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by Inanpi Hidayati

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Indicator Film of Natural Coloring of Butterfly Pea (*Clitoria ternatea* L.) as Detection of Beef Damage

Hermawan Seftiono [1]*, Qorry Aina [1], Inanpi Hidayati Sumiasih[1]

[1] Trilogi University, Jakarta, Indonesia

Abstract

Beef is a food source with a high protein content that would be ideal for microbes to flourish. Microbes would reduce the quality of a product. Therefore, an indicator on smart packaging would be needed to detect the quality of a product. The indicator film in this study used butterfly pea flower extract which contains a natural coloring agent, anthocyanin. The objective of this study was to obtain the indicator film with the best concentration of PVA, chitosan, and butterfly pea flower extract as the natural dye, to study the response of indicator film color, pH, and thickness, to the beef pH and TVBN, and to calculate the total microbes as the determinant of the beef quality. The study consisted of three steps, namely, the extraction of butterfly pea flower, the making of indicator film, and the application of indicator film on beef packaging. The best indicator film was obtained with the formulation of PVA and chitosan of 20:80 with the addition of 5 ml of butterfly pea extract. The color change was from blue to yellowish-green with °hue of 137.81±19.31o. The thickness of indicator film in 48hours of storage decreased from 0.171±0.042 to 0.136±0.043. The pH of beef increased after 8 hours of storage from 5.726±0.011 to 7.540±0.351. The TVBNof beef after 8 hours of storage had exceeded the threshold of 30.815±5,602 which indicates that it was not safe for consumption. The TPC of beef from the 8 hours of storage had exceeded the maximum number of 7.338±0.035 log CFU/g.

Introduction

Beef consisted of water, fat, protein, carbohydrate, vitamin and several minerals (Prasetyo et al. 2013; Komariah et al. 2009). The chemical compositions of beef are 77.65% water. 14.7% fat and 18.26% protein (Prasetyo et al. 2013). The high protein content in beef causes microbes to grow and multiply so that it can reduce product quality. Efforts to increase the shelf life of beef to slow down the quality degradation due to contamination can be done by storing at low temperatures, using natural preservatives, and good packaging.

The current innovation of packaging is smart packaging. Smart packaging is a packaging system that can monitor temperature, freshness, the presence of microbes, and product shelf life (Ahmed et al. 2018). Smart packaging has a pH indicator that can use natural or synthetic dyes. This study used butterfly pea flower as a natural dye in smart packaging because currently its utilization has not been maximized compared to its big potential to be used as a natural pH indicator. Butterfly pea flower has a color pigment

called anthocyanin which can be useful as an indicator of changes in pH. Based on research conducted by Vankar & Jyoti (2010), the anthocyanin levels in the butterfly pea flower are 227.42 mg/kg.

Based on this information, the use of butterfly pea flower as a natural dye in indicator films needs to be developed. The presence of a natural dye in indicator film on smart packaging plays a role in detecting the quality of the product directly which is proportional to its color change. The objective of this study was to obtain an indicator film with the best concentration of polyvinyl alcohol (PVA), chitosan, and butterfly pea flower extract as the natural dye, to study the color response of the indicator film on changes in color, pH, thickness, Total Volatile Base Nitrogen (TVBN) of beef, and to calculate the total microbes as a determinant of beef quality.Based on this information, the use of butterfly pea flower as a natural dye in indicator films needs to be developed. The presence of a natural dye in indicator film on a smart packaging plays a role in detecting the quality of the product directly which is proportional to its color change. The objective of this study was to obtain an indicator film with the best concentration of polyvinyl alcohol (PVA), chitosan, andbutterfly pea flower extract as the natural dye, to study the color response of the indicator film on changes in color, pH, thickness, Total Volatile Base Nitrogen (TVBN) of beef, and to calculate the total microbes as a determinant of beef quality.

