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Brittany Adams
University of Arkansas, Fayetteville

N. Hettiarachchy
University of Arkansas, Fayetteville

M. G. Johnson
University of Arkansas, Fayetteville

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Controlling *Listeria monocytogenes* on ready-to-eat poultry products using carboxymethyl-cellulose film coatings containing green tea extract (GTE) combined with nisin and malic acid

Brittany Adams[*], *N.S. Hettiarachchy*[†], *and M.G. Johnson*[§]

ABSTRACT

The ability to control *Listeria monocytogenes* on ready-to-eat poultry products using carboxymethyl-cellulose film coatings containing green tea extract (GTE), malic acid (M), nisin (N), and their combinations was evaluated. The antimicrobials (GTE: 1.0%, nisin: 10,000 IU/g, malic acid: 1.0%) were incorporated alone or in combination into a carboxymethyl cellulose film coating. Pre-inoculated, fully cooked chicken pieces (~1g, 1cm x 1cm x 1cm) were coated with the film solution. The coated chicken pieces were stored at 4°C and the inhibitory activity against *Listeria monocytogenes* was evaluated at 0, 7, 14, 21, and 28 days. The highest inhibitory activity was found in the sample containing GTE, nisin, and malic acid in combination with a reduction of 3.3 log CFU/mL. These data demonstrate that GTE—combined with nisin and malic acid and incorporated into a carboxymethyl-cellulose film coating, multiple-hurdle technology—is effective in inhibiting *L. monocytogenes* growth on fully cooked chicken pieces at 4°C. Research in the area of finding natural antimicrobials to aid in the prevention of food-borne illnesses is necessary to improve safety and shelf life of products such as ready-to-eat meats. This project provides an effective combination of natural anti-microbials to control *L. monocytogenes* in ready-to-eat chicken pieces.

[*] Brittany Adams is a senior majoring in food science.

[†] N. Hettiarachchy, faculty mentor, is a university professor in the Department of Food Science.

[§] M.G. Johnson, a committee member, is a professor in the Department of Food Science.

MEET THE STUDENT-AUTHOR

Brittany Adams

I graduated from Jonesboro High School in 2003 and enrolled at the University of Arkansas in the fall as a food science major. I currently serve as the president of the Food Science Club and am a student member of the Institute of Food Technologists. I am also an active member of the IFT college bowl team for the University of Arkansas. I have received various honors and awards including the Presidential Scholar Award and the John W. White Outstanding Student Award.

I began working for Dr. Hettiarachchy during my freshman year, conducting research in the area of utilizing proteins and anti-microbial plant extracts to inhibit pathogens, which led me to this research project. In 2004, I competed in the Gamma Sigma Delta Undergraduate Research competition and placed 1st in the poster category. I again competed in 2005 and received 1st place in the poster category as well as 2nd place in the Oral Presentation category. I also competed in the Ozark Food Processors Association undergraduate poster competition and received 2nd place for my research in natural anti-microbials for food safety. Once again in 2007, I competed in the Gamma Sigma Delta Oral Presentation and placed 3rd. I plan to enroll in graduate school in the fall in the Department of Food Science and become director of research in Laboratory R&D.

INTRODUCTION

The growing popularity of refrigerated ready-to-eat (RTE) meat products necessitates development of additional pathogen hurdles including chemicals, natural antimicrobials, and novel processing technology to ensure a safe product (Pszczola, 2002). Frequent outbreaks of food-borne illnesses stimulate even greater demand. In 2005, *Listeria monocytogenes* contamination caused 3,086,104 lbs of meat products to be recalled. In 2006, the recall total was 48,346 lbs, while the recall amount for January 2007 was 17,395 lbs. On February 18, 2007, a South Carolina company recalled chicken breast strips due to *Listeria* contamination, with a total of 2.8 million pounds of product being affected (FSIS/USDA, 2007). *L. monocytogenes* can cause serious illness, especially for unborn fetuses and immuno-compromised adults, including the elderly and pregnant women (Lorber, 1990).

