



Effect of casein-chitosan edible coating on the physicochemical and microbiological characteristics of broiler meat at storage 8° C

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ABSTRACT

Microbial contamination and fat oxidation may cause physical and chemical changes that can reduce the quality of broiler meat. The objective of this study was to determine the ability of casein chitosan edible coatings in maintaining the quality of broiler meat stored in certain storage time under refrigeration (8 °C), in terms of water activity (A_w), cooking loss, organoleptic properties (i.e. color, aroma and possible deviations), physicochemical properties (i.e. moisture content, water holding capacity/WHC, pH, lipid content, color), Total Plate Count (TPC), and microbial properties (i.e. *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* sp.). The materials used were broiler breast fillets and casein chitosan edible coating. The research was conducted using a Completely Randomized Design (CRD) based on 5 variations in storage time treatments i.e. at 0 h; 24 h; 72 h; 120 h; and 168 h in 4 replications. Edible coating casein chitosan on broiler meat under storage showed significant on A_w , WHC, pH, lightness, TPC, *S. aureus*, and *E. coli*. The application of casein-chitosan as an edible coating could be suitable to assure the safety of food products such as chicken meat at the range of storage time studied.

Introduction

Broiler meat is a highly nutritious food ingredient. It has a nice taste and aroma, soft texture, and relatively affordable prices; therefore it is preferred by many people. However, broiler meat has several weaknesses, especially its perishable nature. Edible coating can extend the shelf life of meat by reducing moisture and solute migration, gas exchange, reaction rates, and oxidative respiration as well as reducing physiological disorders (Raghav et al., 2016). Edible coating can improve the quality and appearance of broiler meat by preventing changes in aroma and antimicrobial content (Huber and Embuscado, 2009). Edible coating has transparent appearance, thus the appearance of broiler meat will not change (Fernández-Pan et al., 2014).

Polysaccharides, proteins, resins, lipids and their combinations are commonly used options for edible coating formulations. Polysaccharides and proteins are known to form films with good mechanical properties, but with poor permeability, whereas lipids and resins may form brittle films with better permeability (Marelli et al., 2016). The

main material used for edible coatings in this study was a combination of polysaccharides and proteins because of their good mechanical properties and poor permeability to gases and moisture, which may prevent damage to broiler meat. Casein has a special characteristic that is difficult to be broken down by high heat. Protein based edible coatings at low relative humidity have a strong O₂ barrier property (Raghav et al., 2016). Casein-based edible coatings are desirable for food applications and potentially for protection against the surrounding environments (Galgano et al., 2015). Caseins are soluble and can form films that are resistant even at high temperatures to denaturation and/or coagulation, and so the protein film remains stable over a wide range of temperatures, pH and salt concentrations (Shendurse, 2018).

The perspective of material packaging could be stability and shelf life, such as permeability to water vapor, O₂, CO₂, ethylene, thermal stability, behavior at different relative humidity's, and solubility (Alvarez-Pérez et al., 2015). Polysaccharide's key advantages are abundance, availability, low cost, Thermo-process ability and

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non-toxicity (Cazón et al., 2017). Owing to the hydrophilicity of polysaccharides, however, Polysaccharide-based films have low water vapor barrier properties (Mikkonen et al., 2007).

Coatings with chitosan, it has been researched by (Kanatt et al., 2013) as a microbial obstacle in meat products. On other hand, the potential applications of chitosan as bio-preservative (Duan et al., 2010). The mainly antimicrobial of chitosan shows against many pathogenic and spoilage microorganisms (gram positive and gram-negative bacteria), molds and yeasts (Kong et al., 2010). The antimicrobial activity of chitosan is widely depending on deacetylation degree, molecular weight, pH value, and type of microorganism (Dutta et al., 2009). Ricotta cheese were coated with chitosan/whey protein edible film and stored under modified atmosphere at 4 °C and this has suggested a potential utility of chitosan/whey protein coatings to extend shelf-life of fresh dairy product (Di Pierro et al., 2011).