Materials

The study was conducted from May to July 2019 at the Laboratory of Microbiology and Biochemistry, Department of Food Technology, Faculty of Bioindustry, Trilogi University, Jakarta, and at Testing Laboratory of the Bogor Agricultural Postharvest Research and Development Center.

The materials used in this study were categorized into four types namely the materials to extract butterfly pea flower dye, the materials to make the film/ packaging, the materials for applying the indicator film, and the materials for analysis. The materials used for extracting the color from butterfly pea flowers were butterfly pea flowers obtained froma garden in the Kedung Halang region and distilled water. The materials for making the indicator film were chitosan polyvinyl alcohol (PVA), acetic acid, distilled water, and glycerol.The materials for applying the indicator film were beef obtained from Lenteng Agung market, plastic wrap, and styrofoam. The materials for analysis were aluminum foil, Whatman's filter paper no. 1, Rofa Labolatorium Centre's Vaseline, Nitra Chemical's 7% TCA solution, Rofa Labolatorium Centre K2CO3, Merck's ethanol 97%, Merck's HCL 1.5 N, Merck's HCl 0.02 N, Pudak Scientific's boric acid 3%, Nitra Chemical's bromocresol green (BCG), Pudak Scientific's methyl red (MR), Merck's peptone water (BPW) buffer media, and Merck's plate count agar (PCA).

Tools for extracting the butterfly pea flower were Excalibur dehydrator, Kern analytical balance, Thermo TA288 thermometer, stove, pan, and stirrer. The tools used to make the indicator film are Kern analytical balance, beaker glass, Stuart hot plate, magnetic stirrer, Thermo TA288 thermometer, measuring cup, and plastic mold (size 12x12cm). The tools used for analysis were Memmert incubator, Hirayama autoclave, Agilent Technologies pH meter, Tricle Brand screw micrometer, TCR 200 chromameter, Memmert oven, beaker, mortar and pestle, Bunsen, test tube, petri dish, burette, stative, vortex mixer. ZX3, Erlenmeyer, Conway dish, micropipette, and UV-Vis spectrophotometer.

Methods

Butterfly Pea Flower Extraction (Sinha et al. 2012 modified)

The butterfly pea flowers were first dried using a dehydrator at 60 °C for 1 hour (modified from the previous method without drying), and then the flowers were cleaned and weighted to get 5 grams. In a saucepan, 250 mL of distilled water and 5 grams of flowers were added then bring to 80 °C for 5 to 10 minutes. After extraction, the flowers were separated and the extract was used for the next step.

The Making of Indicator Film (Nofrida 2013 modified)

The indicator film was made by using chitosan-acetate, PVA, and glycerol. The composition used was the combination of 3% PVA (w/v) and 3% chitosan-acetate (w/v) with the addition of glycerol as a plasticizer of 1% (v/v) of the total solution volume. The tested factor was the addition of the dye, using 5, 10, 15, and 20 mL of dye/100 mL of film solution. The dye used was the butterfly pea flower extract.

In the first step, PVA was dissolved with distilled water at 80 °C for 30 minutes using a magnetic stirrer. Next, the chitosan was dissolved in a 1% acetic acid solution. The dissolved PVA solution was added with dissolved chitosan with a volume ratio that can be seen in Table 2. The next step was to add 1% glycerol and then homogenize it by stirring, then add 5, 10, 15, or 20 mL of natural dyes from butterfly pea flower per 100 mL of film solution. The homogeneous film solution was poured into 12x12 cm plastic molds and dried at room temperature (25±3 °C) with a modified time of 48 hours, while research conducted by Nofrida (2013) used 24 hours.

The Application of Indicator Film on Beef Packaging (Octavia 2015 modified)

Beef cutlet of 60 gram was put on styrofoam and covered with cling plastic wrap with 3 x 3 cm of indicator film attached to it on the inside. The beef was then stored at a modified room temperature of (25 ± 2) °C for 48 hours. The storage temperatures in the study by Octavia (2015) were in room temperature of (25 ± 2) °C and cold storage of (4 ± 2) °C. The observation at (25 ± 2) °C was conducted at 0, 8, 24, 32, and 48 hours to observe the color changes of the indicator film.

