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Original Article

Chitosan Edible Coating as Decontaminant During Water Thawing of Frozen Broiler Carcasses

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ABSTRACT

The present study was designed to evaluate the effects of chitosan edible coating applications on the sensory, physicochemical, and bacterial load on chicken during thawing. Edible coatings prepared with 0.5 &1% chitosan in acetic acid (0.5%), was used as thawing solution. The frozen chicken was submerged for 2 hrs in tap water as control, 0.5 &1% chitosan treatments. Chitosan 1% coating improves tenderness and in turn overall acceptability scores. The result of shear force analysis emphasized the tenderness sensory scores of cooked broilers. Application of chitosan coat reduces pH value, APC, Psychrotrophic count, with significant (p<0.05) reduction in thawed broilers with 1% chitosan coat. Therefore, commercial chitosan can be applied in water thawing of frozen broiler to improve the quality characteristics economically. The results demonstrate that the application of chitosan can be an effective method for reducing contamination in chicken meat during thawing.

INTRODUCTION

Broiler production industry has become the main source of protein supplement in human diet worldwide. With tremendous production of broilers, freezing practice is an essential technique used to extend broiler carcasses shelf life, as well as ensure their safety and quality. However, the thawing practices conducted before frozen broilers cooking remains a major challenge facing food processor in mass catering. Freezing-thawing process is complex, where it encompasses transfer of heat and series changes (physical and chemical) in meat. Furthermore, frozen meat quality is significantly affected by thawing process (Akhtar *et al.,* 2013 and Oliveira. *et al.,* 2015). Thawing is performed conventionally with cold water, room temperature, refrigerator, and microwave, while ohmic and high pressure is considered as novel techniques of thawing. Thawing with cold water is a rapid and cheap technique excessively used in thawing frozen chicken at catering and demonstrated less tissue change than other thawing methods (Oliveira *et al.,* 2015). Freezing technique effectively control microbial spoilage, where, the microbes become inactive, however, their activity is recovered during process of thawing, because much time is required than freezing and thawing process is less uncontrolled in term of temperature loss, so certain areas can expose to more favourable microbial growth conditions than others in meat. However, the high availability of moisture and nutrients (proteins, vitamins and minerals) resulting from thawing and exudates formation (due to cellular damage) provide all essential requirement to microbe growth. Therefore, prompt spoilage of frozen-thawed meat in comparing fresh meat, may be due to synergistic effect of regain of microbial activity at thawing step and tissue damage resultant from frozen storage period.

Worthily, appearance, texture, flavour, color and nutritive value are influenced by thawing (Leygonie *et al.,* 2012).

Recently chitosan has emerged as a natural safe bactericidal substance that can be added to food. Chitosan is a polysaccharide and has the ability to form edible coating, easily obtained from chitin (shellfish wastes) deacetylation (Sánchez-Ortega *et al.,* 2014). The antimicrobial activity characteristic of chitosan edible coating enables it to be applied in preservation of freshness in meat; as it is an effective agent against both spoilage bacteria and pathogens in food (López-Mata *et al.,* 2015).

Therefore, the objective of the current study is to assess the use of chitosan during thawing with tap water (at room temperature in mass catering restaurant) on sensory attributes, physicochemical characteristics and microbiological load in chicken meat, during thawing.

MATERIALS AND METHODS

Experimental design

The experiment was conducted in three repetitions in different separate times, and each analysis was performed in triplicate.

Materials

Chitosan coat preparation

Commercial Chitosan flaks produced from shrimp wastes was obtained from a local producer (Chito-MAX) with an average molecular weight of 40-170 kDa 85-90% deacetylation and 15 -200CP viscosity. Chitosan coat solution was prepared by dissolving 0.5 & 1% chitosan in 0.5% acetic acid.

Application of chitosan coat during thawing of frozen broilers

Forty-five frozen, locally produced broilers carcasses (15 for each treatment) at central catering were used. Frozen broilers were immersed in chitosan coat solutions for 90 minutes after the removal of their plastic packages at room temperature. The coated thawed broilers were allowed to dry for 15 minutes at room temperature before examination. Three treatments were prepared: tap water (Ch 0%) as control, 0.5% chitosan (Ch0.5%) and 1% chitosan (Ch1%). The coated broilers were examined for their sensory attributes, physicochemical parameters, and microbiological load.

Sensory evaluation

Raw and cooked samples of all treated groups were examined for their sensory attributes using a hedonic scale of nine-point (9 = extremely like; and 1 = extremely dislike). The sensory attributes of raw (color, odor, texture and overall score) and cooked (color, flavor, juiciness, tenderness and overall score) broiler samples were analyzed by a trained panelists team consisting of 29 members of faculty staff members and postgraduate students, their age ranged from 25 to 53 years of age.