Based on the description above, it is necessary to conduct research on the quality of broiler meat coated with casein chitosan edible coating which is stored in certain storage time under temperature of 8 °C. The quality parameters evaluated includes the physicochemical and microbial qualities.

Research Methods

Materials

The materials used were broiler breast fillets (Fiesta), casein chitosan edible coating and 0.1% peptone solution, PCA media for TPC testing. The tools needed in the application of casein chitosan edible coating on broiler breast fillets with a brush, filter tray, tongs. The study also used instrument such as cooking loss testing, sensory assessment sheets, and gel ice/blue ice for organoleptic testing. Water activity (Aw) was measured using Aw meter (Rotronic). Water bath, analytical balance (HR-300) and plastic were used for cooking loss test. Cool box and thermometer for organoleptic tests which include color, aroma and possible deviations. Mortar, pestle, 100-mL Erlenmeyer (Pyrex), test tube (Pyrex), micro pipette, petri dish, incubator and colony counter for TPC test.

Experimental set-up

The research was conducted using a Completely Randomized Design (CRD) with 5 storage treatments storage at different time (i.e. 0 h; 24 h; 72 h; 120 h; and 168 h). All experiments were performed in 4 replications.

The production of casein chitosan edible coatings

The production of casein chitosan edible coatings refers to (Fabra et al., 2011) and (Di Pierro et al., 2011) with modifications to the materials and manufacturing process. First, the process of making edible coatings was conducted by dissolving casein using distilled water with 1 g/100mL and dissolving chitosan solution, then, two solutions mixed. Then, casein solution and chitosan solution were then heated for 30 min at 50 °C. Liquid 0.05% beeswax was added to the solution. Solution was mixed using hand mixer for 10 min. The solution was applied to the broiler meat by using brush.

Water activity test

The tool used to measure water activity (Aw) is the Aw meter. The way the Aw meter works is as follows: the Aw meter is calibrated by entering the BaCl₂.2H₂O₂ liquid. Closed and waited for 3 minutes until the number on the reading scale Aw becomes 0.9. The meter is opened and the sample holder is cleaned. The sample is inserted and the instrument is blown, wait up to 3 minutes. After 3 minutes, the Aw scale is read and recorded. Pay attention to the temperature scale for the correction factor. The value of water activity is calculated using following formula:

$$Aw = PSA + (PST-20) \times 0.002 \dots \dots \dots (1)$$

Where:

PSA = Initial scale reading

PST = temperature scale reading

Cooking loss test

Cooking loss was measured through 3 stages (i.e. sample weighing phase, cooking phase and measurement phase). The meat sample was weighed as much as 70-100 g and then put into a heat-resistant plastic bag and then heated in water at 75 °C for 50 min prior to weighing again.

Organoleptic analysis

The first step was to prepare list of questions to be answered by 10 trained panelists. By using Scoring Test (modified SNI 01-2346-2006), broiler meat with casein chitosan edible coating were assessed for color, aroma and deviations that might occur. Panelists determined parameters including odors, deteriorations, colors, and deviations (refer to appearance and slice surface) on a scale of 1-4 (normal to abnormal).

Moisture content

Measurement of moisture content was performed using the oven method (AOAC, 2005). The empty crucibles were previously dried in an oven at 100-105°C for 30 min or until a permanent weight was obtained. The crucibles were cooled in a desiccator for 30 min then weighed. Samples were weighed as much as 5 g (b1), then dried in an oven at a temperature of 100-105°C until a constant weight was reached (about 8-12 h). The sample was cooled in a desiccator for 30 min then weighed (b2). Calculation of moisture content is performed as follows:

$$\text{Moisture content (\%)} = \frac{b1-b2}{g \text{ sample}} \times 100\% \dots\dots\dots(2)$$

Water holding capacity (WHC)

WHC determined with the Hamm method (Soeparno, 2009). 0.5 g sample was pressed on Whatman filter paper No. 42 placed between two glass plates with a weight of 35 kg for 5 min. Draw wet area from the sample on a paper and transfer on graph paper. From the image, wet area was obtained after reducing with the area covered by meat (of the total area) (3), then determined moisture content (4), finally calculated WHC (5).