Analysis Methods

The main research carried out in this study included testing the pH of the butterfly pea flower extract, measuring the anthocyanin content (Less & Francis 1972 in Nofrida 2013), testing the thickness of the indicator film (Nofrida 2013), color analysis of the indicator film (Hunter 1958 in Octavia 2015; Nofrida 2013); The analysis of meat quality degradation includes the pH test of the beef (Mega et al. 2009), the Total Plate Count (TPC) test (BSN 2008), and the Total Volatile Basic Nitrogen (TVBN) test (BSN 2009).

Results and Discussion

Chemical Characteristic of Butterfly Pea Flower Extract

The butterfly pea flower is one of the flowers with the potential as a natural dye source. The extract of butterfly pea flower can be used as a natural dye in the making of indicator film due to its anthocyanin content. The chemical characteristic of butterfly pea flower extract had been analyzed by measuring the pH and its anthocyanin content.

The analyzed butterfly pea flower extract analyzed had a pH value of 5.838 and the produced color was purplish-blue. Analysis of the pH aims to see the degree of acidity of the butterfly pea flower extract. The acidity level of the butterfly pea flower extract can affect the stability of the anthocyanin compound. The results of the pH analysis obtained of the butterfly pea flower extract are in the normal pH range because based on the determinationof the pH route carried out by Nikijuluw (2013) at pH 5 to 7 anthocyanin has stable color as at neutral pH, which was blue so that at that pH it can be used as an indicator film.

The result for anthocyanin content of butterfly pea flower was 218.323 mg/kg. The study by Vankar & Jyoti (2010) obtained higher anthocyanin content which was 227.42 mg/kg. This was due to the difference in the extraction method and the difference in the solvent used. The extraction process of butterfly pea flower used by Vankar & Jyoti (2010) was maceration methods which kept in room temperature in the dark using methanol solvent and acidified using 0.1% HCl, while in this study the method used was hot maceration by applying heat at 80 °C for 5 to 10 minutes using distilled water as solvent.

This study used the hot maceration method because the materials and the technique needed were simple. In addition, the butterfly pea flower is polar so it will be easily dissolved with water in the heating process. The methanol solvent maceration method would get a more concentrated extract color, but the extraction process is quite longbecause of the evaporation process to evaporate the methanol in the solution. In addition, the evaporation process is feared to leave residual methanol which can affect the further analysis process.

Determination of the Best Formulation

The objective of this study was to obtain the best indicator film formulation of the PVA and chitosan composition with the addition of butterfly pea flower extract as a natural dye. Based on the previously determined formulation, the next step was to apply theindicator film. The application was to study the color changes in butterfly pea flower dyed indicator film.

The observation results of indicator film in 48 hours showed that there was a color change in the film with 5 ml butterfly pea flower extract while in the film with an additional of 10, 15, and 20 mL of extract did not show any changes. The more concentration of butterfly pea flower extract in the indicator film, the more vibrant the color and resulted in less observable color change. The five formulations (F1-F5) of indicator film showed that thebest formulation was F1 with the composition of PVA: chitosan of 20:80 (Figure 1).

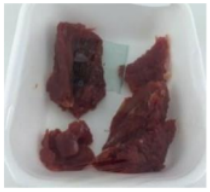
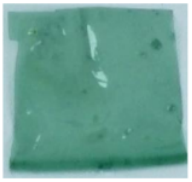
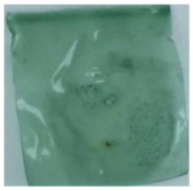
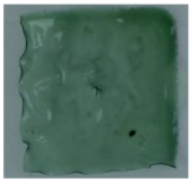
The best formulation which was F1 showed color changes from blue to yellowish-green. The color change in indicator film occurred because of the protein degradationprocess of beef. The result of the degradation process was the volatile base that would evaporate and react with indicator film (Riyanto et al. 2014).

