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Application of edible coating made of rabbit bone Gelatin and Gambir (*Uncaria gambier* Roxb.) extract for improving the shelf life of beef sausages

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ABSTRACT
The increase in rabbit breeding and consumption would result in bone waste that leads to environmental the accumulation and pollution. Rabbit bones can be used as an alternative source of gelatin, which can used as an edible coating material. Edible coatings can be applied to beef sausage, which is prone to deterioration. The addition of gambir extract can act as an antioxidant and antibacterial agent to maintain the quality of beef sausage during storage. This study aimed to determine the effect of gambir extract on an edible coating made of rabbit bone gelatin and its application to beef sausage during storage. The results showed that the addition of gambir extract at concentrations of 0.1%, 0.2%, and 0.3% increased the antioxidant activity in the edible coating with a range of 21 – 50%RSA. In addition, the gambir extract was also able to inhibit the growth of *E. coli* and *S. aureus*. Edible coatings were applied to beef sausages stored at 4 – 7 °C for 15 days. Based on the results of hardness, pH, total plate count, peroxide value, and thiobarbituric acid number test of beef sausage, it was shown that the application of an edible coating of rabbit bone gelatin containing gambir extract was better in maintaining the quality of beef sausage during storage. Sensory evaluation was performed on beef sausage on day 0 using 9 trained panelists. The sensory attributes tested were gambir odor, gelatin odor, bitter taste, and sour taste. Sensory evaluation using a difference test revealed no significant difference ($p>0.05$) between sausages without coating and those coated with the film containing 0.3% gambir extract.

Keywords:
Application of Edible Coating, Rabbit Bone Gelatin, Gambir Extract, Shelf Life, Beef Sausages.

Introduction

Currently, rabbit meat is produced and consumed regularly by people in continents, such as Africa, America, Asia, and Europe (Siddiqui et al., 2023). According to the statistics of the Food and Agriculture Organization of the United Nations (FAOSTAT), the total world production of rabbit meat will reach 899.726 tons in 2020. The high number of rabbit meat products will increase the waste produced. One of the wastes from rabbit meat production is bones. Bone waste is a global problem that, if not properly managed, will lead to an accumulation of waste and environmental pollution. One of sustainable development goals is ensuring responsible production and consumption. Therefore, the alternative would be to use waste in a circular economy approach. Rabbit bone has the potential to be used as a source of gelatin to increase its value because it contains the amino acids glycine, proline, and hydroxyproline (Li et al., 2021; Wulandari et al., 2022).

Gelatin is preferred in many applications due to its clarity and bland taste, such as confectionery, meat products, fruit juices, and coatings for fruits and meats (Evans, 2019). According to Ciannamea et al. (2018), its high availability and biocompatibility of gelatin make it suitable for use as food packaging. The use of edible coatings on food products can limit the growth of pathogenic microorganisms, slow lipid oxidation, and prevent moisture loss to extend shelf life (Cardoso et al., 2016; Umaraw et al., 2020). Gelatin edible coatings can be applied to frozen foods due to their ability to form a gel and insoluble in cold water, dissolving only at temperatures above room temperature.

Meat sausage is a frozen meat product with a high nutritional value that is susceptible to both oxidative and microbiological damage. Oxidative damage occurs due to processing and storage, while microbiological damage can be caused by pathogenic microbes such as *E. coli*, *Salmonella sp.*, and *Staphylococcus sp.* (Lestari et al., 2020). An alternative to maintain the quality and extend the shelf life of beef sausage is the use of edible coatings containing antioxidants.

Gambir is a type of plant that exhibits antioxidant activity, as indicated by IC_{50} of 27.4 µg/mL in distilled water solvent (Apea-Bah et al., 2009). The antioxidants in gambir are safe for consumption (Anggraini et al., 2011). Research on the addition of gambir extract to pure polyvinyl alcohol-based edible film can increase tensile strength, toughness, and decomposition temperature (Abral et al., 2022). The addition of gambir extract to edible coatings of rabbit bone gelatin and applied to beef sausage has the potential for further research. Previous research conducted by Wulandari et al. (2022) only investigated the extraction and characterization of rabbit bone gelatin. Therefore, this research focuses on the utilization and development of rabbit bone gelatin to be used as an edible coating material with the addition of gambir extract and applied to beef sausage.

Methods

The materials used in this study include rabbit ribs from *Rex* and *New Zealand* (NZ) breeds (Magetan, East Java, Indonesia), gambir powder (CV. Rakutken, North Sumatra, Indonesia), glycerol (Progo Mulyo, Yogyakarta, Indonesia), hydrochloric acid (HCl) 37% (Merck, Darmstadt, Germany), ethylenediaminetetraacetic acid (EDTA) (Smart Lab, Tangerang, Indonesia), and beef (Oricow, Yogyakarta, Indonesia).

Extraction of Gelatin from Rabbit Bone

Gelatin extraction was a modification of the study Wulandari et al. (2022). Rex and NZ rabbit bones were boiled at a temperature of 100 °C for 60 minutes. The bones were then cleaned of flesh and dirt, washed with running water, and cut into 1 – 2 cm. Furthermore, the bones were soaked in a 6% HCl solution with a ratio of bone to solution of 1:3 for four days, and the solution was replaced on the second day of soaking. The bones were washed with running water until the pH reached 6 – 7. The bones were rehydrated in 0.25 M EDTA solution with a 1:2 ratio of solution to bone for two days. The bones were rewashed with running water and finally rinsed with distilled water. The bones were then extracted at three different temperature levels (65 °C, 75 °C, and 85 °C) with distilled water at a 1:2 ratio of bone to distilled water. At each temperature step, the bones were extracted for 4 hours, and different distilled water was used at each step. The gelatin solution obtained at each stage was filtered through a 200 mesh sieve and dried in a food dehydrator at 50 °C for 16 hours. The gelatin sheets were ground using a blender to obtain gelatin powder. The ratio of gelatin from *Rex* and New *Zealand* rabbit bones was 3:1.

