Food Research 7 (Suppl. 3) : 107 - 117 (2023)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

Active packaging based on tilapia skin gelatin added with lemon basil leaf extract

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Article history:
Received: 11 November 2022
Received in revised form: 29 June 2023
Accepted: 27 September 2023
Available Online: 31 October 2023

Keywords:
Active packaging,
Lemon basil leaves,
Edible film,
Tilapia gelatin

DOI:
https://doi.org/10.26656/fr.2017.7(S3).14

Abstract

Active packaging can actively change the condition of the packaged food by optimizing its ability to maintain the product. Edible film is easily digested and has zero waste. Gelatin can form a flexible and strong film. The high amino acids in gelatin can increase its ability to form a gel. Tilapia skin (*Oreochromis niloticus*) contains high protein. It is a suitable material to be developed into edible films and active packaging by adding lemon basil leaves (*Ocimum basilicum* L.) as antioxidants and antimicrobials. This study aimed to determine the effect of various concentrations of basil leaf extract on the physical, mechanical, chemical, and antibacterial properties of the gelatin-based edible film of tilapia skin. The treatment used different concentrations of lemon basil leaf extract; 0.2%, 0.4%, 0.6% and 0.8%. The tests include thickness, solubility, tensile strength and elongation, moisture content, water vapor permeability (WVP), antibacterial activity, and antioxidant activity. The results showed that adding lemon basil leaf extract significantly affects the value of solubility, tensile strength, elongation, moisture content, antibacterial activity, and antioxidant activity but was not significantly different on the thickness and WVP parameters.

1. Introduction

Packaging is a preservation method to protect food products from external influences, such as microorganisms, sunlight, moisture, oxygen, impact, friction and insects. It also functioned as a product attraction and facilitated storage and distribution. Packaging can maintain product quality until it is ready for consumption (Ojha *et al*., 2015; Mane, 2016). Nowadays, packaging has been developed into active packaging to optimize its ability to maintain the product. Active packaging is defined as packaging that can actively change the condition of the packaged food product to improve food safety, increase shelf life, maintain flavor and maintain product quality. This active packaging can release or absorb compounds found around or inside the packaging (Prasad and Kochhar, 2014).

Based on their role, active packaging is classified into four categories, such as absorbing, releasing, removing, and temperature-regulating agents. Active packaging as an absorbent agent can absorb oxygen, carbon dioxide, moisture, ethylene and unwanted flavors in the product. Active packaging as a release agent can release ethanol compounds, carbon dioxide, antioxidants, preservatives, sulfur dioxide and certain flavors in the product. Active packaging as a removal agent can reduce cholesterol and lactose levels in the product, and active packaging as a regulator can regulate the temperature around the product (Prasad and Kochhar, 2014). The application of active packaging with antimicrobial compounds has been developed in the United States and Japan for fresh ingredients. The antimicrobial compounds used are spice extracts such as cinnamon, cloves, and oregano (Baghi *et al*., 2022).

Meanwhile, plastic is the most widely used packaging material in various industries. It is strong, light, does not react with acids, water, alkalis, and other chemicals, has a relatively low price, and has an excellent barrier to oxygen and water vapor. However, plastic can pollute the environment because it is

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eISSN: 2550-2166
Published by Rynnye Lyan Resources

composed of synthetic polymers that are difficult to degrade by microorganisms in the soil. Plastic takes 100 to 1000 years to degrade completely. Also, burned plastic will produce carbon emissions that can pollute the air (Ncube *et al*., 2020; Anani and Adetunji, 2021). Therefore, it is necessary to develop biodegradable and edible packaging materials to reduce environmental pollution.

The edible film is a thin protective layer (less than 0.3 mm) made from edible materials. Edible films have several advantages, such as being easily degraded, edible, and easily digested, and protecting products from external influences (Díaz-Montes and Castro-Muñoz, 2021; Su *et al*., 2022). Edible films can be composed of hydrocolloids, lipids, and composites. Edible hydrocolloid film has good mechanical properties and can withstand the rate of oxygen, carbon dioxide, and fat. Polysaccharides and proteins are part of hydrocolloids that can be used as ingredients for edible films (Anandito *et al*., 2012).

Protein-based edible films have better gas, physical, and mechanical barrier properties than polysaccharide and lipid edible films. Proteins have hydrogen bonds with strong intermolecular bonds (Murrieta-Martínez *et al*., 2018). Fish is a source of animal protein and can be used as a constituent of edible films. Fish protein, especially myofibrillar and sarcoplasmic proteins, can provide reasonable flexibility to edible films (Artharn *et al*., 2008). Gelatin is a biopolymer produced from the hydrolysis of collagen proteins in the skin, muscle tissue, and animal bones. Gelatin can form flexible and strong films, increase viscosity, transparent, easily soluble in water, gelling, and easy to digest (Nitsuwat *et al*., 2021). Dry gelatin has a protein content of around 84 - 86% with a high amino acid content. Glycine (Gly) is the highest type of amino acid in gelatin, about 21%. The high content of amino acids in gelatin can increase its ability to form a gel. One of the fishery commodities with high protein content is tilapia (*Oreochromis niloticus*). The tilapia filet industry can produce as much as 60% solid waste consisting of head, bones, skin, entrails, and scales with a protein content of 18%. Among the solid wastes, tilapia skin has the highest yield compared to others (Darmanto *et al.* 2017; Finarti *et al*., 2018; Suryanti *et al*., 2018). Due to its high yield and protein content, tilapia skin can be processed into edible films.