Figure 1. Indicator film with the addition of 5 mL butterfly pea flower extract

Indicator Film Color Change During Storage

The color change of indicator film indicates the quality changes in the product kept inside the smart packaging. The color change in the film occurred because the meat undergoes a decomposition process. As the beef decayed, it produced an unpleasant aroma from the formation of volatile alkaline compounds such as ammonia, dimethylamine, and trimethylamine. Volatile bases were the product of the protein decomposition process into amino acids by bacteria (Iskandar 2014). The gas produced during the decomposition process would interact with the indicator film containing anthocyanins. The anthocyanin compounds in the butterfly pea flower are sensitive to changes in the degree of acidity. This can be indicated by the change in color of the anthocyanin in the butterfly pea extract as the pH change from acidic to alkaline. The volatile compounds produced during the decomposition process are collected in the packaging and cause the pH of the indicator film to change.

Table 1. Color change of indicator film with butterfly pea extract during storage

Duration of Storage (hours)	°Hue Value	Color Range*	Smart Packaging	Indicator Film
0	171,03 ± 4,12	Green		
8	163,84 ± 0,42	Green		
24	151,48 ± 2,20	Yellow to green		
32	151,15 ± 0,53	Yellow to green		

48	137,81 ± 19,31	Yellow to green		

Note: (*) chromatic color range according to Hutchings (1999) in Nofrida (2013)

There was a decline in the °hue value of indicator film after 48 hours of storage. The decline started from hour 0 of 171.03 ±4.12o to hour 8 of 163.84± 0.42o. The °hue value at hour 0 was categorized as green and still green at hour 8. The °hue value continued to decline at hour 24 to 151.48±2.2o, at hour 32 to 151.15±0.53o, and at hour 48 to 137.81±19.3o.

The decline in °hue value of indicator film had a regression equation of y= -0.664x + 169.9 with a strong correlation value (R^2) of 0.978. This showed that the storage time is correlated with the color change of the indicator film. A negative slope value showed a declining graph model during the storage process from hour 0 to hour 48 which can be seen in Figure 2.

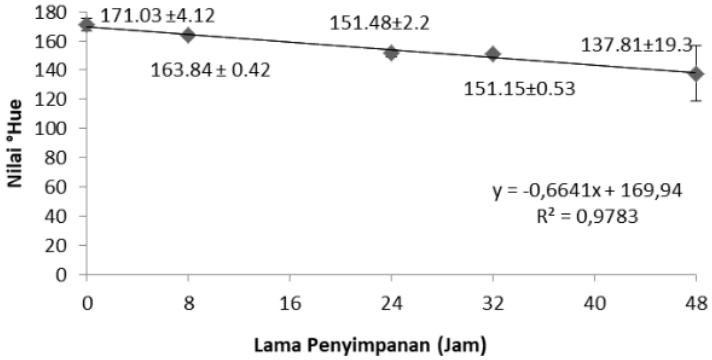


Figure 2 Indicator film °hue value during storage

The total of indicator film color change during storage can be shown with ΔE value by calculating the changes of L*, a*, and b* value from indicator film during storage. The ΔE value obtained (Figure 3) showed that there was a significant increase at hour 0 of 0.7±0.80 to 3.14±1.26 at hour 8, 3.87±0.52 at hour 24, 4.74±0.7 at hour 32, and significantly increase to 7.05±3.55 at hour 48. The regression equation was y= 0.117x + 1.270 with strong correlation value (R2) of 0.937. This showed that duration of storage was correlated with increasing ΔE. A positive slope value showed that the graph model was increasing during the process.

The increasing ΔE value caused the color change of indicator film from green to greenish-yellow for 48 hours. The color change in indicator film during storage showed that the beef underwent a decaying process and produced volatile bases that were interacted with the film.

