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EFFECT OF CHITOSAN AND ALGINATE EDIBLE COATS ON BACTERIOLOGICAL QUALITY OF CHICKEN DRUMSTICKS

By

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ABSTRACT

This study was carried out to investigate the effect of using chitosan and alginate edible coats on the bacteriological quality of chicken drumsticks. Therefore, chicken drumsticks samples were collected from poultry processing plant, transferred immediately to food hygiene and control laboratory then coated with chitosan (2%) and alginate (2%) by dipping for 30 seconds while control uncoated samples were dipped into DW (C) for 30 seconds. All coated and control uncoated chicken drumsticks were stored in freezing storage at -18 ºC for 3 months and exposed to bacteriological examination at zero time (the first day of coating of the samples) and during the freezing storage for enumeration of total aerobic mesophilic bacterial counts, total aerobic psychrotrophic bacterial counts, *S. aureus* and Enterobacteriaceae counts. The results revealed that significant (P<0.05) reduction of all investigated bacterial counts in all chitosan and alginate coated chicken drumsticks as compared with the control uncoated samples. Moreover, the significant reduction rate of the bacterial counts were observed in chitosan coated chicken drumstick as compared with alginate coated chicken drumstick especially in total aerobic mesophilic bacterial counts at zero time, 1st and 2nd month of examination, total aerobic psychrotrophic bacterial counts during the first month of examination and *S. aureus* at zero time of examination and during freezing storage at -18 ºC for 3 months while only at 3rd month of examination in Enterobacteriaceae counts. Therefore, using of chitosan 2% or alginate 2% can provide a relevant antimicrobial activity which improve the stability of fresh poultry meat and could solve some quality and safety issues in poultry meat processing plant.

Keywords:

Chicken meat; chitosan; alginate; bacterial quality.

INTRODUCTION

Poultry meat is greatly accepted by consumers worldwide as compared to the other meat consumption. Increasing the preference and consumption of poultry meat is due to its competitive price and absence of religious and cultural problems. Moreover, poultry meat is very rich in protein, essential amino acid, vitamin and growth factors and lower in fat and cholesterol content **(Vasilatos and Savvaidis, 2013).** However, poultry meat is categorized as a highly perishable food where its meat acts as a perfect medium for microbial growth and its spoilage inflicts an economic problem and health hazard on both the producers and the consumers **(Dal Bosco *et al.*, 2016).** Raw poultry meat and meat products can serve as a source of foodborne pathogens that may accidentally cross-contaminate other foods. Although, elimination of these pathogens from poultry at rearing, shipping, and processing steps remains a great challenge **(Slader *et al.*, 2002).** Moreover, washing of poultry carcass with approved antimicrobial compounds have been achieved limited success because many of foodborne pathogens are hidden in the feather follicles and protect them from the action of these antimicrobials compounds **(Mehyar *et al.*, 2005).** Therefore, the main concern of the food industries is to extend the shelf-life of poultry meat and its products.

Trend of academic researchers and food industries is using edible film and coatings in poultry meat because of the potential benefits of their usage that could substantially improve the quality and safety of fresh poultry meat and they have many opportunities for their application in the food preservation field. Edible film and coatings are of food grade that can be applied by different ways such as dipping, spreading or spraying and after drying forming transparent layer over the food surface **(Han and Gennadios, 2005).** Edible coatings can overcome many problems such as moisture evaporation, reduction of the commercial weight, changes in texture and color and dripping problem during poultry meat storage **(Bazargani-Gilani *et al.*, 2015).** Chitosan is a polysaccharide found in the shells of crab and shrimps and the cell walls of fungi. It has the ability to form edible and biodegradable films providing mechanical protection and oxygen barrier **(Zhang *et al.*, 2018).** It exhibits a broad-spectrum antimicrobial action against both Gram-negative and Gram-positive bacteria **(Prashanthand Tharanathan, 2007).** Moreover, it can be used to improve the quality and safety poultry meat through retarding the microbial growth and oxidative rancidity **(Souza *et al.*, 2018).**

