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Effect of Bay Leaf Infusion on Microbiological, Chemical and Physical Quality of Chicken Meat

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ABSTRACT

Contamination that decreased chicken meat quality could be prevented using natural preservatives. Bay leaves (Syzygium polyanthum) contain volatile fatty acids, tannin, and flavonoid that possess bacteriological and fungicidal activity as well as preventing bacterial spore growth. The purpose of this study was to determine the effect of fresh bay leaf infusion on microbiological, chemical, and physical qualities of chicken meat. This study used bay leaves, water, chicken meat, eight strain of bacteria. chemicals and materials for the analysis of chicken meat. The experiment consisted of two steps, the first was the antibacterial properties of bay leaves and the second was the application of bay leaf infusion for chicken meat. Eight bacteria was used for the bacterial inhibition of bay leaf at the concentration of 0, 5, 10, 15 and 20%. The experiment on antibacterial properties of bay leaf (Syzygium polyanthum) used one way randomized design with five concentration treatments, while the application of bay leaf infusions on chicken meat using factorial completely randomized design 2x5 (2 types of soaking and 5 observation time). At the second step, chicken meat was divided into 2 groups, the first group was soaked in water only and the second group was soaked in 15% bay leaf infusion. They were then stored for 0 (control), 2, 4, 6, and 8 hours at the room temperature. Each treatment was repeated four times. The microbiological, chemical, physical qualities of chicken meat were observed. The results showed that bay leaf infusion had the ability to inhibit the growth of bacteria (P<0.05). The highest growth inhibition was found on Leuconostoc mesenteroides, whereas the lowest growth inhibition was on Pseudomonas putida. Bay leaf infusion influenced the number of bacteria in chicken meat. The concentration of 15% bay leaf infusion could decrease bacterial number amounting to 1 log CFU/mL compared to control. The number of bacteria of chicken meat stored significantly increased during the storage (P<0.05). However, the number of bacteria of chicken meat soaked in bay leaf infusion was significantly lower than the control. Bay leaf infusion has no significant effect on the chemical quality of chicken meat but it did on the physical quality of chicken meat. The conclusion of the study was bay leaf infusion could inhibit the bacterial growth and reduced the amount of bacteria in chicken meat, whereas storage time influenced the microbiological and physical qualities of chicken meat.

Keywords: Bay leaf, Chemical quality, Chicken meat, Microbiology quality, Physical quality

Introduction

Microbes contamination in chicken meat could cause damage and shorten shelf-life. Carcass handling that did not observe hygiene and sanitation whether before, during, or after the slaughter until the sale might give chance to contamination in the carcass. The poor handling of the carcass could affect the decreasing shelflife and the nutrition contained in the meat. The quality of the meat that could be identified through its physical or chemical characteristics have been the prime concern of the consumers. Those conditions would affect the result of the processed chicken meat (Agustina and Widyanigrum, 2012). Pathogenic bacteria that could often be found in chicken meat were Salmonella sp., Escherichia coli 0157:H7, Campylobacter sp., Listeria monocytogenes, Clostridium perfringens, and Staphylococcus aureus (Food Safety Inspection Service, 1996).

The purpose of preserving meat is to extend its shelf-life until before consumption. Preservation could be done through three namely physical, biological, methods. and chemical preservations. Physical preservation involves withering, heating, and coolina processes; biological preservation involves fermentation process through microbes such as the production of salami; while chemical preservation were preservation that involves chemical material (Usmiati, 2009). The use of antimicrobials could inhibit or kill spoilage and pathogenic microbes (Andarwulan *et al.*, 2010).

One type of natural preservative agent that was safe and easy to obtain were bay leaves (Syzygium polyanthum). Bay leaves contain essential oils, tannins, and flavonoids. Bioactive compounds in the bay leaves could have the characteristics of bactericidal, bacteriostatic, fungicidal, and germinal/inhibiting bacterial spore germinal (Suharti et al., 2008). Microbiology test on bay leaves ethanol extract could inhibit the growth of Escherichia coli, Vibrio cholerae, and Salmonella sp. (Dalimartha, 2006). Thorough experiment since the extraction (of the bay leaves) and its application towards the physical, chemical, and microbiological chicken meat has never been performed. This research aimed to evaluate the potential of infusion of fresh bay leaves as antibacterial and its application towards chicken meat to maintain the quality of chicken meat during storage at room temperature.