Preparation of Edible Coating Solution with Addition Gambir Extract

The preparation of the edible coating started with the extraction of gambir powder, which was a modification of the research Kassim et al. (2011). Gambir powder was weighed 0.05 g and dissolved in 50 ml of 80 °C distilled water. The solution was macerated for 30 minutes with periodic stirring. The solution was then centrifuged at 2000 rpm for 30 minutes. The supernatant was stored at 4 °C.

The process of preparing the edible coating solution containing gambir extract was a modification of the research Hamann et al. (2022). Gelatin powder was weighed 3.6 g and dissolved in 30 mL of distilled water. The gelatin solution was heated at a temperature of 60 °C for 30 minutes while stirring with a magnetic stirrer. The gelatin solution was then cooled to 40 °C and glycerol was added at 30% of the weight of the gelatin powder. Stirring was continued for 5 minutes. Next, 1 mL, 2 mL, and 3 mL of 1% concentration gambir extract were added to obtain an edible coating solution containing gambir extract concentrations of 0.1%, 0.2%, and 0.3%, respectively. The edible coating solution was stirred with a magnetic stirrer for 5 minutes to obtain an edible coating solution containing gambir extract.

Antioxidant Activity

The analysis of antioxidant activity was modified from the method of Oliveira et al. (2023), which is based on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging by antioxidants, causing a decrease in absorbance at 515 nm. For each edible coating solution, before mixing with DPPH, the pH was reduced to 1.5 to prevent clumping by adding the HCl solution. Then, 0.1 mL of the edible coating solution was added to a test tube. Then, 3.9 mL of 1 mM DPPH dissolved in methanol was added to the test tube containing the edible coating solution. The solution was homogenized by vortexing and stored in a dark room for 30 minutes. The solution was then read using a spectrophotometer at a wavelength of 515 nm. A blank solution of DPPH and methanol without a sample was used. 100 ppm ascorbic acid was used for comparison.

Antibacterial Activity

Antibacterial activity analysis was performed to determine the ability of gambir extract to inhibit the growth of gram-negative and gram-positive bacteria. The method used was a modification of the study Bubonja-Šonje et al. (2020). The antibacterial growth inhibition zone test was performed using the well diffusion method with *Escherichia coli* and *Staphylococcus aureus* bacteria in the concentration of 10^5 – 10^6 CFU/mL. The inoculum of bacterial strains in the nutrient broth medium was diluted to obtain a bacterial count of 10^5 – 10^6 CFU/mL. The bacterial culture was then spread on nutrient agar media in a petri dish using the spread plate method. A well was then created by making a hole in the agar using a 1 mL micropipette. The edible coating solution was added to the well. The petri dish was then incubated at 37 °C for 24 hours. Chloramphenicol 0.1% was used as a positive control.

Scanning Electron Miscroscopy

The microscopic morphology was studied using a scanning electron microscope (SEM) (Isopencu et al., 2021), with modifications. An edible coating solution of 30 mL was poured into a 13 x 8.5 cm container and dried in a food dehydrator for 12 hours at a temperature of 50 ^{o}C. The SEM was operated at 5.0 kV.

Application of Edible Coating with Gambir Extract on Beef Sausages

The application of the edible coating began with the production of beef sausage. Diced beef of 500 g was placed in a food processor with 200 g of ice cubes and stirred at medium speed for 10 minutes. Then 120 grams of tapioca flour, 50 grams of vegetable oil, 10 grams of salt, 5 grams of sodium tripolyphosphate, and powdered spices such as 5 grams of garlic, 2.5 grams of pepper, 2.5 grams of ginger, 2.5 grams of nutmeg, and 2.5 grams of coriander were added. It was stirred again for 10 minutes. The mixture was then placed in a cellulose sausage casing. After tying the ends of the sausage with food-grade thread, the sausage was steamed for 10 minutes. The half-cooked beef sausage was drained until cold and removed from the casing. The application of the edible coating on beef sausages was done by dipping the beef sausages into the previously prepared edible coating solution, namely the edible coating solution without the addition of gambir extract and the edible coating solution containing 0.1%, 0.2%, and 0.3% gambir extract. Beef sausages without edible coating treatment were used as controls. The beef sausages were dipped in the edible coating solution for 1 minute and then placed in a refrigerator at a temperature of 4 – 7 °C for 5 minutes. The beef sausages were then dipped in the edible coating solution again for 1 minute. Repeated dipping was performed to ensure that the entire surface of the beef sausages was coated with the edible coating solution. The beef sausages were then placed in a plastic container with a lid and stored in a refrigerator at a temperature of 4 – 7 °C for 15 days. Observations and tests of beef sausages were carried out on days 0, 5, 10, and 15 of storage.

Hardness

The hardness of beef sausage during storage was measured using a texture analyzer (TA). The beef sausage was placed in the center of the TA table. The TA needle was then slowly lowered (1 mm/s) until it penetrated the beef sausage. All procedures were performed automatically by the TA and test results were obtained as Fmax data, which is a measure of hardness (N). All tests were performed in triplicate (Shin & Choi, 2021).

pH

The pH of beef sausage during storage was determined by grinding 3 g of beef sausage with 27 mL of distilled water. The sample was then read with a pH meter to obtain the pH value (Hamann et al., 2022).

Total Plate Count

Microbiological analysis of beef sausage during storage using the total plate count (TPC) method. Beef sausage of 5 g was mixed with 45 mL of 0.85% NaCl solution and then ground with a mortar and pestle. A 1 mL sample was taken and diluted to 10^{-4} for microbial enumeration and differentiation. After plating on a petri dish using the pour plate method, the petri dish was incubated at 37°C for 48 hours and the results were determined in log10 CFU/g (colony forming units per gram) (Fallah et al., 2021).

