CHITOSAN COATINGS TO INHIBIT BACTERIAL GROWTH ON CHICKEN DRUMSTICKS

Paul L. Dawson, Inyee Y. Han, Rachel V. Orr, and James C. Acton

Department of Food Science, The South Carolina Agriculture and Forestry Research System, Clemson University, Clemson, South Carolina 29634-0371 USA

Background:

In recent years, environmental pollution has become a major issue of concern. Packaging research has focused more on biodegradable films, including films made from food proteins. Examples of protein films include casein, collagen, corn zein, gelatin, soy protein and wheat gluten (Dawson, et al., 1997). Some advantages of using edible protein films include increasing packaging film biodegradability, enhancing properties of packaged foods (flavorings, colorings), reducing moisture loss, and providing nutritional value from protein. Shelf-life extension of refrigerated poultry meat requires the inhibition of psychrotrophic spoilage organisms. Natural compounds such as nisin and lysozyme have been studied as potential food preservatives that are safe for human consumption. A commercial anti-fungal coating produced from chitosan is sold as a shelf life extender for fresh fruit. Two biocidal films are marketed composed of a chlorinated phenoxy compound and chlorine dioxide. Both films have the biocidal agent residing in the polymer spaces and the agents are released upon food contact or in response to environmental changes (eg. temperature, humidity). Unlike nisin, lysozyme and chitosan, these compounds are not approved for food contact.

Objectives:

The objectives of this research were to determine the inhibitory effects of coating chicken drumsticks with chitosan films on the growth of *Lactobacillus plantarum*.

Methods:

Twenty g of 91% or 93% deacetylated (DA) chitosan (Vanson, Inc., Redmond, Washington, USA) were mixed with 10 ml of acetic acid and 1000 ml of distilled water for 10 min at 60 rpm using a Virtis mixer. This mixture was filtered through 4 layers of cheesecloth under a vacuum. The solution was exposed to a vacuum for 5 min then held for testing. Nisin was added (0.6 g to 400 ml of chitosan solution) for a final concentration of 1.5 g nisin/ml chitosan. The nisin was mixed with the chitosan solution at room temperature using a standard magnetic mixing bar. *Lactobacillus plantarum* was the indicator organism and was grown for 12-18 hours in MRS broth (Bectin Dickinson, Cockeysville, MD, USA) at 37 C in a CO_2 chamber. The inoculum was centrifuged (1500 x g) for 20 min, then the supernatant was decanted. The pellet was resuspended in 10 ml of 0.1% peptone and the pellet washing procedure was repeated twice. Four ml of the final culture was mixed with 400 ml of 0.1% peptone water to be used for inoculation of the drumsticks. Drumsticks were inoculated by dipping in 400 ml of culture for 30 sec then were air-dried in a sterile hood for 5 min.

The drumsticks were weighed then dipped into one of three solutions; distilled water, chitosan, or chitosan + nisin. After dipping the drumsticks were air-dried for 5 min then hung on racks under refrigeration (4 C) for 3 days. The drumsticks were weighed after 3 days then placed in sterile whirlpak bags for rinsing. Twenty ml of 0.1% peptone water was added to the bags and the bags were shaken for 30 sec. The rinse solution was serially diluted and plates were incubated for 48 hr at 37 C under flowing CO_2. All bacterial counts are reported as log CFU/ml. The drumsticks were exposed to 5 treatments including; control, 93% DE chitosan, 93% DA chitosan + nisin, 91% DA chitosan, and 91% DA chitosan + nisin. The experiment was replicated 3 times using duplicate samples within each replication. The data were subjected to ANOVA using a general linear model to determine replication and treatment effects. The replication effect was not significant so replications were pooled and means were separated using the LSMEANS procedure of SAS (1990).

Results and Discussion

Two levels of DA chitosan were used due to their differences in viscosity. Viscosity of the two solutions was determined to be 30 centipose (91% DA) and 345 centipose (93% DA). The more viscous solution (93% DA) appeared to be more cohesive to itself and less adhesive to the drumstick than the 91% DA solution. This affected the uniformity of the coating and may have altered the antimicrobial effectiveness of the two solutions. Although not quantitated, the 93% DA chitosan solution appeared to lose more of the coating during the drying phase than the 91% DA solution. Chicken drumstick weight losses during storage ranged from 9.2% for the 93% DA chitosan to 10.7% for the control. Only the 93% DA solution significantly ($P<0.05$) reduced weight loss during storage compared to the control samples (Table 1). It is expected that any coating would reduce moisture loss and thus weight loss during extended storage.

The 91% DA chitosan solution significantly ($P<0.05$) reduced the population of *L. plantarum* compared to the controls and the 93% DA solutions (Table 2). Both solutions using 91% DA chitosan reduced the log CFU/ml counts 2 logs compared to control and 0.5 to 1 log compared to the 93% DA chitosan whether nisin was present or not. Chitosan has been shown to have an antimicrobial effect when added directly to minced red meat (Darmadji and Izumimoto, 1994). Nisin has been shown to inhibit bacterial growth in films (Padgett et al. 1998) and in packaged chicken (Natrajan and Sheldon, 1995). The added nisin did not reduce bacterial counts when used with either the 93% or 91% DA chitosan. It is possible that the chitosan may have bound or interacted with the nisin to reduce it's efficacy since these same levels of nisin have been shown to inhibit *L. plantarum* (Padgett et al. 1998).

Conclusion:

Chitosan film coatings are inhibitory on the surface of chicken drumsticks. Furthermore, the addition of nisin to chitosan film coatings does not increase the overall inhibitory effect of the coating. Determination if this inhibitory affect can be extended to a broad range of spoilage and pathogenic bacteria will require more research.

References:

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Table 1. Percent weight loss of chicken drumsticks coated with 91% and 93% deacetylated chitosan solutions.

Treatment	Weight Loss (%)
Control	10.7^{b}
91% deacetylated chitosan	$10.0^{a,b}$
91% deacetylated chitosan + nisin	$10.1^{a,b}$
93% deacetylated chitosan	9.2^{a}
93% deacetylated chitosan + nisin	9.5^{a}

^{a,b}means with different superscripts significantly differ ($P<0.05$). n=6

Table 2. Log colony forming units (CFU)/ ml of *Lactobacillus plantarum* from inoculated chicken drumsticks coated with 91% and 93% deacetylated chitosan solution with and without added nisin.

Treatment	Log_{10} CFU/ml
Control	3.2^{a}
91% deacetylated chitosan	2.0^{c}
91% deacetylated chitosan + nisin	2.0^{c}
93% deacetylated chitosan	2.8^{b}
93% deacetylated chitosan + nisin	2.5^{b}

^{a,b,c}means with different superscripts significantly differ ($P<0.05$). n=6