Materials and Methods

Materials

Materials used were the breast part of chicken broiler carcass of the Cobb strain aged 35 days that were taken from the chicken slaughterhouse in Bantul with average weight of kg/chicken, bay leaves 1.5 (Syzygium polyanthum), aquadest, Buffered Peptone Water (BPW), H₂SO₄, Plate Count Agar (PCA), Nutrient Agar (NA), Nutrient Broth (NB), Mueller Hinton Agar (MHA), and filter paper. The bacteria used in the experiment were Staphylococcus aureus (FNCC 047), Listeria monocytogenes (FNCC 0156), Pseudomonas aeruginosa (FNCC 063), Pseudomonas putida, Lactobacillus plantarum, Leuconostoc mesenteroides, and Salmonella typhimurium (FNCC 0734), Escherichia coli (ATCC) that were obtained through Center Of Food And Nutrition Studies UGM.

Methods

Producing bay leaf infusion. Producing bay infusion was carried out according to leaf Farmakope Indonesia (Direktorat Jenderal Pengawasan Obat dan Makanan, 1995), while the choosing of bay leaves referred to Kharismawati et al. (2009). The concentration of bay leaf infusion made were 0, 5, 10, 15, and 20%. The concentration of 5% were made by adding 5 g of fresh bay leaves with 100 mL of water, while concentration 10% were made by adding 10 g of fresh bay leaves with 100 mL of water, and so on. Bay leaves were weighted, washed, placed into the pan and added with aquadest according to the concentration mentioned before. The mixture of bay leaves and water were heated to 90°C for 15 minutes. The bay leaf solution was then filtered and stored for further using.

The preparation of bacterial suspension. The initial test suspension were made identical with turbidity of 0.5 Mc Farland (turbidity mixture of Barium sulfate and HCI) or proportional to the number of bacteria 1 x 10^8 CFU/g or 250-300 colonies in solid media (Cavalieri *et al.*, 2005). Some bacterial colonies were taken and then diluted so that the concentration corresponds to a concentration of 0.5 Mc Farland.

Testing the inhibition of bay leaf infusion against bacteria. The bacterial inhibition test was performed by the disc diffusion method from Kirby Bauer (Cavalieri *et al.*, 2005). The bacteria used in the inhibition test of bay leaf were gram-positive bacteria (*Lactobacillus plantarum*, *Leuconostoc mesenteroides*, *Listeria monocytogenes*, *Staphylococcus aureus*) and gram-negative bacteria (*Pseudomonas putida*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Escherichia coli*).

Determining concentration of bay leaf infusion

Ten g of chicken breast sample each were put into polyethylene (PE) plastic. Each pack of chicken meat was added with 1 mL suspension of Pseudomonas putida bacteria with а concentration of 5 x 10⁵ CFU/g and 10 mL of bay leaf infusion with concentrations of 0, 5, 10, 15, and 20%. Chicken meat was then stored in a refrigerator 24 temperature for hours. Observations were made 24 hours after treatment. The concentration of fresh bay leaf infusion with the lowest total amount of microbes was used for application in the next step.

Analysis of microbiological, chemical, physical and qualities of chicken meat soaked in bay leaf infusion. The concentration of bay leaf infusion that produced the lowest number of Pseudomonas putida bacteria was 15%, this meant that the concentration of bay leaf infusion used for the analysis of microbiological, chemical, physical qualities of chicken meat was 15%. Chicken meat was soaked in two types of solution, namely water and bay leaf infusion, with a concentration of 15% for 20 minutes, drained and then placed (display) in an open and closed place for 0, 2, 4, 6, and 8 hours. The parameters observed were chemical, physical and total plate count (TPC) of chicken meat. Analysis of chemical quality of chicken meat included water content, protein content and fat content of chicken meat using near infrared spectroscopy (NIRS) (Rotua et al., 2017). Analysis of physical quality of chicken meat consisted of pH, water holding capacity, cooking loss and tenderness. pH measurement referred to the method of Bouton et al. (1972). The water holding capacity is determined by the method (Soeparno, 2015). Hamm The measurement of cooking loss and tenderness of chicken meat was carried out according to Soeparno's method (2015), specifically for the measurement of tenderness was carried out using a Warner-Bratzler meat shear press. The total plate count (TPC) of chicken meat referred to the

Indonesian National Standard SNI 2897: 2008 (SNI, 2008).

