

Use of Edible Film Incorporated with Parijoto Fruit Extract (*Medinilla speciosa* Blume) to Inhibit Microbiological and Oxidative Damages of Sausages

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Abstract

Background and Objective: Edible film is one of the solutions for food packaging that carry antimicrobial and antioxidant compounds, usually found in *Medinilla speciosa* fruits. The objective of this study was to assess effects of *Medinilla speciosa* fruit extract on physical, chemical and bioactivity of edible films as well as effects of coating on sausage quality during storage.

Material and Methods: The edible film included 2% w w⁻¹ chitosan, 2% w w⁻¹ sorbitol and *Medinilla speciosa* fruit extract. Variations of *Medinilla speciosa* extract included 0, 2.5, 5 and 10% (w w⁻¹), while the storage temperature included 4 and 27 °C. Seven parameters of edible film characteristics were assessed, including tensile strength, elongation, water vapor permeability, antimicrobial and antioxidant activities, surface microstructure and Fourier-transform infrared response. Parameters assessed in storage treatment included total plate count, yeast mold count and thiobarbituric acid reactive substances. Data were analyzed using Kruskal-Wallis test. Organoleptic characteristics were analyzed using Friedman test and SPSS Software.

Results and Conclusion: Results showed that the higher the concentration of *Medinilla speciosa* extract was, the higher the value of tensile strength, water vapor permeability and antioxidant activity and lower the elongation value were. The film control with 0% *Medinilla speciosa* extract was the only film that met Japanese standard for the water vapor permeability value, including 6.74 gm⁻² h⁻¹. Furthermore, shelf life of sausages coated with edible films revealed that the higher the concentration of *Medinilla speciosa* extract was, the lower the total plate count, yeast mold count and thiobarbituric acid reactive substances values were. Study demonstrated that the *Medinilla speciosa* extract edible film inhibited microbiological and oxidative damages. On Day 15 of storage, sausages coated with edible films with 10% *Medinilla speciosa* extract included lower total plate count, yeast mold count and thiobarbituric acid reactive substances values, respectively including 2.4 ± 0.02 log CFU g⁻¹, 1.3 ± 0.08 log CFU g⁻¹ and 13.38 ± 0.22 mg malonaldehyde kg⁻¹ sausage, compared to the control film. Organoleptic assessment showed no major differences in consumer acceptance. In conclusion, edible film with 10% *Medinilla speciosa* extract is the best physical, chemical and bioactivity film. Moreover, this film extends the sausage shelf life.

Conflict of interest: The authors declare no conflict of interest.

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1. Introduction

Sausages are types of preserved food commonly found in the market. As preserved foods, sausage coatings such as edible film should be able to preserve quality of the sausages. Edible film is a thin layer of edible materials used to increase product durability and consumer interest. Furthermore, edible film is a technological development in food packaging that is environmentally friendly because it can be consumed [1]. One of the basic ingredients for preparing edible films is chitosan. Chitosan contains polymers based on natural

ingredients, which is biodegradable and safe for consumption [2]. Alkaloids, flavonoids, saponins and tannins from plants can be added to edible films as antimicrobial, antifungal and antioxidant agents [3]. Antimicrobial and antifungal compounds control microbiological damages from pathogenic microorganisms or fungi [3,4]. Meanwhile, antioxidant compounds play roles in inhibiting oxidation process by binding to free radicals. One of the plants that contain several secondary metabolites with

antimicrobial, antifungal and antioxidant activities is Parijoto plant [4,5,6]. Parijoto, with the scientific name of *Medinilla (M.) speciosa* Blume, is one of the typical plants on the skirts of Mount Muria, Kudus City, Indonesia. *Medinilla speciosa* fruit includes high anthocyanins such as flavonoids as well as other phytochemicals such as alkaloids, saponins and tannins with potentials as antimicrobial and antioxidant compounds [5-8]. Biodegradable edible films incorporated with *M. speciosa* are suggested as potential solution reducers for plastic wastes in the environment. Extending other potentials of *M. speciosa* automatically increases price of *M. speciosa*; hence, economic aspects for the local people, especially *M. speciosa* farmers, increase as well. In this study, effects of adding *M. speciosa* fruit extract into edible films for sausage coating were investigated. Novelty of this study included incorporation of *M. speciosa* extract into the edible films. Objectives of the study included to assess effects of *M. speciosa* fruit extract on physical, chemical and bioactivity characteristics of the edible films and to investigate effects of edible film coating on the storage quality of sausages.

2. Materials and Methods

2.1. Materials

Sample collection

The *M. speciosa* fruits were harvested from Dawe Village, Gunung Muria, Kudus City, Indonesia, and extracted using 90% ethanol maceration method [9]. Chitosan and sorbitol with food standard were provided from CV Hista Labora, Indonesia. Other materials included Mueller-Hinton agar/MHA (Himedia, India), potato dextrose agar/PDA (Oxoid, UK), peptone dilution fluid/PDF (Oxoid, Germany), plate count agar/PCA (Oxoid, UK), 2,2-diphenyl-1-picrylhydrazyl/DPPH (Sigma-Aldrich, Germany), thio-barbituric acid/TBA (Merck, Germany) and trichloroacetic acid/TCA (Merck, Germany).