The active packaging can also be developed from tilapia skin gelatin by adding antioxidant and antimicrobial substances to the coating. One natural active substance with antimicrobial and antioxidant activity is lemon basil leaves or Kemangi leaves. Lemon basil (*Ocimum basilicum* L.) is a medicinal plant used as a natural insecticide, essential oil, and vegetable. The use of lemon basil leaves as a natural preservative has not been widely known, even though it contains cineol and linalool as antibacterial and antifungal agents (Kačániová *et al*., 2022). In addition, it also contains flavonoids that act as natural antioxidants by capturing free radical molecules. Other chemical compounds found in lemon basil leaves include tannins, steroids, essential oils, xylose, molludistin, pentose, ursolic acid, and methyl homoanisic acid (Riyani *et al*., 2021).

The research is conducted to develop active packaging on protein-based edible films from tilapia skin gelatin. Lemon basil leaf extract was added to the edible film as an active substance with antibacterial and antioxidant activity. Therefore, this study aimed to determine the effect of various concentrations of basil leaf extract (*Ocimum basilicum* L.) on the physical, mechanical, chemical, and antibacterial properties of the gelatin-based edible film of tilapia skin (*Oreochromis niloticus*).

2. Materials and methods

2.1 Materials

The material used in this study was tilapia skin gelatin purchased online from the marketplace. Lemon basil leaves from a traditional market in Cepogo, Central Java, Indonesia. Chemicals such as 96% ethanol from (Merck, Germany), glycerol, and distilled water were obtained from PT. Cipta Kimia, Indonesia.

2.2 Basil leaf extract production

The extraction of lemon basil leaf was performed by modifying the method described by Adam and Omer (2015). Fresh lemon basil leaves were withered for 48 hrs at room temperature, then dried in a cabinet dryer at 45℃ for 2 hrs. The dried leaves were then reduced in size using a blender (Maspion, Indonesia) and sieved through a 20-mesh sieve. The simplicia was then macerated with 96% ethanol (Merck, Germany) with a ratio of 1:10 simplicia and solvent for 24 hrs. The extract was then filtered through Whatman Filter paper No. 1. Finally, the solvent was evaporated using a rotary evaporator (Tmax Equipments Ltd, China). The thick extract of lemon basil leaves was stored in dark glass bottles covered with aluminum foil at 5℃.

2.3 Active packaging production

The active packaging was performed by modifying the method described by Santoso *et al*. (2020). Around 6% of lemon basil leaf extract with a concentration of 0%, 0.2%, 0.4%, 0.6% and 0.8% (v/v) respectively were

https://doi.org/10.26656/fr.2017.7(S3).14

added to this study. The active packaging was made by mixing 6% (w/v) tilapia gelatin, 15% (v/v) glycerol, and 6% lemon basil leaf extract added with distilled water until 100 ml, heated at 40℃, and stirred until homogeneous using a magnetic stirrer. The mixture was poured onto a glass plate with a diameter of 10 cm and then baked at 45℃ for 15 hrs. After drying, the edible film is packaged in airtight packaging and stored at room temperature.

2.4 Thickness analysis

Thickness analysis was carried out using a manual screw micrometer (Mitutoyo, Japan) with an accuracy of 0.001 mm at five different points of edible film samples; each of four sides and the center of the edible film with a diameter of 3.2 cm. The value of the thickness of the edible film was obtained by averaging the thickness of five points (Chae dan Heo, 1997).

2.5 Solubility analysis

The edible film sample was shaped into a square of 3×3 cm; then, the piece was placed in a crucible with known dry weight and dried using an oven at 105℃ for 30 mins. Then, weighed the sample to determine the initial dry weight (W_0), followed by immersion using distilled water for 24 hrs. The insoluble samples were then dried in an oven at 105℃ for 2 hrs. After that, the piece was left in a desiccator for 10 mins and weighed the final dry weight (W_1). The solubility value of the edible film is the percentage difference between W_0 and W_1 divided by W_0 (Gontard, 1993).

2.6 Analysis of tensile strength and elongation

The analysis was carried out using the ASTM D 638 method using the Zwick Z020 assay (ASTM, 1996). The sample was formed according to the test equipment. Then run the machine at a constant speed until the maximum force data, surface area, and sample length was obtained when disconnected.

$$\text{Tensile strength (Mpa)} = \frac{\text{Maximum force}}{\text{Surface area}}$$

$$\text{Elongation} = \frac{\text{sample length at the break} - \text{start length of the sample}}{\text{start length of the sample}} \times 100\%$$

2.7 Moisture content analysis

The moisture content analysis (AOAC, 2005) was started by drying the empty porcelain crucible using an oven at 105℃ for 30 mins, then cooled in a desiccator for 15 mins and weighed. Next, 2 g of sample were put into a crucible to determine the initial weight, then dried in an oven at 105℃ for 12 hrs and desiccated for 15 mins. After that, weigh the sample until it obtained a constant weight. The weight of the piece was declared stable if there was no change in weight after several times of drying. Moisture content was calculated by dividing the weight of water lost during heating by the initial weight of the sample multiplied by 100%.