Statistical analysis. Data of bacterial properties, chemical, physical and microbiological test results were analyzed by using analysis of variance. Data of the application of fresh bay leaf infusion of chicken meat were analyzed by analysis of variance of the completely randomized design of the factorial pattern 2×5 with the type of soaking type and the storage time factor. The significant different means among the treatments were further analyzed using Duncan's new Multiple Range Test (DMRT) at a 95% confidence level (a = 0.05) (Steel and Torrie, 1993). All data analyzes use the SPSS 24 program.

Results and Discussion

Inhibitory of bay leaf infusion against bacteria

The ability of bay leaf infusion to inhibit bacteria was presented in Table 1. From the analysis of the data it was found that all bay leaf concentration treatments were significantly different from the control (P<0.05). The 5% concentration treatment was significantly different from the 10%, 15% and 20% concentration treatments, whereas the three treatments 10, 15 and 20% showed no significant difference. This is consistent with Bukhori (2017) study which stated that the higher the concentration of an antibacterial agent the higher the antibacterial strength.

The results showed that *Leuconostoc mesenteroides* (Gram positive) were sensitive and *Pseudomonas putida* (Gram negative) were more resistant to bay leaf infusion than other seven bacteria tested. Gram-positive bacteria had a larger inhibitory diameter zone than Gramnegative bacteria. The difference in sensitivity of this group of bacteria was caused by Gramnegative bacteria having a unique outer membrane and periplasm which is not found in Gram-positive bacteria (Ceylan and Fung, 2004). Usmiati and Rahayu (2011) stated that Gramnegative bacterial resistance by antibacterial activity was higher than Gram-positive. The structure of cell wall membranes composed of lipopolysaccharides, lipoproteins, and phospholipids. The antimicrobial mechanism of essential oils is to disrupt the cell membrane phospholipid layer which causes an increase in permeability so that it loses the constituent elements of the cell. Antibacterial substance damaged the cell wall and cytoplasmic membrane of sensitive bacteria, while resistant bacteria suffered damage at the cell wall (Naufalin *et al.*, 2010).

The effect of bay leaf infusion soaking on total bacteria of chicken meat

The total bacteria of chicken meat soaked in various concentrations of bay leaf infusion was presented in Table 2. The results showed that the highest total bacteria was obtained in the control chicken meat samples. Water was not good extraction solvent, it was not optimal in extracting active compounds such as tannins, flavonoids, alkaloids, and glycosides that function as antibacterial. Suliantri et al. (2008) showed that extraction of active compounds in plants using water had the lowest inhibitory ability of bacteria compared to ethanol and ethyl acetate. Bay leaf infusion significantly decreased the number of bacteria of chicken meat (P<0.05). 15% bay leaf infusion significantly lowered the number of bacteria. However, there was not any different in the number of bacteria of chicken meat at the bay leaf infusion with concentrations of 5, 10 and 20%. This result is similar to the study conducted by Afrianti et al. (2013) which showed that the total bacterial of chicken meat soaked in extract of senduduk leaves (Melastoma) leaves at the concentration of 15% was lower than the total microbes of chicken meat soaked in leaf extract of senduduk leaves 20%. The concentration of fresh bay leaf infusion used for the further analysis was 15%.

The effect of storage time on the quality of chicken meat soaked in 15% bay leaf infusion

Total bacteria of chicken meat. The effect of storage time on the total bacteria of chicken meat soaked in 15% bay leaf infusion was presented at Table 3. The results showed that