Test organism

Escherichia (E.) coli and *Staphylococcus (S.) aureus* were provided by Department of Microbiology, Faculty of Medicine, Universitas Sebelas Maret, Indonesia, and *Aspergillus (A.) niger* was provided by Department of Microbiology, Faculty of Medicine, Universitas Gadjah Mada, Indonesia.

2.2 Methods

Edible Film

Edible film was prepared based on previous studies [10]. The *M. speciosa* fruit was extracted based on a protocol by Milanda et al. [4]. Briefly, 50 g of dried *M. speciosa* fruit were mixed with 90% ethanol at a ratio of 1:10. Maceration was carried out for two days and re-maceration was carried out for several times to preserve color of the filtrate. Filtrate

was poured into a rotary evaporator at 60-80 °C until the extract was dense. Then, 2% chitosan solution was mixed with 2% sorbitol for 10-15 min using magnetic stirrer and then heated at 50-60 °C. The *M. speciosa* extract with various treatments (0, 2.5, 5 and 10%) was added to the solution before the solution was solid. This was mixed at 50-60 °C for 30 min using magnetic stirrer. Then, 28 ml of the edible film solution were poured into a Petri dish with a film thickness of ± 0.25 cm, cooled down at room temperature and dried at 35 °C for 24 h using oven. Edible film with no *M. speciosa* extract (0%) was used as control.

Sausage Preparation

Sausages were prepared from chicken meat, oil (10%), tapioca flour (5%), salt, garlic (1%), eggs (6%), white pepper (0.35%), nutmeg (0.1%) and ice cubes (10%). Chicken meat was grounded with the ice cubes at the first minute; then, other ingredients were mixed for 15 min. Sausage dough was formed and steamed for 45 min [11]. Prepared sausages were manually coated with edible film based on various treatments under biological safety cabinet. Sausages coated with edible films were used for total plate count (TPC), yeast and mold counts (YMC) and thiobarbituric acid reactive substances (TBARS) analysis.

Characterization of Edible Film with *Medinilla speciosa* Extract

Edible film characterization was assessed through its tensile strength (TS), elongation, water vapor permeability (WVP) and Fourier-transform infrared (FTIR). Moreover, bioactivity of the edible films was assessed through their antimicrobial and antioxidant activities.

Film Tensile Strength and Elongation. Tensile strength and elongation of the film were assessed using mechanical universal testing machine (AND MCT-2150, Japan). Edible film width and thickness were measured before the assessments.

Water Vapor Permeability. The WVP method was carried out according to Sobral et al. (2001) [12], based on ASTM E96-80 assay and a modified time test of 24 h. Water vapor transmission was calculated using the Eq. 1:

$$WVP = \frac{\Delta W}{t \times A} \quad \text{Eq. 1}$$

Where, W was the weight change of the edible film after 24 h; t was time (24 h) and A was the surface area of the film (m^2).

Fourier-transform infrared. The edible film was prepared in 0.5-mm thick KBr pellets by mixing 3–5 mg of the film (extra fine) with 200 mg of dried KBr. The FTIR spectra of 4000-400 cm^{-1} were recorded using Shimadzu FTIR-8201 (Shimadzu, Japan). The edible film for FTIR was edible film control and edible film with 2.5% *M. speciosa* extract represented edible film with *M. speciosa* extract.



Surface Structure. The edible film surface structure was investigated using scanning electron microscopy (SEM) (Fei Quanta 250, USA). The SEM magnification was 500 μm .

Antimicrobial Activity. The antimicrobial method was carried out according to Gomes et al. (2019) [13]. The MHA, *E. coli* and *S. aureus* were used in antibacterial activity assessment. Antifungal activity assessment used PDA and *A. niger*. Antibacterial samples were then incubated at 37 °C for 24 h, while antifungal samples were incubated for 3–5 days at room temperature (± 27 °C). Clear zones under the films indicated antimicrobial activity [13].

Antioxidant Activity. The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was used to assess the antioxidant activity. Blank solution was prepared using 2 ml of methanol PA and 1 ml of 50 ppm DPPH. All sample solution were incubated for 30 min at room temperature in dark. Absorbance was measured at 516–517 nm using UV-VIS spectrophotometer. The IC_{50} was calculated referring to the curve equation of inhibition proportion [14].

Quality of Sausage Coating with Edible Film during Storage

Sausages were stored at 27 and 4 °C for 15 days. Sausages were assessed for TPC, YMC and TBARS analysis at Days 0, 3, 6, 9, 12 and 15. Organoleptic characteristics were assessed on Day 0 to investigate consumer acceptance of the edible films with or without the extract.

Total Plate Count. The TPC was carried out using pour plate method. Briefly, 10 g of the sausage from each sample were mashed and mixed with 90 ml of PDF to prepare a dilution ration of 10^{-1} . These were mixed until 10^{-3} dilutions with a ratio of 1:10 were prepared. Then, 1 ml of each sample dilution was poured into Petri dish and incubated at 36 °C for 24–48 h. Numbers of bacteria and fungi were calculated as CFU g^{-1} [15].

Yeast and Mold Count. The YMC method was carried out according to Ahmad and Srivastava (2007) [16]. Sausages were prepared with 10^{-1} dilutions by mashing 5 g of the sausage and mixing the mashers with 45 ml of PDF. Then, 1 ml of each sample was poured into a Petri dish including solid PDA media. The sample was spread in the Petri dish and incubated at 25–27 °C for five days. Yeasts and molds were enumerated from the average number of colonies on a plate with a sample dilution factor.

