ISSN (electronic): 2302-4399 http://jurnal.fp.unila.ac.id/index.php/jthp/index

Development of antibacterial dual active food packaging based on super water absorbent and ethanol emitter

[Pengembangan kemasan pangan aktif ganda antibakteri berbasis super water absorbent dan etanol emitter]

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Submitted: 25 October 2024, Accepted: 27 February 2025, DOI: 10.23960/jtihp.v30i2.133-143

ABSTRACT

One way to extend shelf life and maintain the quality of fresh chicken meat is to implement an active packaging system. This research aimed to develop active packaging in the form of pads used in chicken meat with dual functions: Super Water Absorbent (SWA) as an adsorbent system and Ethanol Emitter (EE) as an antibacterial release system. The packaging in this study was first carried out by making SWA/EE with a ratio of 0, 4, and 2 (b/v). Furthermore, SWA/EE granules were inserted into non-woven PP bags to form an adsorbent active packaging pad. The experimental design used was a Randomized Group Design (RGD) with 2 factors, namely SWA/EE pad ratio 0, 4, and 2 (b/v) with non-pad as control and storage time (0, 1, 3, 5, and 7 days). Each treatment was repeated 3 times. The results showed that the SWA/EE ratio 4 (w/v) had better ethanol release and water absorption rate compared to other treatments. SWA/EE treatment with SWA/EE ratio of 4 (b/v) showed a significant difference on the anti-bacterial activity of S. aureus (34.58 mm) and total bacterial growth on storage days 3, 5, and 7, with the lowest value on day 3 (1.52 log CFU/g). SWA/EE treatment did not significantly different on the antibacterial activity of E. coli and total bacterial growth at day 1 storage. Packaging with an SWA-EE ratio of 4 (w/v) has the potential to be a double active packaging that can be used on fresh chicken meat.

Keywords : absorbent pads, active packaging, antibacterial, ethanol emitter, super water absorbent

ABSTRAK

Daging ayam segar memiliki umur simpan yang pendek karena kandungan gizinya yang tinggi menyebabkan mikroorganisme pembusuk dapat tumbuh dengan mudah. Salah satu cara untuk memperpanjang umur simpan dan menjaga kualitas adalah dengan menerapkan sistem kemasan aktif. Penelitian ini bertujuan untuk mengembangkan kemasan aktif dalam bentuk bantalan *(pad)* yang digunakan pada daging ayam dengan fungsi ganda. *Super Water Absorbent* (SWA) sebagai sistem penjerap dengan *Ethanol Emitter* (EE) sebagai sistem pelepas anti bakteri. Pembuatan kemasan pada penelitian ini dilakukan dengan membuat SWA/EE dengan nisbah sebesar 0, 4, dan 2 (b/v). Selanjutnya butiran SWA/EE dimasukkan ke dalam kantung PP *non-woven* sehingga diperoleh kemasan aktif berbentuk *absorbent pad*. Rancangan percobaan yang digunakan adalah Rancangan Acak Kelompok (RAK) dengan 2 faktor yaitu pad SWA/EE nisbah 0, 4, dan 2 (b/v) dengan non pad sebagai kontrol serta lama waktu penyimpanan (0, 1, 3, 5, dan 7 hari). Masing-masing perlakuan diulang 3 kali. Hasil penelitian menunjukkan bahwa perlakuan nisbah SWA/EE 4 (b/v) memiliki uji pelepasan etanol dan laju serap air yang lebih baik dibandingkan dengan perlakuan lain. Perlakuan SWA/EE dengan nisbah SWA/EE 4 (b/v) berbeda secara signifikan terhadap aktivitas antibakteri *S. aureus* (34,58 mm) dan pertumbuhan total bakteri pada penyimpanan hari ke 3, 5, dan 7, dengan nilai terendah pada hari ke 3 (1,52 log CFU/g). Perlakuan SWA/EE Tidak berbeda secara signifikan terhadap aktivitas antibakteri *E. coli* dan pertumbuhan total bakteri pada penyimpanan hari ke 1. Kemasan dengan nisbah SWA/EE sebesar 4 (b/v) berpotensi sebagai kemasan aktif ganda yang dapat digunakan pada daging ayam segar.

