F	C	900.0	3	6.8	PG	NA
21	E	G	D	F	C	866.7	3	6.8	PG	NB
21	E	G	D	F	C	866.7	3	5.6	BHA	NB
21	E	G	D	F	C	866.7	3	4.5	BHT	NB
18	E	G	D	F	H	833.3	3	6.8	LG	NB
18	E	G	D	F	H	833.3	3	5.6	LQ	NB
18	E	G	D	F	H	833.3	3	5.6	LG	NB
15	E	G	I	F	H	766.7	3	6.8	LQ	NB
15	E	G	I	F	H	766.7	3	4.5	PG	NA
13	J	G	I	F	H	733.3	3	4.5	LT	NB
13	J	G	I	K	H	700.0	3	4.5	TBHQ	NA
11	J	L	I	K	H	666.7	3	4.5	LA	NB
11	J	L	I	K	H	666.7	3	6.8	TBHQ	NB
9	J	L	I	K		633.3	3	5.6	PG	NB
9	J	L		K	M	566.7	3	6.8	TBHQ	NA
9	J	L		K	M	566.7	3	5.6	TBHQ	NB
9	J	L		K	M	566.7	3	4.5	BHA	NA
5		L		K	M	533.3	3	4.5	BHA	NB
5		L			M	500.0	3	4.5	LG	NB
5		L			M	500.0	3	4.5	LQ	NB
2					M	400.0	3	4.5	TBHQ	NB
2					M	400.0	3	4.5	PG	NB

Table 19: The Minimum Value of Minimum Inhibitory Concentration (MIC) of Monolaurin and/or Selected Antioxidants on 16 Bacteria Tested

Legend identical to Table 1.

Minimum of MIC

Rank	Grouping			Mean(ppm)	Repeat	pH	Reagent	Medium
54		A		866.7	3	5.6	BHT	NA
53	B	A		766.7	3	6.8	BHT	NB
53	B	A		733.3	3	6.8	BHT	NA
51	B	C		600.0	3	5.6	BHT	NB
50	D	C		500.0	3	5.6	LAU	NB
49	D	E		400.0	3	6.8	LT	NB
48	F	E		300.0	3	6.8	LT	NA
47	F	E	G	233.3	3	5.6	PG	NA
47	F	E	G	233.3	3	6.8	LAU	NB
47	F	E	G	233.3	3	6.8	LG	NB
47	F	E	G	233.3	3	6.8	LAU	NA
43	F		G	200.0	3	5.6	LQ	NB
43	F		G	166.7	3	6.8	LG	NA
43	F		G	166.7	3	5.6	LA	NB
43	F		G	166.7	3	6.8	LA	NA
43	F		G	166.7	3	6.8	LQ	NB
43	F		G	133.3	3	5.6	LT	NB
43	F		G	133.3	3	6.8	PG	NA
43	F		G	133.3	3	6.8	LQ	NA
43	F		G	133.3	3	5.6	PG	NB
43	F		G	133.3	3	5.6	LG	NB
43	F		G	133.3	3	6.8	BHA	NA
32			G	100.0	3	4.5	LA	NA
32			G	100.0	3	4.5	LAU	NA
32			G	100.0	3	4.5	BHT	NB
32			G	100.0	3	5.6	LG	NA
32			G	100.0	3	5.6	LAU	NA
32			G	100.0	3	5.6	LQ	NA
32			G	100.0	3	5.6	LA	NA
32			G	100.0	3	5.6	LT	NA
32			G	100.0	3	4.5	BHA	NA
32			G	100.0	3	4.5	BHA	NB
32			G	100.0	3	4.5	BHT	NA
32			G	100.0	3	5.6	TBHQ	NA
32			G	100.0	3	5.6	TBHQ	NB
32			G	100.0	3	4.5	LA	NB
32			G	100.0	3	6.8	BHA	NB
32			G	100.0	3	4.5	LAU	NB
32			G	100.0	3	4.5	LG	NA
32			G	100.0	3	4.5	LG	NB
32			G	100.0	3	4.5	LQ	NA
32			G	100.0	3	4.5	LQ	NB
32			G	100.0	3	4.5	LT	NA
32			G	100.0	3	4.5	LT	NB
32			G	100.0	3	4.5	PG	NA
32			G	100.0	3	4.5	PG	NB
32			G	100.0	3	4.5	TBHQ	NA
32			G	100.0	3	4.5	TBHQ	NB
32			G	100.0	3	5.6	BHA	NA
32			G	100.0	3	5.6	BHA	NB
32			G	100.0	3	6.8	PG	NB
32			G	100.0	3	6.8	TBHQ	NA
32			G	100.0	3	6.8	TBHQ	NB

