Antioxidant Activity of Banana *Kepok Kuning* (*Musa paradisiaca* L.) and Cavendish (*Musa acuminata* Colla, AAA) Peel Extracts and the Potential as Chicken Meat Preservative

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ABSTRACT

Bananas are a type of fruit that is consumed, with the peel being discarded as waste. However, the peel contains phytochemical compounds, including phenolic and flavonoids, which act as natural antioxidants. Therefore, this study aimed to determine the antioxidant activity of banana peel of *Kepok Kuning* (KCE) (*Musa paradisiaca* L.) and Cavendish (CCE) (*Musa acuminata Colla*, AAA) extracts, as well as eluviate their preservative potency. The sample was extracted using 80% methanol, and the antioxidant activities of KCE and CCE were evaluated using DPPH and FRAP. The extracts and the control antioxidant butylated hydroxyl toluene (BHT) were tested for their preservatives potency on chicken meat at concentrations of 5%, 10%, and 15%, respectively. After 8 days of incubation in the refrigerator, the color change was recorded and analyzed using ImageJ, while free fatty acid contents were determined by titration. The results showed that 15% KCE and 15% CCE exhibited higher antioxidant activities than 0.1% BHT. There was no significant difference in the L (lightness) and b (yellowness) values between the treatments, but variations were evident in the a (redness) values. FFA values were significantly different between treatments, with untreated control registering the highest value at 0.41%, and the KCE 15% and CCE 15% concentrations showing the lowest values at 0.12%. Considering these results, both extracts could be developed as preservatives for chicken meat.

Keywords: Banana peel, natural preservatives, antioxidants, phenolic, flavonoids

INTRODUCTION

Banana (*Family musaceae*) is an important food plant, and has been domesticated for thousands of years (Delange et al., 2019). This plant ranks second in terms of global cultivation and consumption. Furthermore, the total gross production has reached 117 million tons per year. World banana producers from 2018-2021 were dominated by India, China, Indonesia, Ecuador, Brasil, and Philippines (Atlasbig, 2021). The higher production does not represent the rank of countries as it relates to importation. According to FAO in Banana Market Review 2021, Ecuador, the Philippines, and Costarica have become the three highest exporters of banana worldwide (FAO, 2022).

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The banana varieties most commonly consumed in Indonesia are *Kepok Kuning* (*Musa paradisiaca* L.) and Cavendish (*Musa acuminata Colla*, AAA) (ProMusa, 2017). *Kepok Kuning* is consumed in various processed for such as fried banana, chips, and drink. Meanwhile, Cavendish, a popular cultivar worldwide, is generally eaten as a fresh table fruit.

Banana consumption results in the disposal of the fruit peel as organic waste. According to Niz (2014), the peel constitutes approximately 12% of the total fruit mass, providing biomass for further use. Furthermore, it contains pectin, starch, and various phytochemical compounds, particularly flavonoids and phenolics (Xi et al., 2014). The phenolics in banana peel have been shown to exhibit antioxidant and antibacterial properties, suggesting that they might be developed as functional foods (Vu et al., 2018).

Food industry still has issues regarding safe and effective preservation. Damage to food products, specifically processed meat items such as nuggets, sausages, and corned beef, is primarily caused by lipid oxidation. This phenomenon is often exacerbated by industry processes, including meat refining and adding salting during production. For instance, in the production procedure of meatloaf, the lipid membrane interacts with the metal oxidation catalyst, whereas the inclusion of salt leads to heightened lipid oxidation in meat (O'Neill et al., 1999; Rhee et al., 2001). To address this issue, industries have resorted to the addition of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). These compounds have shown efficacy in preventing lipid oxidation in food items. However, due to health concerns, the use of these synthetic antioxidants was reduced, prompting the exploration of safer alternatives in the form of natural antioxidants.

A prevailing trend favoring a health-conscious lifestyle has prompted a shift towards the consumption of natural products over chemical alternatives (Templeton et al., 2016). Flavonoids are the primary natural antioxidants of plants (Lewis et al., 1999), and their usage as natural antioxidants can mitigate lipid oxidation-induced harm in meat-derived food products. Several studies reported the potential of banana peel as a source of antioxidants (González-Montelongo et al., 2010; Rebello et al., 2014) and antimicrobes (Aboul-Enein et al., 2016). Additionally, the transformation into jelly product has been documented by (Lee et al., 2010), but studies have explored the viability of banana peel as a meat preservative. Therefore, this study aimed to evaluate the potency of natural antioxidants from banana peel to preserve chicken meat.

