Application of bioindicator film from tapioca/agar/mulberry (Morus alba L.) to detect sausage spoilage

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Abstract. In this study, development of a bioindicator film to detect sausage spoilage has been evaluated. The objectives of this research were to develop a bioindicator film from blended tapioca, agar, and mulberry juice and to assess its performance in detecting sausage spoilage. Different concentrations of agar (3, 5, 7% by weight of tapioca) and crude extract of anthocyanin (CEA) from mulberry (10, 15, 20% by total volume of suspension) were formulated in this experiment beside glycerol as the plasticizer. The screening of the best bioindicator film was determined statistically according to multiple criteria decision making method prior sausages wrapping. Selected biofilm, 2% tapioca + 7% agar + 20% CEA, was able to detect an increase of microbial counts and pH variations as a consequence of sausage spoilage exhibited by color alteration of biofilm from dark red at 0 h to red-green at 24 h, and more greenness at 48 and 72 h.

Keywords: biofilm, sausage, spoilage, mulberry, bioindicator

1. Introduction

Food poisoning cases are one of the scary problems not only in Indonesia, but also throughout the world. In March 2018, three Australian people were killed after eating melons contaminated with listeria bacteria [1]. Moreover, in October 2012, about four Indonesian elementary school students also experienced food poisoning who one of them was died after consuming contaminated sausages [2]. Indonesian National Agency of Drug and Food Control (BPOM) (2017) reported that there are 19 food poisoning incidents in January - March 2017 [3], which are commonly caused by not safe meat-based foods including sausage, meatballs, etc.

Recently, development of intelligent packaging which the condition of packaged foods could be sensed, recorded, traced and monitored [4,5], gains a lot of attentions. Utilization of blended tapioca, agar, and mulberry open the opportunity in improving intelligent packaging, namely bioindicator film which has advantages not only as intelligent, but also as eco-friendly packaging. Anthocyanin contained in mulberry, which could change its color in the pH variation, is the key substance in the bioindicator film fabrication. In this study, its unique properties is utilized to indicate microbial metabolites in food product. That mechanism is based on the ability of microbiological growth in inducing a pH change [6].
Several harmful bacteria metabolites used as a deterioration indicator of meat-based product are NH₃, amines, and other basic compounds which could increase pH near to 7.0-8.0 [7]. Pseudomonas spp., S. aureus, and E. coli represent harmful microorganisms for meat-based product spoilage.

Moreover, tapioca is prospective polymer biomaterial as anthocyanin carrier matrix in biofilm production which is supported by Indonesia as the second world’s largest cassava producer after Nigeria [8]. Agar is also combined with tapioca to enhance the poor physical properties of tapioca-based biofilm. Several researchers have studied that either agar/tapioca-based or agar-based films were able to improve biofilm mechanical, physical, and thermal properties [9,10]. Application of bioindicator film is expected that is able to easily predict the deterioration level of packed food with nondestructive method during shipment and retail display. This study were aimed to develop bioindicator film from blended tapioca, agar, and mulberry juice and to assess its performance in detecting sausage spoilage.

2. Experimental

2.1. Materials
Commercial grade tapioca flour (Rose brand), mulberry, and fresh beef meat were obtained from a local wholesale market in Malang, Indonesia. Commercial agar powder with specification; particle size 80 mesh, moisture 20%, and yellowish color, was obtained from supplier Golden Agar Sentosa (Surabaya, Indonesia). Glycerol obtained from Merck (Germany) is added into biofilm solution as the plasticizer.

2.2. Sausage preparation
A food processor was used to crush beef meat. Later crushed meat is mixed with 5% tapioca flour, 10% egg whites, 10% chopped ice cubes, 2% garlic, 0.5% white pepper, 0.5% coriander, and 1% salt (all the ingredients percentage based on 100% of meat). The blended dough was poured into a stuffer and afterwards filled into cylindrical sausage casings. Subsequently, the pieces of sausages were cooled to the ambient temperature (28-29°C) after boiled at a temperature of 70-80°C for 30 min. Then, they were cut ±5 cm in length and wrapped with bioindicator film as long as 0, 24, 48, and 72 h for testing.