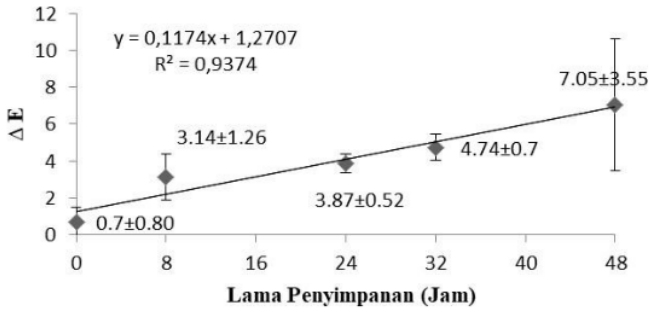


Figure 3. Value of indicator film

Indicator Film Thickness During Storage

The indicator film thickness was measured to observe the changes during storage. The volume of solution and the size of the mold affected the film thickness (Setiautami 2013). When using molds of the same size could produce different thicknesses depending on the solution volume used. The higher the volume, the thicker the film produced.

The indicator film thickness changes during storage were shown in Figure 4. The thickness at hour 0 was 0.171 ±0.042 mm and then kept declining up hour 48 to 0.136±0.043 mm. The indicator film thickness had a regression equation of y = -0.0007 + 0.169 with a strong correlation value (R2) of 0.915. This showed that the storage duration correlated with film thickness. A negative slope value showed that the graph model was declining from hour 0 to hour 48.

The decline in thickness showed that the film was getting thinner. According to Jabbar (2017), the thickness was affected by the film resistance from water vapor, gas, and volatile compound transmission. The thinning of indicator film was caused by the process of water vapor transmission from the product. This process would cause the environmental conditions inside the packaging to become moist so that the indicator film was getting thinner due to interaction with water vapor. In addition, chitosan which was used as the base material for making this indicator film cannot hold water vapor well which causes the film to decompose and causes the indicator film to thin during the storage process (Fehragucci 2012).

Furthermore, Ridhawati (2016) stated that the concentration of plasticizer can affect water vapor transmission. The plasticizer used in this indicator film was glycerol. The addition of glycerol as a plasticizer could increase the permeability of indicator film so that evaporated water could get through the film easily and cause the thinning of indicator film.

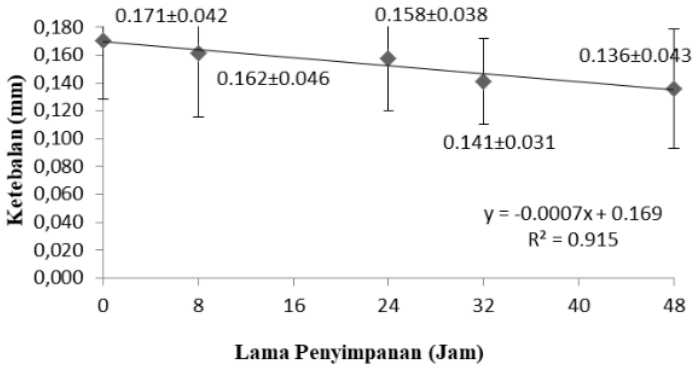


Figure 4. Indicator film thickness during storage

Beef pH Value During Storage

The pH value is an indicator to determine the level of acidity of the beef meat. Analysis of pH became an important factor in determining the quality of beef because the pH value can show the decrease in the quality of stored beef. In addition, analysis of pH in the use of smart packaging was the benchmark for the level of quality changes of beef with changes in the color of the indicator film.

The graph of changes in the pH value of beef is shown in Figure 5. The pH value of beef at hour 0 was 5.761±0.034 and decline to 5.726±0.011 at hour 8. The decline in beef pH was due to the anaerobic glycolysis process that change glycogen into lactic acid (Kurniawan et al. 2014). This process would continue until the glycogen reserves in the meat tissue were depleted. This study was similar to Pangestika (2017) which showed that the pH value of meat decreased at hour 8, from pH 7 to 5.6.