Alginate is one of the hydrophilic biopolymer that has a coating function due to its colloidal properties therefore, used as thickener and gel forming substance **(Acevedo, 2013).** Alginate application for coating or films in food can extend their shelf life, preserve their functional

properties, and reduce undesirable changes such as weight loss during the storage. There is limited available information related to comparing the antibacterial effect of chitosan and alginate edible coats and their use to improve the quality and safety of chicken meat. Therefore, the present study was designed to assess the effect of using chitosan and alginate edible coats on the bacteriological quality of chicken drumsticks.

MATERIAL AND METHODS

1.1. The study design:

A three independent replicates at different times were conducted to investigate the effect of using of chitosan (2%) and alginate (2%) edible coats on the bacterial quality of chicken drumsticks. All coated and control uncoated chicken drumsticks samples were stored at 18 °C for 3 months and their bacterial quality were evaluated periodically every month.

1.2. Ingredients preparation:

Chicken drumsticks (30 kilo) were obtained from poultry processing plant and transferred immediately to the laboratory of Food Hygiene and Control Department, Faculty of Veterinary Medicine, Cairo University. Chitosan of high molecular weight (2%, CH) (Batch 12913CJ, Sigma-Aldrich, USA), was dispersed into 0.5% lactic acid solution (Panreac, Barcelona, Spain). The alginate (2%) edible coat was prepared immediately before use, where 2 grams of sodium alginate (Morgan chemicals, Egypt) were blended with 100 ml DW in lab blender for several minutes till the formation of gel.

1.3. Treatments and chitosan edible coat application:

Chicken drumsticks were divided into 3 groups as follow: first group was prepared by dipping chicken drumsticks into DW for 30 seconds as control samples (C). The 2nd group was prepared by dipping chicken drumsticks into chitosan (2%) edible coat for 30 seconds. While, the 3rd group was prepared by dipping chicken drumsticks into alginate (2%) edible coat for 30 seconds.

1.4. Investigations

1.4.1. Bacteriological examination

1.4.1.1. Preparation of food homogenate:

Sample homogenate was prepared by homogenizing ten grams from each sample in 90 ml of 0.1% peptone water (Oxoid CM9) for 1.5 min. using stomacher (Lab blender 400). From the original homogenate, tenfold decimal dilutions were prepared using the same diluents **(APHA, 1992)**. The following bacteriological investigations were performed.

1.4.1.2. Enumeration of total aerobic mesophilic bacterial count:

From each dilution, 0.1 ml was aseptically spread over the surface of double sets of standard plate count agar plates (Oxoid CM 463) using sterile bent glass spreader. The plates were incubated at 32°C for 48 hours. The average count of the duplicate plates was enumerated and the mesophilic bacterial count/g was calculated **(Swanson *et al.*, 1992).**

1.4.1.3. Enumeration of total aerobic psychrotrophic bacterial count:

One hundred µl from each dilution of the prepared sample homogenate were aseptically spread onto the surface of double sets of standard plate count agar plates (Oxoid CM 463). The inoculated plates were incubated at 7°C for 7 days and the number of psychrotrophic bacteria/g was calculated **(Cousin *et al.*, 1992).**

1.4.1.4. Enumeration of S. aureus count:

One hundred µl from each of the previously prepared sample homogenate was aseptically spread onto the surface of double sets of Baird-Parker agar plates (Oxoid CM 145). Inoculated plates were incubated at 37°C for 48 hours .Typical colonies (black, shiny, smooth, convex, 1-1.5 mm with narrow white margin and surrounded by a clear extending into opaque medium) were enumerated and recorded as presumptive *S. aureus* count **(Bailey and Scott, 1982).** Suspected *S. aureus* colonies were purified and identified biochemically.

