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APPLICATION OF EDIBLE COATING OF DURIAN SEED STARCH CHITOSAN COMPOSITES WITH KESUM LEAVES EXTRACTS ON MICROBIOLOGICAL QUALITY AND TVB-N OF BEEF SAUSAGE

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ABSTRACT

The edible coating is one alternative that has the potential to extend the shelf life of sausages. This study aimed to determine the effect of the type of packaging and the storage times of beef sausages at frozen storage. The study using the factorial completely randomized design (CRD) with two factors. The first factor is the type of packaging (K_1: edible coating durian seeds starch-chitosan; K_2: edible coating durian seeds starch-chitosan with kesum leave extract; K_3: plastic LDPE). The second factor is storage time (L_0: 0 months; L_1: 1 month; L_2: 2 months; L_3: 3 months; L_4: 4 months) with four replication. The research variable observed was the sausage quality like TPC (Total Plate Count) and TVB-N (Total Volatile Bases). The result showed that the treatment of type packaging, storage time, and the interaction had a very significant effect on TPC with the lowest value K_3L_0 (2.31 x 10^4 CFU/g) and the highest value K_1L_4 (11.79 x 10^4 CFU/g). And, it had a significant effect on TVB-N with the lowest K_1L_0 (9.19 mg/N/100 g) and the highest value K_1L_4 (50.93 mg/N/100 g). Edible coating of durian-chitosan seed starch with kesum leaf extract can extend the shelf life of beef sausage for four months at freezing temperature based on the TPC value of 9.65.10^4 cfu/g and TVB-N value 23.96 mg N/100 g.

Key words: Edible coating; kesum leave extract; TPC; TVB-N.

INTRODUCTION

Sausage is a processed meat product that has high nutritional value. Based on SNI 01 3820-1995, sausages contain a minimum of 13% protein, a maximum of 25% fat, and a maximum of 8% carbohydrates. High nutrition is a factor that supports the growth of microorganisms in meat and processed meat products. Meat and processed products contaminated with microbes that exceed the threshold will experience physical and chemical damage. In addition to damage by microorganisms, sausages can also be damaged due to fat oxidation during the storage process. Fat oxidation during sausage storage can result in rancidity in food products to reduce product quality and freshness of sausage products. One alternative to maintain the quality and extend the shelf life of sausage products is to use edible coatings

The edible coating is a thin layer made of edible material formed over food components (coated on the surface of the packaged material), which functions as a mass transfer inhibitor (moisture, oxygen, fat, and solutes) and or as a carrier for food ingredients or additives, and or to improve food handling (Krochta and Johnston, 1997). The use of edible coatings has the potential to reduce the use of synthetic packaging materials. The main ingredient components of edible coatings are hydrocolloid compounds, a group of polysaccharides. This is because polysaccharides can protect against gas seepage, and the flavor is quite good (Julianti, 2006). One of the potential ingredients for making edible coatings from the polysaccharide group is starch.

Starch is one type of polysaccharide that is abundantly available in nature, is biodegradable, easy to obtain, and inexpensive. Starch can be obtained by extracting from plants rich in carbohydrates such as sago, cassava, corn, wheat, and sweet potatoes, and can also be removed from fruit seeds such as jackfruit seeds, avocado seeds, and durian seeds. Durian seeds that have been considered waste by humans due to lack of it turn out to be used as an ingredient in the manufacture of bioplastics. One of the starches that can be used in the manufacture of edible coatings is durian seed starch. Seed Durian has enough starch content high so that it has potential as an alternative substitute for materials that require properties starch (Cornelia et al., 2013). Durian seeds can be used as low-cost source of hydrocolloids at different kinds of food (Sistanto et al., 2017). Seed starch Durian has similarities with flour tapioca, which has a high starch content of amylose and amylopectin. However, durian seed starch has a higher amylose content than tapioca starch. The amylose content in durian seed starch is 26%, while the amylose in tapioca starch is 20% (Wirawan et al., 2016). However, its hydrophilic nature causes edible coatings from starch to be not an excellent barrier to water vapor. Therefore, it needs to combine with more hydrophobic materials, one of which is chitosan.

Chitosan is a biopolymer from D-glucosamine that is produced from the deacetylation of chitin using strong alkali. The ability of chitosan to inhibit bacterial growth and has the advantage of being biodegradable. This ability makes chitosan an alternative material in product packaging

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to maintain quality and extend durability and coating (edible coating) on processed livestock products, namely sausage. The edible coating made from chitosan composite durian seed starch and kesum leaf extract has good characteristics in minimizing gas exchange (Lestari et al., 2020).