$$mgH_2O = \left(\frac{\text{wet area (cm}^2\text{)}}{0.0948} \right) - 8.0 \dots\dots\dots(3)$$

$$\text{Moisture content (\%)} = \left(\frac{W2-W3}{W2-W1} \right) \times 100\% \dots\dots\dots(4)$$

$$\text{WHC} = \text{Moisture cont. (\%)} - \frac{mgH_2O}{300mg} \times 100\% \dots\dots\dots(5)$$

pH

The pH value was measured using a pH meter. This test refers to the method conducted by Mega et al., (2009).

Lipid content

The lipid content was measured using Soxhlet Extraction method. In this test, the filter paper was cut with a size of 15 ×15 cm, and placed cotton on top of the filter paper. Then, weighed the filter paper, cotton, and 1 gram of sample. The sample was placed in filter paper and coded then put in the oven at 105° C for 6 h then cooled in desiccator for 15 min. Samples were extracted using Soxhlet for 3-4 h with diethyl ether solvent. The sample was removed from the Soxhlet device and aerated until to eliminate the diethyl ether odor. The samples were then dried using oven at 105° C for 2 h. The filter paper was cooled in desiccator then weighed.

Percentage of lipid content calculated by the formula below (Azizah et al., 2017) :

$$\% \text{ Lipid content} = \frac{W1-W2}{W} \times 100\% \dots\dots\dots(4)$$

Where:

W1 = filter paper after oven 1

W2 = filter paper after oven 2

W = sample weight

Color

Samples were prepared and the liquid was placed in a glass. The color reader was turned on. The target readings for L*, a*, b* were determined. The results listed are on the color reader screen (Yuwono and Susanto, 1998)

Total plate count (TPC)

The sample was weighed as much as 5 g and crushed with mortar until smooth and homogeneous. The mashed sample was placed into an Erlenmeyer containing 45 mL of 0.1% peptone solution and stirred until homogeneous (dilution 10⁻¹), then prepared the dilution series of 10⁻², 10⁻³ to 10⁻⁴ by dropping as much as 1 mL of diluent solution 10⁻¹ into a test tube containing 9 mL of 0.1% peptone solution (diluent 10⁻²) and so on until diluent 10⁻⁴. After that, poured approximately 15 mL of PCA media evenly until it covers the surface in a petri dish. Then, pipetted as much as 1 mL of 10⁻⁴ diluent solution and pour into the petri dish evenly, and leave it to freeze, then the petri dish was flipped. These steps were also repeated for dilutions 10⁻⁵ and 10⁻⁶ (pour plate method). The sample was incubated at 37° C for 48 h using the incubator. Finally, the number of bacterial colonies was calculated using a colony counter (Turkoglu et al., 2003).

Staphylococcus aureus

Weighed the sample as much as 5 g and crush it with a mortar until smooth and homogeneous. The refined sample was placed in Erlenmeyer which contains 45 mL of 0.1% peptone solution and stirred until homogeneous (10⁻¹ dilution). Prepare a series of dilutions c by pipetting 1 mL of 10⁻¹ dilution solution into a test tube containing 9 mL of 0.1% peptone solution (10⁻² dilution). Poured about 15 mL of BPA media evenly to cover the inner surface of the petri dish and let it solid, then pipetted 0.1 mL of 10⁻¹ dilution solution and pour it over the agar surface / media that has solid evenly, left frozen, then petri dish was turned over,

similarly for the 10^{-2} dilution (spread plate method). Then, the samples were incubated in an incubator with a temperature of 37°C for 48 h. The number of bacterial colonies that grow was calculated with a colony counter.

Escherichia coli

Prepared 10^{-1} , 10^{-2} , and 10^{-3} dilution suspensions were used to determine the amount of *E. coli*. Total of 1 mL of each dilution was placed into a separate sterile petri dish in duplicate. Then petri dish was poured with sterile Eosin Methylene Blue Agar (EMBA, Himedia M022-500G) media. After the media solidified, it was incubated in an upside-down position for 24-48 h at 37°C . After 24-48 h, *E. coli* colonies were counted.