Thiobarbituric Acid Reactive Substances. The TBARS method was carried out according to Farbod et al. (2015) [17] with modifications. Briefly, 2 g of each sausage were mashed and mixed with 18 ml of 5% TCA and centrifuged for 30 s. Then, 5 ml of the supernatant were mixed with 5 ml of TBARS (0.02 M) solution and heated at 90 °C for 30 min using water bath. Samples were cooled down to 20 °C and assessed at 532 nm using spectrophotometer. The blank solution was made from a mixture between 5 ml of TBARS solution and 5 ml of TCA solution. The TBARS value (mg malonaldehyde kg^{-1} sausage) was calculated using Eq. 2:

$$\text{TBARS value} = \frac{3}{\text{sample weight (g)}} A \times 7.8 \quad \text{Eq. 2}$$

Where, A was the absorbance; 3 was the iod number (degree of oil/grease unsaturation) and 7.8 was the TBARS number of mg malonaldehyde kg^{-1} sausage.

Organoleptic characteristics. Organoleptic characteristics were used to assess consumer assessment of the product. Naturally, sausages with good quality include standard texture, flavor and smell [18]. In this study, sausages were cut into small pieces (1 or 2 bites) and then served to 30 panelists. Variable assessments for the food color, smell, taste and texture were scored using hedonic scale (5 = very good, 4 = good, 3 = neutral, 2 = bad and 1 = very bad).

Statistical Analysis

In this study, completely randomized experimental method was used with three repetitions and a significant level of 95%. The SPSS Software v.22 (SPSS, Chicago, USA) was used to analyze data. Data of tensile strength, elongation, WVP, antioxidant activity, TPC, YMC and TBARS analysis were analyzed using Kruskal-Wallis test. Although data were quantitative, the normality test showed that data were not normally distributed. Kruskal-Wallis test was used to show if the different concentrations of *M. speciosa* extract were effective. Organoleptic data were analyzed using Friedman test to show if various concentrations of *M. speciosa* extract affected the consumer acceptance.

3. Results and Discussion

Characterization of the edible films is shown in Tables I and II. Addition of *M. speciosa* extract included significant effects on TS value and elongation of the edible film. The higher the concentration of *M. speciosa* extract was, the higher the TS value and the lower the elongation value of the edible film were (Table 1).

Table 1. Characteristics of the chitosan edible film with *Medinilla speciosa* extract

Treatment	Tensile strength (MPa)	Elongation (%)	Water Vapor Permeability ($\text{g m}^{-2}\text{h}^{-1}$)	IC_{50} ($\mu\text{g ml}^{-1}$)
Control	0.84 $\pm$ 0.01 ^a	185.26 $\pm$ 5.50 ^b	6.74 $\pm$ 0.16 ^a	311.50 $\pm$ 9.70 ^b
Film with extract 2.5%	0.92 $\pm$ 0.09 ^{ab}	109.41 $\pm$ 2.14 ^{ab}	7.22 $\pm$ 0.24 ^{ab}	128.45 $\pm$ 4.30 ^{ab}
Film with extract 5%	0.94 $\pm$ 0.03 ^{ab}	93.79 $\pm$ 0.50 ^{ab}	9.53 $\pm$ 0.77 ^{ab}	82.20 $\pm$ 2.20 ^{ab}
Film with extract 10%	1.09 $\pm$ 0.01 ^b	73.94 $\pm$ 0.60 ^a	13.44 $\pm$ 1.50 ^b	52.98 $\pm$ 1.30 ^a

Means with different superscripts in the same column (lower case letter) differed significantly ($p < 0.05$)



These show that addition of the *M. speciosa* extract concentration resulted in stronger but less elastic edible films. Physical quality of the edible film with *M. speciosa* extract was good because TS and elongation values of all edible films included the JIS (Japanese Industrial Standard), including minimum TS value of 0.39226 MPa and minimum elongation of 70% [19]. Similar results have been reported in previous studies [20] as TS values increased and the film elongation decreased when grape seed procyanidins and green tea polyphenols were added to the films. Adding *M. speciosa* extract led to a further compact structure of the film that enhanced the TS value but decreased the molecular mobility of the film; thus, elongation decreased as well [21]. Increase and decrease of TS and elongation values could be caused by the reaction of *M. speciosa* extract phenolic compounds with other compounds in the films. Phenolic compounds are known to crosslink with amino acids or other protein molecules to affect physical and mechanical characteristics of the edible films [14]. Phenol interaction between *M. speciosa* extract with other components in edible film might decrease the plastisizer effect and increase TS [20]. In this study, increases in WVP value were significantly affected by differences in the concentration of *M. speciosa* extract in the films (Table 1). The control film with 0% extract of *M. speciosa* was the unique film that included the JIS, reported as $7 \text{ gm}^{-2} \text{ h}^{-1}$ [19]. This was possibly due to the absence of -OH groups from *M. speciosa* extract that could increase the value of WVP on the three other films. Increases in WVP values of films are believed to depend on the availability of polar (-OH) groups in polymers [22]. The control film included -OH group less than that the other films with *M. speciosa* extract did; thus, its WVP value could include the JIS. Presence of -OH bonds can be verified by the FTIR results (Table 2); where, FTIR of the control edible film and edible film with *M. speciosa* extract showed that peaks disappeared after adding the *M. speciosa* extract. Number of the peaks decreased from nine peaks in films without extract to six peaks in films with the *M. speciosa* extract. For example, the peak at 1064.75 nm in films without