Peroxide Value

The peroxide value of beef sausage during storage was modified from the study by Cesa et al. (2012), which refers to ISO 3960:2007. The minced beef sausage was weighed 1 g and placed in a beaker. Then 20 mL of glacial acetic acid solvent and chloroform (2:1) and 1 mL of 70% saturated potassium iodide were added. The sample was incubated for 5 minutes in a dark room. The sample was then filtered through filter paper. The resulting filtrate was mixed with 20 mL

Aquadest and 1 mL 1% starch indicator. The sample turns blackish blue. The sample was then titrated with 0.01 N sodium thiosulfate ($Na_2S_2O_3$) until the sample became clear.

Thiobarbituric Acid Reactive Substances (TBARS)

The TBARS analysis of beef sausage during storage was a modification of the study Katsanidis and Zampouni (2023). Beef sausage of 10 g was added to 50 ml of distilled water and then crushed with a mortar and pestle. The sample was then placed in a distillation flask and rinsed with 47.5 mL of distilled water. HCl 4 M 2.5 mL was added to the distillation flask. The distillation flask was heated until 50 mL of distillate was obtained. The distillate obtained was stirred until evenly distributed and 5 mL was transferred to a closed test tube. Then 5 mL of 0.02 M TBA reagent was added. The test tube containing the sample was heated in boiling water for 35 minutes. The blank was prepared using 5 mL of distilled water and 5 mL of TBA reagent with the same treatment as the sample. After heating, the test tube was cooled and the absorbance of the sample (D) was measured at a wavelength of 528 nm with the blank solution as the zero point. TBA values are expressed in mg malondialdehyde/kg sample.

Sensory Evaluation

Sensory evaluation was performed on beef sausage on day 0 using 9 trained panelists. The sensory attributes tested were gambir odor, gelatin odor, bitter taste, and sour taste.

Statistical Analysis

Data were analyzed using a completely randomized design and compared using one-way and two-way ANOVA with Duncan's multiple range test at a significance level of $p \leq 0.05$ using SPSS software version 27. All experiments were performed in at least three replicates.

Results and Discussions

Antioxidant Activity

Table 1. Antioxidant activity of edible coating

Concentration of gambir extract (%)	Antioxidant activity (%)
0	9.01 ± 0.67^{a}
0.1	21.95 ± 0.64^{c}
0.2	35.99 ± 0.86^{d}
0.3	50.51 ± 0.44^{e}
Ascorbic acid 0.01	18.89 ± 0.02^{b}

Data are expressed as mean±standard deviation (n=3). Lowercase differences indicate statistical differences in one-way ANOVA with Duncan's test ($p \leq 0.05$) between treatments on the same parameter.

The antioxidant activity test method used in this study employs the DPPH method, which aims to determine the ability of edible coating solutions containing gambir extract to scavenge free radicals. The antioxidant activity of the edible coating solution is shown in Table 1. Edible coating solution without gambir extract was used as a control and 0.01% ascorbic acid solution as a comparison. Based on Table 1, it is known that the higher the concentration of gambir extract in the edible coating solution, the higher the antioxidant activity. The high and low antioxidant activity can be caused by the amount of gambir powder filtrate in the matrix (Santoso et al., 2019).

Gambir extract is a natural antioxidant whose antioxidant properties come from its rich content of phenolic compounds. The most abundant phenolic component found in gambir extract is catechin (Munggari et al., 2022). The results also showed that the edible coating solution without gambir extract had antioxidant activity, although it was very low. Research by Azizah et al. (2023) stated that gelatin naturally has antioxidant activity due to several amino acids such as glycine and proline in the peptide bond formed during film formation.

Antibacterial Activity

The antibacterial activity test using the well diffusion method was designed to determine the ability of the edible coating solution with the addition of gambir extract to inhibit the growth of *E. coli* and *S. aureus* bacteria. The antibacterial activity of the edible coating solution is shown in Table 2. Edible coating solution without gambir extract was used as a control and 0.1% chloramphenicol solution was used for comparison. Based on Table 2, it is known that the edible coating solution without or with the addition of gambir extract can inhibit the growth of *E. coli* and *S. aureus* bacteria. This inhibitory ability may be due to secondary metabolites, including polyphenols, contained in the catechins in gambir extract (Abral et al., 2022). Catechins can damage the plasma cell membrane, which causes the loss of intracellular components, resulting in leakage of cell components and bacteria will die (Monica & Husna, 2022). The antibacterial activity of the edible coating solution without the addition of gambir extract may be due to the presence of oligopeptide chains from gelatin hydrolysis, which are thought to have antibacterial activity due to the presence of side-chain amino groups, namely hydrophobic or nonpolar aliphatic R groups such as glycine and proline (Jang et al., 2008; Pereda et al., 2011).

Table 2. Antibacterial of edible coating

Concentration of gambir extract (%)	Inhibition zone (cm)	
	E. coli	***S. aureus***
0	3.10 ± 0.17^{a}	2.80 ± 0.17^{ab}
0.1	3.17 ± 0.06^{ab}	2.67 ± 0.21^{a}
0.2	3.33 ± 0.21^{ab}	2.73 ± 0.21^{a}
0.3	3.43 ± 0.12^{b}	2.77 ± 0.12^{ab}
Chloramphenicol 0.1	4.00 ± 0.10^{c}	3.07 ± 0.06^{b}

Data are expressed as mean±standard deviation (n=3). Lowercase differences indicate statistical differences in one-way ANOVA with Duncan's test ($p \leq 0.05$) between treatments on the same parameter.

