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EDIBLE COATING CHITOSAN AS AN ANTIMICROBIAL IN THE THAWING PROCESS OF FROZEN BROILER CHICKEN CARCASSES WITH DIFFERENT SHELF LIFE

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Abstract
This study aims to determine the best concentration of chitosan edible coating for use as an antimicrobial agent during the thawing process of frozen broiler chicken carcasses with different shelf lives. This study used a completely randomized factorial design (CRFD) comprising two factors: shelf life differences (Factor 1) (1, 2, and 3 months) and chitosan edible coating concentration (Factor 2) (0, 1 %, 1.5%). There were nine treatments, each replicated five times. The total microbial count, pH, percentage of drip loss, and cooking losses of each sample were evaluated. The data were analyzed using Two Way Analysis of Variance and the Duncan Multiple Range Test (DMRT) for further testing. The results showed that frozen chicken carcasses treated with chitosan edible coating during thawing had a lower pH (P<0.05) compared to those without chitosan. The use of 1.5% chitosan edible coating demonstrated the best results (P<0.05) in inhibiting microbial growth, reducing drip loss, and cooking loss of the frozen chicken carcass storaed for 1, 2, and 3 months. In conclution, the application of chitosan edible coating proves effective as an antimicrobial during thawing.
Keywords: Antimicrobial, Broiler, Chitosan, Thawing

EDIBLE COATING KITOSAN SEBAGAI ANTIMIKROBIAL PADA PROSES THAWING KARKAS AYAM BROILER BEKU DENGAN UMUR SIMPAN YANG BERBEDA

Abstrak
Penelitian ini bertujuan untuk mengetahui jumlah konsentrasi edible coating kitosan yang terbaik pada saat thawing karkas ayam broiler beku dengan umur simpan yang berbeda. Penelitian ini menggunakan Rancangan Acak Lengkap Faktorial (RALF) yang terdiri dari dua faktor yaitu Faktor ke-1: perbedaan pada umur simpan (1, 2, dan 3 bulan) dan faktor ke-2: konsentrasi edible coating kitosan (0, 1%, 1,5%) dengan 9 perlakuan dan lima ulangan. Analisis yang dilakukan, yakni analisis jumlah total mikroba, pH, persentase drip loss, dan susut masak. Data yang diperoleh dianalisis menggunakan Two Way Analysis of Variance dan uji lanjut uji Duncan Multiple Range Test (DMRT). Hasil penelitian menunjukkan karkas ayam beku yang menggunakan edible coating kitosan saat thawing memiliki pH lebih rendah (P<0,05) daripada tanpa kitosan. Penggunaan edible coating kitosan 1,5% menujukkan hasil terbaik (P<0,05) dalam menghambat pertumbuhan mikroba, menurunkan nilai drip loss dan susut masak karkas ayam beku selama penyimpanan 1, 2 dan 3 bulan. Disimpulkan bahwa penggunaan edible coating kitosan efektif sebagai antimicrobial selama thawing.
Kata Kunci: Antimicrobial, Broiler, Kitosan, Thawing

INTRODUCTION

Chicken meat, widely consumed for its animal protein content, holds a prominent place in the Indonesian diet. According to Central Statistics Bureau (BPS) data (2022), the average consumption of broiler meat reaches 6.04 kg per capita per year in the Indonesian national household group. Poultry consumption is the first-ranked consumption of meat in Indonesia, followed by beef and pork. The affordability, tender texture (Ilham et al., 2018), protein richness, low fat, and low cholesterol content (Bourre, 2005) contribute to the popularity of chicken meat in Indonesia.

Recognized as a perishable product (Miwada, 2015), chicken meat presents storage challenges. According to the USDA (2003) guidelines, chicken meat can only be stored for two hours at room temperature (25^0C), and 60 minutes at 32^0C due to its high water content (68-75%). This substances and moisture-rich environment creates an ideal medium for the growth and proliferation of destructive microorganisms (Miwada, 2015). The growth of such microorganisms can be prevented or inhibited by preservation methods. Preservation measures typically involve freezing broiler chickens, resulting in frozen chicken or frozen broiler chicken products.