extract was linked to the presence of C-O in C₃-OH [23]; however, this peak disappeared in films with *M. speciosa* extract. Reducing and capping agents were respectively shown at peaks of 484.15 and 580.6 nm in films without the extract and those of 442.68 and 532.38 nm in films with the extract; contributing to the stabilization [24]. The peak at 1637.64 nm in films without the extract and 1638.6 nm in films with *M. speciosa* extract demonstrated the C=C alkene. Presence of C=C bonds could be caused by a molecular chain scission [25]. Peaks decreased in FTIR could be caused by several factors. When more than one substance was mixed, physical blends and chemical interactions between the substances could change the spectrum peak characteristics. Chemical interactions of the substances could decrease the number of peaks presented on the FTIR graphs [26].

The WVP value greater than the JIS was affected by the hydrophilic characteristics of the chitosan and sorbitol and surface of the edible films seen using SEM. Surface of edible films with *M. speciosa* extract was hollower and hence water vapor transmission occurred more quickly (Figure 1). Rough surface of the edible films with *M. speciosa* extract could be formed by the extract distribution in the edible film matrix [26]. The *M. speciosa* extract could also reduce hydrogen bonds in the films and increase distances between the molecules; resulting in pores in the matrix and surface of the films [21]. Pores on the edible films with *M. speciosa* extract revealed that films included further heterogeneous structures. Moreover, the *M. speciosa* extract in the films disrupted the hydrophilic region in the film matrix, forming pores and increasing the WVP value [21]. All the edible films included antimicrobial activities against *E. coli*, *S. aureus* and *A. niger* (Figure 2). The antimicrobial activity was shown by the clear zone of inhibition under the films. The clear zone under the edible films did not show different strengths of their antimicrobial activities; hence, it was necessary to assess the films as sausage coatings to demonstrate differences in their antibacterial and antifungal activities. Different strengths of the edible film antimicrobial activity could be demonstrated by the number of microbes that grew.

Table 2. List of Fourier-transform infrared peaks of the edible films with and without *Medinilla speciosa* extract, 4000-400 cm^{-1}

Functional group	Wave number (cm^{-1})	
	Edible film without extract	Edible film with <i>M. speciosa</i> extract
reducing and capping agent	484.15	442.68
reducing and capping agent	580.6	532.38
C-H	630.75	-
C-O of C ₃ -OH	1064.75	-
O-H bending	1282.72	1230.64
CH ₂ bending	1413.88	-
C=C	1637.64	1638.6
C=O (amide I band)	2077.42	2078.39
-OH, -NH ₂	3445.98	3449.84

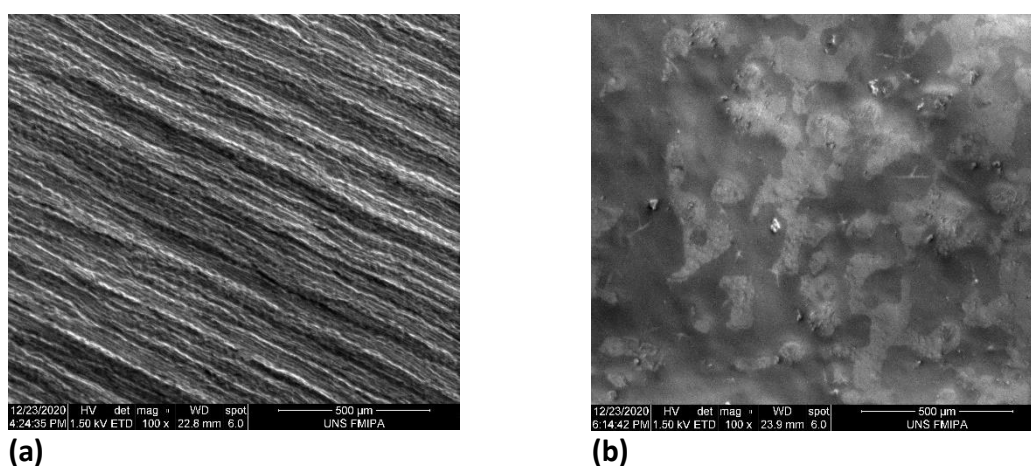


Figure 1. Scanning electron microscope magnification at 500 µm of (a) edible film without *Medinilla speciosa* extract and (b) edible film with *Medinilla speciosa* extract