Physicochemical analysis

pH value

Determination of the pH was done according to Harold *et al.* (1981). Ten g of the sample was thoroughly homogenized with 100 ml of previously boiled distilled water and then cooled to 25°C and left to stand for 10 minutes. Using a pH meter (Jenway, 3310), and electrode each sample was measured three times; the pH value was recorded as an average of the three readings.

Instrumental color measurement

Broiler breast and thigh colour characteristics as: Lightness (L^*) (dark (0) to light (100)), the redness (a^*) values ((+), reddish to (-) greenish, the yellowness (b^*) values ((+), yellowish to (-) bluish) were assessed by using Chroma meter (Konica Minolta, model CR 410, Japan). Calibration was performed to Chroma meter as the manufacturer instruction (white plate and light trap). Color was expressed using the CIE L^*, a^*, and b^* color system (CIE, 1976). A total of three spectral readings were taken for each sample.

Shear force

Chicken meat samples (six core samples for each treatment) were analyzed for shear force (kgf/cm^3) using steaks of 2x2x2 cm, prepared from each breast and thigh chicken meat cooked at core temperature (72 °C). The samples were removed parallel to muscle fibers direction by hand- held coring device (1.3cm diameter). Each core was sheared once with Warner-Bratzler shear force (WBSF) device attached to an Instron Universal Testing machine (Model 2519-105, USA) with a 55-kg tension / compression load cell and a cross head speed of 200 mm /min. An average shear force value was calculated and recorded for each sample as described by Xiong *et al.* (2006).

Microbiological examination

The technique recommended by APHA (2001) was applied for bacterial counts as follows. Twenty-five grams of the chicken samples were mixed into sterile stomacher polyethylene bags with 225 ml of sterile 0.1% peptone water (Oxoid, BO0619), homogenized for one minute at room temperature. Then ten-fold serial dilutions were prepared using maximum recovery as diluent.

Aerobic mesophilic count

One hundred microliters from the original food homogenate and the prepared dilutions were separately pipetted into duplicated dry sterile standard plate counts agar (Oxoid, CM325). The inoculum was spread using a sterile glass spreader. Inoculated plates were left to dry for at least 15 min prior to inversion. Plates were incubated at 35±1° C for 48 hours, then average number of colonies were counted and the APC /g of the sample was calculated.

Psychrotrophic count

The same surface plate method previously mentioned in mesophilic count, was applied but the inoculated plates were incubated at 7 ºC for 7-8 days.

Coliforms count

Coliforms count was performed following the procedure established by Schang *et al.* (2016). All samples were analyzed according to guidelines from the manufacturer (IDEXX), including method blanks and spikes. All samples were diluted at 1:10 before the addition of the Colilert reagents. Quanti-trays were sealed and then incubated for 24 hours at 35±1°C. The trays were then compared to comparators, and positive wells were counted and transformed to determine the most probable numbers (MPNs) using the provided IDEXX MPN charts.

Statistical analysis

Data are presented by mean ± standard deviation of three samples. For bacteriological count, data were analyzed after logarithmic transformation. One-way analysis of variance (ANOVA) is applied for each parameter using SPSS software (release 20, IBM CO), and significance is tested at α = 0.05. Comparisons between treatments for each parameter were performed using LSD (least significance difference).

RESULTS AND DISCUSSION

Sensory examination

The sensory characteristics, especially color, are the dominant factor of meat purchasing decision, where consumers prefer to use color as the freshness and wholesomeness indicator (Mancini & Hunt, 2005). Mean values of raw and cooked sensory scores for control and chitosan coated frozen thawed broilers are illustrated in figures 1 & 2. Application of chitosan 0.5% and 1% coating on broilers carcasses during thawing proved no significant differences (p<0.05) in color, odor of raw samples compared to the control group, however, texture score for raw thawed broilers coated with 1% chitosan showed slight slimness on broilers skin, this may be referred to the viscous nature of the chitosan solution used. However, after cooking no slimness could be recognized by assessors. The results recorded by Taher *et al.* (2018) agreed with the achieved result. Significant reduction of the overall acceptability score (p<0.05) of raw thawed broiler coated with 1% chitosan as a reflection of adverse effect was noticed on texture score, with no significant differences (p<0.05) between control and 0.5% chitosan coated broilers. On the other hand, significant higher overall acceptability scores of cooked 1% chitosan coated broiler samples in relation to other groups are observed, due to marked improvement in tenderness scores in the same group. No significant change (p<0.05) in color, flavor and juiciness scores among examined cooked samples of treated and control groups could be sensed. Nearly similar results are recorded by Kanatt *et al.* (2008) and Jafari *et al.* (2017) who found no significant differences in sensory properties of cooked chitosan coated chicken fillets and uncoated control samples. Also, Antoniadou *et al.* (2019) and Ruíz-cruz (2019) stated that chitosan coating had no adverse effect of sensory characteristics.

