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Edible chitosan films on ready-to-eat roast beef for the control of *Listeria monocytogenes*

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Abstract

The use of chitosan as an edible film was evaluated for its antimicrobial activity against *Listeria monocytogenes* (LM) on the surface of ready-to-eat (RTE) roast beef. *L. monocytogenes*, decimally diluted to give an initial inoculation of $>6.50\log$ CFU/g, was inoculated onto the surface of RTE roast beef cubes, and air-dried. The samples were dipped into chitosan (high or low molecular weights) solutions dissolved with acetic or lactic acid at 0.5% (w/v) or 1% (w/v) then bagged and refrigerated at 4 °C. The bacterial counts were determined on days 0, 7, 14, 21, and 28. The samples were spread plated onto modified Oxford agar plates and incubated at 37 °C for 48 h. An initial 6.50 log CFU/g of *L. monocytogenes* inoculated onto the surface of the non-coated RTE roast beef increased too $>10\log$ CFU/g by day 28. On day 14, *L. monocytogenes* counts were significantly different for all the chitosan-coated samples from the control counts by 2–3 log CFU/g and remained significantly different on day 28. Our results have shown that the acetic acid chitosan coating were more effective in reducing *L. monocytogenes* counts than the lactic acid chitosan coating. Our study indicated that chitosan coatings could be used to control *L. monocytogenes* on the surface of RTE roast beef.

Keywords: Chitosan; *Listeria monocytogenes*; Ready-to-eat meats

1. Introduction

Listeria monocytogenes, a Gram-positive rod, is a bacterium that can cause illness in a variety of food products. One food product of great concern is the refrigerated, ready-to-eat (RTE) foods contaminated with *L. monocytogenes* (Levine et al., 2001). Eating foods contaminated *L. monocytogenes* normally causes the disease listeriosis which is more serious for elderly adults and adults with compromised immune systems and can cause meningitis (Roberts and Greenwood, 2003). In pregnant women, the disease may cause spontaneous abortions or stillborn babies (Anonymous, 2003).

The heightened demands by consumers for better quality and improved freshness of food products have given rise to the development and implementation of edible films. Edible films or coatings are defined as continuous matrices that can be prepared from proteins, polysaccharides, and lipids (Cagri et al., 2004). The use of edible films helps to maintain product quality, enhance sensory properties, improve product safety, and increase the shelf life of various RTE food products.

Chitosan, derived by the deacetylation of chitin (poly-β-(1→4)-*N*-acetyl-D-glucosamine), is a major component of the shells of crustaceans such as crab, shrimp, and crawfish (No et al., 2002). Chitosan has been reviewed for commercial application in the biomedical, food, and chemical industries (Knorr, 1984; Muzzarelli, 1977). Due to its biological activities, such as the antimicrobial activity (Tsai et al., 2004; No et al., 2002; Tsai and Su, 1999; Chen et al., 1998; Fang et al., 1994; Sekiguchi et al., 1994; Sudarshan et al., 1992; Kendra and Hadwiger, 1984), antitumor (Tokoro et al., 1988; Suzuki et al., 1986), and hypocholesterolemic functions (Sugano et al., 1980), chitosan and its oligomers have received considerable

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attention. Chitosan is a good candidate for an antimicrobial film due to its film-forming properties (Darmadji and Izumimoto, 1994).

Lactic acid, 2-hydroxypropionic acid, is a weak organic acid. In the food industry, lactic acid can be used for the acidification potential, pH regulation of sodium and potassium lactates, reduction of water activity, synergism with antioxidants and antimicrobial activity (Bogaert and Naidu, 2000). Acetic acid, methanecarboxylic acid, is a colorless liquid with a pungent odor and a sour taste that is generally listed as vinegar. It is a low cost, generally recognized as safe (GRAS) substance which serves as an excellent solvent for organic compounds. When acetic acid, lactic acid, citric acid, and hydrochloric acids were tested on *L. monocytogenes*, acetic acid reduced the numbers of this pathogen more effectively than other acids (Young and Foegeding, 1993; Ita and Hutkins, 1991).

This study evaluated the antimicrobial effect of chitosan, as an edible film, that was dissolved in lactic acid or acetic acid against *L. monocytogenes* on RTE roast beef.

2. Materials and methods

2.1. Culture growth conditions

L. monocytogenes, strain V7, serotype 1/2a obtained from the Centers for Disease Control and Prevention, Atlanta (CDC), GA, USA, was used during this study. The *L. monocytogenes* culture was grown for 18 h in Brain Heart Infusion (BHI) Broth (Becton–Dickinson, Sparks, MD, USA) at 37 °C. The pure cultures were stored at −70 °C and sub-cultured twice in BHI Broth at 37 °C for 24 h before being used.