Kata kunci : antibakteri, bantalan penyerap, *ethanol emitter*, kemasan aktif, *super water absorbent*

ISSN (electronic): 2302-4399 http://jurnal.fp.unila.ac.id/index.php/jthp/index

Introduction

Chicken meat is one of the most popular meats for Indonesians. The average consumption of Indonesian people is 7.46 kg per capita/year. This value increased from the previous year of 7.15 kg per capita/year (Kementan RI, 2023). Chicken meat is also relatively affordable and rich in nutrients with high biological value, relatively low fat and cholesterol content (Gurunathan et al., 2022). However, chicken meat has a disadvantage, which it can release fluids naturally. This causes a decrease in quality because it spoils the appearance and supports the proliferation of spoilage microorganisms and pathogens (Millan & Sirante, 2020). One way to reduce the causes of spoilage and also maintain food quality and safety is to implement an active packaging system (Dirpan et al., 2023).

Active packaging is packaging in which the product, packaging and environment interact synergistically to extend product shelf life, improve safety and sensory properties while maintaining food quality (Gogliettino et al., 2020). Active packaging is one of the current packaging trends. Active packaging can be classified into two systems, they are an absorbent and a release system (Vasile and Baican, 2021). Super Water Absorbent (SWA) as an absorbent system is a polymer that can absorb water. According to the results of research by Lertsarawut et al. (2021) SWA with cassava starch-KOH-acrylic acid composition and synthesized using gamma irradiation, then washed with ethanol to remove residual KOH and acrylic acid. This SWA is called cassava starch SWA. Cassava starch SWA has a development ratio in water with a ratio value of 543 g/g. Ethanol Emitter (EE) as a release system releases ethanol as an antimicrobial into the packaging (Mousavi and Mahmoudpour, 2024).

Various studies on active packaging have been reported, including the combination of active packaging as absorbent pads soaked in cationic polymer solutions of various concentrations that function as antibacterials and placed under fresh beef products (Castrica et al., 2020). The study showed that the pad with the active substance mixture was able to delay the growth of all microorganisms studied only on day 3 compared to the control group. Huang et al. (2019) reported the use of N-halamine compounds in pads on fresh beef and also Lee et al. (2024) reported the use of bacteriophages in active packaging on chicken meat. The use of ethanol in active packaging in mulberry fruit was also reported (Choosung et al., 2019). Likewise Mugasundari & Anandakumar (2022) reported that the technology of using ethanol as an antimicrobial is effective against mold, inhibits the growth of yeast and bacteria, and can also extend the shelf life of bread products.

Currently, no one has reported the use of cassava starch SWA in absorbent pads as a sorbent material combined with ethanol emitters as an antibacterial releaser for chicken meat storage. It is reported that active packaging in the form of absorbent pads in the meat industry is still limited in its ability to absorb liquid (drip loss) from meat products (Wang et al., 2022). Previous studies have reported that the incorporation of clove essential oil (EO) into wood fiber-based absorbent pads reduces their water absorption capacity. This reduction is attributed to the excessive formation of pores within the wood fibers, which diminishes their ability to retain absorbed water (Jiang et al., 2024). The wood fiber-based absorbent pads with concentrations of 2% and 3% polyvinyl (PVA) solution have a greater development ratio in water compared to concentrations of 1%, 4%, 5%, and 6% polyvinyl (PVA) solution with a maximum ratio value of 10 g/g. This contrasts with cassava starch-based SWA, which not only exhibits high water absorption capacity but also effectively retains the absorbed water, with a retention capacity of up to 543 g/g (Lertsarawut et al., 2021).

The purpose of this research is to develop active packaging in the form of absorbent pads in the form of pads with dual functions in one package, namely cassava starch SWA as a water absorption system which is expected to absorb and retain chicken meat drip loss water so that it can maintain its quality. The combination of Ethanol Emitter (EE) as an antibacterial release system, which is expected to inhibit bacterial growth and thus extend its shelf life.