Microstructure

Scanning electron microscopy is a high-resolution examination of the morphological structure of the surface of edible coating solutions printed in films. The microstructure of the edible coating solution at 10.000x magnification is shown in Figure 1. From the image, it can be seen that the edible coating containing 0.3% gambir extract is denser and the crack lines are smoother than the control edible coating or no gambir extract. This shows that the gambir extract is evenly distributed in the solution, making the solution more homogeneous. According to the study by Zhang et al. (2019), the rabbit skin gelatin film with gambir extract added has a relatively smoother surface and homogeneity that is not easily damaged and shows a clearer layer than the control. The increased interaction between protein and polyphenols may contribute to the formation of a denser film structure.

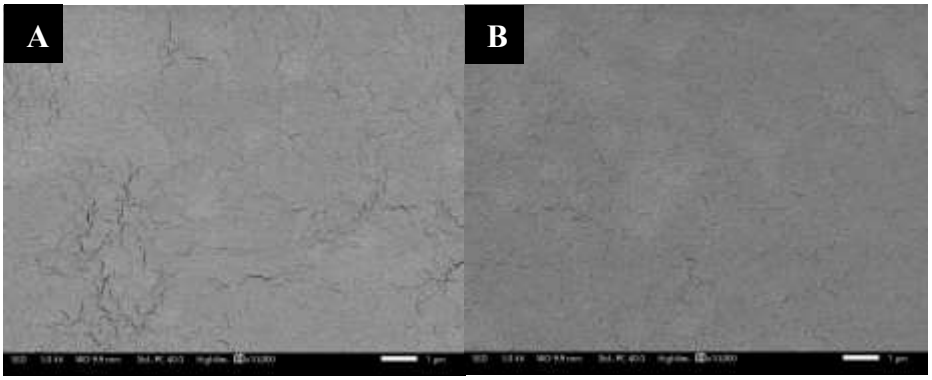

Figure 1. Scanning electron miscroscope of edible coating, (A) edible coating without gambir extract, (B) edible coating containing gambir extract 0.3%

Quality of Beef Sausages during Storage

Hardness

The hardness of beef sausage without or treated with an edible coating containing gambir extract during 15 days of storage at 4 – 7 °C is shown in Figure 2. The hardness value of beef sausage indicates its water and fat retention capacity during the dough preparation stage and its stability during storage. Based on Figure 2, it is known that the hardness of beef sausage increased with the length of storage time, except for beef sausage treated with the edible coating without the addition of gambir extract and beef sausage treated with an edible coating containing 0.2% gambir extract on day 15. It was also found that on the 15th day of storage, beef sausage without edible coating was harder than beef sausage treated with edible coating. The increase in hardness may be due to surface water loss, syneresis, and a decrease in the water-binding capacity of the beef sausage during storage, increasing the hardness of the beef sausage. The use of gelatin as an edible coating material can maintain the texture of beef sausage during storage. Protein has the functional property of absorbing and retaining water, which allows the product's texture to remain compact (Zhang et al., 2021).

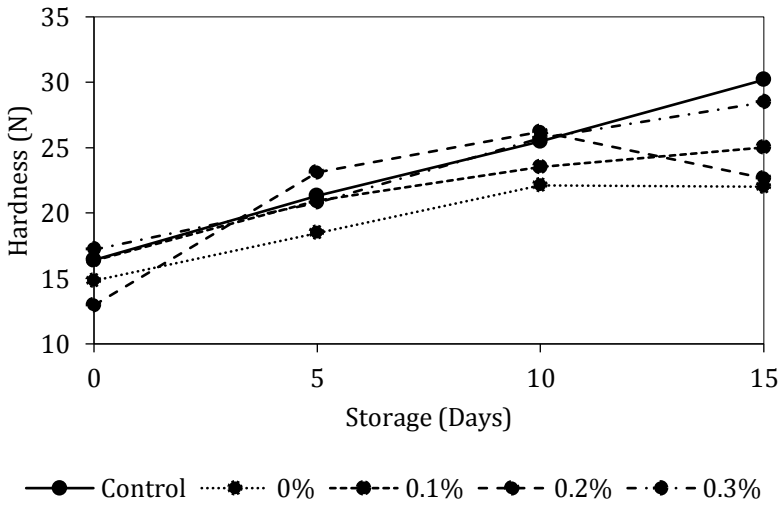

Figure 2 The hardness of beef sausage during storage

pH

The pH value of beef sausage without or treated with an edible coating containing gambir extract during 15 days of storage at 4 – 7 °C is shown in Figure 3. The pH value is related to the texture, quality, and safety of beef sausage during storage. Based on Figure 3, it is known that the pH value of beef sausage without or with edible coating treatment tends to increase with the length of storage time. The increase in pH of animal products during storage is due to the accumulation of volatile bases ammonia and trimethylamine produced by endogenous enzymes or microbes (Venkatachalam & Lekjing, 2020). The results also showed that beef sausage treated with edible coating during storage had a slower rate of pH increase compared to the control treatment. This may be due to the addition of antibacterial compounds in the coating material, which may inhibit the growth of spoilage bacteria and volatile base nitrogen-producing microorganisms (Venkatachalam & Lekjing, 2020).