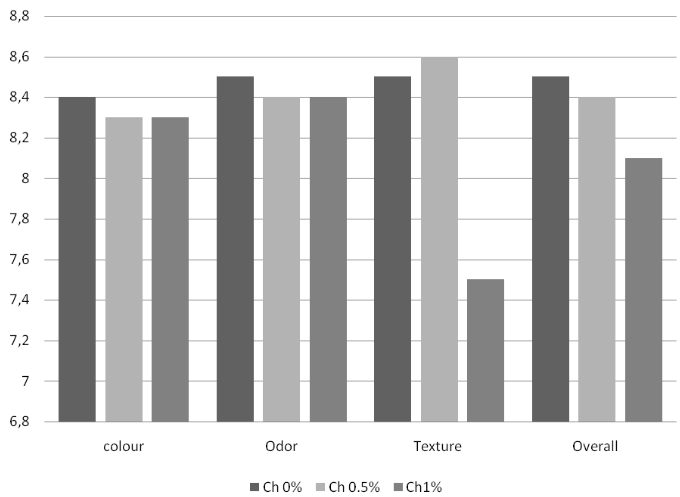

Figure 1 – Sensory attributes of frozen broilers coated with chitosan film during water thawings (Raw Samples). Ch0%: tap water, Ch0.5%: 0.5% chitosan, Ch1%: 1% chitosan.

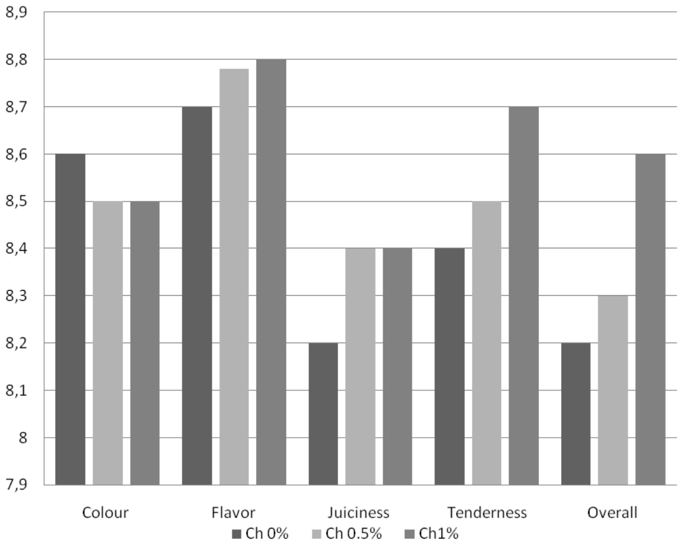

Figure 2 – Sensory attributes of frozen broilers coated with chitosan film during water thawings (cooked Samples). Ch0%: tap water, Ch0.5%: 0.5% chitosan, Ch1%: 1% chitosan.

Physicochemical examination

pH, shear force kgf/cm³ and instrumental color of control (Ch0%) and chitosan coated (Ch 0.5% & Ch 1%) broilers' thigh and breast are presented in table 1 & 2 respectively.

pH value is an indicator for the keeping quality of meat where it affects bacterial growth and therefore is used to assess the shelf life and quality of the products (Hathout *et al.,* 2010). pH value of thawed untreated broilers are 5.93 for breast samples and 6.2 for thigh, similar results were recorded by Fernandes *et al.* (2016) who stated that pH value of chicken meat ranged between 5.7 and 5.9 for breast and 6.4 for thigh. While lower pH values for breast and thigh (5.12 &5.73) in thawed broilers was recorded by Ahmed (2018). Significant reduction (p<0.05) of pH values are observed in breast and thigh samples of chitosan coated broilers (Ch0.5% & Ch1%) compared to the control non treated group, with no significant difference among Ch0.5% & Ch1% groups for breast or thigh, this is referred to acetic acid solution (0.5%) applied to dissolved chitosan coating solution. Findings of Ruíz-cruz, *et al.* (2019) were in agreement with the achieved results. The pH value of all examined broilers depicted the agreement of permissible limit stated by ESS (1090/2005).