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Materials and method

Materials and tools

This study used the main ingredients of cassava starch SWA granules from the Research Center of Radiation Process Technology Research (PRTPR)-BRIN, EE consisting of ethanol (Merck, food grade) and sodium stearate (Techno Pharmachem, food grade), chicken breast fillet (freshmart, Jakarta), Plate Count Agar (PCA), Chromocult Coliform Agar (CCA), Baird Parker Agar (BPA), Sodium Agar (NA), Buffered Peptone Water (BPW) (Oxoid, England), *Eschericia coli (E. coli)* bacteria, *Staphylococcus aureus (S. aureus)* bacteria. The packaging was made of non-woven polypropylene (PP).

The tools used were analytical balance (Chyo JL-180), incubator (Memmert, Germany) refrigerator (Haier, China), sealer machine, magnetic stirrer, vortex, oven, laminary air flow, hot plate (IKA C-MAG HS 7), and other glassware for chemical analysis (Pyrex, France).

Research method

This research used an experimental method with a form of factorial design. The experimental design carried out in this study was a Randomized Group Design (RGD) with 2 factors, namely the use of SWA/EE pads and the length of storage time. The first factor consisted of 4 treatments, namely non-pad, SWA/EE active packaging (pad) with a ratio of 0 (b/v), pad with SWA/EE mixture formula with a ratio of 4 (b/v) and pad with SWA/EE mixture formula with a ratio of 2 (b/v). The second factor consisted of 5 storage times, namely 0, 1, 3, 5, and 7 days. Each treatment was repeated 3 times, resulting in a total of 60 experimental units.

Data were analyzed using Excel 2016 software and IBM SPSS version 20. Data processing using Excel software is the ethanol release rate data in the form of fractional release curves of SWA/EE active packaging and water absorption rate. Antibacterial activity data and microbiological testing were statistically analyzed using one-way ANOVA ($\alpha = 0.05$) and if significantly different, Tukey HSD further test was conducted.

Preparation of SWA and EE mixture

The mixing of SWA and EE begins with the preparation of EE, namely ethanol with sodium stearate heated at 70°C until transparent (Mu et al. 2017). The addition of sodium stearate is done to bind ethanol and reduce the volatility of ethanol so that it is expected to have slow release properties and become EE. In this study, 3 SWA/EE treatments were used, namely 0 (b/v), 4 (b/v) and 2 (b/v) (Table 1). Furthermore, to get a SWA/EE ratio of 4 (b/v), 15 g of SWA was mixed with 3.75 ml of ethanol and 0.1 g of sodium stearate. To get a SWA/EE ratio of 2 (b/v) was done by weighing 15 g of SWA, mixed with 7.5 ml of ethanol and 0.2 g of sodium stearate (Figure 1a).

Table 1. Mixed formula of super water absorbent (SWA) and ethanol emitter (EE)

SWA/EE ratio (b/v)	Weight of SWA (g)	Weight of Sodium Stearate (g)	Ethanol (ml)
0	15	-	-
2	15	0.2	7.5
4	15	0.1	3.75

Preparation of active packaging

Active packaging was made from PP non-woven with a size of 1.5 x 1.5 cm for water absorption rate and antibacterial activity tests and a size of 15 x 8 cm for microbiological tests on chicken meat. A pair of PP non-woven of this size was sealed on three sides using a sealer machine. A homogenized SWA/EE mixture of 0.2 grams was put into one of the open sides and then the package was sealed (Figure 1b).

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Figure 1. Illustration of the SWA/EE mixture (a) and its active packaging using PP non-woven bags (b)

Chicken packaging on active packaging

Chicken breast fillets weighing 250 g were placed on the surface of SWA/EE active packaging at ratios of 0 (w/v), 2 (w/v), and 4 (w/v) in plastic food containers with covers. Sample preparation was carried out in a Laminar Air Flow (LAF) under sterile conditions. Scissors, tweezers, plastic food containers, and work surfaces were sterilized using 70% alcohol. Food containers containing samples were stored in a refrigerator at 4±1°C with each storage time treatment (0, 1, 3, 5, and 7 days) (Castrica et al., 2020).