Table 20: The Effects of Nutrient Broth and Nutrient Agar on 16 Bacteria Tested

Legend identical to Table 1.

Organism	Grouping	Mean(ppm)	Repeat	Medium
	A	348.15	81	NB
Acinetobacter calcoaceticus				
	B	241.98	81	NA
	A	430.86	81	NA
Bordetella bronchiseptica				
	A	423.46	81	NB
	A	724.69	81	NA
Citrobacter freundii				
	B	514.81	81	NB
	A	958.02	81	NA
Enterobacter aerogenes				
	B	713.58	81	NB
	A	716.05	81	NA
Escherichia coli				
	B	580.25	81	NB
	A	775.31	81	NA
Hafnia alvei				
	B	613.58	81	NB
	A	918.52	81	NA
Klebsiella pneumoniae				
	B	701.23	81	NB
	A	443.21	81	NA
Morganella morgannii				
	A	430.86	81	NB
	A	732.10	81	NA
Proteus mirabilis				
	B	580.25	81	NB
	A	491.36	81	NB
Providencia stuartii				
	A	476.54	81	NA
	A	562.96	81	NA
Pseudomonas fluorescens				
	B	380.25	81	NB
	A	540.74	81	NA
Salmonella molade				
	B	466.67	81	NB
	A	790.12	81	NA
Serratia marcescens				
	B	703.70	81	NB
	A	430.86	81	NA
Shigella sonnei				
	A	412.35	81	NB
	A	255.56	81	NB
Staphylococcus aureus				
	A	228.40	81	NA
	A	344.44	81	NB
Streptococcus faecalis				
	B	292.59	81	NA
	A	578.94	81	NA
Mean				
	B	497.53	81	NB
	A	1000.00	81	NA
Maximum				
	B	809.88	81	NB
	A	198.77	81	NB
Minimum				
	A	177.78	81	NA

Table 21: The Effects of 3 Different pH values (6.8, 5.6, and 4.5) on 16 Bacteria Tested

Legend identical to Table 1.

Organism	Grouping	Mean(ppm)	Repeat	pH
	A	390.74	54	6.8
Acinetobacter calcoaceticus	A	329.63	54	5.6
	B	164.81	54	4.5
	A	598.15	54	6.8
Bordetella bronchiseptica	B	538.89	54	5.6
	C	144.44	54	4.5
	A	759.26	54	5.6
Citrobacter freundii	A	712.96	54	6.8
	B	387.04	54	4.5
	A	916.67	54	5.6
Enterobacter aerogenes	A	894.44	54	6.8
	B	696.30	54	4.5
	A	748.15	54	5.6
Escherichia coli	A	718.52	54	6.8
	B	477.78	54	4.5
	A	805.56	54	5.6
Hafnia alvei	B	735.19	54	6.8
	C	542.59	54	4.5
	A	890.74	54	5.6
Klebsiella pneumoniae	A	874.07	54	6.8
	B	664.81	54	4.5
	A	614.81	54	5.6
Morganella morganii	B	537.04	54	6.8
	C	159.26	54	4.5
	A	875.93	54	5.6
Proteus mirabilis	A	827.78	54	6.8
	B	264.81	54	4.5
	A	629.63	54	6.8
Providencia stuartii	A	625.93	54	5.6
	B	196.30	54	4.5
	A	657.41	54	5.6
Pseudomonas fluorescens	B	562.96	54	6.8
	C	194.44	54	4.5
	A	574.07	54	5.6
Salmonella molade	A	572.22	54	6.8
	B	364.81	54	4.5
	A	864.81	54	5.6
Serratia marcescens	A	846.30	54	6.8
	B	529.63	54	4.5
	A	553.70	54	5.6
Shigella sonnei	A	548.15	54	6.8
	B	162.96	54	4.5
	A	314.81	54	6.8
Staphylococcus aureus	A	288.89	54	5.6
	B	122.22	54	4.5
	A	427.78	54	6.8
Streptococcus faecalis	B	366.67	54	5.6
	C	161.11	54	4.5
	A	650.69	54	5.6
Mean	A	636.92	54	6.8
	B	327.08	54	4.5
	A	977.78	54	5.6
Maximum	A	957.41	54	6.8
	B	779.63	54	4.5
	A	250.00	54	6.8
Minimum	A	214.81	54	5.6
	B	100.00	54	4.5