Based on the obtained results, the pH value from hour 8 to hour 48 increased from 5.726±0.011 to 7.540±0.351. The increase in pH value was due to the formation of volatile bases compounds from the decomposition process of protein (Azizah 2015). The increase of pH showed the rigor mortis phase had stopped and had entered the post rigor phase. The post rigor phase is characterized by the formation of aroma and the meat becomes soft again (Anggraeni 2005).

The beef pH value had regression equation of y = 0.039x + 5.597 with strong correlation value (R2) of 0.971. This showed that storage duration is correlated with beef pH value. A positive slope value showed that the graph model was increasing from hour 8 to hour 48.

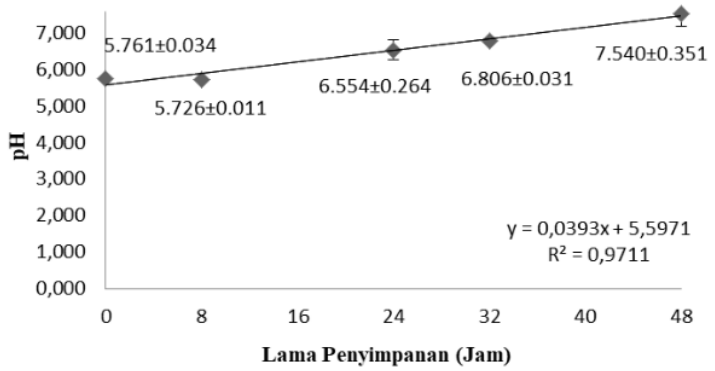


Figure 5. Beef pH value during storage

Beef TVBN Value During Storage

The freshness of beef can be determined by the Total Volatile Base Nitrogen (TVBN) test. The principle of the Total Volatile Base Nitrogen (TVBN) test was to evaporate the volatile base nitrogen such as amino-, mono-, di-, and trimethylamine during storage (Hasnedi 2009). The presence of those compounds caused the unsavory odor of beef during storage at a temperature of 25 °C with RH 50%. The storage temperature affects themicrobial activity which caused the formation of volatile compounds from meat (Heising 2014).

The value of beef Total Volatile Base Nitrogen (TVBN) during 48 hours of storage in temperature of 25 °C and RH 50% showed in Figure 6. The TVBN value increased during storage. The first measurement at hour 0 showed a TVBN value of 16.808±6.496 mg N/100 g, which increased to 30.815±5.602 mg N/100 g at hour 8. The increase of Total Volatile Base Nitrogen (TVBN) continued to hour 48 which was 58.829±10.728 mg N/100 g. The value of beef TVBN had a regression equation of y = 0.829x + 20.64 with a strong correlationvalue (R2) of 0.968. This showed that storage duration was correlated with beef TVBN. A positive slope showed that the graph model increased during storage from hour 0 to hour 48.

The increase of Total Volatile Base Nitrogen (TVBN) was due to the increase in activity of microbes that decompose protein compounds into amino acids which produce volatile base compounds such as ammonia due to the deamination of amino acids during decomposition (Cristiana et al 2007). In addition, trimethylamine (TMA) compounds are produced by the degradation of decomposition bacteria (Jinadasa 2014). The increase in these compounds correlated with the deterioration of beef quality and the odor produced when the meat entered the rotten phase.

Based on the study by Byun et al. (2003), the limit for TVBM for beef was 20 mg N/100g. The obtained TVBN value at hour 8 had already passed the threshold which was 30.815±5.602 mg N/100 g that indicates that beef had already entered the rotten phase and was not suitable for consumption.