Test for temperature effect on ethanol release rate

The test of temperature effect on ethanol release rate refers to Mu et al. (2017). SWA that has been mixed with ethanol with 2 different ratios, namely SWA/EE ratios of 4 (b/v) and 2 (b/v), each placed in a beaker glass without a lid and weighed the initial weight (M0). Then each beaker glass containing SWA-EE mixture will be placed in an incubator at 5°C, 15°C, and 35°C, every 30 minutes for 5 hours weighed (Mt). The percentage of ethanol released into the air (fractional release) will be calculated based on the formula according to the following equation:

$$\text{Fractional release (\%)} = \frac{(Mo-Mt)}{Mo} \text{ x } 100\% \text{ .....(1)}$$

where Mo is the initial amount of ethanol gel (g), Mt is the weight of SWA, EE, and beaker glass after storage at a certain temperature and time (g).

Water absorption transmission rate test

Analysis of the water absorption transmission rate of double active packaging using a modified gravimetric method (Meindrawan et al., 2016). The cup that had been placed with the active packaging was weighed using an analytical balance, then put into a desiccator that had been adjusted to 100% RH using a sterile solution of distilled water. Cups were weighed periodically every 24 hours for 7 days. The water absorption transmission rate (g/m^{2}h) was calculated from the slope of the line resulting from regression analysis of weight as a function of time.

$$\text{Water absorption transmission rate test} = \frac{Slope}{area} \quad \text{.....(2)}$$

Antibacterial activity test

The antibacterial activity test in this study used the agar diffusion method based on Pranoto et al. (2005) with modifications. The test bacteria were *S. aureus* and *E. coli*. SWA/EE active packaging measuring 1.5 × 1.5 cm from each different ratio was placed on NA agar media, which had previously been spread with 0.1 ml of test microorganism culture containing 10^6 CFU/ml. Petri dishes were then incubated in an incubator for 24 hours at 37°C. After the incubation period, an inhibition zone will appear, and the area of the inhibition zone is measured. The inhibition zone area is calculated as the clear zone area formed.

Total plate count (ALT)

A sample of 1 g chicken breast fillet was homogenized for 3 minutes with 9 ml Buffered Peptone Water (BPW) in a sterile bag (as the initial 10^{-1} dilution). After that, 1 ml of the 10^{-1} dilution suspension was

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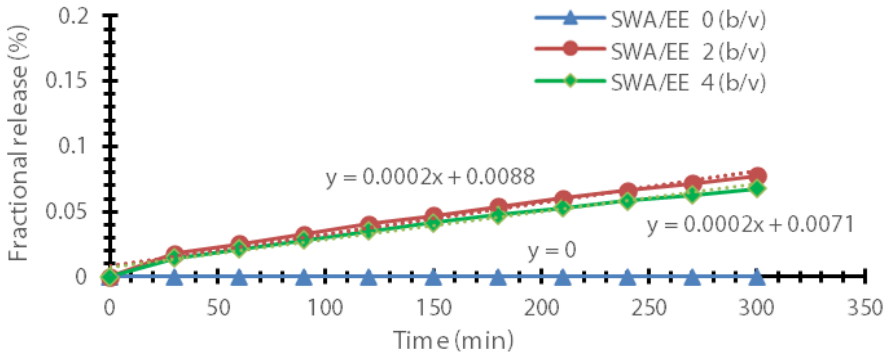
transferred into the 9 ml BPW solution with a pipette. Homogenized using a vortex to obtain a 10^{-2} dilution. The next step is to make dilutions up to 10^{-5} following the same method.