2.2. Preparation of chitosan solution

Chitosans with molecular weight (MW) of 470 or 1106 kDa were designated as having a low or high MW, respectively. Chitosan with the MW of 470 kDa was purchased from Keumho Chemical (Seoul, Korea) while the chitosan with MW of 1106 kDa was purchased from Premix Ingredients (Avaldsnes, Norway). The low MW chitosan was prepared from shrimp shells, while the high MW chitosan was prepared from crab shell. The chitosan came in a powder form and was stored at freezer temperatures until dissolved. Chitosan was dissolved in 1% (v/v) lactic or 1% (v/v) acetic acid individually at 1% (w/v) on dry basis and diluted to 0.5% (w/v) with distilled water (No et al., 2002). All solutions were adjusted to a pH of 5.6. The control samples were coated with lactic acid or acetic acid solutions without chitosan.

2.3. Treatment of cooked roast beef samples with chitosan solution

An overnight *L. monocytogenes* culture was decimally diluted to 6.5 log CFU/g. One milliliter of the *L. monocytogenes* culture was inoculated onto the 5-g cubed roast beef samples. The cultures were allowed to air dry on the cubed roast beef samples for 10 m under a laminar flow hood. The samples were then dipped into the different types of chitosan solution for 30 s. The cubed roast beef samples were allowed to air dry for 1 h under the laminar flow hood before placing into sterile Whirl-Pack® bags.

The samples were refrigerated at 4 °C and bacterial counts determined at days 0, 7, 14, 21, and 28 by adding 5 ml of phosphate buffer saline (PBS) to each bag, stomaching for 2 min, making serial dilutions and plating the dilutions onto modified Oxford agar with a selective supplement (cycloheximide, colistin sulfate, acriflavine, cefoyetan, and fosfomycin, each at 20 mg/l) (Oxoid, Hampshire, England).

The plates were incubated at 37 °C for 48 h and colony counts expressed as log CFU/g. The only bacteria present on the media were the added cells of *L. monocytogenes*, strain V7, serotype 1/2a. This was confirmed by plating the control non-inoculated cubed beef samples for 48 h at 37 °C which exhibited no bacterial growth. This was done to ensure that no other *Listeria* species were present.

2.4. Statistical analysis

The inhibitory effects of the ACS treatments against *L. monocytogenes* strain V7 on the surface of the cubed cooked roast beef at 4 °C were analyzed using one-way ANOVA ($\alpha = 0.05$) (JUMPIn version 4.0.3, SAS Institute Inc., Cary, NC, USA). All experiments were repeated three times with two replications per experiment.

3. Results

The *L. monocytogenes* strain used during this study was able to grow on the surface of the RTE roast beef product. The initial level of 6.50 log CFU/g increased to over 10 log CFU/g on the control samples at day 21. This study along with others done in our lab have shown that *L. monocytogenes* cultures can increase to high CFU/g levels on RTE roast beef products (Beverly and Janes, 2006; Beverly, 2004).

The log CFU/g of the controls and those samples coated with chitosan showed no significant difference at day 0 (Table 1). However, by day 7, all chitosan treatments were significantly different from the control, but not from one another. By day 14, there were no significant differences between the chitosan treatments, but the treatments were significantly different from the control and lactic acid treatments. On day 14, there was a 1.40–1.65 log reduction in *L. monocytogenes* counts with all chitosan treatments when compared to the control. The greatest reduction on day 14 was observed for the low MW chitosan at 0.5% (w/v) in lactic acid. The *L. monocytogenes* counts on the RTE roast beef were reduced about 1 log CFU/g when treated with lactic acid by days 21 and 28. The most effective chitosan coating was the low MW chitosan at 1%

Table 1
Listeria monocytogenes 1/2a recovered from the surface of ready-to-eat roast beef coated with chitosan dissolved in lactic acid at 4 °C for 28 days

Chitosan treatment[a]	log CFU/g[b]				
	Day 0	Day 7	Day 14	Day 21	Day 28
Control	6.65±0.44 A	8.11±1.31 A	9.29±0.67 A	10.53±0.18 A	10.72±0.21 A
Lactic acid	6.07±1.02 B	6.85±1.55 A	9.07±0.67 AB	9.42±0.15 C	9.44±0.15 D
High MW 1%	5.75±0.43 B	6.60±0.80 A	7.79±0.55 C	10.10±0.17 B	10.03±0.25 B
Low MW 1%	6.05±0.77 B	6.81±0.92 A	8.03±0.56 B	9.47±0.22 C	9.52±0.17 D
High MW 0.5%	5.66±.059 B	6.64±0.97 A	7.89±0.60 C	10.13±0.23 B	10.14±0.32 BC
Low MW 0.5%	6.09±0.65 B	6.15±1.10 A	7.64±0.55 C	9.67±0.11 C	9.70±0.16 C

[a]Different concentrations of chitosan solutions used to treat *L. monocytogenes* inoculated ready-to-eat roast beef samples. Controls are samples without chitosan coating. Lactic acid is samples coated with lactic acid and without chitosan.
[b]All analyses were based on three separate experiments with each mean±S.D. being average of three determinations. Means within each vertical column followed by the same letter are not significantly different ($P>0.05$) from each other.