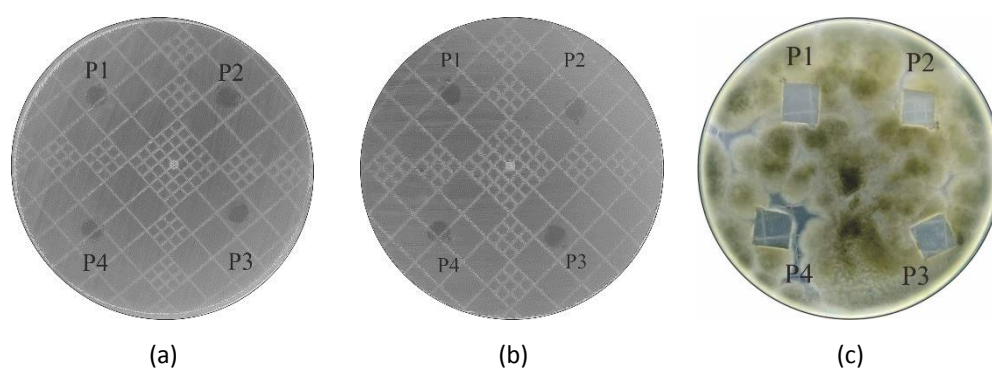


Figure 2. Antimicrobial activity of the edible film with 0% (P1), 2.5% (P2), 5% (P3) and 10% (P4) *Medinilla speciosa* extracts against (a) *Escherichia coli*; (b) *Staphylococcus aureus* and (c) *Aspergillus niger*

Antimicrobial activity of the control edible film might be caused by the chitosan, while antimicrobial activity of the three other films might be caused by the chitosan and antimicrobial agents of the *M. speciosa* extract [5]. Previous studies [13] have shown that chitosan film included antibacterial activity against *E. coli* and *S. aureus*. Chitosan molecules that are not soluble in water can precipitate and stack on the surface of microbial cells to form layers and block the channels, which are dangerous for the survival of microbial cells [27]. Previous studies [5,28] hypothesized that the antimicrobial activity of *M. speciosa* fruits was due to the presence of alkaloids, flavonoids, tannins and saponins. However, active compounds acted as antimicrobial agents in *M. speciosa* were not explicitly characterized. Alkaloids could inhibit and disrupt peptidoglycans and hence formation of the bacterial cell walls was not completed. Flavonoid groups in the plant extracts have shown high potentials of the antimicrobial activity [29]. Tannins led cell walls wrinkle to shrink, damaging cell wall permeability, while saponins could damage cell membrane permeability, lowering cell wall surface tension [5].

The edible film with 10% *M. speciosa* extract showed the best antioxidant activity with the lowest IC_{50} value, compared to three other films (Table I). Statistical analysis revealed significant effects of *M. speciosa* extract on IC_{50} value. The IC_{50} value of the edible films with 10% extract was included in the strong category of $52.98 \mu\text{g ml}^{-1}$, while concentrations of 5, 2.5 and 0% (control) included IC_{50} values of $82.2 \mu\text{g ml}^{-1}$ (strong), $128.45 \mu\text{g ml}^{-1}$ (moderate) and $311.5 \mu\text{g ml}^{-1}$ (weak), respectively. This antioxidant activity is hypothesized to be affected by flavonoids, saponins, tannins and anthocyanins in *M. speciosa* extracts [7,30]. Flavonoids are known as natural antioxidants that belong to the class of polyphenolic compounds. Saponins and tannins (and their derivatives) act as primary antioxidants or free radical scavengers [28]. Moreover, antioxidant activity of the extract is not only based on the number of phenolic compounds but also is based on its characterization and structure. Furthermore, antioxidant activity of the extract is possibly due to the presence of other phytochemicals such as pigments or ascorbic acid [29]. The control film included antioxidant activities due to the

presence of amino groups in chitosan that could interact through ionic interactions with free radicals [30]. In this study, storage quality of sausages was revealed by the total microbial (bacteria and fungi) count and TBARS value. Higher concentrations of *M. speciosa* extract in films resulted in decreases in the number of microbes in sausages (Tables 3 and 4). Sausages stored at 4 °C showed good storage qualities because they included the maximum limits of TPC and YMC, which were respectively less than 4 and 2.3 log CFU g⁻¹[31]. Edible film coated sausages with 10% extract included the best inhibition schemes of microbiological damages during storage, either at 4 or 27 °C. Statistical analyze for TPC and YMC showed significant effects of *M. speciosa* extract addition on decreasing microbial population that grew at 4 or 27 °C. Moreover, concentration of *M. speciosa* extract in edible films was directly proportional to the antimicrobial compounds such as alkaloids, flavonoids and saponins in the film. Alkaloids interfere with the formation of peptidoglycan in the microbial cell walls and function of the fungal mitochondria, leading to cell death [32]. The functional system of flavonoids is almost similar to that of saponins, damaging permeability of the microbial membranes [33]. Phytochemical compounds such as alkaloids, flavonoids,

saponins and tannins in *M. Speciosa* extract act as antimicrobials. Higher *M. Speciosa* extract concentrations include phytochemical compounds and hence can inhibit microbiological damages, prolonging sausage shelf life. Previous studies [34] have revealed similar results regarding correlations of the plant extract concentrations in films with antimicrobial activities of the films.

Further, edible film coating sausages with 10% extract included the lowest TBARS value, followed by the edible film coated sausages with 5, 2.5 and 0% extracts (Table 5), verifying that higher concentrations of the *M. speciosa* extract in films prevent sausage oxidative damages. Statistical analysis exhibited significant effects of the *M. speciosa* extract addition on the TBARS value of sausages.