2.3. Bioindicator film preparation
Tapioca flour (2% by total volume of suspension), glycerol (30% by weight of tapioca flour), and agar powder (3%, 5%, 7% by weight of tapioca flour) were blended in distilled water with constant stirring at temperature 28-30°C for ± 20 min. Subsequently, the solution was gelatinized (90°C) for 10 min until its color turned to completely transparent. After it was cooled (28-30°C), the crude extract of anthocyanin (CEA) contained in mulberry juice (10%, 15%, 20% by total volume of suspension) were mixed to biofilm solutions using constant stirring for 10 min. Then, 30 ml of the biofilm solutions were poured into casting plates (7 x 15 cm) and putted in an air-circulating oven at 50°C (14 - 15 h). After they were dry, the cast biofilms were cooled at 28-30°C, peeled, and stored inside a plastic bag. Mulberry juice was made by crushing mulberry (500 g) in distilled water (1 L) and filtered using Whatman paper. Following show the formulations of bioindicator films, namely: (1) 2% tapioca flour + 3% agar + 10% CEA, (2) 2% tapioca flour + 3% agar + 15% CEA, (3) 2% tapioca flour + 3% agar + 20% CEA, (4) 2% tapioca flour + 5% agar + 10% CEA, (5) 2% tapioca flour + 5% agar + 15% CEA, (6) 2% tapioca flour + 5% agar + 20% CEA, (7) 2% tapioca flour + 7% agar + 10% CEA, (8) 2% tapioca flour + 7% agar + 15% CEA, (9) 2% tapioca flour + 7% agar + 20% CEA.

2.4. Mechanical properties of biofilm
Microcomputer Controlled Universal Testing Machine was used to measure the biofilm mechanical properties (tensile strength (TS) and elongation at break (EAB)) according to ASTM D 638 - 99 (1999) [11]. The biofilm was formed into 2 x 7cm. Initial grip was set at 17 mm with tensile speed of 20 mm/min. The calculation of biofilm tensile strength and elongation followed this equation respectively:

$$TS \ (N/cm^2) = \frac{F}{A}$$

where F is the force maximum at rupture of the biofilm (N); A is initial cross-section area (mm²).
where \( L_0 \) is initial gage length; \( L \) is the final length.

2.5. Water vapour transmission rate (WVTR) of biofilm

WVTR of the biofilm was measured based on ASTM D1249-90 method (1993) with slight modifications [12]. Samples of biofilm were cut in a cylindrical shape with diameter of 30 mm, placed on the surface of the cups filled with silica gel and coated with paraffin wax around the cups circumference. The WVTR of each biofilm was recorded in glass desiccators containing saturated KCl solution (RH 75%) and measured at 25 ± 2°C. WVTR was determined by following this equation:

\[
\text{WVTR} = \frac{\Delta w}{A \Delta t}
\]

Where \( \Delta w \) is difference of weight during storage (g); \( A \) is film area exposed to water (m\(^2\)); \( \Delta t \) is difference of time during storage (24 h).

2.6. Bioindicator film on sausage spoilage

The biofilm was cut and formed with dimension 6 x 7 cm. Afterwards, the sausages were wrapped inside the biofilm and stored in a room with temperature 28-30°C throughout the test period (0-72 h). The color changes of biofilm were determined each 24 h using CIE (\( L^* \), \( a^* \), \( b^* \)) color system from instrument colorimeter. Total color difference (\( \Delta E \)) of samples were determined by converting the value of \( L^* \), \( a^* \), \( b^* \) using this equation [13]:

\[
\Delta E = (\Delta L^*^2 + \Delta a^*^2 + \Delta b^*^2)^{1/2}
\]

Where \( \Delta L^* \), \( \Delta a^* \) and \( \Delta b^* \) are the difference of measurement values between different time intervals (24, 48, 72 h) and initial sample (0 h).

2.7. pH and microbial count of sausage

Measurement of sausage pH and microbial count were performed gradually every 24 h. “Hanna” pH meter was used to determine sausage pH which was calibrated at pH 4 and 7 prior to measurement. Bacteriological Analytic Method (1992) was adopted to count the total plate count of microbial in log CFU/g (colony forming units per g of sample) after incubation at 37°C for 48 h [14].

2.8. Statistical analysis

The best biofilm was selected by Multiple Criteria Decision Making (MCDM) prior sausages wrapping [15]. Moreover, the statistical analyses were determined using analysis of variance (ANOVA) with SPSS (Statistical Product and Service Solutions) software 16.0. The significant difference between treatment was further tested using Duncan’s Multiple Range Test (DMRT) which was considered at 95% probability.

3. Result and Discussion

3.1. Mechanical properties of biofilm

Figure 1 shows the results of the measurement of mechanical properties of biofilm. The mechanical properties of biofilm are critical point in terms its performance ability to protect the integrity of food. This study revealed that the incorporation of CEA decreased the elongation at break of the biofilm and tended not to influence the tensile strength. Previous researchers suggested that the particles incorporation may cause morphological structural changes of the polymers [16]. In addition, the addition of agar increased both biofilm’s tensile strength and elongation at break at only at concentration 7% and 5% respectively. Previous studies reported that the enhancement of tensile strength affected by agar due to the starch-agar molecules hydrogen bonds resulting more compact structures, while the enhancement of elongation was associated with water holding capacity [9,17].
Figure 1. Mechanical properties of biofilm. Means followed by different letters indicate statistically significant differences (p < 0.05).