Salmonella sp.

Salmonella sp. test was performed based on previous method described in (Sartika et al., 2016). Chicken meat samples were weighed 1 g and homogenized in 10 mL of sterile aquadest. Samples were diluted using sterile BPW at dilutions of 10^{-1} to 10^{-9} . Each dilution result was taken as much as 1 mL sample and poured into XLD media so that in a sterile petri dish then homogenized. The sample was incubated at an incubator at 37°C for 24 hours. Colonies of growing bacteria were observed and counted using colony counter.

Statistical analysis

The data were analyzed using analysis of variance (ANOVA) and the LSD test was performed to determine significant difference between the treatments (Yitnosumarto, 1991).

Results and Discussion

Water activity

The analysis results of variance on water activity showed a very significant differences on A_w broiler meat ($P < 0.01$), as can be seen in Table 1. The coating can decrease A_w because casein chitosan edible coating has antimicrobial properties preventing microbes from breaking down bound water to be free water content. At storage up to 120h, there was a decrease in the value of A_w , and no significant differences at 168 h, but above the minimum growth requirements for food microbes. A_w of broiler meat increases over time due to an enzymatic process in meat protein, thus may reduce water retention capacity (Grau et al., 2011). Chitosan has primarily characterised which molecular weight 100-1000 kDa and can be applied as a material to inhibit the growth of bacteria or

fungi more effectively (Goy et al., 2009). Most moulds grow at a pH range of 3 to 8 and some can grow at very low water activity levels (0.7–0.8). Generally, aerobic microbes require a high water activity for growth (0.95 or higher) (Rawat, 2015).

Moisture content

The analysis results of variance on moisture content did not show significant differences ($P > 0.05$), as can be seen in Table 1. The mean value indicates that meat coated with edible coating and stored at refrigerator temperature ($\sim 8^{\circ}\text{C}$) until the 168 h has relatively the same moisture content and is as the standard moisture content in chicken meat. This is comparable to the opinion of (Ouattara et al., 2001), which states that edible coatings can be made from a variety of materials including polysaccharides, protein, and lipid. The main mechanism or the role of edible coating on food is to improve quality and to extend shelf life which acts as a barrier to oxygen and water, thus slowing down bacterial growth. This is also because one of the materials used in edible coating is casein which has hydrophobic characteristics, hence it can prevent the water in the meat from evaporating or decreasing. Casein is highly hydrophobic but due to the presence of high number of phosphate and sulfur-containing amino acids and carbohydrates in case of casein, they are quite soluble in aqueous solvents. Casein tends to associate with other protein and some ligands, according to the hydrophobic characteristics of micelles (Yuksel et al., 2010).

Lipid content

The analysis results of variance on lipid content did not show significant differences ($P > 0.05$), as can be seen in Table 1. The mean value indicates that meat coated with edible coating and stored at refrigerator temperature ($\sim 8^{\circ}\text{C}$) until the 168 h has relatively the same lipid content. A Film based on casein possess low oxygen permeability and good strength for food packaging purposes, but have low flexibility and susceptible moisture (Shendurse, 2018). Casein is easily formed into a casein-lipid emulsion which has a good gas and moisture barrier to prevent oxidation (Broumand et al., 2011). The use of chitosan as an edible coating that can delay the occurrence of lipid oxidation has been investigated by (Cardoso et al., 2016) where chitosan can delay lipid oxidation through ion chelation properties and/or through lipid complexation, hence lipid content as long as storage can be maintained. The average value of the lipid content of the samples studied was lower than what has been reviewed in (Bogosavljević-Bošković et

al., 2010) mentioning breast lipid in chickens at 3.9- 8.4%.