METHODS

Banana Peel Preparation

Banana peel was prepared following the method outlined by Fatemeh et al. (2012) with slight modification. The varieties of the peel, namely *Kepok Kuning* and Cavendish, were collected from ripe bananas, using the entire yellow color skin to determine the ripeness. After cleaning the peel, it was cut into small pieces of ±3 cm long and then dried in a 40 °C oven for 6-8 days until a constant weight was reached. Finally, the sample was powdered using a blender, allowed to pass 60 mesh sieves, and stored at 4 °C until the following experiment.

Banana Peel Extraction

A total of 20 g banana peel powder was macerated with 200 mL of 80% methanol overnight with periodic shaking (Fatemeh et al., 2012; Ahmed et al., 2016). The mixture was filtered with gauze, centrifuged at 3000 g for 15 minutes, then the extracts were collected. The solvent was removed using a vacuum evaporator and stored in a dark bottle inside a refrigerator maintained at 4 °C. For preservation treatment of chicken meat, extracts were dissolved in aquadest to reach the desired concentrations of 5, 10, and 15%. In this subsequent sections of this study, *Kepok Kuning* and Cavendish peel extract are referred to as KCE and CCE, respectively.

Chicken Meat Preservation Treatment

Broiler chicken breast weighing 1 kg was finely chopped and divided into 5 treatments groups, including 1). Control: the meat remained unaltered, devoid of any additive, 2). Salt: the meat was mixed with 2% salt, 3). BHT: the meat was blended with 2% salt and 0.1% BHT, 4). KEP: the meat was combined with 2% salt, alongside the incorporation of *Kepok Kuning* banana peel extract at concentrations of 5%, 10%, and 15%, and 5). CAV: the meat was blended with 2% salt and supplemented with Cavendish banana peel extract at concentrations of 5%, 10%, and 15%. Each meat sample treated with extract or salt and BHT was allowed to stand for 30 minutes at room temperature. Subsequently, about 100 g sample was kept in a zip bag and then stored at 4 °C for 7 days (Devatkal et al., 2014). To ensure robust results, all experiments were conducted in triplicate for each treatment variant.

Free Radical Scavenging Measurement

The measurement procedure for assessing free radical scavenging activity was conducted using a DPPH assay, aligning with the methodology detailed by Devatkal et al. (2014). Approximately 1 mL banana peel extract was added into 5 mL of 250 mM DPPH solution,

then stored in a dark room/dark bottle for 20 minutes. The mixture was subjected to spectrophotometric analysis at a wavelength of 517 nm. Finally, the DPPH activity was expressed in the form of percent inhibition using Equation 1.

$$(\%)\ inhibition\ = \frac{Abs.of\ control - Abs.of\ sample) \times 100}{Abs.of\ control} \quad (1)$$

Free Fatty Acid (FFA) Analysis

A quantity of 2 g meat sample was added with 30 mL chloroform, and was vigorously shaken for 5 minutes until achieving homogenous consistency. The mixture was let to settle, and the solution formed was collected. Subsequently, the solution was titrated using 0.01 N potassium hydroxide (KOH), with the addition of phenolphthalein indicator beforehand. The titration process was halted upon the appearance of a light pink color. The value of free fatty acids was expressed in percentage and was calculated based on its equivalence to oleic acid. This choice of oleic acid is attributed to its proportional representation of the acid concentration within the sample. Finally, aquadest without samples were used as blanks (Mir et al., 2017), and the FFA value was calculated using Equation 2.

$$FFA\ (\%)\ = \frac{(0.1 \times vol.of\ 0.1N\ alc.KOH \times meq\ wt.of\ oleic\ acid \times 0.282) \times 100}{Sample\ weight\ (g)} \quad (2)$$

Ferric Reducing Antioxidant Power (FRAP) Analysis

The FRAP assay was performed using the OxiSelect FRAP Assay Kit (Cell Biolabs). The analysis was conducted according to the instructions provided by the company. Firstly, a 15 mL solution of 1x buffer assay was prepared by adding 12 mL distilled water into 3 mL of 5 times buffer assay. The reagent was prepared by mixing 4 mL of 1x buffer assay with 500 µL colorimetric probe and 500 µL Iron Chloride, after which the mixture was vortex for 1 minute. Finally, the standard solution was prepared by diluting the Iron (II) standard solution into a series of 125, 62.5, 31.3, 15.6, 7.8, 3.9, and 0 µL concentrations. The measurement process involved adding 100 µL of the sample, control, blank, and standard solution to 100 µL reagent in a 96 well microplates. After mixing by pipetting up and down, incubation was performed for 10 minutes at room temperature. Finally, the absorbance was recorded at a wavelength of 600 nm using a microplate reader (Halim et al., 2022), and FRAP value was calculated using Equation 3.

$$Csam\ =\ (ABS\ \times Cst)/Ast \quad (3)$$

Where ABS, Ast, Csam, and Cst, represents Absorbance of sample, Absorbance of standard, Sample concentration, and Standard concentration, respectively.