3.2. WVTR of biofilm
WVTR can be defined as the ability of the biofilm to allow passage of water vapour which the results are represented in Figure 2. Overall, the addition of both agar and CEA to tapioca flour based biofilm increase the WVTR of the biofilm. These results indicated that the addition of agar component in the solution was solubilized well resulting the enhancement the biofilm integrity. Moreover, the effect of CEA can be supposed because the formation of tortuous pathways contributed by CEA for water vapour molecules passing through the matrix of the biofilm. However, those values were still not able to achieve a good film characteristic in referring to Japanese Industrial Standard (JIS) which required the maximum WVTR value of a good film to be 10 g.m^{-2}.24h^{-1} [18].

Figure 2. WVTR of biofilm. Means followed by different letters indicate statistically significant differences (p < 0.05).

3.3. Bioindicator film on sausage spoilage
The screening of the best bioindicator film was determined statistically according to MCDM before wrapping on sausages which was obtained in formulation #4 (2% tapioca flour + 5% agar + 10% CEA). It has characteristic as the following: tensile strength = 3.54 N/cm^2, elongation at break = 7.62 %, and WVTR = 52.21 g.m^{-2}.24h^{-1}. The total color difference (ΔE) which is visually perceptible (ΔE > 1) and clinically acceptable (ΔE > 3.3), refers to the ability of the human eye to differentiate color without a necessary sensory analysis panel [6, 19, 20]. Figure 3 shows that bioindicator film #4 wrapping sausage has significant changes during storage started at 24 h compared to 0 h. It is visually represented with
Figure 4. This bioindicator film showed an alteration of color which is able easily to distinguish them every 24 h of storage gradually. At 0 h, bioindicator film showed a dark red color, while at 24 h it turned into a red-green color. At 48 and 72 h it was altered to a more greenness color. The alteration of color mainly due to the role of anthocyanin contained in CEA from mulberry juice. Commonly, at acidic pH (1.0-3.0) anthocyanins is in the form of the flavylum cation and the colorless carbinol base, which shows the color of purple and red and afterwards the quinoidal blue species appear at pH 2.0 - 4.0. Greater pH to 6.0 and 5.0 undergoes hydration providing a colorless carbinol pseudobase and chalcone. At greater alkaline environment, which is pH > 7.0 and pH > 8.0, a purple quinoidal anhydrobase and a deep blue ionized anhydrobase are arised respectively. Subsequently, at higher pH, a yellow chalcone is resulted by opening of the inner pyran ring of carbinol [21,22,23].

Figure 3. Color difference of bioindicator film #4, pH, microbial counts of sausages stored at 28°C for 72 h. Means followed by different letters indicate statistically significant differences (p < 0.05).

An increase of sausage pH during storage is associated with the metabolite products of microbes which degradate the meat proteins. Those are converted by microbial enzymes resulting in ammonia and amines, increasing pH [7, 24]. As shown in Figure 3, the pH of the sausages covered by biofilm gradually increased until the last storage time. This pH enhancement coincides with microbial counts (Figure 3), ΔE and color changes observed visually. Microbial counts reached greatly by approximately 5 logarithmic cycles up to more than 10⁹ CFU/g at the last storage which allowed to form undesirable compounds such as off-odors, slime, and gas. Mono-, di-, and trimethylamines, ethyl- and propyl-compounds, ammonia, H₂S, alcohols, and short chain fatty acids are the compounds which in spoiled meat products [25, 26]. High protein content of sausage induced the growth of many types of microorganisms that caused it to be highly perishable food product [27, 28]. Indonesia National Agency
of Drug and Food Control (BPOM) regulated that the maximum microbial counts of sausage which is not allowed to consume is not higher than 5 log CFU/g [29].

![Figure 4. Bioindicator film #4 color response in contact with representative sausages during storage](image-url)

### 4. Conclusion

Bioindicator films were prepared using casting technique with a mixing solution of 2% tapioca flour, glycerol (30% by weight of tapioca flour), and different amounts of agar and CEA. MCDM method recommended that the best biofilm was bioindicator film #2 (2% tapioca flour + 3% agar + 15% CEA), which has characteristics: tensile strength = 3.54 N/cm², elongation at break = 7.62 %, and WVTR = 52.21 g.m⁻².24h⁻¹. Sausage quality degradation could be indicated using that bioindicator film through color changes. This study suggest that this bioindicator film may be used as cutting edge smart packaging without destructive method for perishable food products. However, this research still need further improvement of physical and mechanical properties of biofilm and the anthocyanin stability during storage.

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