The dilution suspensions that have been made are each taken as much as 0.1 ml and put into Petri dishes in triplicate. Petri dishes that have contained sample suspensions are poured with Plate Count Agar (PCA) at 45°C as much as 15-20 ml. Then rotate the cup in the form of a figure eight so that the media and suspension are well mixed, then let the cup stand until the media solidifies, the cup in an inverted state is incubated for 24-48 hours at 37°C (Firdausyi et al., 2022).

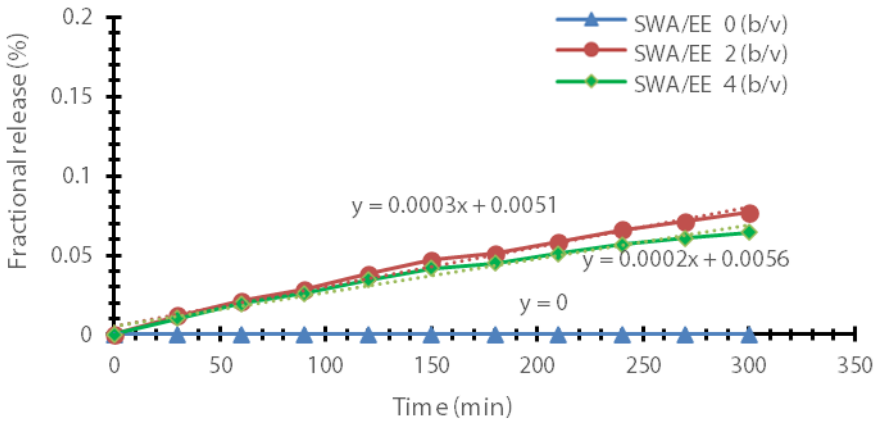
Result and discussion

Temperature effect on ethanol release rate

Release behavior data of SWA/EE active packaging was obtained by conducting gravimetry on each SWA/EE ratio treatment of 0, 2, and 4 (b/v). Weighing was done every 30 minutes for 5 hours. The gravimetric results were analyzed for fractional release using equation (1), and then a fractional release curve was obtained. Figure 2 shows the fractional release of each active package at 5°C, 15°C and 35°C. The increase in temperature resulted in a controlled release of SWA and EE released into the air faster. This is in accordance with the research of Mu et al. (2017) who reported that the release efficiency of ethanol gel is mainly influenced by temperature.



(a)



(b)

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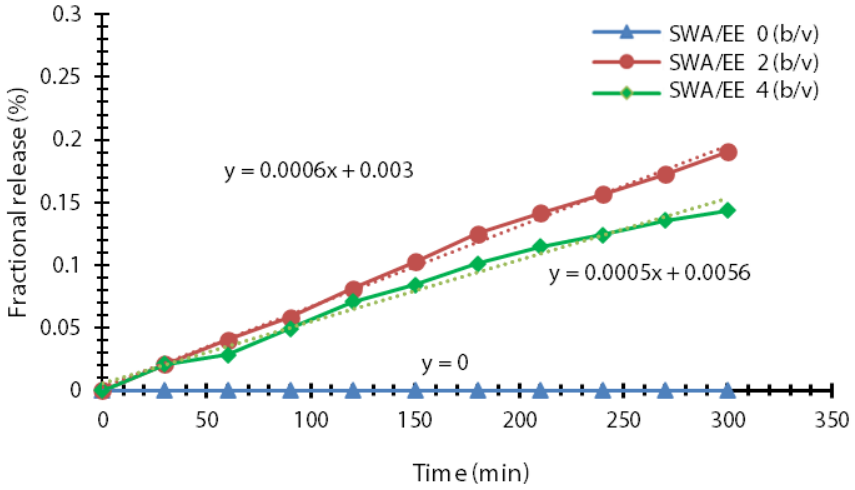

(c)

Figure 2. Fractional release of dual active packaging (pad) SWA/EE at 5°C (a), 15°C (b), and 35°C (c)

The ethanol release rate increases as the temperature increases. This shows that low temperature can slow down the ethanol release rate. The ethanol release rate as affected by temperature is shown in Figure 2. The ethanol release rate increases with increasing temperature and Relative Humidity (RH) (Kita et al., 2018). The ethanol vaporization process is an endothermic reaction, where after the ethanol gel absorbs heat, causing the molecular motion of ethanol and sodium stearate to increase, so that the spatial network structure of the ethanol gel becomes loose, and more liquid ethanol molecules turn into gas (Mu et al., 2017).