2.4.1.5. Enumeration of Enterobacteriaceae count:

One hundred µl from each of the previously prepared sample homogenate was aseptically spread onto the surface of double sets of Violet Red Bile Glucose Agar (Oxoid CM 1082). The inoculated plates were incubated at 37 ºC for 24 hours. Typical colonies red to dark purple colonies were enumerated **(ISO, 1979).**

1.4.2. Statistical analysis:

All data were analyzed using SPSS statistics 17.0 for windows, expressed as mean±SE and compared using one-way analysis of variance (ANOVA). The significance was determined using least square difference test (LSD) procedure and the main effects were considered significance at the $P<0.05$ level.

RESULTS AND DISCUSSION

Table (1): Bacterial counts (Log_{10} CFU/g) of chicken drumsticks coated with chitosan (2%) edible coats.

Treatments	Storage period (months)			
	0-time	1st month	2nd month	3rd month
Total aerobic mesophilic bacterial count				
Control	3.52[a]±0.09	3.75[a]±0.26	4.71[a]±0.02	5.18[a]±0.02
Chitosan	<2.00[b]±0.00	<2.00[b]±0.00	2.61[b]±0.17	2.83[b]±0.17
Total aerobic psychrotrophic bacterial counts				
Control	2.91[a]±0.24	2.99[a]±0.29	3.95[a]±0.31	4.26[a]±0.26
Chitosan	<2.00[b]±0.00	<2.00[b]±0.00	2.17[b]±0.17	2.47[b]±0.26
***S. aureus* counts**				
Control	3.20[a]±0.24	3.20[a]±0.17	3.30[a]±0.19	3.89[a]±0.06
Chitosan	<2.00[b]±0.00	<2.00[b]±0.00	<2.00[b]±0.00	<2.00[b]±0.00
Enterobacteriaceae counts				
Control	2.20[a]±0.20	2.53[a]±0.29	2.87[a]±0.13	3.36[a]±0.29
Chitosan	<2.00[b]±0.00	<2.00[b]±0.00	<2.00[b]±0.00	<2.00[b]±0.00

[a–b]Means with different superscripts within the same row for each parameter are significantly (*P*<0.05) different.

***Values represent the mean of 3 independent replicates± SE.**

Table (2): Bacterial counts (Log_{10} CFU/g) of chicken drumsticks coated with alginate (2%) edible coats

Treatments	Storage period (months)			
	0-time	1st month	2nd month	3rd month
Total aerobic mesophilic bacterial count				
Control	3.90[a]±0.11	4.10[a]±0.00	4.51[a]±0.28	6.20[a]±0.42
Alginate	2.17[b]±0.09	3.13[b]±0.30	3.74[b]±0.01	4.15[b]±0.06
Total aerobic psychrotrophic bacterial counts				
Control	3.00[a]±0.00	4.17[a]±0.13	4.30[a]±0.09	4.84[a]±0.31
Alginate	2.00[b]±0.00	2.53[b]±0.30	3.17[b]±0.17	3.67[b]±0.26
***S. aureus* counts**				
Control	2.70[a]±0.06	3.84[a]±0.03	3.97[a]±0.03	4.47[a]±0.15
Alginate	2.00[b]±0.00	2.60[b]±0.30	3.14[b]±0.08	3.47[b]±0.06
Enterobacteriaceae counts				
Control	2.10[a]±0.10	2.92[a]±0.03	3.20[a]±0.38	3.16[a]±0.22
Alginate	<2.00[b]±0.00	<2.00[b]±0.00	<2.00[b]±0.00	1.33[b]±0.66

[a–b]Means with different superscripts within the same row for each parameter are significantly (*P*<0.05) different.

***Values represent the mean of 3 independent replicates± SE.**

Table (3): Bacterial reduction rates (Log_{10} CFU/g) of chicken drumsticks coated with chitosan (2%) and alginate (2%) edible coats.