2.8 Water vapor permeability analysis

The ASTM (1996) was used. The edible film sample was spread over a permeation cell tube containing silica gel (0% RH). Next, put the permeation cell into a desiccator having a saturated NaCl solution (70% RH) at 30℃. Weighed the permeated cells every 24 hrs to obtain five points. Then, the thickness of the edible film was measured using a micrometer screw. Then graphed data of changes in weight and time to determine the value of the water vapor transmission rate (WVTR). Used the following equation to analyze the WVP value:

$$\text{WVP} = \frac{\text{WVTR}}{\text{Ps}(\text{RH1} - \text{RH2})} \times \delta\text{m}$$

Where Ps was the saturated water vapor pressure at the experimental temperature (30℃), RH_1 was the desiccator's relative humidity, RH_2 for the permeation cell, and m for the average thickness of the edible film.

2.9 Analysis of antibacterial activity

The antibacterial activity was analyzed by inoculating *Staphylococcus aureus* and *Escherichia coli* bacteria in nutrient broth (NB) media and then incubating at 37℃ for 24 hrs (Mulyadi *et al*., 2016). Next, put 0.1 mL of the bacterial suspension into a petri dish containing nutrient agar (NA) media. The petri dish was then rotated and allowed to solidify. In the agar diffusion method, the solid media was perforated using a cork borer and added 100 L of lemon basil leaf extract. In contrast, the paper disk method was done by placing a sample of the edible film with a diameter of 5 mm on NA media containing 1 mL of the solidified bacterial suspension. Furthermore, the samples were incubated at 37℃ for 24 hrs and measured the diameter of the inhibition zone formed around the wells and edible film samples.

2.10 Analysis of antioxidant activity

The analysis begins by dissolving the film into distilled water at 40℃ while stirring. Next, the sample was diluted in a series of 0, 5, 10 and 15 dilutions. Next, 1 mL of each dilution was added with 7 mL of methanol and 2 mL of DPPH solution with a concentration of 20 mg/L. The solution was then homogenized using a vortex and incubated in the dark for 30 mins. The absorbance was measured at 517 nm using a spectrophotometer. The results then calculated the percentage of inhibition against DPPH (Santoso *et al*., 2020).

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2.11 Statistical analysis

This research was done in triplicate, and data were analyzed using ANOVA. The significant difference was then further tested by using Duncan.

3. Results and discussion

3.1 Thickness

The results of the thickness analysis are shown in Figure 1. Based on Figure 1, the thickness value of active edible film packaging ranged from 0.229 to 0.237 mm. The control sample was the lowest at 0.229 mm, while the highest at 0.237 mm on 0.8% lemon basil leaf extract. The analysis showed an increase in the thickness value but no significant difference as the leaf concentration was added. All thickness values in this study followed the standards set by the Japan Industrial Standards (1975), with the highest permissible thickness of the edible film was 025 mm. The results in this study concurred with Hemalatha *et al*. (2017) in that the lemon basil extract in edible films made from chitosan showed an increase in the thickness value along with the extract concentration. However, the growth that occurred was not significantly different.

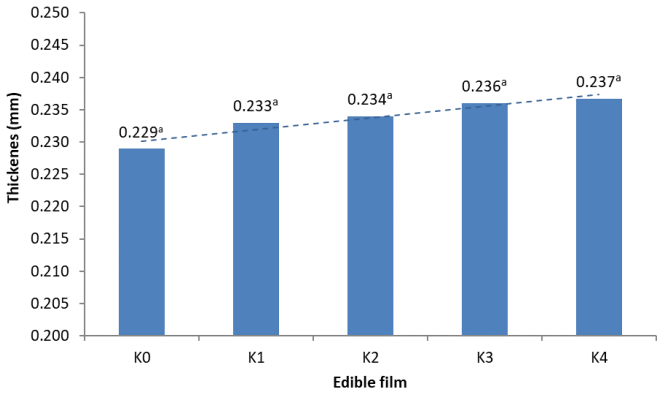

Figure 1. The thickness of active packaging. Values with different superscript are statistically significantly different ($p<0.05$). K0 = Control, K1 = 0.2% of leaf extract (w/v), K2 = 0.4% of leaf extract (w/v), K3 = 0.6% of leaf extract (w/v), K4 = 0.8% of leaf extract (w/v).

Different results were shown by Nurdiani *et al*. (2022), which showed that the thickness value increased along with the addition of mangrove extract. The increase in thickness was due to the increase in total dissolved solids in the edible film. The higher the leaf extract concentration added, the greater the total dissolved solids in the film solution. Total dissolved solids are influenced by the mineral composition of the materials used to make edible films (Mulyadi *et al*., 2016). In addition, according to Hemalatha *et al*. (2017), the increase in the thickness value is influenced by the increase in the hydration layer of the polymer on the edible film, causing a thickening effect.