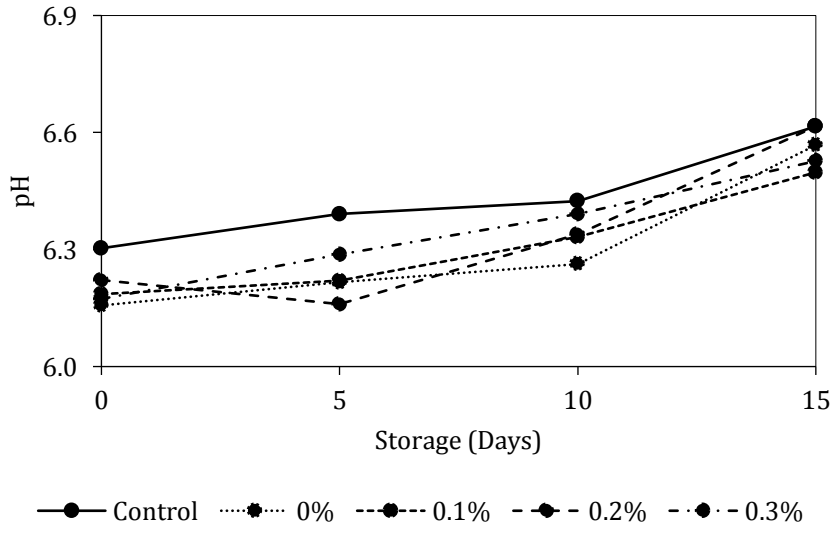

Figure 3 pH of beef sausage during storage

Total Plate Count

The total plate count (TPC) of beef sausage without or treated with an edible coating containing gambir extract during 15 days of storage at 4 – 7 °C is shown in Figure 4. The TPC test is designed to provide information on the safety of beef sausage during storage. Based on Figure 4, it is known that the TPC value of beef sausage of all treatments increased with the length of storage time. The results also showed that during storage, beef sausage treated with edible coating containing gambir extract had a lower TPC value compared to beef sausage without edible coating or beef sausage treated with edible coating without gambir extract. This is due to the antibacterial properties of gambir extract in the edible coating solution. Gambir extract contains flavonoids and alkaloids that act as antibacterial agents, causing damage to the bacterial plasma cell membrane, suppression of nucleic acid biosynthesis, and disruption of electron transport (Munggari et al., 2022; Cheng et al., 2023). The TPC value of beef sausage containing gambir extract on day 0 to day 15 of storage is still in an acceptable category based on International Microbiological Criteria (Ireland's Guidelines). While beef sausage without edible coating and beef sausage treated with edible coating had an unsatisfactory TPC value based on IMC on the 15th day of storage. The microbiological quality of meat sausages based on IMC is $< 10^3$ = satisfactory, 10^3 – $\leq 10^4$ = acceptable and $\geq 10^4$ = unsatisfactory.

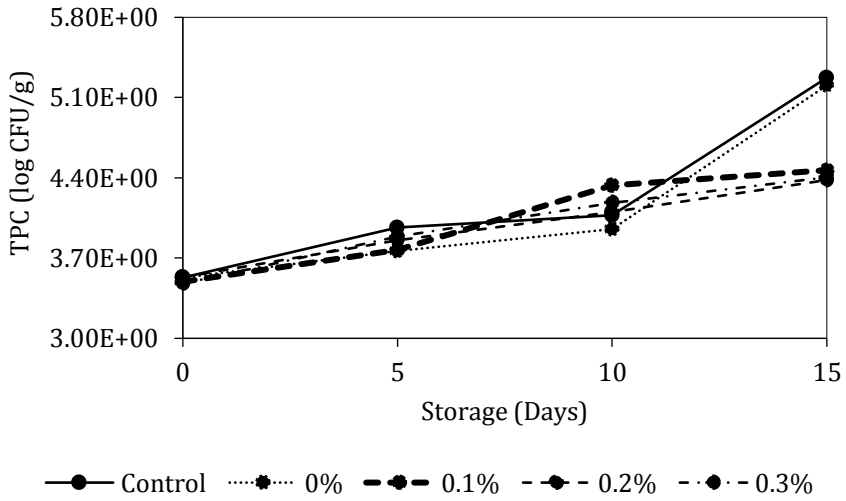

Figure 4. Total plate count of beef sausage during storage

Lipid oxidation: Peroxide Value and Thiobarbituric Acid Reactive Substances

Lipid oxidation is a free radical chain reaction that occurs during processing. The initial oxidation products are unstable peroxides that are easily oxidized to composite products such as ketones and aldehydes. Lipid oxidation can be measured by peroxide value (PV) and thiobarbituric acid reactive substances (TBARS). The PV and TBARS of beef sausage without or with treated edible coating containing gambir extract during 15 days of storage at 4-7 ^{o}C are shown in Table 3.

Table 3. Peroxide value and TBARS of beef sausage during storage

Storage (Days)	**Control (without edible coating)**	**Coating with edible coating**	**Coating with edible coating with 0.1% gambir extract**	**Coating with edible coating with 0.2% gambir extract**	**Coating with edible coating with 0.3% gambir extract**
Parameters	Peroxide value (m_{eq}/kg)				
0	0.07 ± 0.02^{Ba}	0.10 ± 0.01^{Aa}	0.02 ± 0.01^{Aa}	0.12 ± 0.02^{Ab}	0.03 ± 0.01^{Aa}
5	0.23 ± 0.01^{Bb}	0.07 ± 0.03^{Aa}	0.11 ± 0.01^{Ac}	0.09 ± 0.012^{Aab}	0.10 ± 0.03^{Aa}
10	0.11 ± 0.02^{Ba}	0.07 ± 0.00^{Aa}	0.07 ± 0.00^{Ab}	0.07 ± 0.02^{Aa}	0.06 ± 0.01^{Aa}
15	0.32 ± 0.06^{Bc}	0.08 ± 0.02^{Aa}	0.06 ± 0.02^{Ab}	0.06 ± 0.02^{Aa}	0.05 ± 0.02^{Aa}
Parameters	Thiobarbituric acid reactive substances (mg MDA/kg)				
0	0.43 ± 0.06^{Aa}	0.59 ± 0.01^{Cb}	0.51 ± 0.01^{Ba}	0.53 ± 0.01^{Ba}	0.60 ± 0.03^{Ba}
5	0.45 ± 0.02^{Aa}	0.53 ± 0.03^{Ca}	0.53 ± 0.02^{Ba}	0.51 ± 0.01^{Ba}	0.54 ± 0.02^{Ba}
10	0.55 ± 0.01^{Ab}	0.71 ± 0.02^{Cc}	0.61 ± 0.03^{Bb}	0.56 ± 0.02^{Bb}	0.51 ± 0.10^{Ba}
15	0.55 ± 0.01^{Ab}	0.62 ± 0.02^{Cb}	0.65 ± 0.07^{Bb}	0.62 ± 0.01^{Bc}	0.56 ± 0.02^{Ba}