Thawing, the final step in processing frozen products, is a critical procedure for preparing frozen food. In the meat industry, the thawing process often uses water and room temperature (Zhang et al., 2017). Practically, thawing poses a significant challenge for frozen product processors or consumers. Improper thawing can negatively impact the texture, taste, color (Lygonie et al., 2012), and nutritional value (Akhtar et al., 2013; Zhang et al., 2017) of meat. Moreover, thawing process influences microbial activity. Dormant pathogenic microbes within frozen meat can reactivate during the thawing process (Mahmoud et al., 2021), leading to meat contamination (Dewi et al., 2016). Microbial contamination will reduce meat quality, resulting in spoilage (Doulmotioni, 2012). According to the National Standards Agency (SNI, 2010), broiler chicken meat must adhere to specific microbial limit, such as a maximum *Escherichia coli* count of 1×10^2 CFU/g, a maximum *Staphylococcus aureus* count of 1×10^4 CFU/g, and complete absence or negativity for *Salmonella* (BPOM, 2016).

Recently, edible coatings have emerged as food surface treatments, designed to prevent contamination by pathogenic bacteria and maintain meat quality (Kenawi et al., 2011). The edible coating forms a transparent, thin, and consumable layer on the food surface (Han and Gennadios, 2005). Edible coatings act as an oxygen barrier, limit moisture, and preserve the aroma and taste of volatile foods, enhancing the quality, safety, and functionality of the coated food (Han, 2002).

Chitosan, a highly sought-after edible coating in the food industry, is valued for its physicochemical properties, biocompatibility, biodegradability, nontoxicity, antimicrobial, and antifungal activity (Yu et al., 2017). The antimicrobial content in chitosan is effectively combat spoilage or pathogenic bacteria in food (Lopez et al., 2015). Chitosan is made from shrimp shell and crab shell waste from the fishery waste, and therefore safe for consumption. In poultry meat products, chitosan is utilized to extend shelf life by preventing oxidation and microbial growth (Eldaly et al., 2018). Based on Alhuur et al. 's research (2019), fresh broiler carcass dipped in a 3% solution of chitosan edible coating for 10 minutes before being stored at cold temperatures, shows a longer shelf life and reduced pathogenic bacteria count.

Previous research has explored the effect of chitosan edible coating on the thawing process. Mashat et al. (2022) reported that using 1% chitosan in the thawing of frozen broiler carcasses demonstrated potential antibacterial effects and improved meat sensory characteristics. However, this research did not reveal whether shelf life influences the performance of chitosan edible coating. Therefore, this research aims to determine the effect of shelf life and concentration of chitosan edible coating as an antimicrobial during the thawing of frozen broiler carcasses.

MATERIALS AND METHODS

Materials

This study used broiler carcasses weighing 0.9 – 1 kg obtained procured from 45 poultry slaughterhouses (5 for each treatment). The commercial chitosan edible coating was obtained from local producers in Indonesia with (Chimultiguna brand). The tools used included freezer storage, knives, basins, pans and stoves, distilled water, labels, aluminum foil, and Ziplock plastic bag.

Experimental Design

This study adopted a completely randomized factorial design (CRFD) with two factors: differences in shelf life (1, 2, and 3 months) and the concentration of chitosan in edible coating (0%, 1%, 1.5%). The design resulted in 9 treatments, each replicated five times.

Research Procedure

The cleaned broiler chicken carcasses were put into ziplock-type PP plastic and then stored in a freezer storage with a temperature of -20℃ until the internal temperature of the chicken was at least -12℃. Three groups with varying shelf life of 1, 2, and 3 months were established. Chitosan powder, derived from local production shrimp waste, was dissolved in 0.5% acetic acid to create an edible coating solution. The edible chitosan coating used in this study was 0.1% and 1.5% of the chicken carcass weight. After reaching the specified storage time, the frozen chicken was removed from the freezer and removed from the plastic. The chicken was thawed by immersing the chicken in a chitosan edible coating solution for 90 minutes at room temperature. The treatment for 0 Concentration of chitosan edible coating, the chicken was thawed by immersing in water for 90 minutes at room temperature, followed by a 15-minutes drying period.