Natural antimicrobial agents are effective and inexpensive and can be alternative, practical, and feasible measures to ensure microbial safety of food. Edible films may serve as carriers for such antimicrobial agents as well as act to provide a controlled release of these agents over an extended period of time. Additionally, edible film coatings may prevent moisture loss and maintain freshness (Eswaranandam et al., 2004; Lungu and Johnson, 2005).

An increasing interest in and demand exist for identifying natural antimicrobials, especially of plant origin, that are safe, economical, and effective. The increasing resistance of pathogens to antibiotics further enhances this demand for utilizing plant extracts as alternatives. Plant extracts are a prime source for natural antimicrobials. It is primarily the phenolic compounds in plant extracts, such as green tea extract, that yield antimicrobial and antioxidant activities (Ahn et al., 2004). These phenolic constituents in natural extracts have been shown to be individually effective against bacterial pathogens (Ho et al., 2001; Ahn et al., 2004; Aziz et al., 1998). Epicatechin and catechin phenolic constituents were found to have effective inhibitory activities against pathogenic bacteria (Ho et al., 2001).

Teas are traditionally used in production of beverages

that are recognized for their health benefits, including antioxidant, anti-inflammatory, anti-carcinogenic, platelet-aggregation inhibition, and metal-chelation properties (Yang and Wang, 1993; Bagchi et al., 1998.)

Phenolic compounds in green tea extract (GTE) are believed to be responsible for the compounds' antimicrobial and antioxidant activities (Kim et al., 2004; Ahn et al., 2004). Faculty mentor Hettiarachchy's laboratory has demonstrated the antioxidant activity of tea and grape seed extracts in a model system and in irradiated chicken (Rababah et al., 2004). GTE is commercially available and is used in a variety of food products.

Organic acids, which are naturally present in fruits and vegetables, also act as antimicrobials. Malic acid is a low-cost organic acid naturally found in apples. It has proven affective in killing up to 2.8 Log CFU/mL of *L. monocytogenes* when incorporated in a soy-protein film (Eswaranandam et al., 2004; Hettiarachchy and Eswaranandam, 2007).

Nisin is a bacteriocin produced through the fermentation of *Lactococccus lactis* bacteria. Bacteriocins can be incorporated into food products to control the growth of other microorganisms (Montville and Matthews, 2005). Nisin has been given GRAS (generally recognized as safe) status as a safe biological food preservative (Federal Register, 1988). Janes et al. (2002) demonstrated that nisin is effective in controlling growth of Gram-positive organisms such as *L. monocytogenes*.

MATERIALS AND METHODS

Listeria monocytogenes (v7 serotype 1/2a) was obtained from Dr. Johnson at the Center for Food Safety and Quality research laboratory, University of Arkansas. A commercial sample of nisin, Nisaplin (Alpin & Barrett Ltd., Trowbridge, Wilts., England), was used. Nisin potency was determined by the method of Janes et al. (2002). Commercial green tea extract was obtained from Jarrow Formulas® (Los Angeles, Calif.). Malic acid was purchased from Baker (Phillipsburg, N.J., U.S.A).

Evaluating inhibitory activity against Listeria monocytogenes. A loop of *Listeria monocytogenes* (v7 serotype 1/2 a) was taken from a frozen stock culture stored at –70°C and activated in fresh BHI for 24 h at 37°C in an incubator. For each test, 1.0 mL of the culture was centrifuged (14,000 rpm, 20 min), and the supernatant was decanted. Test solutions in BHI broth containing natural extracts and their combinations were added to the pellet. Nisin (10,000 IU, Franklin et al., 2004), / green tea extract (10 mg/ml) / malic acid (10 mg/ml) and their combinations were added to BHI broth and inoculated with *Listeria monocytogenes* suspensions of 10^6 CFU/ml (colony-forming units per milliliter). Samples were then incubated at 37°C for 24 h and *Listeria* counts determined at 3 h intervals using spread-plate techniques. Results were evaluated to determine the ability of each of the antimicrobial combinations to inhibit *Listeria monocytogenes* in a 24 h time period (Fig. 1).