Edible coating of durian seed starch composite chitosan as sausage coating material can be improved its functional properties by adding certain natural ingredients that have antimicrobial and antioxidant activity as preservatives. One of the natural ingredients that contain antimicrobial compounds is kesum leaves. Kusum leaf (Polygonum minus Huds) is an endemic plant of West Kalimantan, which has a distinctive taste and aroma.

The local community knows this plant as a spice Bubur Padas, a typical West Kalimantan food. Based on the results of phytochemical screening research, the methanol fraction of kesum leaf extract contained phenolic compounds, terpenoids-steroids, flavonoids, and alkaloids (Wibowo, 2009). The major combinations of kesum leaves, according to (Baharum *et al*., 2010) consist of dodecanal (43.47%), decanal (16.263), and 1-decanol (12.68%). Wibowo (2009) has investigated the antimicrobial bioactivity of kesum essential oil obtained from the methanol and diethyl ether fractions of the methanol extract of kesum leaves capable of inhibiting the growth of E. coli and Bacillus subtilis bacteria.

The edible coating composite of durian seed starch and chitosan with kesum leaf extract applied to sausages has never been studied. Therefore, this study aims to determine the effect of the beef sausage packaging on microbiological quality and the impact of frozen beef sausage storage time on microbial quality. The combination of edible coating of durian seed starch and chitosan with the addition of kesum leaf extract as an antimicrobial is expected to improve the quality and extend the shelf life of sausages.

MATERI AND METHODE

Material

The tools used are analytical balance, Erlenmeyer (Pyrex), measuring cup, hot plate stirrer (IKA C-MAG HS7), stirring rod, test tube, Conway dish, petri dish (Pyrex), beaker (Pyrex), autoclave (GEA), stove, wire loop, bunsen lamp, cotton, matches, pestle and mortar, micropipettes (Eppendorf, Germany), oven (Memmert UN 55 Universal Oven, Germany), laminar airflow (Biobase BBS-V1800), incubators (Memmert IN 55 Universal Incubator, Germany), evaporators (Rotary Evaporator IKA RV 10 Digital V, Germany), tweezers, refrigerators, and gloves. The materials used were chitosan, durian seed starch, kesum leaves, glycerol (Merck, Germany), ethanol (Merck, Germany), distilled water, PCA (Plate Count Agar) media (Merck, Germany), filter paper (Whatman®), physiological salt, aluminum foil, label paper, and silica gel.

Methode

The study used an experimental Completely Randomized Design (CRD) factorial pattern with two treatment factors. The first factor is the type of sausage packaging consisting of 3 levels: K_1: Durian seed starch edible coating packaging-chitosan, K_2: Durian seed starch edible coating packaging chitosan-kesum leaf extract, and K_3: LDPE plastic packaging. The second factor is storage time consisting of 5 levels, namely: L_0: 0 months of storage, L_1: 1 month of storage, L_2: 2 months of storage, L_3: 3 months of storage, and L_4: 4 months of storage. Each combination is repeated four times.

Durian seeds starch preparation

The manufacture of durian seed starch refers to the method (Lestari *et al*., 2020). Durian seeds were clean from the outer sheath and the epidermis. Durian seeds were cut and weighed 500 g and after addition of 1 L water were blended with a food processor (Panasonic MK-F800, Indonesia) for 5 min (ratio durian seeds and aquadest 1:5). After storage for 24 h and decantation, the durian seed homogenate was filtered using Whatman® filter papers, and then the durian seed homogenate was deposited in 1 L of water for 3 d, replacing the settling water every 24 h. On the fourth day, the water was removed by filtering using Whatman® and the starch slurry was separated. The starch slurry was dried in oven (Memmert UN 55 Universal Oven, Germany) at 50 °C for 24 h until moisture content was 12 %. The dried starch deposits were crushed using a Panasonic MK-F800 food processor for 1 min and mesh sieved with size 10.

Kesum leaf extracts preparation

Kesum leaf extract is made by extraction using the maceration method, referring to the (Lestari *et al*., 2020), Kesum leaf extracts were obtained by using a maceration method with ethanol. Fresh chopped kesum leaves (± 0.5 cm) were weighed. They were soaked with 96 % ethanol (Merck, Germany) at room temperature for 24 h. The ratio between materials and solvent was 1:50. The filtrate was obtained from the pulp, then it was macerated with ethanol for 24 h during 4 x 24 h. Separation of the solvent from filtrate was conducted using rotary vacuum evaporator (Rotary Evaporator IKA RV 10 Digital V, Germany) in 100 rpm at temperature 80°C and the process was terminated after the ethanol was entirely evaporated.