The analysis was carried out on frozen meat coated with chitosan edible coating. The total bacterial count, drip loss, cooking loss, and pH were analyzed. Sample analyses were conducted at the Laboratory of the Faculty of Agriculture, University of Graha Nusantara, and the Laboratory of the Faculty of Agriculture, University of North Sumatra.

Drip loss

The drip loss was determined by the method by Augustyńska-Prejsnar et al. (2018). The quantity of leakage was determined by analyzing the weight disparity before freezing and after thawing. The drip loss percentage was calculated by the formula. W1, which represents the sample weight before freezing (in grams), and W2, which signifies the sample weight after thawing (in grams).

$$\text{Drip loss (\%)} = \left[\frac{W1 - W2}{W1}\right] \times 100\%$$

Cooking Loss

Samples were pierced with a bimetal thermometer, boiled until reaching an internal temperature of 81°C. The meat samples were removed and allowed to stand until they reached a constant weight (Ulupi et al., 2018). Cooking losses were calculated by the following formula, where, W1 = weight before cook; W2 = weight after cook.

$$\text{Cooking loss (\%)} = \left[\frac{W1 - W2}{W1}\right] \times 100\%$$

pH Measurement

Meat pH was measured by SNI (1992) using a calibrated HANNA pH meter. The pH meter was inserted into the meat and then waited until the pH value on the pH meter remains. Meat pH measurements were carried out three times and the average value was recorded.

Total Microbial Count

The total plate count method was used in calculating the total bacterial count, and dilution was carried out to 10^{-6} according to the National Agency of Drug and Food Control reference (BPOM, 2016). The total microbial count was determined by the method used by Fardiaz (1992). All treatments were carried out in duplicate, then the number of colonies that grew in the dish was multiplied by the dilution factor and the total number of bacteria was produced by the formula:

$$\text{Total microbial count} = \frac{\text{The number of bacteria}}{(\text{Volume} \times \text{Dilution factor})}$$

Data Analysis

The research data were analyzed using the Two Way ANOVA (Analysis of Variance) method and to determine whether there were differences between treatments, the Duncan Multiple Range Test (DMRT) was carried out at a significance level of α=0.05. Data were analyzed using SPSS version 21.

RESULTS AND DISCUSSION

Total Microbial Colonies

Table 1 displays the total bacterial count of frozen chicken thawed with chitosan edible coating. The results indicated a significant reduction ($P < 0.05$) in the total number of bacteria at shelf life of 1, 2, and 3 months with the use of chitosan edible coating during thawing. Bailey et al. (2000) noted that shelf life affects microbes number, with longer shelf life leading to higher microbes count in frozen chicken. Chitosan can inhibit microbial growth in frozen products up to a shelf life of 6 months (Karsli et al., 2021) by preventing microbial growth (Lopez Mata et al., 2015). The antimicrobial action of chitosan involves the formation of a positive charge, allowing it to associate with negatively charged components on microbial cell surfaces, disrupting cell membrane structure and inhibiting growth (Rubio et al., 2018). Petrou et al. (2012) reported that coating with chitosan showed inhibits both gram-positive and gram-negative bacterial growth making it an effective antimicrobial against spoilage and pathogenic bacteria in frozen chicken carcasses.

The total microbial count provides essential information for determining quality, shelf life, contamination, and hygiene in the production (BPOM, 2006), handling, and storage (El Nasri, 2015) process. According to Indonesian National Standard (SNI) No: 7388-2009, the maximum limit for microbial contamination in chicken meat is $1x10^6$ colonies/g or equivalent to 6 log CFU/g. The total microbial count in this study was still below the maximum limit for microbial contamination, indicating that frozen chicken carcasses at a shelf life of 1, 2, and 3 months, thawed with chitosan edible coating, were processed under hygienic conditions during production, handling, and storage.