In fact, TBARS value demonstrates the product rancidity based on the quantity of malonaldehyde (MDA), which is the end product of the lipid peroxide reaction, causing sausage rancidity. High MDA also shows oxidative stress in the product [7]. The *M. speciosa* fruit extract included a strong antioxidant activity with an IC₅₀ value of 6.52 ppm [6]. In preventing oxidative damages to sausages, antioxidant compounds in *M. speciosa* extract which can play roles include flavonoids, saponins, tannins and anthocyanins [7,29,34].

Table 3. Microbial profiles of the sausages coated with edible films of *Medinilla speciosa* extract at various temperatures and storage times

Temperature	Edible film	Day 0 (log CFU g ⁻¹)	Day 3	Day 6	Day 9	Day 12	Day 15
ROOM 27°C	Control (without extract)	1.00±0.22 ^{bdA}	2.4±0.01 ^{bdA}	2.89±0.15 ^{bdAB}	3.34±0.08 ^{bdBC}	5.6±0.13 ^{bdCD}	5.64±0.05 ^{bdD}
	Extract 2.5%	1.00±0.1 ^{abdA}	1.00±0.2 ^{abdA}	2.53±0.08 ^{abdAB}	2.65±0.05 ^{abdBC}	5.41±0.01 ^{abdCD}	5.56±0.03 ^{abdD}
	Extract 5%	1.00±0.23 ^{abdA}	1.00±0.14 ^{abdA}	2.40±0.09 ^{abdAB}	2.58±0.07 ^{abdBC}	5.41±0.05 ^{abdCD}	5.43±0.01 ^{abdD}
	Extract 10%	1.00±0.14 ^{adA}	1.00±0.1 ^{adA}	2.40±0.05 ^{adAB}	2.40±0.04 ^{adBC}	4.40±0.03 ^{adCD}	5.40±0.05 ^{adD}
COLD 4°C	Control (without extract)	1.00±0.13 ^{bcA}	1.00±0.1 ^{bcA}	1.00±0.23 ^{bcAB}	2.40±0.02 ^{bcBC}	2.62±0.07 ^{bcCD}	2.95±0.04 ^{bcD}
	Extract 2.5%	1.00±0.13 ^{abcA}	1.00±0.23 ^{abcA}	1.00±0.22 ^{abcAB}	2.40±0.05 ^{abcBC}	2.51±0.09 ^{abcCD}	2.97±0.03 ^{abcD}
	Extract 5%	1.00±0.22 ^{abcA}	1.00±0.08 ^{abcA}	1.00±0.13 ^{abcAB}	2.40±0.09 ^{abcBC}	2.46±0.10 ^{abcCD}	2.51±0.05 ^{abcD}
	Extract 10%	1.00±0.10 ^{acA}	1.00±0.13 ^{acA}	1.00±0.10 ^{acAB}	2.40±0.03 ^{acBC}	2.40±0.06 ^{acCD}	2.4±0.02 ^{acD}

Means with different superscripts in the same row (upper case letter) and column (lower case letter) differed significantly ($p < 0.05$)

Table 4. Yeast and mold counts of the sausages coated with edible films of *Medinilla speciosa* extract at various temperatures and storage times

Temperature	Edible film	Day 0 (log CFU g ⁻¹)	Day 3	Day 6	Day 9	Day 12	Day 15
ROOM 27°C	Control (without extract)	1.00±0.22 ^{bdA}	2.90±0.03 ^{bdAB}	3.18±0.18 ^{bdBCD}	3.18±0.08 ^{bdCDE}	3.18±0.10 ^{bdDE}	3.18±0.10 ^{bdE}
	Extract 2.5%	1.00±0.10 ^{abdA}	1.30±0.08 ^{abdAB}	2.74±0.04 ^{abdBCD}	2.76±0.05 ^{abdCDE}	3.18±0.08 ^{abdDE}	3.18±0.10 ^{abdE}
	Extract 5%	1.00±0.22 ^{abdA}	1.00±0.14 ^{abdAB}	2.32±0.1 ^{abdBCD}	2.61±0.05 ^{abdCDE}	2.56±0.05 ^{abdDE}	3.18±0.07 ^{abdE}
	Extract 10%	1.00±0.14 ^{adA}	1.00±0.13 ^{adAB}	1.30±0.07 ^{adBCD}	2.24±0.04 ^{adCDE}	2.36±0.10 ^{adDE}	2.92±0.02 ^{adE}
COLD 4°C	Control (without extract)	1.00±0.13 ^{bcA}	1.00±0.14 ^{bcAB}	1.18±0.03 ^{bcBCD}	1.30±0.08 ^{bcCDE}	2.40±0.09 ^{bcDE}	2.68±0.10 ^{bcE}
	Extract 2.5%	1.00±0.13 ^{abcA}	1.00±0.10 ^{abcAB}	1.18±0.07 ^{abcBCD}	1.18±0.03 ^{abcCDE}	1.70±0.09 ^{abcDE}	1.88±0.01 ^{abcE}
	Extract 5%	1.00±0.23 ^{abcA}	1.00±0.17 ^{abcAB}	1.00±0.14 ^{abcBCD}	1.00±0.13 ^{abcCDE}	1.18±0.03 ^{abcDE}	1.78±0.07 ^{abcE}
	Extract 10%	1.00±0.10 ^{acA}	1.00±0.19 ^{acAB}	1.00±0.15 ^{acBCD}	1.00±0.17 ^{acCDE}	1.00±0.14 ^{acDE}	1.30±0.08 ^{acE}