Edible coating preparation

The edible coating was produced by used (Ban *et al*., 2006) method with modifications. The edible film was manufactured from ingredients containing 3% of durian seeds starch, 0.8% of chitosan, 0.6 % of glycerol, and 120 mL of distilled water. Chitosan was dissolved into 10% acetic acid, and durian seed starch and chitosan were weighed and dissolved into 120 mL of distilled water. The solution was heated at temperature 70°C and stirred using a hotplate magnetic stirrer until it reached the gelatinization and then added glycerol until homogenous. After the solution reached cold condition, the kesum leaf extracts in different concentration was added (0 % (K0); 0.2 % (K1); 0.4 % (K2); 0.6 % (K3); 0.8 % (K4); 1.0 % (K5) and 1.2% (K6) the solutions were then mixed. The edible coating solution is ready to be applied as a sausage coating.

Edible coating aplication on sausage

Application of edible coating with dyeing method beef sausage on edible coating. The dyeing beef sausage on the edible coating twice for 1 min until the edible coating distributed all surface sausage. Then the sausages beef is hung and dried in a drying box. The beef sausage was packed in LDPE plastic with a thickness of 0.8 mm and stored at freezing temperature (-10 ± 2°C) for four months.

Total Plate Count (TPC)

Total bacteria were analyzed using the Total Plate Count method based on (Fardiaz S, 1993) and (Yusuf *et al*., 2016), modified. Samples of beef sausage were crushed as much as 25 grams and put into 225 ml of sterile 0.1% peptone water buffer so that a dilution of 10^{-1} (P1) was obtained. 1 ml suspension from P1 was transferred with a sterile pipette into 9 ml of sterile 0.1% peptone water buffer medium to obtain a dilution of 10^{-2} (P2), in the same way, the dilution was carried out up to a dilution of 10^{-10} (P10).

Furthermore, from each dilution of 10^{-4}, 10^{-5}, and 10^{-6}, 1 ml was taken to be put into a sterile petri dish with a Duplo system, then 10-15 ml of Plate Count Agar (Merck, Germany) medium was poured into making it sterile and homogenized by sliding the cup.

Horizontally or form a figure eight and allowed to solidify. The next step was incubated (Memmert IN 55 Universal Incubator, Germany) at 37°C with the cup upside down for 24-48 h. Colony counts were carried out using a colony counter.

Total Volatile Base Nitrogen (TVB-N)

Total Volatile Base on beef sausage was calculated using Kjeldahl distillation (Huang *et al*., 2014) and (Amongsari *et al*., 2020) with modification. A total of 10 g of the sample was crushed and put in a glass beaker containing 100 mL of distilled water. The sample was allowed to stand for 30 min with stirring every 10 min, and then the solution was filtered through filter paper. 5 mL of the filtrate was alkalized by adding 5 mL of MgO 10 g/L. The solution was then distilled using Kjeldahl distillation for 5 min.

The distillate was absorbed with 10 mL of boric acid 20 g/L to which 5-6 drops of methyl red methyl blue (MMMB) indicator had been added and titrated with 0.01 N HCL until a purplish-blue color changed. where V1 is the titration volume for the tested sample (mL), V2 is the titration volume of blank (mL), and c is the actual concentration of HCl (mol L1), m is the weight of ground pork sample (g).

$$\text{TVB-N (mg/100g sample)} = \frac{(V1-V2) x\ c\ x\ 14}{m\ x\ 5/100} \text{ x } 100$$

Data analysis

The research data obtained were analyzed using analysis of variance (ANOVA). If there is a differencein effect between treatments, then proceed with Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

TVB-N-N (*Total Volatile Base Nitrogen*)

Based on analysis of variance (ANOVA), show that the type of packaging, storage time, and the combination of packaging types – the length of storage of beef sausage have a very significant effect on TVB-N levels ($p<0.01$). The average value of TVB-N in various types of packaging is in the range of 14.11 (K_3) – 30.70 (K_1), for various storage periods, it is in the range of 9.39 (L_0) – 31.56 (L_4), while the interaction between types of packaging – duration storage range 9.19 (K_1L_0) – 50.93 (K_1L_4).

Figure 1 shows that the TVB-N value of beef sausage during frozen storage has increased both in edible coating packaging without EDK (K_1), edible coating with EDK (K_2), and LDPE plastic packaging (K_3).