Table 1. Water activity, moisture, and lipid content of casein chitosan edible coating on broiler meat

Storage Time (h)	Aw	Moisture Content (%)	Lipid Content (%)
0	0.95±0.002 ^c	74.75 ± 0.40	2.21 ± 0.45
24	0.94±0.002 ^c	75.40 ± 1.88	2.41 ± 0.88
72	0.93±0.006 ^b	73.99 ± 0.61	2.18 ± 0.23
120	0.92±0.005 ^a	74.89 ± 0.35	1.76 ± 0.26
168	0.93±0.006 ^b	73.89 ± 0.33	2.39 ± 0.37

Note: Different notation showed very significant differences (P<0.01)

Water holding capacity (WHC)

The results of the analysis of variance on WHC showed very significant differences (P<0.01), as can be seen in Table 2. The coating of broiler meat with casein chitosan which stored until 168 h would control WHC. Casein chitosan edible coating controlled the chemical and structural attributes of the muscle tissue as they are influenced by the hydrophilic property’s transformation of muscle to meat. According to Varela and Fizsman (2011) stated that higher WHC values were observed relative to control values of hydrophilic properties of the samples. Xia et al. (2018) reported the WHC increases as it increased hydrophobic interactions and hydrogen bonds, but decreased disulfide bonds.

pH

The results of the analysis of variance on pH showed very significant differences (P<0.01), as can be seen in Table 2. A decrease in pH occurs because lactic acid is formed by the anaerobic breakdown of glycogen which produces lactic acid. Soeparno (2009) stated that the release of lysosomal enzymes into the cytoplasm and intracellular spaces of the degradation that occurs after cell death. Calpain and cathepsin enzymes work synergistically against myofibrillar proteins.

The change in pH of broiler meat can be affected because the protein solution shifts the charge on the ionizable group and changes the protein conformation by revealing or hiding the water-binding site. Hassanzadeh et al. (2017) and Sharafati Chaleshtori et al. (2016) stated that chitosan-coated samples had lower pH values than uncoated samples; moreover, the application of chitosan coating in chicken meat samples could stabilize the pH value during storage.

Cooking loss

The results of the analysis of variance on cooking loss showed no significant differences (P> 0.05), as can be seen in Table 2. Casein chitosan edible coating could withstand water loss until the end of the storage period at 168 h. The higher cooking losses were due to loss of water and other volatile components that are volatile at refrigerator temperatures (Risnajati, 2010). During storage, the water content in food can change due to differences in humidity with the environment (Triwarsita et al., 2013). Edible coating is a mechanically strong matrix with hydrophobic groups to provide low permeability to water vapor (Marelli et al., 2016).

Table 2. WHC, cooking loss, and pH of casein chitosan edible coating on broiler meat

Storage Time (h)	WHC	Cooking Loss	pH
0	17.61 ± 0.63 ^c	33.65± 1.16	5.01 ± 0.13 ^b
24	15.50 ± 0.17 ^d	32.71±1.42	4.70 ± 0.05 ^{ab}
72	14.12 ± 0.69 ^c	31.51±0.33	4.95 ± 0.10 ^b
120	9.74 ± 0.73 ^b	30.66±2.82	4.59 ± 0.12 ^a
168	8.42 ± 0.25 ^a	29.57±3.17	4.40 ± 0.27 ^a

Note: Different notation showed very significant difference (P<0.01)

Table 3. Color of casein chitosan edible coating on broiler meat

Storage Time (h)	<i>L</i> *	<i>a</i> *	<i>b</i> *
0	71.10 ± 1.82 ^b	17.23 ± 0.85 ^a	24.24 ± 0.99
24	70.70 ± 1.82 ^b	17.35 ± 0.82 ^a	24.00 ± 0.99
72	69.35 ± 1.64 ^b	17.89 ± 0.75 ^a	23.71 ± 0.99
120	67.97 ± 1.43 ^a	18.39 ± 0.63 ^{ab}	19.20 ± 0.58
168	65.07 ± 1.51 ^a	19.20 ± 0.58 ^b	22.90 ± 0.87

Note: ^{a,b} showed very different on *L** (P<0.01), ^{a,f} showed different on *a** (P<0.05)