Table 2
Listeria monocytogenes 1/2a recovered from the surface of ready-to-eat roast beef coated with chitosan dissolved in acetic acid at 4 °C for 28 days

Chitosan treatment[a]	log CFU/g[b]				
	Day 0	Day 7	Day 14	Day 21	Day 28
Control	6.65±0.45 A	8.11±1.31 A	9.29±0.67 A	10.53±0.18 A	10.72±0.21 A
Acetic acid	6.34±0.62 AB	6.93±1.42 AB	8.73±0.58 AB	9.36±0.16 B	9.40±0.11 B
High MW 1%	5.90±0.73 B	6.41±0.52 AB	6.87±0.95 C	7.88±0.06 D	7.93±0.07 D
Low MW 1%	5.91±0.53 B	5.48±0.95 B	7.39±0.70 BC	7.35±0.22 E	7.41±0.11 E
High MW 0.5%	5.98±0.76 B	6.03±0.44 B	7.25±0.93 C	8.43±0.12 C	8.50±0.04 C
Low MW 0.5%	5.94±0.65 B	5.95±0.43 B	7.39±0.67 BC	8.38±0.16 C	8.37±0.17 C

[a]Different concentrations of chitosan solutions used to treat *L. monocytogenes* inoculated ready-to-eat roast beef samples. Controls are samples without chitosan coating. Acetic acid is samples coated with acetic acid and without chitosan.
[b]All analyses were based on three separate experiments with each mean±S.D. being average of three determinations. Means within each vertical column followed by the same letter are not significantly different ($P>0.05$) from each other.

(w/v) in lactic acid that had about 1 log CFU/g reduction at day 28.

The effects of the different MW chitosans at 1.0% or 0.5% (w/v) in acetic acid had no significant differences between the treatments and the control at day 0 (Table 2). On day 7, there was greater than a 2.5 log reduction in *L. monocytogenes* counts on the roast beef coated with the low MW 1% acetic acid chitosan in comparison to the control. The other chitosan treatments on day 7 had a significant 2 log CFU/g reduction in *L. monocytogenes* counts when compared to the non-treated control. The chitosan treatments on day 14 were not significantly different from one another. Day 14 showed a 2.0–2.5 log reduction in *L. monocytogenes* counts for the 1% acetic acid chitosan treatments when compared to the control. By day 21, the 1% acetic acid chitosan solutions had significantly lower counts than the chitosan 0.5% in acetic acid. This trend was also seen on day 28 of the study.

4. Discussion

During the past year, several RTE products have been recalled due to *L. monocytogenes* contamination (CDC, 1999). No et al. (2002) found that chitosan generally showed a stronger bactericidal effect against Gram-positive bacteria than Gram-negative bacteria. However, our study found that *L. monocytogenes* was able to grow on the surface of the RTE roast beef regardless of the chitosan treatments used. Comma et al. (2002) also observed the ability of *L. monocytogenes* to grow on the surface of cheese regardless of chitosan treatments. This could be due to the antimicrobial activity of chitosan films which decreases over time due to the decreased availability of amino groups on chitosan (Cagri et al., 2004; Comma et al., 2002).

The antifungal and antimicrobial activities of chitosan are believed to originate from its polycationic nature (Kim et al., 2003; Roller and Covill, 2000). The antimicrobial action of chitosan is hypothesized to be mediated by the electrostatic forces between the protonated amino group (NH_2) in chitosan and the negative residues at cell surfaces (Tsai and Su, 1999). The number of protonated amino groups (NH_2) present in chitosan increases with increased degrees of deacetylation (DD) which influences antimicrobial activity (Tsai et al., 2002). Liu et al. (2004) state that the bactericidal activity of chitosan is caused by the

electrostatic interaction between NH_3^+ groups of chitosan and the phosphoryl groups of the phospholipid components of the cell membrane. This interaction action was observed in this study due to *L. monocytogenes* being negatively charged (Briandet et al., 1999).

Furthermore, the antibacterial effect of chitosan and its oligomers are dependent on the MW (Jeon et al., 2001; Liu et al., 2001; Uchida et al., 1989), which our study observed. The differences in antimicrobial activity may also be due the preparation methods used to convert chitin to chitosan. This change would then cause differences in the deacetylation and the distribution at acetyl groups, chain length, and the conformational structure of chitin and the chitosan molecule. All these factors will affect the characteristics of chitin and chitosan (Tsai et al., 2002; Terbojevich et al., 1992).

Chitosan has proven to be a suitable matrix to form an edible film. Our study demonstrated that edible chitosan films dissolved in acetic acid or lactic acids could be useful as coatings to control *L. monocytogenes* on the surface of RTE roast beef.

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