Total Phenolic Content (TPC) Analysis

Analysis of TPC was conducted using the Folin Ciocalteau method developed by Singleton and Rosi (1965) with some modifications as outlined by Ilmiah et al. (2018). Both KCE and CCE were dissolved in methanol to achieve a final concentration of 1 mg×mL^{-1}. Subsequently, 1 mL of either KCE or CCE solutions was added to 0.5 mL Folin Ciocalteau reagent (1:1) along with 1 mL Na_2CO_3 (7.5%) and 2.5 mL distilled water. Following this, the mixture was allowed to stand at room temperature for 1 hour before absorbance measurements were taken at 650 nm. For the calibration curves preparation, gallic acid was used as a standard, with concentrations ranging from 10-160 mg×mL^{-1}, and intervals of 20 mg×mL^{-1}. Finally, the calculation of TPC was performed in terms of gallic acid equivalents (mg GAE×g^{-1} of extract).

Total Flavonoid Content (TFC) Analysis

A colorimetric test with aluminum chloride was used to measure TFC (Ilmiah et al., 2018). In this method, 1 mL sample was combined with 0.5 mL of 2% $AlCl_3$ and 2.5 mL distilled water. The sample was gently mixed and incubated for 10 minutes at 37 °C before measuring the absorbance at 425 nm. Quercetin was employed as a standard for the preparation of calibration curve, and the concentration ranged between 20-100 µM×mL^{-1} (20, 40, 60, 80, and 100 µM×mL^{-1}). The determination of TFC was calculated in terms of equivalent quercetin derived from the standard curve (mg QE g×g^{-1} of extract).

Color Change Determination

The procedure for color change determination closely followed the methodology outlined by Hidayati et al. (2018). In this process, meat samples were photographed using a Canon 600D camera, ensuring uniform conditions with one another. The position and lighting of lamps remained constant throughout the photography session. Subsequently, the captured image was analyzed using the ImageJ software, where the L (lightness), a (redness), and b (yellowness) values were extracted. For the L score, a value of 100 and 0 represented maximum and minimum brightness (dark), respectively. The presence of red and green gradients indicated positive and negative values. Positive and negative b values showed yellow and bluish colors, respectively. Finally, the test was conducted at intervals of 2 days over a span of 8 days.