Table 22: The Effects of 9 Antimicrobial Combinations of Monolaurin and/or Selected Antioxidants on 16 Bacteria Tested

Legend identical to Table 1.

Organism	Grouping			Mean (ppm)	Repeat	Reagent
		A		627.78	18	BHT
		B		388.89	18	LT
	C	B		350.00	18	LAU
	C	B	D	322.22	18	LA
Acinetobacter calcoaceticus	C	E B	D	272.22	18	LQ
	C	E F	D	238.89	18	LG
		E F	D	194.44	18	PG
		E F		155.56	18	TBHQ
		F		105.56	18	BHA
		A		722.22	18	BHT
	B	A		655.56	18	LT
	B			611.11	18	LAU
		C		472.22	18	LA
Bordetella stuartii		D		311.11	18	LQ
		D		305.56	18	LG
		D		266.67	18	BHA
		D		261.11	18	PG
		D		238.89	18	TBHQ
		A		916.67	18	BHT
		A		911.11	18	LAU
		A		883.33	18	LT
		B		644.44	18	LA
Citrobacter freundii	C	B		572.22	18	LQ
	C			505.56	18	LG
		D		394.44	18	BHA
		D		388.89	18	TBHQ
		D		361.11	18	PG
		A		983.33	18	LT
		A		983.33	18	BHT
		A		972.22	18	LA
		A		961.11	18	LAU
Enterobacter aerogenes		B		811.11	18	LG
	C	B		788.89	18	BHA
	C	B		738.89	18	LQ
	C	D		683.33	18	PG
		D		600.00	18	TBHQ
		A		977.78	18	LAU
		A		961.11	18	BHT
		A		938.89	18	LT
		B		650.00	18	LQ
Escherichia coli	C	B		605.56	18	LA
	C	D		538.89	18	LG
	E	D		450.00	18	TBHQ
	E			361.11	18	BHA
	E			350.00	18	PG
		A		933.33	18	BHT
		A		900.00	18	LAU
		A		900.00	18	LT
		B		744.44	18	LG
Hafnia alvei	C	B		694.44	18	LA
	C	D		594.44	18	PG
	E	D		566.67	18	LQ
	E	F		477.78	18	BHA
		F		438.89	18	TBHQ