-	Concentration of bay leaf infusion (%)							
Type of bacteria	0	5	10	15	20	50	100	
Gram-positive bacteria								
Lactobacillus plantarum	0.6ª	3.53 ^b	4.40 ^b	5.66 ^b	3.80 ^b	3.39 ^b	6.26 ^{bc}	
Leuconostoc mesenteroides	0.6ª	4.64 ^b	6.48 ^b	6.43 ^b	4.6 ^b	4.37 ^b	13.38°	
Listeria monocytogenes	0.6ª	2.05 ^b	3.47°	3.83°	4.63 ^d	3.63°	7.88 ^e	
Staphylococcus aureus	0.6ª	2.42 ^b	4.70 ^c	4.53°	5.13°	5.77°	5.72°	
Gram-negative bacteria								
Pseudomonas putida	0.6ª	0.30 ^a	2.34 ^b	2.73 ^b	2.93 ^b	3.55°	7.44°	
Pseudomonas aeruginosa	0.6ª	3.25 ^b	3.87 ^b	3.97 ^b	4.30 ^b	7.62°	10.23 ^d	
Salmonella typhimurium	0.6ª	1.65 ^b	2.18 ^b	3.77°	4.40 ^c	6.81 ^d	8.87 ^e	
Escherichia coli	0.6ª	2.87 ^b	4.52 ^b	4.42°	4.39 ^b	2.88 ^b	4.52 ^{bc}	

Table 1. Inhibitory diameter of bay leaf infusion against bacteria (mm)

a, b, c, d, e Different superscripts on the same line show the real difference (P<0.05)

Replication			Concentration	(%)	
	0	5	10	15	20
1	6.91	7.16	7.08	5.95	7.00
2	6.98	6.85	6.56	5.93	6.61
3	6.86	6.94	7.15	6.13	6.82
4	7.14	7.19	6.78	6.04	7.03
5	7.18	5.78	6.74	6.15	6.76
Average	7.01±0.06 ^b	6.79±0.26 ^b	6.86±0.11 ^b	6.04±0.04 a	6.84±0.08 ^b

Table 2. Total bacteria of chicken meat soaked in bay leaf infusion for 24 hours (log CFU/mL)

a, b Different superscripts on the same line show the real difference (P<0.05)

Tabel 3. Effect of soaking in 15% bay leaf infusion and storage time on chicken meat total bacteria (log CFU/mL)

Treatment	Storage time (hours)							
	0	2	4	6	8	Average		
Bay leaf	5.87	5.97	6.30	6.52	6.84	6.30±0.39 ^p		
Water	5.93	6.16	6.46	6.82	7.21	6.52±0.519		
Average	5.90±0.04 ^a	6.07±0.14 ^b	6.38±0.11°	6.67±0.21 ^d	7.03±0.27 ^e			

^{a, b, c, o, e} Different superscripts on the same column show real differences (P<0.05)

soaking in bay leaf infusion gave a significant effect the total bacteria of chicken meat (P<0.05). The total bacteria of broiler chicken soaked in bay leaf was 6.30 log cfu/mL, whereas with water soaking of 6.52 log cfu/mL. The lower bacterial counts in chicken meat soaked in fresh bay leaf infusion due to active compounds of essential oils. tannins, flavonoids which acted to inhibit bacteria. Flavonoids acted as antibacterial by disrupting the metabolic function of bacteria. The mechanism of action of flavonoids was to disrupt the activity of peptidoglycan transpeptidase so that the formation of bacterial or viral cell walls is disrupted and cells undergo lysis (Suliantri et al., 2008). The ability of tannins as an antimicrobial is suspected because tannins will bind to bacterial cell walls so that they will activate the ability to attach to bacteria, inhibit growth, protease enzyme activity and can form complex bonds with polysaccharides (Cowan, 1999).

The storage time significantly influenced the total chicken meat bacteria (P<0.05). The lowest total bacteria was at 0 hours (5.90 log cfu/mL), while the highest number was at the 8th hour of observation (7.03 log cfu/mL). The longer storage time caused the lowering level of freshness of the meat and led to spoilage. This was because the environmental conditions (26-28°C) were favorable for the development of mesophyll microbes. Besides that, there was degradation of complex compounds due to autolysis. Autolysis was damage to tissues and organs through chemical processes caused by intracellular enzymes. The process of autolysis occurred due to the influence of enzymes released by dead cells. The nucleoprotein in chromatin was first affected by cytoplasm, then the cell wall would be destroyed which resulted in softening of the tissue (Naufalin et al., 2010). Data analysis showed that there was an interaction between the soaking of bay leaf infusion and the storage time on the total number of microbes in chicken meat.

Chemical quality of chicken meat. The effect of 15% bay leaf infusion soaking and storage time on the chemical quality (water, protein and fat contents) of chicken meat was presented in Table 4.