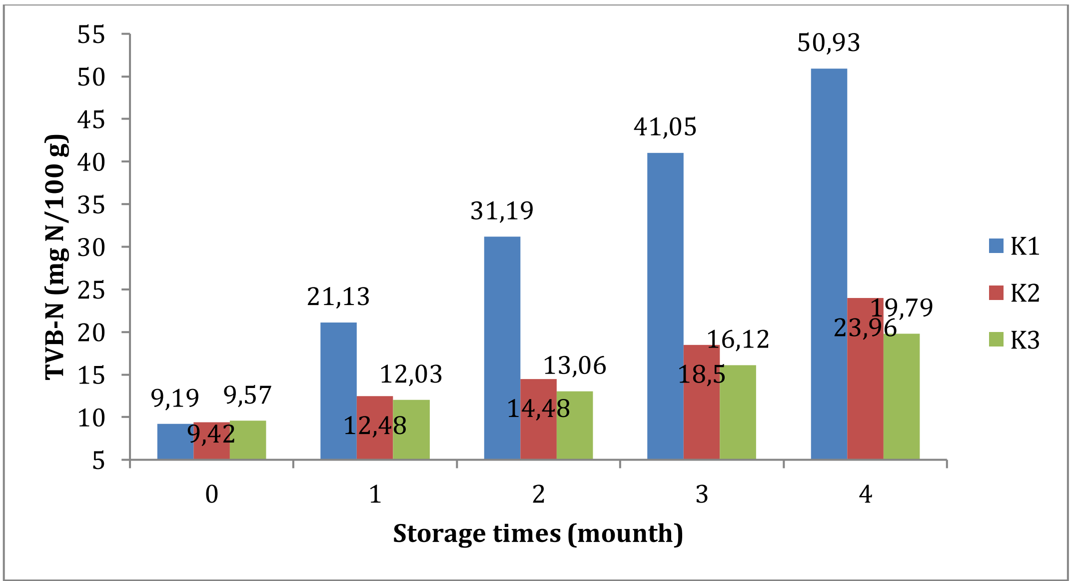

Figure 1. Effect of packaging type on TVB-N at various ages storage

The TVB-N value of edible coating packaged sausages without EDK (K_1) increased from 9.19 to 50.93 mg N/100 g, the TVB-N value of edible coating packaged sausages with EDK (K_2) increased from 9.42 to 23.96 mg N/100 g, the TVB-N value of synthetic plastic packaged sausage (K_3) increased from 9.57 to 19.79 mg N/100 g. The results of testing every month during storage, the TVB-N value of sausage packaged with the edible coating without EDK (K_1) was significantly higher than sausage packaged with edible coating with EDK (K_2) and synthetic packaging (K_3). The increase in TVB-N values is related to the activity of proteolytic enzymes (produced by bacteria) in breaking down proteins into simple nitrogen compounds and breaking down trimethylamine oxide to trimethylamine (Darmawati et al., 2021). The TVB-N value of edible coating packaging with EDK (K_2) is almost the same as the TVB-N value of synthetic packaged sausage (K_3). Adding kesum leaf extract to the edible coating can inhibit damage to beef sausage.

The maximum TVB-N value limit contained in the ingredients is 30 mg/100 g, the freshness value of meat and its processed products is <15 mg/100 g for fresh meat, 15–30 mg/100 g for rotting meat, and >30 mg /100 g for rotten meat (Xiao et al., 2014). Overall, sausages with edible coating packaging with EDK (K_2) and synthetic plastic packaging (K_3) have TVB-N values that have not passed the maximum limit until the fourth month. In contrast, the TVB-N value of edible coating packaged sausages without EDK (K_1) has exceeded the maximum limit in the second month.

TPC (*Total Plate Count*)

Based on the study results, it was shown that the type of packaging treatment, storage time, and combination of packaging types - storage time on beef sausage had a very significant effect on TPC ($p<0.001$). The average value of TPC for various types of packaging is in the range of 5.62 x 10^4 cfu/g (K_3) – 6.85 x 10^4 cfu/g (K_1), for various storage periods in the range of 2.44 x 10^4 cfu/g (L_0) – 10.31 x 10^4 cfu/g (L_4), while the interaction between the type of packaging and storage time ranged from 2.31 x 10^4 cfu/g (K_3L_0) – 11.79 x 10^4 cfu/g (K_1L_4).

At the beginning of storage, the number of microbes in each sausage was still relatively low, ranging from 2.65 to 2.37x10^4 cfu/g. This is because the beef sausage has undergone a heating process during processing so that the microbes contained in the sausage meet the limits of SNI 01-3820-1995, which is 1x10^5 colonies/g or 5 log cfu/g.