Means with different superscripts in the same row (upper case letter) and column (lower case letter) differed significantly ($p < 0.05$)

Table 5. Thiobarbituric acid reactive substances analyses of the sausages coated with edible films of *Medinilla speciosa* extract at various temperatures and storage times

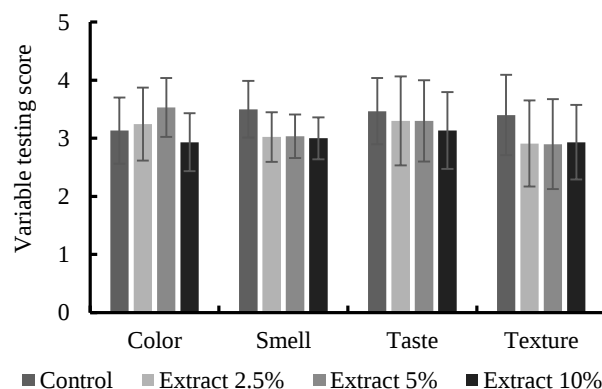
Temperature	Edible film	Day 0 (mg malonaldehyde kg ⁻¹ sausage)	Day 3	Day 6	Day 9	Day 12	Day 15
COLD (4 °C)	Control (without extract)	4.73±0.04 ^{cdA}	7.31±0.13 ^{cdAB}	9.80±0.08 ^{cdBC}	11.08±0.23 ^{cdC}	17.28±0.45 ^{cdDE}	20.96±0.20 ^{cdE}
	Extract 2.5%	4.77±0.01 ^{bcdA}	6.90±0.21 ^{bcdAB}	8.99±0.38 ^{bcdBC}	8.26±0.22 ^{bcdC}	10.63±0.28 ^{bcdDE}	15.67±0.32 ^{bcdE}
	Extract 5%	4.88±0.22 ^{abcdA}	6.71±0.16 ^{abcdAB}	7.87±0.25 ^{abcdBC}	8.10±0.21 ^{abcdC}	8.63±0.25 ^{abcdDE}	15.56±0.29 ^{abcdE}
	Extract 10%	4.73±0.02 ^{adA}	4.75±0.15 ^{adAB}	5.75±0.23 ^{adBC}	7.78±0.17 ^{adC}	8.05±0.31 ^{adDE}	13.38±0.22 ^{adE}
ROOM (27 °C)	Control (without extract)	4.73±0.03 ^{ceA}	8.27±0.17 ^{ceAB}	13.52±0.13 ^{ceBCD}	15.91±0.09 ^{ceCD}	23.05±0.04 ^{ceDE}	23.62±0.17 ^{ceE}
	Extract 2.5%	4.77±0.01 ^{bceA}	6.69±0.05 ^{bceAB}	13.52±0.08 ^{bceBC}	15.58±0.26 ^{bceC}	16.25±0.56 ^{bceDE}	22.18±0.10 ^{bceE}
	Extract 5%	4.88±0.23 ^{abceA}	5.73±0.17 ^{abceAB}	10.35±0.17 ^{abceBC}	11.60±0.07 ^{abceC}	15.91±0.44 ^{abceDE}	17.84±0.03 ^{abceE}
	Extract 10%	4.73±0.02 ^{aeA}	5.64±0.13 ^{aeAB}	6.10±0.10 ^{aeBC}	7.18±0.11 ^{aeC}	10.18±0.26 ^{aeDE}	14.82±0.22 ^{aeE}

Means with different superscripts in the same row (upper case letter) and column (lower case letter) differed significantly ($p < 0.05$)

Those antioxidant compounds can inhibit formation of oxygen free radicals [14]. Correlations between the food shelf life and microbial growth in preventing sausage oxidative damages might be due to the phyto-chemical compounds in the film. Previous studies on *M. speciosa* extract revealed that the *M. speciosa* extract included phytochemical compounds such as alkaloids, flavonoids, saponins, tannins and anthocyanins as antimicrobial [4,5] and antioxidant [6,7] agents. Decreases of peaks in FTIR indicated that the *M. speciosa* extract bound with other components in the film matrix [35], automatically carrying *M. speciosa* phytochemical compounds. Concentration of *M. speciosa* extract in the film effected the shelf life of the sausages due to the existence of the antimicrobial and antioxidant activities [34]. One of the major mechanisms of the *M. speciosa* antimicrobial activity was linked to its phytochemical hydrophilic and hydrophobic sites that cooperated to penetrate to the microbial cell, leading to the cell death [36].

In this study, organoleptic characteristics were assessed only on Day 1 of the experiment because TPC and YMC values of the sausages at the end of the shelf life did not include the standard, which was more than 4 log CFU g⁻¹ for TPC and 2.3 log CFU g⁻¹ for YMC [31]. Tables III and IV show that the control sausage passed the standard at 27 °C on Day 3 of the storage. Limited shelf-life of the sausages coated with edible films with *M. speciosa* extract concentrations of 2.5 and 5% included three days and was extended to Day 9 for 10% concentrations. At 4 °C, shelf life of the control sausage was limited until Day 12 of storage, shorter than that of sausage coated with edible films of *M. speciosa* extract, which was below the standard until Day 15 of storage. Responses of 30 respondents to the organoleptic assay variables of sausages coated with or without *M. speciosa* extract are listed in Figure 3. Significance values of the Friedman test for color, smell, taste and texture respectively included 0.511, 0.068, 0.117 and 0.281 ($p > 0.05$), meaning that addition of *M. speciosa* extract into edible films were not significantly different in consumer acceptance. It was possibly caused by similar sausage ingredients as only the

coatings were different. Use of films with *M. speciosa* extract did not lead to dislike of the product by the consumers. The *M. speciosa* fruit extract in the film coating was acceptable by the respondents because *M. speciosa* fruit was a typical fruit of Mount Muria, Kudus City, Indonesia, and was commonly consumed directly [7].