Organism	Grouping		Mean(ppm)	Repeat	Reagent
		A	1061.10	18	LAU
		A	1011.10	18	BHT
	B	A	961.10	18	LT
Klebsiella pneumoniae	B		872.20	18	LA
		C	694.40	18	LQ
	D	C	638.90	18	PG
	D	C	633.30	18	BHA
	D		555.60	18	TBHQ
		A	750.00	18	BHT
		A	711.11	18	LAU
		A	700.00	18	LT
		B	405.56	18	LA
Morganella morganii	C	B	322.22	18	LQ
	C		305.56	18	LG
	C		294.44	18	TBHQ
	C	D	238.89	18	PG
		D	205.56	18	BHA
		A	744.44	18	BHT
		A	738.89	18	LAU
	B	A	716.67	18	LT
	B	A	694.44	18	LA
Proteus mirabilis	B	A	655.56	18	BHA
	B	C	611.11	18	LG
	B	C	611.11	18	LQ
	B	C	611.11	18	PG
		C	522.22	18	TBHQ
		A	716.67	18	LT
		A	716.67	18	BHT
		A	688.89	18	LAU
		B	550.00	18	LA
Providencia stuartii	C	B	477.78	18	LG
	C	D	400.00	18	LQ
	E	D	294.44	18	PG
	E		277.78	18	BHA
	E		233.33	18	TBHQ
		A	638.89	18	BHT
	B	A	561.11	18	LA
	B	A	550.00	18	LAU
	B	A	550.00	18	LT
Pseudomonas fluorescens	B	C	455.56	18	LQ
		C	422.22	18	LG
	D	C	400.00	18	BHA
	D	C	361.11	18	TBHQ
	D		305.56	18	PG
		A	805.56	18	BHT
	B	A	766.67	18	LAU
	B		694.44	18	LT
		C	477.78	18	LQ
Salmonella molade	D	C	422.22	18	LG
	D	C	383.33	18	LA
	D		366.67	18	PG
	D		361.11	18	TBHQ
		E	255.56	18	BHA

Organism	Grouping		Mean (ppm)	Repeat	Reagent
	A		927.78	18	LAU
	A		922.22	18	BHT
B	A		888.89	18	LT
B	C		777.78	18	LG
Serratia marcescens	D	C	738.89	18	LA
D	C	E	672.22	18	PG
D		E	661.11	18	BHA
	F	E	616.67	18	LQ
	F		516.67	18	TBHQ
	A		738.89	18	BHT
	B		622.22	18	LT
	B		600.00	18	LAU
	C		438.89	18	LA
Shigella sonnei	D	C	344.44	18	LQ
D	C		338.89	18	LG
D	E		261.11	18	PG
D	E		238.89	18	TBHQ
	E		211.11	18	BHA
	A		700.00	18	BHT
	B		244.44	18	LAU
C	B		216.67	18	PG
C	B		205.56	18	LQ
Staphylococcus aureus	C	B	D	200.00	18	LT
C	B	D	194.44	18	LA
C		D	155.56	18	LG
C		D	144.44	18	BHA
		D	116.67	18	TBHQ
		A	872.22	18	BHT
		B	522.22	18	PG
		C	300.00	18	LT
D		C	277.78	18	LAU
Streptococcus faecalis	D		E	222.22	18	LG
D		E	211.11	18	BHA
		E	188.89	18	LA
F		E	166.67	18	LQ
F			105.56	18	TBHQ
		A	815.28	18	BHT
		B	704.86	18	LAU
		B	693.75	18	LT
		C	546.18	18	LA
Mean		D	483.68	18	LG
E		D	462.85	18	LQ
E		F	410.76	18	PG
G		F	378.13	18	BHA
G			348.61	18	TBHQ
		A	1077.80	18	LAU
B		A	1055.60	18	BHT
B		A	1005.60	18	LT
B			994.40	18	LA
Maximum		C	894.40	18	LG
		C	855.60	18	LQ
D		C	844.40	18	BHA
D			772.20	18	PG
		E	644.40	18	TBHQ

Organism	Grouping			Mean(ppm)	Repeat	Reagent
		A		527.78	18	BHT
		B		211.11	18	LAU
	C	B		188.89	18	LT
	C	B	D	155.56	18	LA
Minimum	C		D	138.89	18	LG
	C		D	133.33	18	LQ
	C		D	133.33	18	PG
			D	105.56	18	BHA
			D	100.00	18	TBHQ

Table 23: Aerobic Psychrotroph Counts * (Colony Forming Unit/gram) in Ground Pork at 1, 3, 5, and 7 Day Intervals.