Water content. The results showed that soaking in fresh bay leaf infusion and storage time did not have a significant effect on chicken meat water content. The average water content of chicken meat soaked in fresh bay leaf infusion was 74.6%, while that soaked in water was 72.30%. This might be because bay leaves did not have humectant properties that hold water in in the meat. Hiariey (2015) stated that one way to reduce water activity is to add additives that have high water holding capacity (humectants). Moisture and water content usually affect the growth of microorganisms. The results showed that soaking chicken meat in bay leaf infusion was able to maintain the water content in chicken meat. Water content in meat ranging from 60-75% can last a long time during storage (Winarno, 1991).

Protein content. The soaking of chicken meat in bay leaf infusion did not have a significant effect on the protein content of chicken meat, nor did the storage time. The average protein content of chicken meat soaked in bay leaf infusion was 23.47%, while the protein content of chicken soaked in water was 22.95%. Chicken meat protein content in this study are within the normal range. Supadmo (1997) stated that the protein content of broiler chicken meat is 18.16 – 23.42%. The storage time did not have significant effect on the protein content of chicken meat. This was in agreement with Vergiyana *et al.* (2014) study that protein content of chicken meat did not change during the short duration of storage time.

Fat level. The soaking in bay leaf infusion and storage time did not significantly influence fat content of chicken meat. The mean fat content of chicken meat soaked in bay leaf infusion was 4.94%, while the fat content of chicken meat soaked in water was 4.61%. Water content tends

Variable	Treatment		Averagens				
		0	2	4	6	8	
Water	Bay leaf	72.86	72.64	72.24	71.98	71.87	72.32±0.43
(%)	Water	72.49	72.34	71.77	71.92	71.78	72.06±0.33
. ,	Averagens	72.68±0.27	72.49±0.21	72.01±0.04	71.95±0.04	71.82±0.06	
Protein	Bay leaf	23.19	23.05	23.25	23.60	24.27	23.47±0.49
(%)	Water	22.86	22.59	22.61	23.04	23.68	22.95±0.44
	Averagens	23.03±0.24	22.82±0.33	22.93±0.45	23.32±0.42	23.97±0.42	
Fat	Bay leaf	4.55	4.85	4.86	4.77	5.65	4.94±0.42
(%)	Water	4.37	4.13	4.55	4.67	5.36	4.61±0.46
()	Averagens	4.46±0.13	4.49±0.51	4.70±0.22	4.72±0.07	5.50±0.21	

Tabel 4. Effect of soaking in 15% bay leaf infusion and storage time on chemical quality of chicken meat

ns : non-significant

to keep preventing the occurrence of fat oxidation thus preventing chemical changes, including fat. Aberle *et al.* (2012) stated that the higher the water content, the lower the fat content. Water content is positively correlated with protein content, protein content is negatively correlated with fat content.

Physical quality of chicken meat

The effect of 15% bay leaf infusion soaking and storage time on the physical quality (pH value, water holding capacity (WHC), cooking loss, and tenderness) of chicken meat was presented in Table 5.

pH value. Soaking in 15% bay leaf infusion and storage time did not have any significant effect on the pH value of chicken meat. The average pH value of chicken meat soaked in water was 5.99, while for chicken meat soaked in bay leaf infusion was 5.94. The pH values of chicken meat during storage time of 0, 2, 4, 6, and 8 hours were 5.97, 5.92, 5.98, 5.92, and 6.03 respectively. The pH values of chicken meat were in the range of pH values of food products recommended by the Indonesian National Standard that is 6 - 7 (SNI, 2008). The pH value of chicken meat was affected by the handling of the bird prior to slaughter as well as the treatment of chicken meat. pH of the bay leaf infusion at various concentration was in the range of 4.8 - 5.74 that relatively similar to the pH of chicken meat. Muchtadi (2010) stated that chicken meat had the pH values at the range of 5.8-5.9.