Treatments	Storage period (months)			
	0-time	1st month	2nd month	3rd month
Total aerobic mesophilic bacterial counts				
Chitosan	3.52[a]±0.09	3.75[a]±0.26	2.10[a]±0.15	2.35[a]±0.15
Alginate	1.73[b]±0.02	0.97[b]±0.30	0.77[b]±0.27	2.05[a]±0.36
Total aerobic psychrotrophic bacterial counts				
Chitosan	2.91[a]±0.24	2.99[a]±0.29	1.78[a]±0.14	1.79[a] ±0.00
Alginate	1.00[b] ±0.00	1.64[b] ±0.17	1.13[a] ±0.08	1.17[a] ±0.05
***S. aureus* counts**				
Chitosan	3.20[a]±0.24	3.20[a]±0.17	3.30[a]±0.19	3.89[a]±0.06
Alginate	0.70[b]±0.06	1.24[b]±0.27	0.83[b]±0.05	1.00[b]±0.09
Enterobacteriaceae counts				
Chitosan	2.20[a]±0.20	2.53[a]±0.29	2.87[a]±0.13	3.36[a]±0.29
Alginate	2.10[a]±0.10	2.92[a]±0.03	3.20[a]±0.38	1.83[b]±0.44

[a–b]Means with different superscripts within the same column for each parameter are significantly (*P*<0.05) different.

***Values represent the mean of 3 independent replicates± SE.**

Coating of chicken drumsticks with chitosan (2%) and alginate (2%) resulted in significant (*P*<0.05) reduction of total aerobic mesophilic bacterial counts, total aerobic psychrotrophic bacterial counts, *S. aureus* and Enterobacteriaceae counts at zero time of examination and during freezing storage at -18 ºC for 3 months as compared with control uncoated samples (Table 1,2). It is also clear that total aerobic mesophilic bacterial count was under detectable limit (< 2 Log_{10} CFU/g) in chitosan coated samples at zero time and the first month of examination and start to increase gradually with the freezing storage (2.83 Log_{10} CFU/g). Total aerobic mesophilic bacterial count of alginate coated samples was 2.17 Log_{10} CFU/g at zero time of examination and 4.15 Log_{10} CFU/g at the 3rd month of freezing storage. Total aerobic psychrotrophic bacterial count of chitosan coated samples was under detectable limit (< 2 Log_{10} CFU/g) at zero time and the first month of examination and reaches 2.47 Log_{10} CFU/g at the end of freezing storage (3 months). Total aerobic psychrotrophic bacterial count of alginate coated samples was 2.00 Log_{10} CFU/g at zero time of examination and 3.67 Log_{10} CFU/g at the 3rd month of storage. *S. aureus* count of chitosan coated samples was under detectable limit (< 2 Log_{10} CFU/g) at zero time and during freezing storage at -18 ºC for 3 month meanwhile it was 2 Log_{10} CFU/g and reaches 3.47 Log_{10} CFU/g at the 3rd month of

freezing storage in alginate coated samples. Enterobacteriaceae count was under detectable limit (< 2 Log_{10} CFU/g) in chitosan and alginate coated samples at zero time and during freezing storage at -18 ºC for 3 month except in the 3rd month of freezing storage in case of alginate coated samples (1.33 Log_{10} CFU/g). (Table 3) summarized the bacterial reduction rates (Log_{10} CFU/g) of chicken drumsticks coated with chitosan (2%) and alginate (2%) edible coats. The significant reduction rate of the bacterial counts were observed in chitosan coated chicken drumstick as compared with alginate coated chicken drumstick especially in total aerobic mesophilic bacterial counts at zero time, 1st and 2nd month of examination, total aerobic psychrotrophic bacterial counts during the first month of examination and *S. aureus* at zero time of examination and during freezing storage at -18 ºC for 3 months while only at 3rd month of examination in Enterobacteriaceae counts.