Reagents (ppm)	0 Time	1 Day#	3 Day#	5 Day#	7 Day#
Contorl	8.9×10^{3}	2.6×10^{5} a	2.2×10^{7} a	8.3×10^{8} a	9.4×10^{8} a
TBHQ 200		2.5×10^{5} a	5.0×10^{6} b	5.7×10^{8} ab	9.0×10^{8} ab
TBHQ 400		1.7×10^{5} b	6.8×10^{6} b	3.7×10^{8} bcd	6.8×10^{8} bc
TBHQ 600		6.6×10^{4} c	2.7×10^{5} b	3.4×10^{8}bcde	5.4×10^{8} cd
Monolaurin 200		2.3×10^{5} ab	4.2×10^{6} b	4.5×10^{8} cb	8.2×10^{8} ab
Monolaurin 400		5.5×10^{4} c	6.0×10^{5} b	4.3×10^{7} ed	4.0×10^{8} dc
Monolaurin 600		4.2×10^{4} c	2.4×10^{5} b	3.7×10^{7} e	3.0×10^{8} e
LAU + TBHQ 200		6.4×10^{4} c	7.0×10^{5} b	2.1×10^{8} cde	4.9×10^{8} cde
LAU + TBHQ 400		4.2×10^{4} c	4.3×10^{5} b	2.8×10^{7} de	2.9×10^{8} e
LAU + TBHQ 600		3.7×10^{4} c	5.5×10^{4} b	8.9×10^{6} e	2.8×10^{7} e

The statistical comparison is under daily basis, which having the same letter are no significant difference.
* Plates were incubated at 7° C for 10 days before counting.

Table 24: Fecal Coliform Counts * (Colony Forming Unit/gram) in Ground Pork at 1, 3, 5, and 7 Day Intervals.

Reagents (ppm)	0 Time	1 Day	3 Day	5 Day #	7 Day #
Control	ND**	ND	ND	81 a	290 a
TBHQ 200	ND	ND	ND	53 b	225 ab
TBHQ 400	ND	ND	ND	34 c	195 abc
TBHQ 600	ND	ND	ND	24 c	140 bcd
Monolaurin 200	ND	ND	ND	33 c	70 cde
Monolaurin 400	ND	ND	ND	ND d	41 de
Monolaurin 600	ND	ND	ND	ND d	29 de
LAU + TBHQ 200	ND	ND	ND	ND d	ND e
LAU + TBHQ 400	ND	ND	ND	ND d	ND e
LAU + TBHQ 600	ND	ND	ND	ND d	ND e

\# The statistical comparison is under daily basis, which having the same letter are no significant difference.
* Plates were incubated at 45° C for 24 hours before counting.
** ND Non-Detectable.

ANTIMICROBIAL PROPERTIES OF MONOLAURIN AND SELECTED ANTIOXIDANTS IN VITRO AND IN GROUND PORK

by

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B.S., Chinese Culture University, R.O.C. 1980

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ABSTRACT

The antimicrobial properties of monolaurin and selected antioxidants were compared in both in vitro and ground pork. Miniaturized microbioligical method was adopted in the in vitro system where 54 treatments derived from 2 media (nutrient broth and nutrient agar), 3 pH values (6.8, 5.6, and 4.5), and 9 reagent combinations (monolaurin, BHA, BHT, TBHQ, PG, monolaurin with BHA, monolaurin with BHT, monolaurin with TBHQ, and monolaurin with PG) were studied. Among the results of all data combined for the 16 bacterial cultures tested, nutrient agar supported better growth than nutrient broth as a growth medium. Bacterial activity was significantly reduced at pH 4.5 but was not affected at pH 6.8 and 5.6. TBHQ showed the best antimicrobial activity out of 9 reagent combinations tested, and the best combination out of 54 treatments was TBHQ in nutrient broth at pH 4.5. No synergistic effects were observed when combinations of monolaurin and selected antioxidants were tested in vitro.

Psychrotroph and fecal coliform in cold stored ground pork with monolaurin and TBHQ were monitored at 1 day, 3 day, 5 day, and 7 day intervals. Monolaurin and/or TBHQ at 3 concentrations (200, 400, and 600 ppm) were applied to individual meat samples. When compared to TBHQ, monolaurin was more active. The combination of monolaurin and TBHQ gave a greater inhibitory effect than either of the substances alone. Comparatively, concentration factor is less important than the reagent factor in inhibiting bacterial growth.