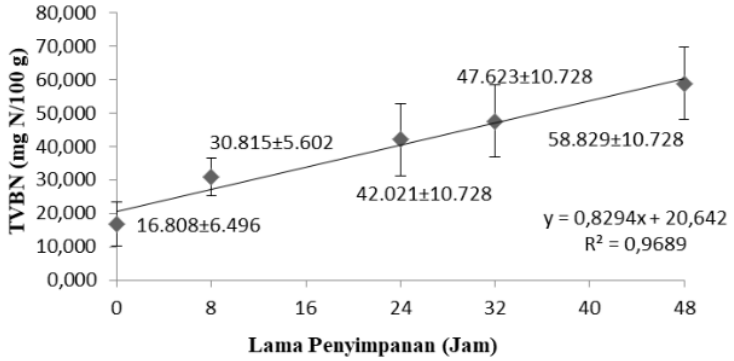


Figure 6 Value of beef TVBN during storage

Beef Total Plate Count (TPC) During Storage

Bacterial activity is responsible for the spoilage of beef during storage. The Total Plate Count (TPC) test was carried out to determine the number of bacteria contained in beef so that the quality of the meat can be determined. The results of the Total Plate Count (TPC) test on beef stored at 25 °C with 50% RH were shown in Figure 7.

The beef TPC value at hour 0 was 5.483±0.067 log CFU/g. The TPC value then increased significantly at hour 8 to 7.338±0.035 log CFU/g. The increase in total bacteria continued to hour 48 of storage which was 10.474±0.196 log CFU/g. Based on that data the regression equation can be obtained, which was y = 0.105x + 6.294 with a strong correlationvalue (R2) of 0.861 (Figure 7). This showed that storage duration was correlated with the number of bacteria on the beef meat. A positive slope value showed that the graph model increased during storage.

An increase in the number of bacteria in beef with increasing storage time indicates a decrease in meat quality (Anggraeni 2012). Parameters that showed the decreasing quality of meat caused by bacteria were changes in color, aroma, texture, formation of a slimy compound, the emergence of gas, and increase in liquid (Dengen 2015). According to SNI-7388-2009, the microbiological requirements contained in beef for consumption should not exceed 1x106 CFU/g or about 6 log CFU/g. The TPC value of beef at hour 8 of storage was 7.338±0.035 log CFU/g, which had exceeded the maximum microbial limit set so that beef was not suitable for consumption anymore because it had been damaged.

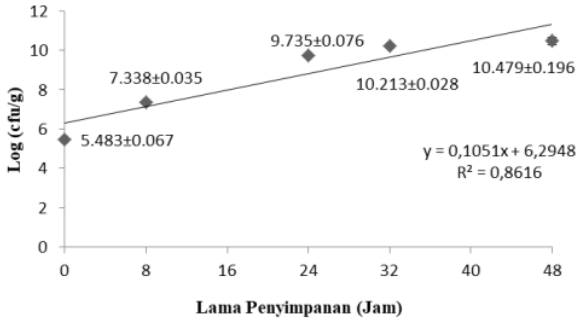


Figure 7. Beef TPC value during storage

Conclusions

Butterfly pea extract can be used as a natural dye in the making of indicator film due to its anthocyanin content of 218.323 mg/kg and pH of 5.838. The best formulation for indicator film was obtained with the composition of polyvinyl alcohol (PVA and chitosan 20:80 with the addition of 5 mL of butterfly pea flower extract. Based on the data obtained the pH of beef increased after 8 hours of storage from 5.726±.011 to 7.540±0.351 which indicates that the beef had already entered the rotten phase. The Total Volatile Base Nitrogen (TVBN) value obtained at hour 8 was 30.815±5.602 mg N/100 g and already exceeded the threshold of 20 mg N/100g. At hour 8 of storage, the beef TPC value was 7.338±0.035 log CFU/g and showed that the number of bacteria had already exceeded the maximum limit of 6 log CFU/g. The TVBN and TPC value at hour 8 of storage showed thatthe beef was not safe for consumption. The application of indicator film on beef packaging showed that there was a correlation between the decline of beef quality with the color change of indicator film. The color change of indicator film in 48 hours of storage was from green to yellowish-green with film thickness changed from 0.171±0.042 mm to 0.136±0.043 mm.

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