Shear force

Shear force value is performed as an objective measurement of meat tenderness. In general, mean values of shear force in breast samples is higher in comparison to thigh samples irrespective to chitosan application, this may be due to high fat content in thigh red muscles compared to white muscles in breast. Significant reduction (p<0.05) in shear force mean values of chitosan coated samples (Ch0.5% & Ch1%) was noticed when compared to the control uncoated group. With no significant differences (p<0.05) between different chitosan concentrations (Ch0.5% & Ch1%) used for coating in breast samples, meanwhile shear force value ofCh1% coated thigh samples was significantly lower (p<0.05) than the Ch0%, this could be clarified by the ability of chitosan to bind water (Knorr, 1983). Consequently, more hydration is available for muscle fiber, in addition to,

Table 1 – physicochemical characteristics of examined Thigh samples.

Treatment	pH	Shear force	Color		
			L*	a*	b*
Ch 0%	6.2^{a} ±0.05	2.86^{a} ±0.495	55.27^{a} ±0.05	14.17^{a} ±0.42	9.14^{a} ±0.82
Ch 0.5%	5.9^{b} ±0.12	2.22^{ab} ±0.650	49.89^{b} ±0.14	14.33^{a} ±0.06	6.59^{b} ±0.06
Ch 1%	5.73^{b} ±0.10	1.63^{b} ±0.425	51.58^{c} ±0.05	13.16^{b} ±0.01	7.67^{c} ±0.07

Data presented as mean ± Standard deviation.

There are significance differences (p<0.05) between means having different letters in the same column.

Ch0%: tap water , Ch0.5%:0.5% chitosan, Ch1%:1% chitosan.

Table 2 – physicochemical characteristics of examined Breast samples.

Treatment	pH	Shear force	Color		
			L*	a*	b*
Ch 0%	5.93^{a} ±0.06	4.07^{a} ±0.274	59.32^{a} ±0.24	11.07^{a} ±0.09	10.33^{a} ±0.13
Ch 0.5%	5.72^{b} ±0.10	3.24^{b} ±0.516	56.77^{b} ±0.45	10.27^{b} ±0.15	12.36^{b} ±0.72
Ch 1%	5.59^{b} ±0.17	2.95^{b} ±0.393	53.89^{c} ±0.21	11.29^{c} ±0.06	6.87^{c} ±0.18

Data presented as mean ± Standard deviation

There are significance differences (p<0.05) between means having different letters in the same column.

Ch0%: tap water , Ch0.5%:0.5% chitosan, Ch1%:1% chitosan.

the tenderizing effect of acetic acid (0.5%) used as solvent for chitosan. Initial lower shear force values for both thigh (1.05 kgf/cm³) and breast (1.82 kgf/cm³) samples are recorded by Ahmed (2018).

Initial higher values of shear force in thigh (2.86 kgf/cm³) and breast (4.07 kgf/cm³) samples may be explained as frozen broilers were subjected to bad freezing temperature and /or bad frozen storage condition before purchasing. Which could result in the formation of large extracellular ice crystals between and within fibers and can damage the microstructure of the meat leading to moisture loss during thawing process, the muscle fibers become less hydrated and less tender, thus, a greater quantity of fibers per surface area seemed to increase the toughness thereby, the shear force of muscle tended to be higher (Lagersted *et al.,* 2008 and Leygonie *et al.,* 2012).

Color

Instrument color attributes (L* a* B*) of coated and uncoated chitosan broilers samples significantly differ. Lightness defines the reflection and absorption relationship of light on meat surface (from 100 for white to 0 for black). Lightness value L* of chitosan coated ranged from 53.89 to56.77 in breast samples, and49.89 to 51.58 in thigh samples. The significant reduction (p<0.05) of lightness in broilers was observed as affected by Ch 1% coating in both breast and thigh samples (from 59.3 in Ch0% to 53.89in Ch1% for breast samples, and from 55.27in Ch0% to 51.58 Ch1% in for thigh samples). Fernandez-Lopez *et al.* (2005) and Damme & Ristic (2013) stated that L* values is affected by many factors such as feeding, sex, breading, cooling, surface water, haem-pigments, and pH. The authors also reported that normal L* of chicken broilers ranged from 49to 53. It's worth to mention that, the application of chitosan coat (Ch1%) improved broilers carcass lightness compared to the control, this could be referred to prevention of moisture loss from broilers surface by the effect of chitosan coating.

Regarding redness value (a*), it was noticed that it´s value in thigh samples was higher than that of breast as expected -regardless to chitosan application- owing to high myoglobin content in thigh than in breast meat. Furthermore, for thigh samples, application of Ch1% coating significantly reduced (p<0.05) a* value, with no significant effect for Ch0.5% coating in relation to the control. Nonetheless, chitosan coating showed various effects on the examined breast samples, where Ch 0.5% significantly reduced (p<0.05) a* value while with the higher concentration (Ch1%) of chitosan a* the value has increased.