The results that were not significantly different in this study were caused by the concentration of the leaf extract, which tended to be low, so it did not affect changes in total dissolved solids and polymer hydration layers on edible films. In addition, according to Nurdiani *et al*. (2022), increasing the concentration of gelatin and glycerol can significantly increase the thickness of the edible film. However, in this study, the concentration of tilapia skin gelatin and glycerol used for each treatment was the same.

3.2 Solubility

The results of the solubility analysis are shown in Figure 2. The result ranged from 25.133–36.319%, with the lowest value found on 0.8% leaf extract and was significantly different from the other treatments. While the addition of basil leaf extract with a concentration of 0.2–0.6% was not significantly different. The decrease in solubility value occurred because of the bond between the non-polar components of the leaf extract with hydrophobic amino acids in gelatin, which increased the hydrophobic components of the film. In addition, the extract is a hydrophobic essential oil, so its addition reduces the hydrophilicity of gelatin and glycerol (Ahmad *et al*., 2012). Adding lemon basil leaf extract resulted in cross-linking between the phenolic compounds in the extract and gelatin. This interaction resulted in a reasonably strong bond and increased the hydrophobic properties of the film (Getachew *et al*., 2021). In this study, there was a decrease in the solubility value, indicating a significant difference from the control by adding 0.8% leaf extract. It happened because the low extract concentration was insufficient to dominate the hydrophilic nature of the interaction between gelatin and the plasticizer that had formed (Hemalatha *et al*., 2017).

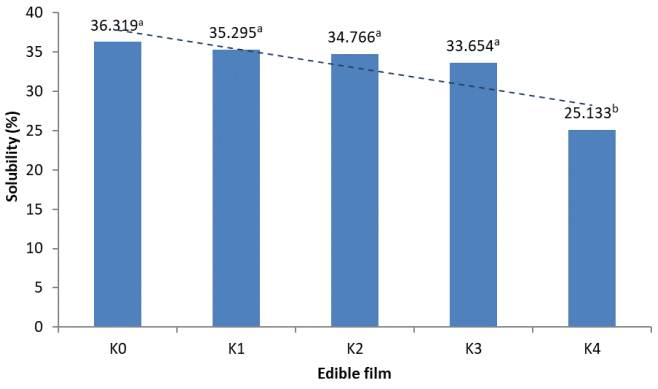

Figure 2. Solubility of active packaging. Values with different superscript are statistically significantly different ($p<0.05$). K0 = Control, K1 = 0.2% of leaf extract (w/v), K2 = 0.4% of leaf extract (w/v), K3 = 0.6% of leaf extract (w/v), K4 = 0.8% of leaf extract (w/v).

The results of this study concurred with the research by Ahmad *et al*. (2012), who added the extracts of

https://doi.org/10.26656/fr.2017.7(S3).14

bergamot and lemongrass to the edible film of flatfish gelatin fish skin (*Aluterus monoceros*). The study showed a significant decrease in the control sample from 97.8% to 88.92% with 25% bergamot extract and 89.16% with 25% lemongrass extract. Another study by Getachew *et al*. (2021) showed a significant decrease in the solubility value from 93.95% in the control sample to 67.21%. Adding 5% coffee grounds extract decreased to 53.81% in the control sample–the addition of the extract concentration of 20%. The research of Hemalatha *et al*. (2017), who added basil leaf extract to chitosan edible film showed a decrease in the solubility value along with an increase in the concentration of the extract used. The result showed that the solubility values ranged from 10.33–16.50%, with significant differences in 0.3% and 0.5% extract. The solubility in this study and that of Hemalatha *et al*. (2017), when compared to other plant extracts, was lower due to the hygroscopic nature of basil leaves than other herbal plants.

3.3 Tensile strength

The results of the analysis of the tensile strength are shown in Figure 3. Based on Figure 3, the value of the tensile strength of active packaging of edible film from tilapia skin gelatin was around 5.012–16.790 MPa and lower than the control sample. The tensile strength value also decreased with the increase in the concentration of the leaf extract used. The lowest tensile strength value of 5.012 Mpa was found on 0.8% leaf extract, and the control sample produced the highest value of 16.790 Mpa. According to the Japan Industrial Standards (1975), an excellent tensile strength has a minimum value of 3.94 MPa. Thus, all samples in this study had tensile strength values that met the standard. The results followed previous studies regarding basil extract's addition to chitosan edible films by Hemalatha *et al*. (2017), which showed a decrease in tensile strength values compared to controls. The tensile strength values ranged from 30.04–39.00 Mpa and found a significant reduction on 0.3% and 0.5%. Another study by Yanwong and Threepopnatkul (2015), who added the mint leaf extract into fish skin gelatin edible films, also showed a decrease in the tensile strength value compared to the control sample. The reduction in tensile strength value occurred significantly with the addition of 10% mint leaf extract, which was 9.2% (33.46 to 30.38 Mpa).