WHC. Soaking in 15% bay leaf infusion did not have any significant effect on WHC of chicken meat. Storage time significantly (P<0.05) affected WHC of chicken meat. There was no interaction between treatments. WHC mean of chicken meat soaked in bay leaf infusion was 36.88%, higher than WHC of chicken soaked in water (33.92%). The bay leaf infusion treatment was able to slow the decline of WHC of chicken meat. The active ingredients in bay leaf infusion such as tannins and flavonoids had an antimicrobial effect (Sumono and Wulan, 2009) thought to secure chicken that has been meat

Variable	Treatment		Averagens				
		0	2	4	6	8	
pН	Bay leaf	5.93	5.88	5.97	5.94	5.98	5.94±0.39
	Water	6.02	5.96	5.98	5.90	6.07	5.99±0.06
	Averagens	5.97±0.06	5.92±0.06	5.98±0.01	5.92±0.03	6.03±0.07	
WHC (%)	Bay leaf	36.13	37.38	36.19	35.04	39.63	36.88±1.75
	Water	33.42	31.96	33.15	34.46	36.61	33.92±1.75
	Average	34.78±1.92ª	34.67±3.83ª	34.67±2.15 ^{ab}	34.75±0.42 ^{ab}	38.12±2.13 ^b	
Cooking	Bay leaf	29.16	30.16	31.35	31.21	32.55	30.89±1.29
Loss (%)	Water	29.94	30.25	30.85	31.52	32.73	31.06±1.11
	Average	29.55±0.56ª	30.20±0.06ª	31.10±0.35 ^b	31.37±0.22 ^b	32.64±0.13°	
Tenderness	Bay leaf	3.36	2.56	2.34	2.23	2.04	2.51±0.51
	Water	3.46	2.73	2.44	2.18	1.99	2.56±0.57
	Average	3.41±0.07 ^b	22.82±0.12 ^{ab}	2.39±0.07 ^{ab}	2.21±0.03 ^{ab}	2.01±0.04ª	

Tabel 5. Effect of soaking in 15% bay leaf infusion and storage time on physical quality of chicken meat

^{a, b, c} Different superscripts on the same line show the real difference (p<0.05)

ns : non-significant

soaked in bay leaf infusion from microbes that can reduce the binding capacity of meat water. This was in agreement with the study of Agustina *et al.* (2017) that bay leaf infusion was able to inhibit the growth of microbes and slow the rate of decrease in the binding capacity of water in pork. Storage duration had significant effect on WHC of chicken meat. The pH of chicken meat tended to rise at the 8 hours of storage time causing WHC to increase. At higher pH, a number of positive charges were released and there was a surplus of negative charges which resulted in rejection of the microfilament and gave more space for water molecules to bind (Soeparno, 2015).

Cooking loss. The results showed that soaking chicken meat in bay leaf infusion did not had significant effect on cooking loss. Storage time had significant effect on cooking loss (P<0.05). There was no interaction between treatments. The percentage of cooking loss in this study was in the range of normal cooking loss in broiler chicken meat, which is between 30 to 37% (Raj, 2003). Cooking loss of chicken meat in this study significantly increased along with the storage time. This was due to probably an increase in the damage of myofibrillar protein. Thus, it was followed by the loss of protein's ability to bind water, which in turn increases the cooking loss. Church and Wood (1992) stated that damage to the structure of meat protein will cause the protein's ability to retain water to be reduced. This is in accordance with research from Risnajati (2010) where the longer the meat is stored in the refrigerator, the higher the value of cooking loss. Soeparno (2015) stated that cooking loss has a negative relationship with the water holding capacity. The low water holding capacity will result in high cooking loss (Risnajati, 2010).

Tenderness. Soaking chicken meat in 15% bay leaf infusion had no significant effect on tenderness, while storage time significantly affected tenderness of chicken meat (P<0.05). There was no interaction between treatments. The tenderness of chicken meat soaked in bay leaf infusion was 2.51 kg/cm², while the tenderness of chicken meat soaked in water was 2.56 kg/cm². This was presumably because bay leaves did not have proteolytic enzymes that could increase meat tenderness. Komariah et al. (2004) stated that the addition of antibacterial compounds had no significant effect on meat tenderness. Storage time affected on tenderness, the longer the storage time, the tenderness of chicken meat decreased. Through the storage time, there were damages and changes in the structure of muscle protein, especially in actin and myosin. Actin and myosin damage caused a decrease in the ability of muscle protein and increased tenderness in meat (Bouton et al., 1972).

Conclusions

The conclusion could be drawn from the experiment was bay leaf infusion had the ability to inhibit bacterial growth and reduced the amount of

bacteria in chicken meat, whereas storage time influenced the microbiological and physical qualities of chicken meat.

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