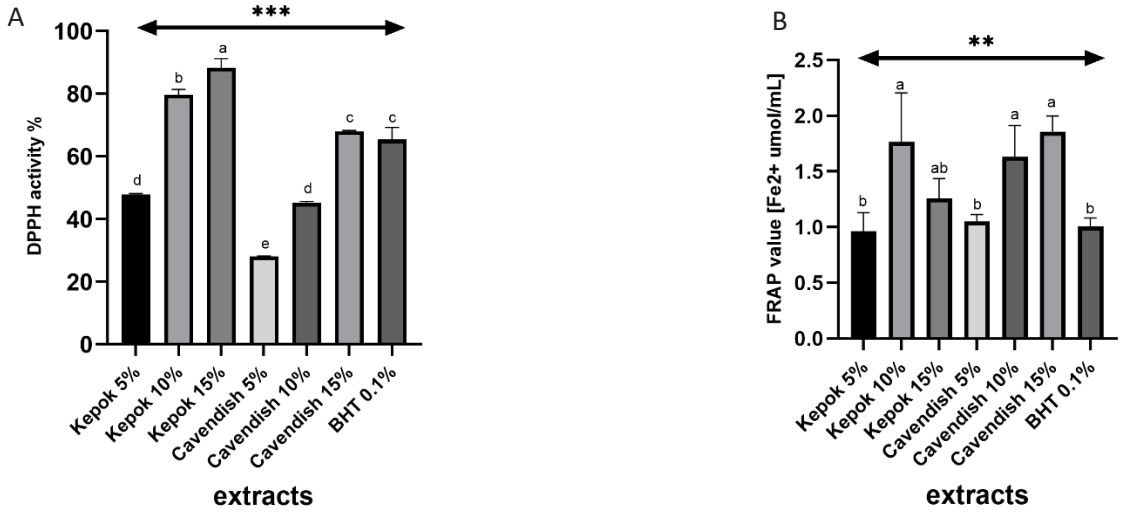

Figure 1. Antioxidant activity of *Kepok Kuning* peel extract, Cavendish peel extract, and 0.1% BHT using A) DPPH and B) FRAP assays. Kep = *Kepok Kuning*, Cav = Cavendish, BHT = butylated hydroxytoluene. The data presented showed the mean value and standard deviations obtained from 3 replicate measurements each. Different letters denoted significant differences at ** $p< 0.001$ *** , $p< 0.0001$

Data Analysis

The results obtained from DPPH, FRAP, FFA, and evaluation of color changes were statistically analyzed with One-way ANOVA with a significance level of 0.05 and continued with DMRT as posthoc to identify the significant difference between groups. This entire statistical analysis was performed using SPSS 16 software. Additionally, all graphics represent the mean±standard deviation produced using GraphPad Prism 9.

RESULTS AND DISCUSSION

Antioxidant activity of Banana Peel Extract

The DPPH antioxidant assay showed that KCE had higher DPPH than CCE at all concentrations. The highest antioxidant activity at 88.81% was observed in the 15% KCE followed by 10% of the same extract. Antioxidant DPPH value of KCE was even higher than 0.1% BHT, a concentration often recommended for food product. The CCE at concentrations of 15% had the same antioxidant activity as 0.1% BHT, as shown in Figure 1.A. During the DPPH assay, the antioxidant compound in the banana peel extract reacted with synthetic radicals, probing its efficiency in capturing free radicals. The mechanism of action of an antioxidant varies, extending beyond free radical scavenging to include roles as reducing agent. The results of the evaluation of antioxidant activity by the FRAP method showed different results from the DPPH. At 5% concentrations, both KCE and CCE had a comparable ability to reduce Fe^{3+} with BHT as the positive control. This was represented by the absence of significant difference in the FRAP values among treatments. At 10-15% concentration of CCE and 15% of KCE, FRAP values exhibited comparative values while exceeding those attributed to BHT. Finally, it was observed that BHT had no reducing activity, as presented in Figure 1.B.

The high antioxidant activity of KCE in scavenging free radicals was derived from TPC and TFC of *Kepok Kuning* peel which was around 1.5 times higher, as shown in Figure 2.

Phenolic compounds exist ubiquitously in plants and receive extensive study due to their potency as natural antioxidants. The aromatic ring equipped with several hydroxyl groups that inhibit the free radical contributes to the antioxidant capacity of the compounds (Soobrattee et al., 2005). The antioxidant activity of these phenolic compounds varies depending on their molecular structure. This contributed to the variations in the antioxidant activity of plant extracts. Flavonoids commonly discovered in plants, including flavones, flavonols, flavanols, and flavanones, have a basic 15-carbon flavan (C6C3C6) structure. Therefore, those possessing an abundance of -OH group accessories have the potential to be better antioxidants. Flavonoids also have the ability to reduce transition metals better than phenolic (Brewer, 2011).

Free Fatty Acid (FFA) Content

The test results showed that chicken meat without treatment had the highest FFA value of 0.412%, and

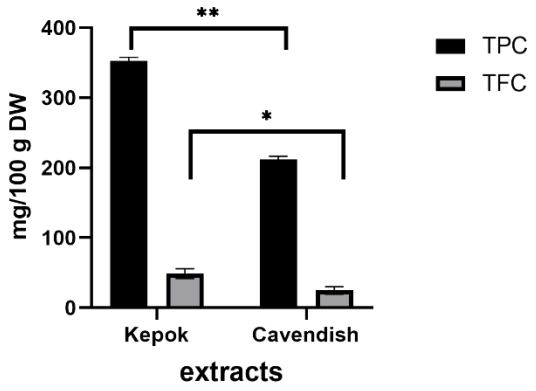

Figure 2. TPC and TFC of *Kepok Kuning* and Cavendish peel extract. Kep: *Kepok Kuning*, Cav: Cavendish. The data presented showed the mean value and standard deviations obtained from 3 replicate measurements each. Different letters were employed to signify significant differences at * $p<0.05$, ** $p< 0.001$

this was followed by 0.279% for samples with 2% salt. The lowest value of 0.119% was observed in meat treated with 15% KEC. Treatment of 15% CCE, showed good value of 0.159% but this remained below the KEC. Additionally, the FFA value of the 15% KCE matched that of a positive control containing 0.1% BHT, as presented Figure 3.