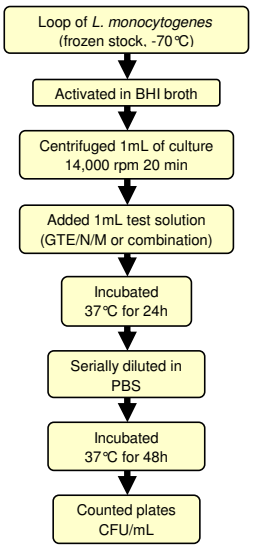

Fig. 1. Flow chart for evaluating inhibitory activity against *Listeria monocytogenes* in broth culture

Preparing films containing antimicrobials. The method for preparing film-forming solutions and films used by Eswaranandam et al. (2004) was followed with slight modifications. The procedure consisted of solubilizing carboxymethyl cellulose (1.75 g) in water (98.25 g), adding glycerol (2.6% w/w) to prevent brittleness, and heating at 90°C for 30 min in a water bath. Nisin (10,000 IU) / green tea extract (10 mg/ml) / malic acid (10 mg/ml) or their combinations were added. The films were cast using the Draw Down instrument from Paul N. Gardner Co., Inc. (Pompano Beach, Fla.) and dried at 50°C and 40% RH for 4 h. The dried films were stored in a dessicator and tested for antilisterial activity (Fig. 2).

Testing antilisterial activity of films. Overnight cultures of *Listeria monocytogenes* (V7 serotype 1/2 a) were inoculated onto 1-cm film discs. Thereafter, 15 µl of the culture was inoculated onto the discs in a Whirl-Pak bag and incubated at room temperature for 1 h. Then 985 µL of phosphate buffer were added to the Whirl-Pak bags and discs were stomached for 2 min. The stomached, inoculated film discs were serially diluted up to 10^4 times using phosphate buffer. They were spread-plated onto *Listeria*-selective agar and incubated for 48 h