Data are expressed as mean ± standard deviation (n=3). Capital letter differences indicate statistical differences in two-way ANOVA with Duncan's further test ($p \leq 0.05$) between treatments during storage. Lowercase differences indicate statistical differences of one-way ANOVA with Duncan's test ($p \leq 0.05$) between storage durations in the same treatment.

Based on Table 3, it is known that beef sausage treated with edible coating without or with the addition of gambir extract has a lower peroxide number up to the 15th day of storage compared to beef sausage without edible coating. The application of the edible coating to beef sausage can provide a protective effect against lipid oxidation. The addition of gambir extract, which is an antioxidant, can prevent damage caused by free radicals through oxidative

mechanisms to maintain the quality of beef sausage during storage. The antioxidant properties of gambir are due to the phenolic compounds it contains (Munggari et al., 2022; Yeni et al., 2014). During storage, beef sausages are also known to experience increases and decreases in peroxide number. The accumulation of high lipid oxidation intermediates is the cause of this phenomenon. However, due to its instability, the peroxide will break down into small molecular substances such as aldehydes and ketones. The more chained the lipid oxidation process is, the more the peroxide will decompose, and the decomposition speed of hydrogen peroxide will be faster than its formation, and the peroxide number value will decrease (Sun et al., 2009; Gu et al., 2017).

The TBARS test is designed to measure the extent of fat oxidation or rancidity in beef sausages during storage. TBARS levels during storage may be associated with lipid hydrolysis, oxidative rancidity, and by-product formation at cold temperatures (Hamann et al., 2022). Table 3 shows that during storage, beef sausage treated with edible coating containing gambir extract was significantly different from beef sausage without edible coating and beef sausage treated with edible coating without gambir extract. Among all beef sausages treated with an edible coating containing gambir extract, an edible coating containing 0.3% gambir extract was the best in suppressing the rate of TBARS value as evidenced by the absence of differences between the TBARS values of beef sausages on day 0 to day 15 storage ($p \leq 0.05$). Similar results have been reported by Shen et al. (2022) regarding the relatively slower increase in TBARS value of pork coated with chitosan-curcumin. Edible coatings containing antioxidant compounds counteract free radicals, maintain antioxidant enzyme activity, and retard oxidation. In this study, the TBARS value of beef sausage during storage was < 1 mg MDA/kg, which means it is still permissible and acceptable (Behbahani & Fooladi, 2018).

Sensory Evaluation

The sensory evaluation aims to determine the effect of edible coating, both with and without the addition of gambir extract, on beef sausage in terms of the test parameters, including gambir aroma, gelatin aroma, bitter taste, and sour taste. The sensory evaluation of beef sausage is shown in Table 4. The sensory evaluation results showed that there was no difference between beef sausage without and with an edible coating containing gambir extract in all test parameters. This is because gelatin, which is the basic material for the production of edible coating, has odorless and tasteless properties (Huda et al., 2014). In addition, gambir extract does not have a distinctive aroma, so it does not affect the taste of food products (Melia et al., 2015). The beef sausage sensory tested by trained panelists had also undergone a ripening process (resteaming for 15 minutes) after treatment, which can cause the gelatin to lose its gelling properties. Rabbit bone gelatin has a melting point of 33 – 35 °C (Wulandari et al., 2022).

Table 4. Sensory evaluation of beef sausage

Concentration of gambir extract	Sensory attributes			
	Odor (gambir)	Odor (gelatin)	Taste (bitter)	Taste (sour)
Control	1.49 ± 0.53[a]	1.84 ± 0.63[a]	1.61 ± 0.67[a]	1.89 ± 0.84[a]
0%	1.58 ± 0.89[a]	1.95 ± 0.84[a]	1.45 ± 0.50[a]	2.13 ± 0.92[a]
0.1%	1.55 ± 0.76[a]	2.20 ± 1.23[a]	1.52 ± 0.67[a]	2.11 ± 0.77[a]
0.2%	1.50 ± 0.63[a]	1.93 ± 0.72[a]	1.44 ± 0.49[a]	2.15 ± 1.05[a]
0.3%	1.51 ± 0.68[a]	2.13 ± 0.89[a]	1.31 ± 0.38[a]	1.82 ± 1.03[a]

Data are expressed as mean±standard deviation (n=9). Lower case differences indicate statistical differences in one-way ANOVA with Duncan's test ($p \leq 0.05$) between treatments on the same parameter.

Conclusion

The addition of gambir extract to the edible coating solution demonstrated its ability as an antioxidant and antibacterial agent. In addition to not altering the aroma and flavor of beef sausage, the application of edible coating containing gambir extract to beef sausage was also able to retard the deterioration of beef sausage during storage, both oxidative damage and microbiological damage. Therefore, rabbit bone gelatin edible coating containing gambir extract can be used as an alternative to extend the shelf life of beef sausage products.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

References

Abral, H., Ikhsan, M., Rahmadiawan, D., Handayani, D., Sandrawati, N., Sugiarti, E., & Muslimin, A. N. (2022). Anti-UV, antibacterial, strong, and high thermal resistant polyvinyl alcohol/*Uncaria gambir* extract biocomposite film. *Journal of Materials Research and Technology*, *16*, 796-804. https://doi.org/10.1016/j.jmrt.2022.01.120

Anggraini, T., Tai, A., Yoshino, T., & Itani, T. (2011). Antioxidative activity and catechin content of four kinds of Uncaria gambir extracts from West Sumatra, Indonesia. *African Journal of Biochemistry Research*, *5*(1), 33-39. http://www.academicjournals.org/AJBR

Apea-Bah, F., Hanafi, M., Dewi, R., Fajriah, S., Darwaman, A., Artanti, N., Lotulung, P. D., Ngadymang, P., & Minarti, B. (2009). Assessment of the DPPH and α-glucosidase inhibitory potential of gambir and qualitative identification of major bioactive compound. *Journal of Medicinal Plants Research*, *3*(9), 736-740.