The decrease in the tensile strength value in this study was caused by the interruption of the basil leaf extract on the hydrogen bonds connecting the proteins in gelatin. The higher the leaf extract concentration, the greater the interruption in hydrogen bonding, resulting in a more brittle film and a lower tensile strength value. It was followed by the statement of Hemalatha *et al.* (2017) that adding basil extract to chitosan can trigger matrix breakdown, which results in a decrease in film cohesion and changes in polymer chain interactions. In addition, the active substance also has a plasticizing ability, reducing block interactions between gelatin chains to produce a film that breaks easily (Yanwong and Threepopnatkul, 2015).

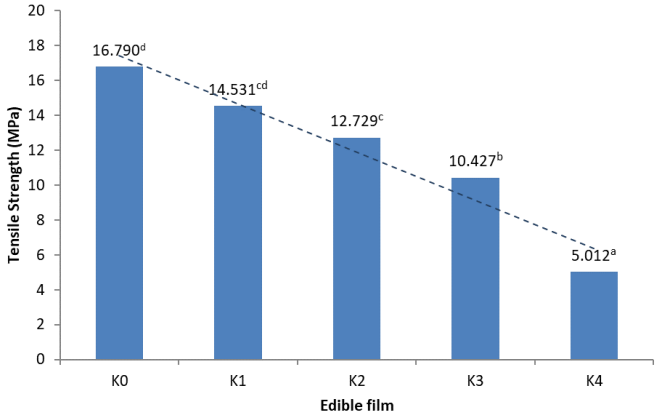

Figure 3. Tensile strength of active packaging. Values with different superscript are statistically significantly different ($p<0.05$). K0 = Control, K1 = 0.2% of leaf extract (w/v), K2 = 0.4% of leaf extract (w/v), K3 = 0.6% of leaf extract (w/v), K4 = 0.8% of leaf extract (w/v).

3.4 Elongation

The results of the elongation analysis of the active edible film from tilapia skin gelatin are shown in Figure 4. Based on Figure 4, the elongation value with 0.2% leaf extract increased significantly compared to the control. However, the elongation value decreased as higher leaf extract concentration were added. The highest elongation value of 107.708% was found on 0.2% leaf extract, and the lowest value of 34.877% on 0.8% lemon basil leaf extract. At the same time, the control sample has an elongation value of 45.806%. Active packaging with the addition of 02% lemon basil leaf extract meets the elongation standard by Japan Industrial Standards (1975), which is a minimum of 70%. In comparison, the other four samples did not meet these standards.

Previous research regarding the addition of citronella extract to fish skin gelatin edible films conducted by Yanwong and Threepopnatkul (2015) showed an increase in the elongation value in the addition of extract by 10% compared to the control. However, it decreased with increasing concentration of the extract used. The elongation value of the control sample was 3.44%, while the 10 - 30% citronella extract decreased from 15.33 to 4.68%. Another study by Ahmad *et al*. (2012) also showed an increase in the elongation value by adding bergamot extract by 5% compared to the control. However, the value decreased as the concentration of the extract increased. The control sample in the form of

https://doi.org/10.26656/fr.2017.7(S3).14

edible film from fish skin *Aluterus monoceros* gelatin has an elongation value of 6.99%. In comparison, adding 5% bergamot extract elongates 8.76% and decreases to 3.06% with the addition of 25% extract.

The decrease in elongation value was due to the bond formed between the compounds in the extract and the protein in gelatin which causes the film to become stiff and inelastic (Yanwong and Threepopnatkul, 2015). In addition, adding essential oils or polymeric compounds in large quantities produces films with various mechanical characteristics, including bond weakening and disordered reactions. However, the addition of extracts at appropriate concentrations can increase the interactions that occur between gelatin proteins. The increase in the elongation value compared to the control can be caused by the presence of extracts in the form of oil droplets on the film matrix, which is easily deformed, thereby increasing its flexibility (Ahmad *et al*., 2012). In comparison, the decrease in elongation value and the increase in the concentration of lemon basil leaf extract was due to the interaction between phenolic compounds and gelatin molecules, which can break the bonds between gelatins. Therefore, the more basil leaf extract was added, the more gelatin bonds were broken, making the film less elastic (Getachew *et al*., 2021).

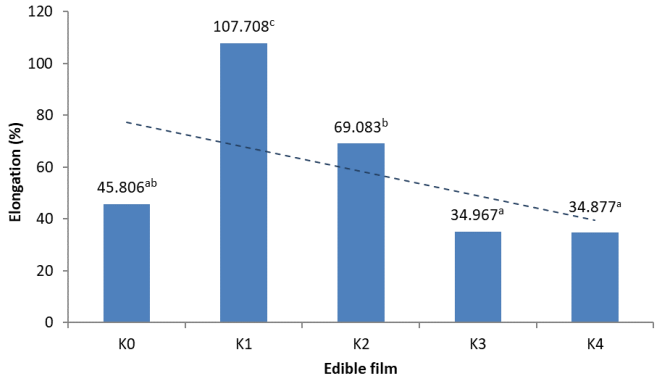

Figure 4. Elongation of active packaging. Values with different superscript are statistically significantly different ($p<0.05$). K0 = Control, K1 = 0.2% of leaf extract (w/v), K2 = 0.4% of leaf extract (w/v), K3 = 0.6% of leaf extract (w/v), K4 = 0.8% of leaf extract (w/v).