The research revealed an interaction between shelf life and the concentration of chitosan edible coating on total microbial colonies. The use of 1.5% chitosan shows stronger antimicrobial properties than 1% which is able to suppress microbial growth from a shelf life of 1 to 3 months. Yilmaz (2020) reported that chitosan possesses antibacterial properties and is an effective antibacterial additive. These results align with Sotoudeh et al.'s (2020) findings that higher chitosan concentration more effectively suppress microbial growth. Mashat et al. (2022) also stated that the use of 1% chitosan showed stronger antimicrobial properties than 0.5% chitosan on frozen chicken carcasses. Darmadji and Izumimoto (1994) reported that 1.5% chitosan reduced microbes number in beef. The total microbial count in this study was higher compared to Mashat et al. (2022), whp reported a microbial count of 4.65 ± 0.60 log cfu/g in frozen chicken carcasses using 1% chitosan.

Table 1. Total microbial count, pH, drip loss, and cooking losses in frozen chicken thawed with chitosan edible coating with different shelf lives.

Parameter	Concentration of chitosan edible coating (%)	Shelf life (months)		
		1	2	3
Total microbes (Log cfu/g)	0	4.73 ± 0.11 ax	4.82 ± 0.05 ay	5.76 ± 0.09 az
	1	4.30 ± 0.03 bx	4.54 ± 0.05 by	5.47 ± 0.17 bz
	1.5	4.15 ± 0.04 cx	4.24 ± 0.04 cy	5.08 ± 0.10 cz
pH	0	5.95 ± 0.20 a	5.90 ± 0.05 a	5.92 ± 0.02 a
	1	5.87 ± 0.05 b	5.78 ± 0.08 b	5.86 ± 0.03 b
	1.5	5.85 ± 0.04 b	5.83 ± 0.04 b	5.80 ± 0.01 b
Drip Loss (%)	0	3.70 ± 0.02 ax –	4.49 ± 0.05 ay	5.96 ± 0.07 az
	1	3.51 ± 0.06 bx	4.19 ± 0.05 by	5.73 ± 0.09 bz
	1.5	3.34 ± 0.09 cx	3.91 ± 0.08 cy	5.62 ± 0.02 cz
Cooking Loss (%)	0	30.95 ± 0.28 ax	33.97 ± 0.91 ay	35.56 ± 0.65 az
	1	30.53 ± 0.74 bx	32.60 ± 0.36 by	34.47 ± 0.14 bz
	1.5	30.04 ± 0.14 cx	31.37 ± 0.30 cy	34.62 ± 0.36 cz

a, b, c: mean values with different superscripts in the same column indicate significant differences (P<0.05);
x, y, z: mean values with different superscripts in the same row indicate significant differences (P<0.05)

pH

The pH value can be used to determine bacterial growth, thus providing insights into meat shelf life and quality (Hathout et al., 2010). In this study, no significant interaction (P > 0.05) was observed between the shelf life of chicken carcasses and the concentration of chitosan used during deep thawing on pH values. This lack of interaction may be attributed to the use of the same concentration, namely 0.5% acetic acid, to dissolve 1% and 1.5% chitosan. Ahmed et al. (2017) highlighted the influence of acetic acid on pH results. Despite findings by Augustyńska-Prejsnar et al. (2018) indicating no significant difference between shelf life and pH value in frozen chicken, Abdel-Naeem et al. (2021) reported that the shelf life of frozen chicken affects pH. The pH value of chicken will decrease during frozen storage (Chen et al., 2016). Factors that affect changes in pH in the frozen chicken are improper thawing processes (Gambuteanu et al., 2013), freezing methods, and storage conditions (Wei et al., 2017).

The pH value of frozen chicken carcasses using chitosan edible coating during thawing had a lower pH (P < 0.05) than without chitosan. Based on the research of Jumaa et al. (2002), lower pH indicates more antimicrobial activity, suggesting that chitosan edible coating with a concentration of 1% and 1.5% can work in suppressing microbial growth. This significant difference in pH may be attributed to the acetic acid solution used in the chitosan edible coating during thawing. Ahmed et al. (2017) also confirmed that the use of acetic acid as a solvent in chitosan edible coatings has a significant effect on the pH of the chicken.

The pH value in this study was higher compared to Mashat et al. (2022), who reported a pH of 5.59 ± 0.17 in frozen chicken breasts thawed with 1% chitosan. However, the pH value in this study was lower when compared to the research by Abdel-Naeem et al. (2021), which used an edible coating of chitosan of 20gr/kg on chicken thighs which resulted in a pH of 6.44 ± 0.00, 6.45 ± 0.01, and 6.46 ± 0.01, in 1, 2 and 3 months shelf life, respectively.