Fresh meat is naturally susceptible to deterioration and decomposition after a given length of storage. It includes numerous lipids that are prone to rancidity and

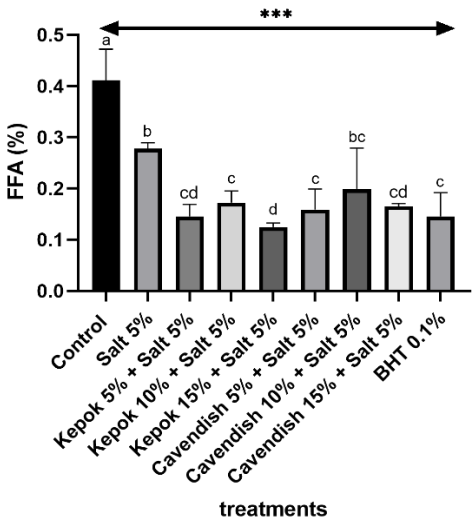

Figure 3. FFA Content of chicken meat treated with several preservatives after 8 days of storage. The data presented showed the mean value and standard deviation of 3 replicates each. Different letters denote significant differences at *** $p< 0.0001$

lipid oxidation. The process of lipid oxidation significantly adds to the degradation of meat and its product quality. During this process, the phospholipids in the meat created various chemicals, including hydroperoxides, aldehydes, and ketones, and increased the release of free fatty acids. However, the initial three compounds mentioned were typically observed at the beginning of the lipid oxidation, exhibiting volatility, thereby making measurement difficult. Therefore, the FFA value in this study was selected as an indicator of the process in the test meat (Calvo et al., 2017; Song et al., 2022). This value represents the amount of FFA hydrolyzed from triacylglycerol, and it is affected by duration storage, humidity, and temperature changes resulting from fat storage and processing. Salt has been as a preservative in meat by the community over a long time. It is also applied in the food industry as a natural preservative along with synthetic preservatives. However, this study proved that the administration of salt alone had not been able to inhibit food quality deterioration. The addition of this substance at a dose of 2% to 3.5% reduced the amount of secondary oxidative production, but at a higher dose of 5%, it promoted lipid oxidation. As a result of this dose-dependent character, the application of salt as a meat preservative necessitates meticulous consideration (Henney et al., 2010).

Antioxidant treatment is reported to chelate free radicals and inhibit lipid oxidation. In addition to inhibiting lipolysis enzymes' action, natural antioxidants such as phenolic compounds also have antimicrobial activity. KEC and CCE showed their ability as antioxidants, which included free radical scavengers (DPPH activity) and reducing agents (FRAP activity). According to Al-Rubeii et al. (2009) natural antioxidant activity was closely correlated with the total phenolic content of an extract. Aboul-Enein et al. (2016) reported that banana peel extract had antimicrobial properties. This was in line with the result of this study, in which 15% KEC + salt treatment showed the most lower FFA value.

Color Change Evaluation

Evaluation of color changes was conducted to determine the effect of lipid peroxidation and various treatments on the sample. The change in the color was an important parameters as it serve as an indicator of meat quality and affects consumer preferences.

Evaluation of color changes was performed utilizing the ImageJ software. Images from cameras in the RGB format were converted to CIELAB coordinates, a space that was commonly used for color measurement in food (Trinderup & Kim, 2015) (Figure 4.). The parameters analyzed were L (lightness), a (redness), and b (yellowness) (Devatkal et al., 2014).

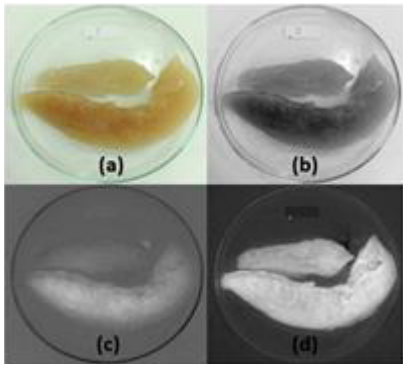

Figure 4. Conversion result of the chicken meat picture from an RGB format to CIELAB on the first day chicken meat: (a) RGB; (b) L filter; (c) a filter; (d) b filter.