3.5 Moisture content

The results of the moisture content are shown in Figure 5. Based on Figure 5, the moisture content of the active packaging was lower than the control sample and decreased along with the increase in the concentration of the leaf extract. The moisture content ranged from 15.872–18.714%, with the highest value on the control sample and the lowest value found on 0.8% leaf extract. The moisture content decreased due to the edible film's increased density and the leaf extract's dry matter content. In addition, the hydrophobic lemon basil leaf extract increased the hydrophobic components formed in the film and reduced its moisture content (Shen *et al*., 2021). Adding lemon basil leaf extract also reduces the interaction between gelatin and glycerol with the surrounding water (Getachew *et al*., 2021). The formation of a strong biopolymer film matrix between the emulsion of leaf extract particles and the hydrophilic part of the gelatin reduces the film's hygroscopic properties (Shen *et al*., 2021). The decreased ability to bind water around the film was also caused by the cross-links formed between the leaf extract phenolic compounds and gelatin protein. Therefore, the film loses its ability to absorb water during storage, decreasing moisture content (Getachew *et al*., 2021).

The results of this study agreed with the research conducted by Shen *et al*. (2021), who added clove extract in the form of Pickering emulsion into edible films made of pullulan and gelatin. The study showed a significant decrease in moisture content from 24.20% in the control sample to 21.00%. Another study conducted by Getachew *et al*. (2021) also showed a significant reduction in water content from 19.46% in the control sample of edible film from tuna skin gelatin to 18.33% with the addition of 15% coffee grounds extract, and it decreased further to 17.19% at the addition of 20% extract.

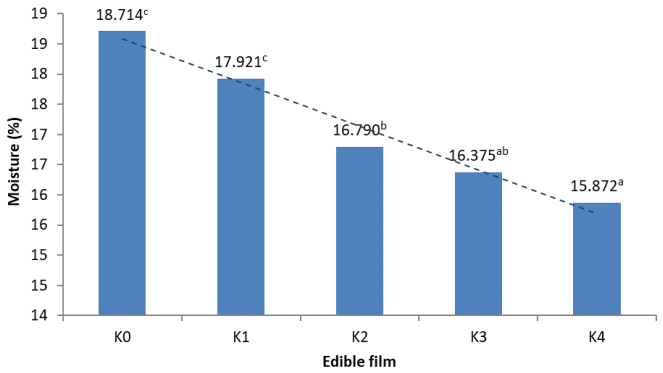

Figure 5. Moisture content of active packaging. Values with different superscript are statistically significantly different ($p<0.05$). K0 = Control, K1 = 0.2% of leaf extract (w/v), K2 = 0.4% of leaf extract (w/v), K3 = 0.6% of leaf extract (w/v), K4 = 0.8% of leaf extract (w/v).

3.6 Water vapor permeability

The results of the WVP analysis of the active packaging of edible film from tilapia skin gelatin are shown in Figure 6. WVP values ranged from 6.610×10^{-5} –7.047×10^{-5} g./m.day.Pa and were not significantly different. It concurred with the results by Hemalatha *et al*. (2017), who showed no significant difference in the addition of basil extract at concentrations of 0.1% and 0.3% into chitosan edible films. The WVP values were

https://doi.org/10.26656/fr.2017.7(S3).14

higher than the control, and the value decreased as the extract concentration increased.

According to Bonilla *et al*. (2012), the WVP value on edible film was influenced by the composition of the hydrophilic and hydrophobic components. The greater the hydrophobic component, the lower the WVP value, so water vapor and other volatile substances are hard to penetrate the packaging. The increase in WVP value at 0.2% leaf extract compared to the control occurred due to the termination of the polymer network by oil droplets from the leaf extract. The break caused a decrease in film cohesion, thereby increasing the activity of transporting water vapor and volatile substances through the film (Hosseini *et al*., 2015). However, as more leaf extract were added, the WVP value showed a decreasing trend compared to the control. The higher leaf extract concentration increases the film's hydrophobic component.

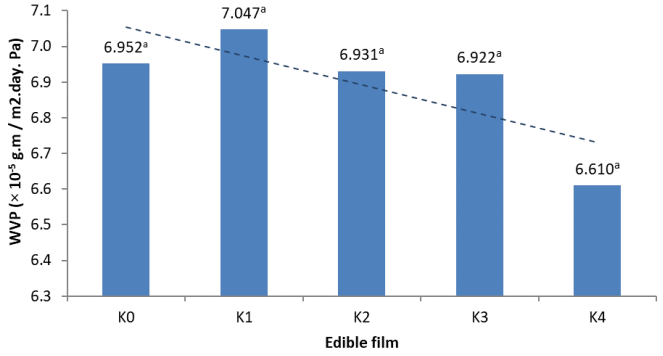

Figure 6. WVP of active packaging. Values with different superscript are statistically significantly different ($p<0.05$). K0 = Control, K1 = 0.2% of leaf extract (w/v), K2 = 0.4% of leaf extract (w/v), K3 = 0.6% of leaf extract (w/v), K4 = 0.8% of leaf extract (w/v).