Microbiological Examination

Mean values of aerobic mesophilic, psychrotrophic and coliforms count (log cfu/g) are illustrated in Table (3) for both coated and uncoated chitosan thawed broilers. Aerobic mesophilic count (AMC) is considered as an index of quality, which gives an idea about the hygienic measures during chicken processing (Aberle *et al.,* 2001).

Table 3 – Bacterial counts (log cfu/g) of examined samples.

Treatment	AMC	Psych C	Coliforms C
Ch 0%	6.00 [a] ±0.60	3.59 [a] ±0.44	3.40[a] ±0.20
Ch 0.5%	5.69 [a] ±0.57	3.44 [a] ±0.53	3.38 [a] ±0.07
Ch 1%	4.65 [b] ±0.60	2.39 [b] ±0.24	3.34 [a] ±0.11

Data presented as mean ± Standard deviation

There are significance differences (p<0.05) between means having different letters in the same column.

Ch0%: tap water , Ch0.5%:0.5% chitosan, Ch1%:1% chitosan.

Chitosan1% coated broilers (Ch1%) showed significantly lower (p<0.05) AMC than control uncoated and 0.5% chitosan coated thawed broilers, this result emphasized the broad spectrum antibacterial activity of chitosan coating against both gram positive and gram negative (Jeon *et al.,* 2002; Fan *et al.,* 2009; Fernandez-Saiz *et al.,* 2010; Ojagh *et al.,* 2010; Souza *et al.,* 2010, Petrou *et al.* 2012). The obtained results were in harmony with that reported by Darmadji & Izumimoto (1994), who stated that 1.5% chitosan coating proved 2log reduction in beef. On the other hand, lower result of AMC was recorded by Jafari *et al.* (2017). It is worth to mention that, The AMC mean values (log cfu/g) for the control uncoated broilers and Ch 0.5% exceeded the permissible limit stated by EES (1090/2005), this proved unhygienic practices are followed during broilers processing and freezing. El Nasri *et al.,* (2015) stated that in general, AMC of foods are not sure indicatives of their safety, nonetheless, it is of supreme importance in judging the hygienic condition under which food has been produced, handled and stored. Psychrotrophic bacterial count of examined chitosan coated thawed broilers were lower in count in comparision to AMC in the same treated groups, however, psychrotrophic bacterial count showed the same pattern as AMC, whereas there was non-significant (p<0.05) difference in their count among the control and Ch1 coating groups. With significant reduction (p<0.05) in their count in 1% chitosan coated group. The present results were in agreement with that achieved by Jafari *et al.* (2017) and Sotoudeh *et al.* (2020). From result pertaining bacteriological analysis, it could be deduced that 1% chitosan coating has more potent antimicrobial effect than 0.5%chitosan coating, where it reduced AMC, psychrotrophic count by nearly 1.35 and 1.20 log, respectively, while 0.5% chitosan coating proved 0.3 & 0.15 log for AMC and psychrotrophiccount, respectively. The results recorded by Ruíz-cruz, *et al.* (2019) were in agreement with the achieved results.

Concerning coliform count, The IDEXX technique is used to quantify their indicator levels because it is convenient of use and low cost. Coliforms were detected in all examined broilers, with no detected significant difference (p<0.05) among of control, Ch0.5% and CH 1% treated broilers. Controversially to AMC and psychrotophic bacterial count, coliforms count showed no reduction level as a function of chitosan coating. Result recorded by Jafari *et al.* (2017), supported the present findings, where he stated that, no significant differences in coliforms count could be detected between chitosan and control untreated chicken fillets. This may be explained by the inability of chitosan to reduce all types of coliforms bacteria (Kanatt *et al.,* 2008). Coliforms are an indicator microorganism and their presence indicate direct or indirect fecal contamination, moreover, coliforms commonly contaminate feather of broilers in farms and may constitute a health risk. The coliform count recorded in the current study is under exceeded acceptable level stated by EES (1090/2005).

CONCLUSION

From the data achieved in the present study it could be concluded that, the application of chitosan proved potential antibacterial and sensory improvement activity. Where, both AMC and psychrotrophic count are reduced by over than one log after thawing. In addition, enhancement to lightness and shear force properties of thawed broilers are found. Beside its natural substance, biodegradation, cause no environment pollution and is valuable in low cost, enroll chitosan as one of the best choices as fast and secure decontaminant agent in water thawing process in mass catering.

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