Stored chicken meat exhibited color and texture changes as the duration progressed. This decrease in quality was observed in all treatments. On day zero right after treatments, the meat samples showed bright flesh color and good texture. However, significant color changes became evident on the 5th day of storage. This was indicated by darken color, and soggy texture, as shown in Figure 5.

The change in lightness on the control sample was observed to be the highest with a ΔL value of 22.08, while 0.1% BHT as a positive control had a ΔL value of 13.065. Comparable values of ΔL were observed on the treatment of 10% and 15% CCE. Furthermore, this extract had better performance in maintaining lightness than KCE, as shown in Figure 5. The same trend was also observed in the Δa value. The control sample showed the highest Δa with a value of 4.22. Unlike the lightness, this redness parameter had more random results, with several samples exhibiting negative Δa, indicating a shift towards green rather than red color. These outcomes were mainly observed in the treatment of salt 2%, BHT 0.1%, KCE 10%, and the highest color change to green was discovered at 5% CCE, as presented in Figure 5. In contrast to the previous parameters, the control sample had the lowest Δb value of 1.531, and the highest Δb value was observed in the treatment with 5% KCE. The positive control with BHT showed a moderate Δb value of 3.57. Finally, meat subjected to KCE treatment had a greater Δb value.

Discoloration is not the only indicator of meat spoilage, as color changes also showed that the decomposition process has begun. The primary mechanisms responsible the spoilage were microbial growth, oxidation, and enzymatic autolysis. These deterioration processes contribute to the color changes. Meat contains a variety of pigments that cause unique colors appearance. The optimum color associated with fresh meat is very unstable and short-lived. In fresh conditions, myoglobin in the flesh gives a fresh red appearance. Over time, the storage duration triggers the oxidation of proteins, leading myoglobin to transform into pigments known as oxymyoglobin. This transformation led to the flesh turning a brownish-red. Furthermore, the decline in meat quality can also

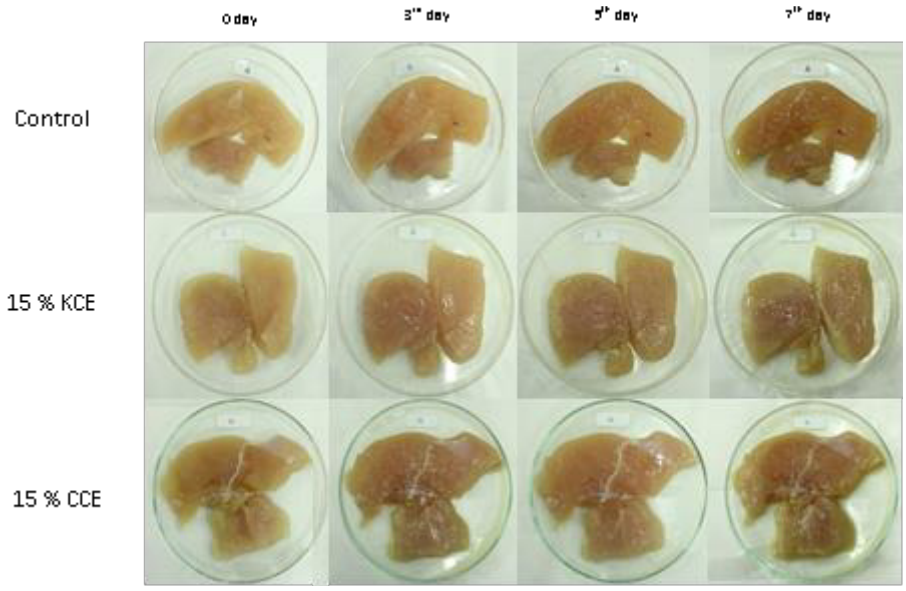

Figure 5. Chicken meat physical appearance from the day of treatment (zero-day) to 7th day stored in the 4 °C

manifest as a greenish discoloration. This phenomenon was attributed to the presence of iron, fat, and other compounds.

CONCLUSION

In conclusion, the antioxidant activity of *Kepok Kuning* peel extract was discovered to be higher than Cavendish peel, as assessed using the DPPH assay. Meanwhile, the Cavendish extract exhibited greater antioxidant activity than *Kepok Kuning* when evaluated through the FRAP assay. *Kepok Kuning* extract at a 15% concentration displayed promising potential as an effective preservative for chicken meat, outperforming other extracts. Furthermore, observations of color changes in the redness of chicken meat across various treatments post-storage indicated significant differences.

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CONFLICT OF INTEREST

The authors declare there is no conflict of interest in the subject, material, and financial of this study.

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