According to Shen *et al*. (2021), adding essential oils increases the hydrophobic properties of the film, also it could increase the intermolecular pressure in the polymer chain of the film. It influences by decreasing the chain fluid condition and the film's free volume. In addition, adding essential oils to the edible film increases the formation of cross-links between phenolic compounds and gelatin proteins, decreasing the film network's water permeability space. Intermolecular interactions also affect hydrogen bonding, electrostatic properties, and hydrophilic interactions, reducing the film's free volume and intermolecular distances. It results in a lower WVP value, indicating it is increasingly difficult for water vapor and other volatile substances to pass through the film (Getachew *et al*., 2021). The declining trend in WVP values that occurred in this study was in agreement with the research conducted by Shen *et al*. (2021), who showed a significant decrease in WVP value from 7.99×10^{-7} g/m.Pa.s in control samples, while the edible film made from pullulan and gelatin was 4.83×10^{-7} g/m.Pa.s. While in 0.2% clove extract, it decreased to 2.36×10^{-7} g/m.Pa.s at a concentration of 0.6%. The insignificant difference in this study occurred due to the low concentration of lemon basil leaf extract used.

3.7 Antibacterial activity

Antibacterial activity was analyzed using the paper disk method on *S. aureus* as gram-positive bacteria and *E. coli* as gram-negative bacteria. Antibacterial activity was determined by measuring the diameter of the inhibition zone formed around the film. The larger the diameter of the inhibition zone, the better its antibacterial activity (Retnaningsih *et al*., 2019). The antibacterial activity of the active packaging is shown in Figure 7. Based on Figure 7, the control sample did not produce an inhibition zone. However, there was an increase in the inhibition zone's diameter as the concentration increased. The inhibition zone formed in *S. aureus* bacteria ranged from 6.883–16.283 mm, while in *E. coli* ranged from 5.450–12.767 mm.

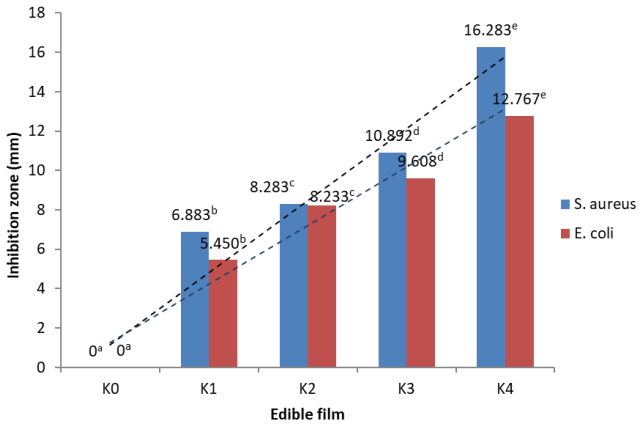

Figure 7. Antibacterial activity of active packaging. Values with different superscript are statistically significantly different ($p<0.05$). K0 = Control, K1 = 0.2% of leaf extract (w/v), K2 = 0.4% of leaf extract (w/v), K3 = 0.6% of leaf extract (w/v), K4 = 0.8% of leaf extract (w/v).

In this study, the diameter of the inhibition zone formed in *S. aureus* was more significant than that of *E. coli* due to differences in cell wall structure between the two bacteria. *E. coli*, a gram-negative bacterium, has a thick cell wall composed of lipoproteins, oligosaccharides, and phospholipids (Synowiec *et al*., 2014). The presence of lipopolysaccharide as a cell wall coating causes inhibition of the diffusion of hydrophobic components in lemon basil leaf extract, making it challenging to penetrate cell walls (Hosseini *et al*., 2015). At the same time, *S. aureus*, a gram-positive bacterium, has a more straightforward cell wall structure composed of peptidoglycan. This structure is easily penetrated by foreign compounds, such as polyphenols in lemon basil leaf extract, to the cytoplasmic membrane (Synowiec *et al*., 2014). According to Ramos *et al*.

https://doi.org/10.26656/fr.2017.7(S3).14

(2016), active compounds at the cytoplasmic membrane can cause damage to protein membranes, leakage of cellular components, and inhibition of nucleic acid synthesis to cytoplasmic clumping. Changes in the structure and composition of these cells cause bacterial death.

The diameter of the inhibition zone formed was getting more prominent as the lemon basil leaf extract concentration was added because the film contained more active compounds that could inhibit bacterial activity. According to Vasilica *et al*. (2020), basil leaves had the most effective compound content in the form of linalool at 64.35%. Meanwhile, according to Suppakul *et al*. (2003), basil leaves from Indonesia contain the most significant compound in the form of eugenol. These two compounds have potent antibacterial activity, but linalool is better than eugenol because its soluble nature makes it easier to break down bacterial cell walls. In addition, Vasilica *et al*. (2020) reported that the other compounds that play a role in antibacterial activity include 1,8 cineole, germacrene, -terpineol, and p-cymene.