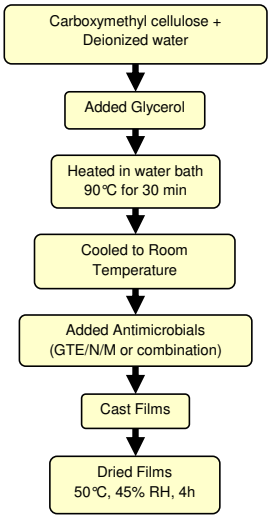

Fig. 2. Flow chart for preparing CMC films

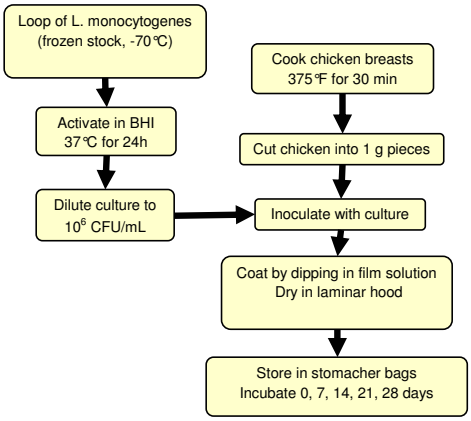

Fig. 4. Flow chart for inoculating and coating chicken pieces

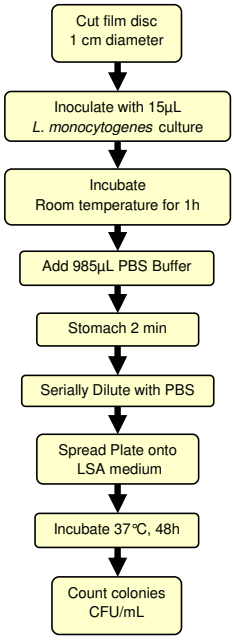

Fig. 3. Flow chart for evaluating anti-listerial activity of CMC films

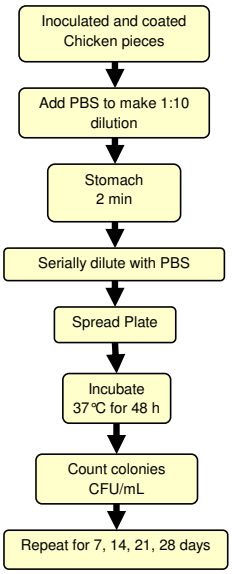

Fig. 5. Flow chart for evaluating anti-listerial activity in meat system

at 37°C. Results were evaluated to determine the log reduction (CFU/mL) given by each antimicrobial film (Fig. 3).

Inoculating with Listeria monocytogenes and coating chicken pieces. Chicken breast products were cooked in a convection oven at 375°F for 30 min and diced to obtain approximately 1-g pieces. These pieces were immersed in 18-h broth cultures of *Listeria monocytogenes* (V7 serotype 1/2 a) containing approximately 10^6 CFU/ml for 30 sec, then removed and allowed to drip free of excess inoculum. This was followed by dipping samples into film-forming solution (carboxymethyl-cellulose) containing green tea extract, nisin, malic acid, or one of their combinations. A total of 21 pieces per d was used (triplicates (3) x 7 treatments including nisin/green tea extract combinations with or without malic acid and a film solution containing no extracts). For controls, 3 pieces per d were used (triplicates). These products were placed into sterile Whirl-Pak bags and refrigerated at 4°C for 28 d and were evaluated every 7 d for inhibitory activity (Fig. 4).

Evaluating antilisterial activity of films containing green tea extract in meat system. Products were removed and evaluated for *Listeria* counts on days 0, 7, 14, 21, and 28. *Listeria* counts were done by placing 9 ml of sterile peptone buffer with 1 g of product (10x dilution) in a Whirl-Pak bag, stomaching, and serially diluting, and the total viable cell counts were determined with the appropriate media (LSA with supplement antibiotic) (EM science, Gibbstown, N.J.) (Fig. 5).

RESULTS AND DISCUSSION

Inhibitory activities of green tea extract, nisin, malic acid, and their combinations in a model system at 37°C.

Fig. 1 shows that *Listeria monocytogenes*, without the addition of antimicrobials (control), grew from an initial level of 6.1 logs CFU/mL to 9.1 logs CFU/mL over 24 h at 37°C in BHI medium. Addition of green tea extract (GTE) or nisin (N) alone allowed *L. monocytogenes* to grow to 9.0 and 8.1 logs CFU/mL, respectively, from the same initial level. Combination of the two (GTE/N) reduced the count to 3.7 logs CFU/mL in 24 h. Malic acid (M) alone reduced the count to non-detectable levels after 9 h while the combinations of M/N and GTE/M reduced them to non-detectable levels after 6 h. The most effective combination was that of GTE/M/N which reduced the counts to non-detectable levels after only 3 h from an initial level of 6.1 logs CFU/mL.

Antilisterial activity of carboxymethyl cellulose films.

Fig. 2 shows log reductions of each antimicrobial combination incorporated in carboxymethyl-cellulose films. Film discs were inoculated with *L. monocytogenes* and incubated at room temperature for 1 h. Control film containing no antimicrobials and film containing only GTE showed very low CFU log reductions of 1.3 and 1.4, respectively. The most effective combination of carboxymethyl-cellulose films was, as before, the combination of GTE/N/M, which gave a log reduction of 4.4 CFU/mL in comparison to the control film.

Inhibitory activity of film solutions on pre-cooked, inoculated, and coated chicken pieces.