Azizah, F., Nursakti, H., & Ningrum, A. (2023). Development of edible composite film from fish gelatin–pectin incorporated with lemongrass essential oil and its application in chicken meat. *Polymers*, *15*(9), 2075. https://doi.org/10.3390/polym15092075

Behbahani, B. A., & Fooladi, A. A. I. (2018). Shirazi balangu (*Lallemantia royleana*) seed mucilage: chemical composition, molecular weight, biological activity and its evaluation as edible coating on beefs. *International Journal of Biological Macromolecules*, *114*, 800-808. https://doi.org/10.1016/j.ijbiomac.2018.03.177

Bubonja-Šonje, M., Knežević, S., & Abram, M. (2020). Challenges to antimicrobial susceptibility testing of plant-derived polyphenolic compounds. *Archives of Industrial Hygiene and Toxicology*, *71*(4), 300-311. https://doi.org/10.2478/aiht-2020-71-3396

Cardoso, G. P., Dutra, M. P., Fontes, P. R., Ramos, A. L. S., Gomide, L. A. M., & Ramos, E. M. (2016). Selection of a chitosan gelatin-based edible coating for color preservation of beef in retail display. *Meat Science*, *114*, 85-94. https://doi.org/10.1016/j.meatsci.2015.12.012

Cesa, S., Casadei, M. A., & Cerreto, F. (2012). Influence of fat extraction methods on the peroxide value in infant formulas. *Food Research International*, *48*(2), 584-591. https://doi.org/10.1016/j.foodres.2012.06.002

Cheng, Y., Xue, F., & Yang, Y. (2023). Hot water extraction of antioxidants from tea leaves—optimization of brewing conditions for preparing antioxidant-rich tea drinks. *Molecules*, *28*(7), 3030. https://doi.org/10.3390/molecules28073030

Ciannamea, E. M., Castillo, L. A., Barbosa, S. E., & Angelis, M. G. D. (2018). Barrier properties and mechanical strength of bio-renewable, heat-sealable films based on gelatin, glycerol and soybean oil for sustainable food packaging. *Reactive and Functional Polymers*, *124*, 25-32. https://doi.org/10.1016/j.reactfunctpolym.2018.02.001

Evans, A. (2019). *Gelatin handbook*. Gelatin Manufacturers Institute of America.

Fallah, M., Rouhi, M., Soltani, M., Mohammadifar, M. A., Bahrami, R., Davachi, S. M., Abbaspourrad, A., & Mohammadi, R. (2021). Physico-mechanical, antimicrobial, and antioxidant properties of gelatin edible films incorporated with olibanum essential oil and sodium hexametaphosphate on the rainbow trout fillet under refrigerated conditions. *Journal of Polymers and the Environment*, *29*, 549-561. https://doi.org/10.1007/s10924-020-02027-9

Gu, X., Sun, Y., Tu, K., & Pan, L. (2017). Evaluation of lipid oxidation of Chinese-style sausage during processing and storage based on electronic nose. *Meat Science*, *133*, 1-9. https://doi.org/10.1016/j.meatsci.2017.05.017

Hamann, D., Puton, B. M. S., Comin, T., Colet, R., Valduga, E., Zeni, J., Steffens, J., Junges, A., Backes, G. T., & Cansian, R. L. (2022). Active edible films based on green tea extract and gelatin for coating of fresh sausage. *Meat Science*, *194*, 108966. https://doi.org/10.1016/j.meatsci.2022.108966

Huda, M. N., Easa, N., & Fazilah, A. (2014). Poultry as an alternative source of gelatin. *Health & the Environment Journal*, *5*(1), 37-49.

Isopencu, G. O., Stoica-Guzun, A., Busuioc, C., Troescu, M., & Deleanu, I. M. (2021). Development of antioxidant and antimicrobial edible coatings incorporating bacterial cellulose, pectin, and blackberry pomace. *Carbohydrate Polymer Technologies and Applications*, *2*, 100057. https://doi.org/10.1016/j.carpta.2021.100057

Jang, A., Jo, C., Kang, K. S., & Lee, M. (2008). Antimicrobial and human cancer cell cytotoxic effect of synthetic angiotensin-converting enzyme (ACE) inhibitory peptides. *Food Chemistry*, *107*(1), 327-336. https://doi.org/10.1016/j.foodchem.2007.08.036

Kassim, M. J., Hussin, M. H., Achmad, A., Dahon, N. H., Suan, T. K., & Hamdan, H. S. (2011). Determination of total phenol, condensed tannin and flavonoid contents and antioxidant activity of *Uncaria gambir* extracts. *Indonesian Journal of Pharmacy*, *22*(1), 50-59.