The results of this study were agreement with the research conducted by Mallick *et al*. (2020), that adding basil extract into starch-based edible films causes an increase in the bacterial inhibition zone. In *E. coli*, the diameter of the inhibition zone is in the range of 3-10 mm, while in *S. aureus*, it is in the range of 6-14 mm. In another study by Synowiec *et al*. (2014), adding basil extract into edible films made from pullulan showed that *S. aureus* was more sensitive to active compounds than *E. coli*. The diameter of the inhibition zone formed in *S. aureus* has a range of 19.4–25.6 mm, while that of *E. coli* has a range of 12.0–13.1 mm.

3.8 Antioxidant activity

The results of the antioxidant activity analysis are shown in Figure 8. Based on Figure 8, the IC_{50} value ranged from 15.023–44.055 g/mL, with the highest value found on the control sample and the lowest value found on 0.8% leaf extract. The IC_{50} value on active packaging was lower than the control and decreased along with the increasing leaf extract concentration. According to Erviana and Malik (2016), the results of this study indicate that all samples have solid antioxidant activity because they ranged from 10-50 g/mL.

The results of this study conformed to the previous studies conducted by Hemalatha *et al*. (2017) that added 0.3% and 0.5% basil leaf extract in chitosan edible films significantly increased antioxidant activity. Similar findings by Synowiec *et al*. (2014), showed that adding basil extract could increase the antioxidant activity of the edible film pullulan at a concentration of 6 mg/cm^2. Nadeem *et al*. (2022) showed that the increase in antioxidant activity, along with the rise in the concentration of basil leaf extract, was caused by the presence of phenolic compounds, like flavonoids, alkaloids, and saponins in the basil leaf extract. Phenolic compounds are secondary metabolites with a phenol structure and several hydroxyl functional groups. In addition, according to Vasilica *et al*. (2020), basil contains other compounds such as eugenol, cineol, pinene, methyl chavicol, d-comphor, and ocimene.

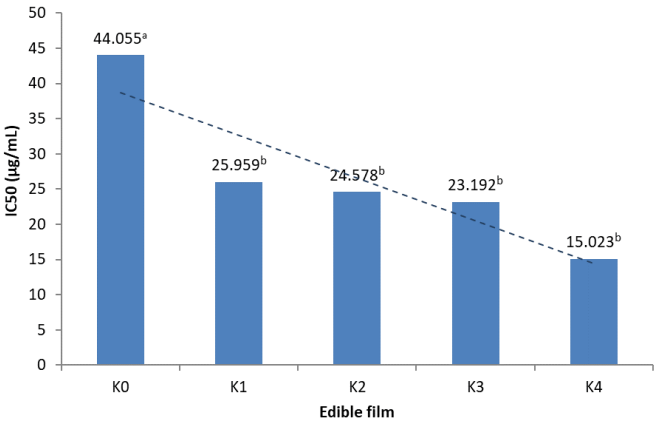

Figure 8. Antioxidant activity of active packaging. Values with different superscript are statistically significantly different ($p<0.05$). K0 = Control, K1 = 0.2% of leaf extract (w/v), K2 = 0.4% of leaf extract (w/v), K3 = 0.6% of leaf extract (w/v), K4 = 0.8% of leaf extract (w/v).

In this study, the control also had antioxidant activity and was influenced by the amino acid content of tilapia skin gelatin which acts as an antioxidant. According to Karami and Akbari-adergani (2019), amino acids such as tyrosine, methionine, proline, lysine, histidine, cysteine, glycine, and tryptophan have good antioxidant activity. Histidine donates protons from the imidazole group; proline act as an antioxidant in the Pro-His-His bond, and cysteine has a sulfhydryl group (SH) that donates hydrogen. Meanwhile, according to Kitts (2021), glycine, a hydrophobic amine group, can reduce free radicals quickly. Bordignon *et al*. (2019) explained that the primary amino acid, glycine, contained in tilapia skin gelatin is the highest, followed by proline, histidine, methionine, and cysteine as an antioxidant.

4. Conclusion

Lemon basil leaf extract added in active packaging of edible film from tilapia skin gelatin significantly affected the value of solubility, tensile strength, elongation, moisture content, antibacterial activity, and antioxidant activity, but not significantly different on the thickness and WVP parameters. Adding lemon basil leaf extract showed an increasing trend of thickness parameters and antibacterial activity. Meanwhile,

https://doi.org/10.26656/fr.2017.7(S3).14

solubility, tensile strength, elongation, water content, WVP, and antioxidant activity decreased. The 0.8% lemon basil leaf extract was the best treatment, resulting in the active packaging of edible film from tilapia skin gelatin.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

This research was funded by Kementrian Pendidikan, Kebudayaan, Riset, dan Teknologi year 2022, Scheme Penelitian Produk Vokasi Unggulan Perguruan Tinggi cooperation with AJS Fish, Sukoharjo, Indonesia. Grant number 1458.1/UN27.22/ PT.01.03/2022.

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