1-g pieces of chicken were inoculated with *Listeria monocytogenes* and coated with a carboxymethyl-cellulose coating alone or in combination with the antimicrobials. The chicken pieces were stored at 4°C, inhibitory activity was evaluated on d 0, 7, 14, 21, and 28, and results are displayed in Fig. 3. The control, which was inoculated but not coated, grew from 7.0 logs CFU/mL to 8.2 logs CFU/mL from d 0 to d 28. The sample that was inoculated and coated with only carboxymethyl cellulose grew from 6.8 to 9.1 logs CFU/mL from d 0 to d 28. The combination of GTE/N reduced the counts by d 28 by 2.0 logs CFU/mL when compared to the control with no coating. The combination of GTE/N/M, however, showed the greatest inhibitory activity at 4°C by reducing counts by 3.3 logs CFU/mL by d 28 when also compared to the control with no coating. This value was statistically significant at $p<0.05$.

It is believed that the structure of the phenolic compounds that are present in green tea extract gives it its antimicrobial properties. The ring structure of the phenolic groups is attracted to the lipid membrane of the pathogenic organism while the hydroxyl groups disrupt membrane potential. Addition of nisin and malic acid enhances activity of green tea extract. The unique structure and positive charge of nisin allow it to form pores in the membrane of Gram-positive organisms. These pores allow rapid flow of ions out of the organism and allow malic acid and green tea extract to enter the cell. Once inside, malic acid reduces the internal pH of the cell while green tea extract binds to important enzymes needed for the cell to function. A decrease in log reduction with ready-to-eat chicken could be due to some interactions of the phenolic-active groups with the food components.

This research demonstrates that a combination of antimicrobials incorporated in carboxymethyl-cellulose films is effective in providing an additional hurdle for the growth of *L. monocytogenes* in ready-to-eat chicken products. This may also be applied to a variety of other foods, including fresh fruits and vegetables.

ACKNOWLEDGMENTS

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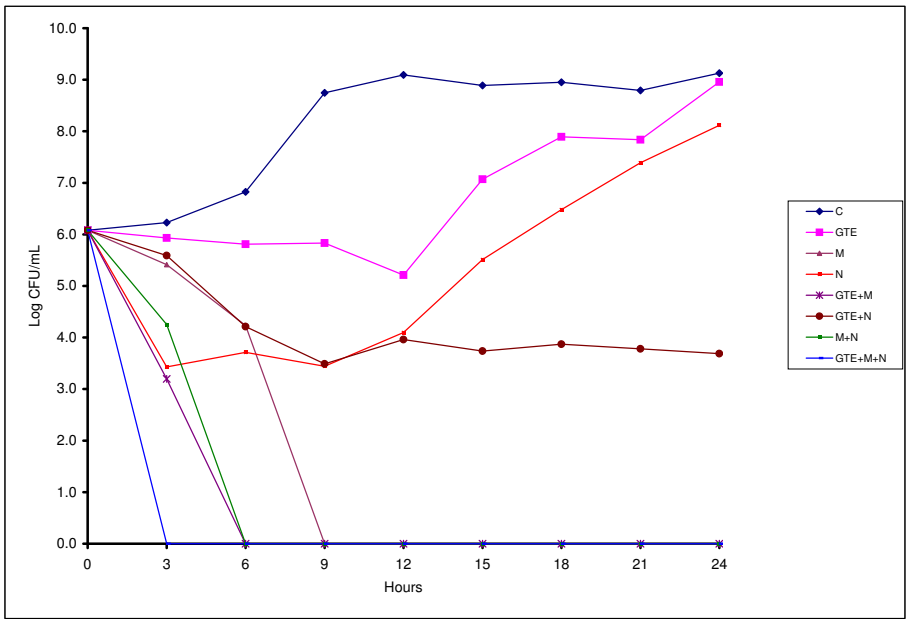
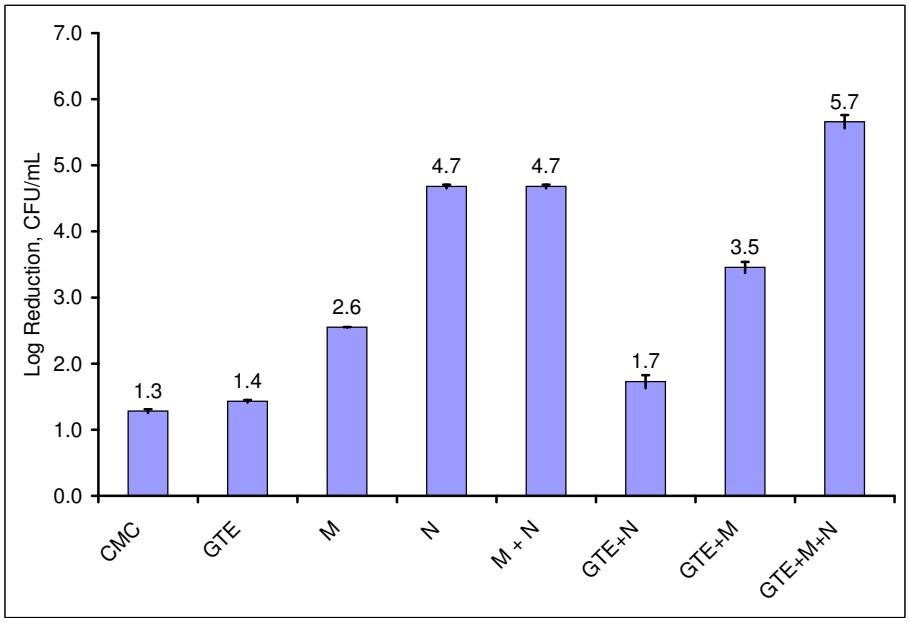