**Figure 3.** Organoleptic characteristics of the sausages coated with edible films with and without *Medinilla speciosa* extract

4. Conclusion

In conclusion, edible film of the *M. speciosa* fruit extract as a sausage film coating included good quality because it could inhibit microbiological and oxidative damages in sausages. Addition of *M. speciosa* fruit extract to the film also included good quality because of its JIS. Although WVP of the edible film with 10% *M. speciosa* extract was above the JIS value, it included the best quality within the three films because it could inhibited the microbial growth and its oxidative damages. The edible film with 10% extract included the best quality based on the shelf-life assessments since the number of bacteria, yeasts and molds and the rancidity value (TBARS analysis) in sausages included the smallest values during the storage. Furthermore, no major differences were reported in consumer acceptances of the edible films with or without the extract.

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6. Conflict of Interest

The authors report no conflicts of interest.

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کاربرد فیلم خوراکی حاوی عصاره میوه پاریجوتو (*Medinilla speciosa* Blume) برای مهار رشد میکروبی و تخریب اکسیداتیو سوسیس

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چکیده

سابقه و هدف: فیلم‌های خوراکی یکی از راهکارها برای بسته‌بندی مواد غذایی حاوی ترکیبات آنتی اکسیدان و ضد میکروبی است، که معمولاً در گیاهان *Medinilla speciosa* یافت می‌شوند. هدف این مطالعه، بررسی اثرات عصاره میوه *Medinilla speciosa* بر فعالیت فیزیکی، شیمیایی و زیستی فیلم‌های خوراکی و نیز اثرات پوشش بر کیفیت سوسیس در مدت نگهداری بود.

مواد و روش‌ها: فیلم خوراکی حاوی $2\% w w^{-1}$ کیتوزان، $2\% w w^{-1}$ سوربیتول و عصاره میوه *Medinilla speciosa* بود. عصاره‌های *Medinilla speciosa* در غلظت‌های گوناگون ۰، ۵، ۲/۵ و ۱۰ درصد وزنی و درجه حرارت نگهداری ۴ و ۲۷ درجه سلسیوس تهیه شد. هفت ویژگی فیلم خوراکی شامل استحکام کششی، افزایش طول، نفوذپذیری بخار آب، فعالیت‌های ضد میکروبی و ضد اکسایشی^۱، ساختار سطحی و پاسخ مادون قرمز تبدیل فوری^۲ بود. ویژگی‌های محصول در مدت نگهداری شامل شمارش کلی میکروبی، شمارش کپک و مخمر و ترکیبات واکنش تیوباریتوریک اسید بود. داده‌ها با آزمون کروسکال والیس آنالیز شدند. ویژگی‌های حسی^۳ با نرم افزار فریدمن و SPSS تجزیه و تحلیل شدند.

یافته‌ها و نتیجه‌گیری: نتایج نشان داد با افزایش غلظت عصاره میوه *Medinilla speciosa*، استحکام کششی، نفوذپذیری بخار آب و فعالیت آنتی‌اکسیدانی افزایش و افزایش طول کمتر بود. فیلم شاهد که فاقد عصاره میوه *Medinilla speciosa* بود، تنها فیلمی بود که با استاندارد ژاپنی نفوذپذیری بخار آب، $6/74\text{ gm}^{-2}\text{ h}^{-1}$ ، انطباق داشت. علاوه بر این، نتایج عمر انباری^۴ سوسیس با پوشش فیلم‌های خوراکی نشان داد در مقایسه با فیلم کنترل، افزایش غلظت عصاره *Medinilla speciosa* موجب کاهش شمارش کلی میکروبی، شمارش کپک و مخمر و ترکیبات واکنش تیوباریتوریک اسید و معادل به ترتیب $2/4 \pm 0/2 \log\text{ CFU g}^{-1}$ ، $1/3 \pm 0/08 \log\text{ CFU g}^{-1}$ و $13/38 \pm 0/22$ میلی گرم مالون آلداید بر کیلوگرم سوسیس شد. بررسی ویژگی‌های حسی تفاوت عمده‌ای در پذیرش مصرف کننده را نشان نداد. در نتیجه، فیلم خوراکی حاوی ده درصد عصاره *Medinilla speciosa* بهترین فیلم به لحاظ فعالیت فیزیکی، شیمیایی و زیستی است. همچنین این فیلم عمر انباری سوسیس را افزایش می‌دهد.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافع مرتبط با انتشار این مقاله ندارند.

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^۱ Antimicrobial and antioxidant activities

^۲ Fourier-transform infrared response

^۳ Organoleptic characteristics

^۴ Shelf life