Figure 1. Discoloration during storages (the result of a gradual change in meat color during storage)

Color

The results of the analysis of variance on color showed a very significant effect (P <0.01), as can be seen in Table 3. The color of meat during the storage process tends to darken, while the pH of meat during storage decreases. The color of chicken meat is influenced by the concentration of myoglobin. Chickens have lower myoglobin concentrations compared to other birds (Mir et al., 2017). Fresh chicken meat color is gray/white (Taran et al., 2015). Color changes during storage can be observed in Figure 1. The use of chitosan with an addition of 1% can improve the color stability on the surface of beef patties (Suman et al., 2010). Meat discoloration results from the conversion of oxymyoglobin (MbO₂) to metmyoglobin (MetMb) and has been shown to interfere with the processes of discoloration and lipid oxidation (Faustman et al., 2010). The main cause of degradation in meat quality during storage and processing is lipid oxidation (Prado et al., 2015).

Organoleptic (color, odors, and deviations)

The results of the analysis of variance on color organoleptically did not show significant differences (P> 0.05) and very significant effect (P<0.01) on organoleptic odors and deviations (appearance and slice surface) showed as can be seen in Table 4. The casein chitosan edible coating can inhibit air contact in broiler meat because casein chitosan is less permeable to oxygen and prevent the oxidation of oxymyoglobin to methemoglobin (darkening the color). According to Cayré et al. (2005) stated that color variations can be related to lactic acid bacteria creating hydrogen peroxide during the storage process or to myoglobin oxidation due to lipid oxidation. In a study by Valencia-Sullca et al. (2018), the Maillard reaction

is correlated with this specific shift in color between the carbonyl group and chitosan amino group.

Based on panelists, they gave good score on odor, appearance, and slice surface of broiler meat with casein chitosan edible coating for 72 h in contrast to 120 h and 168 h which the deviation for each showed slimy, moldy, short shelf-life, with a bad odor. Chaparro-Hernández et al. (2019) found that for a long period of time, the coated chicken displayed a new odor. For all 16 days of storage, the items treated with the chitosan-tomato plant extract edible coatings remained edible.

The appearance, slice surface and odors produced that develop during spoilage in broiler meat muscle. Furthermore, spoilage potential evaluation was carried out by screening the bacterial which is the predominant potential spoilers as: *Enterobacter* and *Acinetobacter* at room temperature, *Pseudomonas* and *Aeromonas* at refrigerated storage and, *Aeromonas* and *Enterococcus* at ice storage (Don et al., 2018). In another study, under chilled temperature conditions, *Pseudomonas fragi* has a high growth rate. The development of spoilage-related off-odors and slime rapidly contributes to consumer rejection of meat products (Ercolini et al., 2010).

Total plate count (TPC)

The results of the analysis of variance on TPC showed very significant differences (P<0.01), as can be seen in Table 5. Casein chitosan edible coating began to decrease its performance at 168 h, hence the TPC showed an increase at the time of storage. Based on the achieved results (Apriliyani et al., 2020), accelerated storage beef with casein chitosan coating (addition catechin) were no differences found on TPC (after 120 h of storage), the chicken meat would be inadequate for consumption. In

addition, different studies by (Sagoo et al., 2002) that chitosan coating's ability to minimize microbial (viable counts) in meat products. The obtained results casein chitosan edible coating has coincided with the amphiphilic design of protein-based films allows active compounds to serve as carriers to maintain the consistency of packaged foods (Wihodo and Moraru, 2013). In addition, the active effect or food quality is determined by the regulation of the release of active and volatile mass transfer through the coating.

Staphylococcus aureus

The analysis results of variance on *S. aureus* showed very significant differences

($P < 0.01$), as can be seen in Table 5. The number of *S. aureus* increased, indicating more protein can be degraded by the bacteria, leading to a lower ability of meat protein to bind water. Chitosan as antimicrobial agent against *S. aureus* because chitosan is a poly 2-amino-2-deoxy- β -D-glucose, is deacetylation chitin, where the acetyl group in chitin is substituted by hydrogen into an amino group with the addition of a high concentration of a strong base solution (Fernandez-Saiz et al., 2010). The use of chitosan as much as 20% in chitosan coating can provide the increased inhibitory ability to the growth of bacteria in tuna meatballs, thus it can last up to 2 days.