Values are means of three different determinations.
GTE = Green tea extract (1%), N = Nisin (10,000 IU/g), M = Malic acid (1%)

Fig.1. *Listeria monocytogenes* inhibitory activity (log CFU/mL) of green tea extract (GTE), nisin (N), malic acid (M), and their combinations in BHI broth at 37°C for 24 h



Values are means of three determinations.
CMC: Carboxymethyl-cellulose film
GTE: green tea extract (1%), N: nisin (10,000 IU/g), M: malic acid (1%)

Fig. 2. Antilisterial activity of carboxymethyl-cellulose films containing green tea extract (GTE), nisin (N), malic acid (M), and their combinations at 25°C for 1 h.

Treatment	L. monocytogenes Log CFU/g		
	(Days)		
	0	14	28
L.m. Control*	7.0±0.0^{a**}	8.1±0.0^{b}	8.2±0.0^{d}
CMC	6.8±0.0^{ab}	9.1±0.0^{a}	9.1±0.1^{ab}
CMC+M	6.5±0.2^{bcde}	7.1±0.0^{e}	9.0±0.1^{ab}
CMC+M+N	6.5±0.3^{bcd}	5.2±0.1^{h}	8.1±0.4^{d}
CMC+N	6.2±0.1^{de}	7.6±0.1^{d}	8.2±0.1^{d}
CMC+GTE	6.7±0.1^{ab}	9.1±0.0^{a}	9.2±0.0^{a}
CMC+GTE+M	6.7±0.3^{abc}	7.9±0.1^{c}	8.9±0.0^{b}
CMC+GTE+M+N	6.1±0.4^{e}	5.5±0.2^{g}	3.7±0.0^{f}
CMC+GTE+N	6.6±0.1^{abcd}	5.9±0.0^{f}	5.0±0.1^{e}

* Lm control: Inoculation of L. monocytogenes without coating.
CMC: Carboxymethyl-cellulose coating without GTE/Nisin/Malic acid, GTE = green tea extract (1%) N = nisin (10,000 IU/g) M = malic acid (1%)

**All means were measurements of three separate experiments in duplicates. Means within a column followed by same superscript are not significantly different ($p<0.05$)

Fig. 3. *Listeria monocytogenes* inhibitory activity of carboxymethyl-cellulose film (CMC) solutions containing green tea extract (GTE), nisin (N), and malic acid (M) in meat system