Drip Losses

Drip loss refers to meat liquid that comes out during frozen storage and thawing (Oliveria et al., 2015). Drip loss shows the magnitude of the decrease in water holding capacity which will have an impact on reducing the weight and quality of the meat (Ali et al., 2016). This study confirmed that the use of chitosan during thawing reduced drip loss. The statistical results indicated a significant interaction (P < 0.05) between the shelf life of chicken carcasses and the concentration of chitosan during thawing on drip loss. Longer shelf life resulted in greater drip loss, however this was mitigated by the use of chitosan edible coating. The 1.5% chitosan concentration produced lower drip loss values compared to 1% at a shelf life of 1 to 3 months. The lowest drip loss occurred in frozen chicken carcasses stored for 1 month with 1.5% chitosan edible coating, while the highest was observed in those stored for 3 months without chitosan coating. These findings align with the research by Azizkhani et al. (2023) who stated that the use of chitosan reduces drip loss in frozen chicken. Yang et al. (2018) also proved significantly lower loss of water droplets in chicken fillets coated with chitosan compared to those without chitosan. The hydrophilic properties of chitosan contribute to its water-binding capacity, that reduces water loss (Varela and Fizsman, 2011). Chitosan also forms a protective layer around the chicken carcass, preventing water loss during thawing (Algarni et al., 2022). Sathivel (2005) noted that chitosan helps reduce moisture loss in meat, maintaining its the shape, texture, and taste.

The drip loss value in this study was lower than that reported by Zheng et al. (2023), who observed a drip loss value of 7.5% in chickens with 1% chitosan concentration coating during frozen storage for 12 days. Khare et al. (2017) found a drip loss of 6.94 ± 0.26 on the 9th day of storage with the use of 1% chitosan during frozen storage. Suwattitanun and Wattanachant (2014) revealed that drip loss is affected by storage time and temperature. Azizkhani et al. (2023) suggested that lower water droplet loss indicates lower microbial activity in meat.

Cooking Loss

Cooking loss is the weight of meat loss during the cooking process (Jama et al. 2008) and is a crucial indicator because it influenced the processed meat product quality (Ouyang et al., 2022). Table 1 illustrates that the cooking loss value of frozen chicken carcass increases with prolonged shelf life. Hassan and Muhamad (2022) confirmed that longer shelf life leads to increased cooking loss in frozen chicken. The highest cooking loss value in this study was observed after 3 months of shelf life. However, the use of chitosan edible coating during thawing can reduce cooking losses. The cooking losses on chicken carcass with 1% and 1.5% chitosan edible coating in this study were lower than those without chitosan at the same shelf life. This reduction in cooking loss might be attributed to chitosan's proven ability to increase the water-holding capacity of meat products. Amoli et al. (2021) reported that chitosan incorporated into meat helps retain more moisture during cooking, thereby reducing losses. Inhibition of protein denaturation by chitosan has also been proven to reduce cooking losses. According to Zhang et al. (2022), by preventing or reducing protein denaturation, chitosan can help maintain the structure and integrity of meat thereby reducing cooking loss.

The value of cooking loss in this study was higher when compared to the findings by Hassan and Muhamad (2022), which had a cooking loss value of frozen chicken at a shelf life of 1, 2, and 3 months of 31.49 ± 1.12%, 32.63 ± 0.95% and 34.23 ± 0.74, respectively. The use of 1.5% chitosan as a coating was also studied by Chang et al. (2023) who obtained a 15.23% cooking loss value for fish balls stored for 7 days in frozen storage. Alam et al. (2018) showed that cooking losses in frozen beef stored for 12 days using chitosan levels coating of 1, 1.5%, and 2% were 21.74 ± 0.006%, 22.03 ± 0.006%, 22.23 ± 0.006%, respectively.

CONCLUSION

The use of 1.5% chitosan edible coating showed the best results in inhibiting microbial growth, reducing drip loss, and cooking loss of frozen chicken carcasses during 1, 2 and 3 months of storage.

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