The obtained results concerning reduction of all investigated bacterial counts of chitosan coated drumsticks were in agreement with those recorded by Eldaly et al. (2018) who observed that dipping of raw chicken fillet in chitosan (1.0%, 1.5%, and 2.0%) caused significant (P<0.05) reduction of the total bacterial count, total Enterobacteriaceae, and total staphylococcus counts during the refrigeration storage (4 ± 1 °C) up to 12th day of storage. Several hypotheses have been proposed the mechanism of antibacterial efficacy of chitosan. The most commonly accepted mechanism is based on the electrostatic interactions between the polycationic charged chitosan and the negatively charged components at the cell surface **(Helander *et al.*, 2001).** Binding of chitosan with the cell wall anionic macromolecules apparently forms an impermeable layer around the cell, which can prevent the transport of nutrients to and from the cell **(Eaton *et al.*, 2008).** Interaction with cell membrane constituents may alter permeability, resulting in leakage of intracellular electrolytes, glucose, enzymes, and other proteinaceous cytoplasmic material **(Tao *et al.*, 2011).** Another possible antibacterial mechanism of chitosan is through chelation of essential nutrients needed for growth **(Dutta *et al.*, 2009).** It was also proposed mechanism of action of chitosan occurs by inhibiting messenger RNA and protein synthesis by penetrating the cell of the microorganism and binding with DNA **(Sudarshan *et al.*, 1992).** In addition, the extent of the antimicrobial activity of chitosan depends on various factors such as deacetylation degree, molecular weight, concentration, water solubility, pH and temperature **(Wang *et al.*, 2004; Holappa *et al.*, 2006).** Moreover, the significant (P<0.05) reduction of total bacterial count of chitosan treated samples was explained by **Zheng and Zhu (2003)** who found that chitosan as a coating

solution or film act as an oxygen barrier around the bacterial cell and thus prevent the growth of aerobic bacteria.

The obtained results concerning decreasing the investigated bacterial counts were in disagreement with the results of **Kargozari *et al.* (2018)** who observed coating of chicken fillet with 3% alginate edible coat resulted in non-significant (P>0.05) decreasing of total mesophilic bacterial counts, total psychrotrophic bacterial counts, LAB and *S. aureus* as compared with control uncoated samples. While, incorporation of alginate coat with coriander seed essential oil (CEO 0, 0.5, 1%) caused significant (P<0.05) reduction of these bacterial counts. Therefore, incorporating CEO agent into the alginate coating matrix could increase the antimicrobial activity of this edible coating and could be used to improve food quality and enhance the shelf life of perishable foods **(Alboofetileh *et al.*, 2014).** Moreover, **Raeisi *et al.* (2016)** reported that sodium alginate incorporated with cinnamon and rosemary essential oils had a significant (P<0.05) reduction of psychrotrophic bacteria, Pseudomonas, LAB and Enterobacteriaceae count in chicken fillet during cold storage time. At the same time, **Khare *et al*. (2016)** studied the microbiological quality of chicken fillets coated with sodium alginate 2%, calcium chloride 3%, citric acid 0.5% and cinnamon oil stored under refrigerated conditions at 4 ºC for 5 days. The results showed significant (P<0.05) decreasing of total bacterial counts as compared with control untreated samples. **Kristam *et al*. (2016)** investigated the effect of coating of chicken meat nuggets with alginate 2% and alginate incorporated with 1% green tea extract (GTE). The nuggets were analyzed at regular intervals of 5days for refrigerated storage (4±1°C) and 15 days for frozen storage period 75 day (−18±1°C). The results showed that, the total bacterial count of chicken nuggets coated with alginate and 1% green tea extract was significantly (P<0.05) lower than alginate coated samples and control uncoated samples.

CONCLUSIONS

From this study it could be concluded that coating of chicken drumsticks with chitosan 2% or alginate 2% edible coats resulted in significant (P<0.05) reduction of total aerobic mesophilic bacterial counts,total aerobic psychrotrophic bacterial counts,*S.aureus* and Enterobacteriaceae counts as compared with control uncoated samples. In addition, the significant reduction rates of all investigated bacterial counts were more pronounced in chitosan coated chicken samples as compared with alginate coated samples. Therefore, chitosan 2% and alginate 2% could be used safely for successful decontamination of chicken carcass at the poultry processing plants.

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