Table 4. Organoleptic (color, odors, and deviation) of casein chitosan edible coating on broiler meat

Storage Time (h)	Color	Odors	Deviations
0	4.00±0.00	4.00 ±0.00 ^b	4.00 ±0.00 ^b
24	4.00±0.00	4.00 ±0.00 ^b	4.00±0.00 ^b
72	4.00±0.00	4.00 ±0.00 ^b	4.00 ±0.00 ^b
120	4.00±0.00	3.80 ±0.41 ^{ab}	3.80 ±0.41 ^a
168	3.90±0.30	3.60 ±0.50 ^a	3.85 ±0.37 ^{ab}

Note: ^a^b showed very significant differences ($P < 0.01$) on organoleptic odors and deviations (appearance and slice surface)

Table 5. TPC from *S. aureus* and *E. coli* of casein chitosan edible coating on broiler meat

Storage Time (h)	TPC (log cfu/g)	<i>S. aureus</i> (log cfu/g)	<i>E. coli</i> (log cfu/g)
0	7.33 ±0.69 ^b	2.01 ± 0.07 ^a	1.95 ± 0.30 ^a
24	6.19 ±0.45 ^{ab}	2.05 ± 0.07 ^a	2.56 ± 0.63 ^a
72	5.48 ±0.61 ^a	2.10 ± 0.04 ^a	3.87 ± 0.99 ^b
120	4.69 ±0.64 ^a	2.27 ± 0.03 ^b	2.57 ± 0.65 ^a
168	5.69 ±1.08 ^{ab}	2.76 ± 0.05 ^c	3.28 ± 0.95 ^b

Note: ^a^b showed very significant differences ($P < 0.01$) on TPC and *S. aureus*, significant effect ($P < 0.05$) on *E. coli*

Table 6. *Salmonella* sp. of casein chitosan edible coating on broiler meat

Storage Time (h)	Replications			
	U1	U2	U3	U4
0	Negative	Negative	Negative	Negative
24	Negative	Negative	Negative	Negative
72	Positive	Positive	Negative	Positive
120	Negative	Positive	Negative	Negative
168	Negative	Negative	Positive	Positive

Escherichia coli

The analysis results of variance on *E. coli* showed significant differences ($P < 0.05$), as can be seen in Table 5. Casein chitosan edible coating cannot protect broiler meat from contamination of *E. coli* because the edible coating as oxygen and water barrier thus slowing down bacterial growth. It does not play a role to stop the process of bacterial growth. This is comparable to the opinion of

(Ouattara et al., 2001) which states that edible coatings are made from various materials including polysaccharides, protein, and lipid.

***Salmonella* sp.**

The analysis results of variance on *Salmonella* sp. can be seen in Table 6. The edible coating can resist *Salmonella* sp. for ~24 hours due to an edible coating solution. The edible coating can extend the

shelf life of animal origin food by blocking water vapor, oxygen, and carbon dioxide and as a carrier of substances to inhibit spoilage pathogenic microorganisms (Sánchez-Ortega et al., 2014). Chitosan has antimicrobial activity, for example *Salmonella* (Gram Negative) is more resistant because of their outer membrane (Fernandez-Saiz et al., 2010), often associated with bacteria that cause foodborne disease (Pui et al., 2011).

Conclusion

The use of casein-chitosan edible coating for 168 h on broiler meat can maintain the value of moisture content, pH, lipid content, color, *S. aureus*, and *Salmonella* sp. for some time. However, it cannot maintain WHC and cannot protect the meat from *E. coli* bacteria. Special treatment is needed before the application of edible coatings to the product as well as the addition of additional ingredients that can reduce or even to eliminate microbes in the products.

Conflict of interest

The authors declare that there is no conflict of interest in this publication.

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