Katsanidis, E., & Zampouni, K. (2023). Development of a novel steam distillation TBA test for the determination of lipid oxidation in meat products. *Foods*, *12*(2), 359. https://doi.org/10.3390/foods12020359

Lestari, R. B., Permadi, E., & Harahap, R. P. (2020). Decrease quality during storage packaged beef sausage edible coating by durian seeds starch-chitosan with the addition of kesum leaf extract. *IOP Conference Series: Earth and Environmental Science*, *478*(1), 012036. https://doi.org/10.1088/1755-1315/478/1/012036

Li, X., He, Z., Xu, J., Zhang, L., Liang, Y., Yang, S., Wang, Z., Zhang, D., Gao, F., & Li, H. (2021). Effect of nanoprocessing on the physicochemical properties of bovine, porcine, chicken, and rabbit bone powders. *Food Science & Nutrition*, *9*(8), 4147-4158. https://doi.org/10.1002/fsn3.2312

Melia, S., Novia, D., & Juliyarsi, I. (2015). Antioxidant and antimicrobial activities of gambir (*Uncaria gambir* Roxb) extracts and their application in rendang. *Pakistan Journal of Nutrition*, *14*(12), 938-941. https://doi.org/10.3923/pjn.2015.938.941

Monica, S., & Husna, Z. (2022). Antibacterial activity test of (+)-katekin and gambir (*Uncaria gambir* Roxb.) against some types of gram-negative bacteria and their mechanism. *International Journal on Health and Medical Sciences*, *2*(2), 1-6. https://journals.iarn.or.id/index.php/HealMed/article/view/26

Munggari, I. P., Kurnia, D., Deawati, Y., & Julaeha, E. (2022). Current research of phytochemical, medicinal and non-medicinal uses of *Uncaria gambir* Roxb.: A review. *Molecules*, *27*(19), 6551. https://doi.org/10.3390/molecules27196551

Oliveira, J. T., Machado da Costa, F., Gonçalves da Silva, T., Simões, G. D., Pereira, E. S., Quevedo da Costa, P., Andreazza, R., Schenkel, P. C., & Pieniz, S. (2023). Green tea and kombucha

characterization: Phenolic composition, antioxidant capacity and enzymatic inhibition potential. *Food Chemistry*, *405*, 135206. https://doi.org/10.1016/j.foodchem.2022.135206

Pereda, M., Ponce, A. G., Marcovich, N. E., Ruseckaite, R. A., & Martucci, J. F. (2011). Chitosan-gelatin composites and bi-layer films with potential antimicrobial activity. *Food Hydrocolloids*, *25*(5), 1372-1381. https://doi.org/10.1016/j.foodhyd.2011.01.001

Santoso, B., Hazirah, R., Priyanto, G., Hermanto, & Sugito. (2019). Utilization of *Uncaria gambir* Roxb. filtrate in the formation of bioactive edible films based on corn starch. *Food Science and Technology*, *39*(Suppl. 2), 610-616. https://doi.org/10.1590/fst.06318

Shen, W., Yan, M., Wu, S., Ge, X., Liu, S., Du, Y., Zheng, Y., Wu, L., Zhang, Y., & Mao, Y. (2022). Chitosan nanoparticles embedded with curcumin and its application in pork antioxidant edible coating. *International Journal of Biological Macromolecules*, *202*, 350-359. https://doi.org/10.1016/j.ijbiomac.2022.02.025

Shin, S. H., & Choi, W. S. (2021). Variation in significant difference of sausage textural parameters measured by texture profile analysis (TPA) under changing measurement conditions. *Food Science of Animal Resources*, *41*(4), 585-598. https://doi.org/10.5851/kosfa.2021.e26

Siddiqui, S. A., Gerini, F., Ikram, A., Saeed, F., Feng, X., & Chen, Y. (2023). Rabbit meat production, consumption and consumers' attitudes and behavior. *Sustainability*, *15*(3), 2008. https://doi.org/10.3390/su15032008

Sun, W., Zhao, H., Zhao, Q., Zhao, M., Yang, B., Wu, N., & Qian, Y. (2009). Structural characteristics of peptides extracted from Cantonese sausage during drying and their antioxidant activities. *Innovative Food Science & Emerging Technologies*, *10*(4), 558-562. https://doi.org/10.1016/j.ifset.2009.07.006

Umaraw, P., Munekata, P. E. S., Verma, A. K., Barba, F. J., Singh, V. P., Kumar, P., & Lorenzo, J. M. (2020). Edible films/coating with tailored properties for active packaging of meat, fish and derived products. *Trends in Food Science & Technology*, *98*, 10-24. https://doi.org/10.1016/j.tifs.2020.01.032

Venkatachalam, K., & Lekjing, S. (2020). A chitosan-based edible film with clove essential oil and nisin for improving the quality and shelf life of pork patties in cold storage. *RSC Advances*, *10*(30), 17777-17786. https://doi.org/10.1039/D0RA02986F

Wulandari, D., Hermiyati, I., Iswahyuni, I., & Tawarniate, A. Z. (2022). Production and characterization of gelatin from rabbit bone as bioplastics material by acid pre-treatment. *World Rabbit Science*, *30*(4), 275-284. https://doi.org/10.4995/wrs.2022.16639

Yeni, G., Syamsu, K., Suparno, O., Mardliyati, E., & Muchta, H. (2014). Repeated extraction process of raw gambirs (*Uncaria gambir* Roxb.) for the catechin production as an antioxidant. *International Journal of Applied Engineering Research*, *9*(24), 24565-24578.

Zhang, X., Ma, L., Yu, Y., Zhou, H., Guo, T., Dai, H., & Zhang, Y. (2019). Physico-mechanical and antioxidant properties of gelatin film from rabbit skin incorporated with rosemary acid. *Food Packaging and Shelf Life*, *19*, 121-130. https://doi.org/10.1016/j.fpsl.2018.12.006

Zhang, Y., Sharan, S., Rinnan, S., & Orlien, V. (2021). Survey on methods for investigating protein functionality and related molecular characteristics. *Foods*, *10*(11), 2848. https://doi.org/10.3390/foods10112848