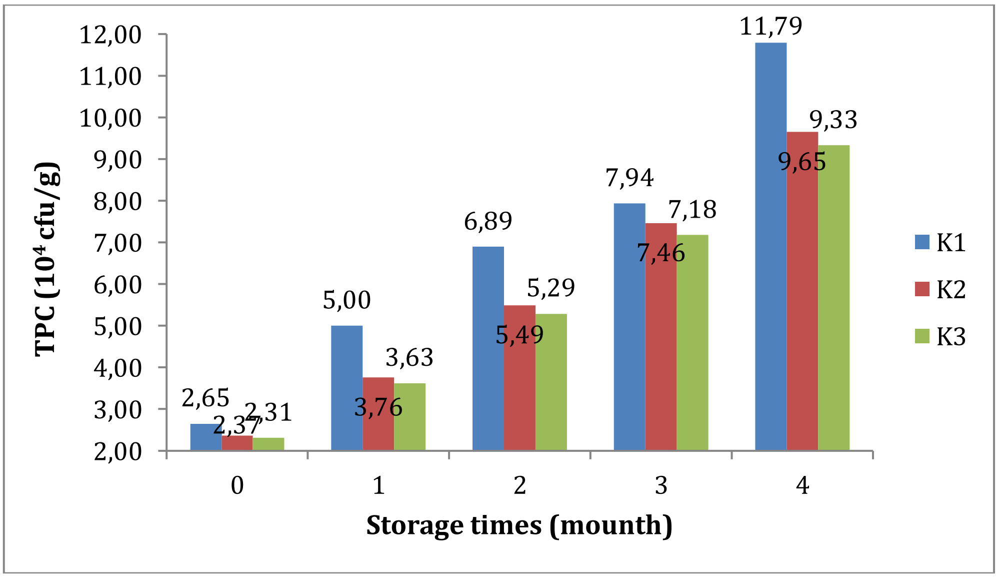

Figure 2. Effect of packaging type on TPC at various ages storage

In Figure 2, the number of bacteria in the three treatments of beef sausage increased with the length of storage time. In the four month, the number of microbes ranged from 9.33 to 11.79 x 10^4 cfu/g. The average TPC value of the K_1 treatment was 11.79x10^4 cfu/g, K_2 of 9.65 x 10^4 cfu/g, and K_3 of 9.33 x 10^4 cfu/g. The results of this study indicate that the edible coating treatment with EDK (K_2) and synthetic plastic (K_3) in the four month of storage still meets the SNI standard. In contrast, the edible coating treatment without EDK (K_1) in the four month of storage does not meet the SNI requirements.

The fluctuating pattern in Figure 2 from the 0 to 3 months of storage is the same as the study (Bhattacharyya and Sinhamahapatra, 2013) on duck meat sausage samples using vacuum and aerobic packaging. The increase in TPC value until the end of the 4th month of storage is probably due to the presence of residual bacteria that have gradually adapted to freezing temperatures (Utami, et al, 2014). The leading causes of food spoilage are microbial growth, enzyme activity, and chemical changes. Damage to food by microbes can cause food and beverages to be unfit for consumption due to a decrease in quality or toxic food. According to Buckle et al (1987) The meat will look slimy, smell bad, and spoil or not suitable for sale if the number of bacteria increases to 10^7–10^8 CFU/cm^2. Based on this, it means that beef sausage in all treatments, both edible coating without EDK, edible coating with the addition of EDK, and synthetic plastic during four months of storage, has decreased in quality but is not considered rotten.

CONCLUSION

Based on the study results, the application of the edible coating on sausages during frozen storage shows that the type of packaging, storage time, and combinations have a very significant effect on the Total Volatile Base Nitrogen (TVB-N) and Total Plate Count. The lowest average TVB-N value for edible coating without kesum leaf extract at 0 months storage (K_1L_0) is 9.19 mg N/100 g, while the highest for edible coating without kesum leaf extract at four months storage (K_1L_4) is 50.93 mg N/100 g. The lowest average TPC value in synthetic plastic at 0 months storage (K_3L_0) was 2.31x10^4 cfu/g, while the highest was in edible coating without kesum leaf extract (K_1L_4), which was 11.79x10^4 cfu/g. Edible coating of durian-chitosan seed starch with kesum leaf extract can extend the shelf life of beef sausage for four months at freezing temperature based on the TPC value of 9.65.10^4 cfu/g and TVB-N value 23.96 mg N/100 g.

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