At SWA/EE ratios of 4 (w/v) and 2 (w/v) at 35°C, the fractional release value is greater, meaning that it releases faster than at 5°C and 15°C. The fractional release values at SWA/EE ratios of 4 (w/v) and 2 (w/v) at 5°C and 15°C have relatively similar values. With the same ethanol release rate, the use of ethanol in packaging with a SWA/EE ratio of 4 (b/v) is more efficient when compared to a SWA/EE ratio of 2 (b/v). At a SWA/EE ratio of 2 (b/v) the 5°C temperature slope is 0.0002, the 15°C temperature slope is 0.0003, and the 35°C temperature slope is 0.0006. At a SWA/EE ratio of 4 (b/v), the 5°C temperature slope is 0.0002, the 15°C temperature slope is 0.0002, and the 35°C temperature slope is 0.0005. As can be seen in Figure 2, fractional release increases with temperature. Fractional release ratio SWA/EE 2 (b/v) is faster than the ratio SWA/EE 4 (b/v). This is in line with the research of Wang et al. (2023) which states that the higher the concentration of ethanol and Essential Oil (EO) mixture added in the pad, the faster the fractional release. This can occur because higher ethanol concentrations can penetrate more polymer structures, thereby increasing polymer development and accelerating ethanol release (Wang et al., 2023).

Water absorption transmission rate

The water absorption transmission rate is one of the important quality parameters of double-activated packaging as it relates to the ability to absorb water to protect the product from the growth of microorganisms. A low water absorption transmission rate causes the packaged food product to deteriorate faster. In the sense that the packaging cannot absorb the liquid that comes out of the fresh food product, so that it will accelerate the damage and growth of microorganisms. High water content makes the product very susceptible to microbial spoilage, thus making it highly perishable (Pettersen et al., 2021).

Figure 3 presents the water absorption transmission rate. The addition of ethanol did not significantly affect the transmission rate, as indicated by the absence of notable differences among treatments. This is in line with research conducted by Wang et al. (2022) who reported no significant difference in absorbing

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liquid on a pad combined with levulinic acid and sodium dodecyl sulfate compared to the control. The factor that most affects the water absorption rate of the pad is the presence of SWA.

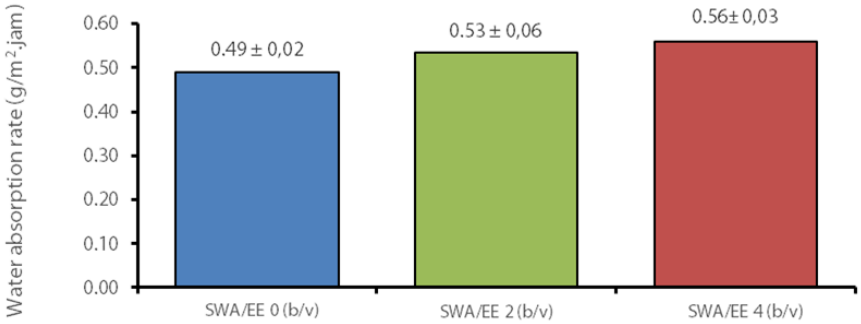

Figure 3. Water absorption transmission rate of active packaging at SWA/EE ratio of 0 (b/v), 2 (b/v), and 4 (b/v)

Antibacterial activity

Antibacterial activity was determined based on the diameter of the clear zone that appeared after incubation at 37°C for 24 hours. The larger the diameter of the clear zone, the greater the zone of packaging inhibition. The resulting clear zone showed that the SWA-EE mixture inhibited the growth of gram-negative bacteria *(E. coli)* (Figure 4a) and gram-positive bacteria *(S. aureus)* (Figure 4b).

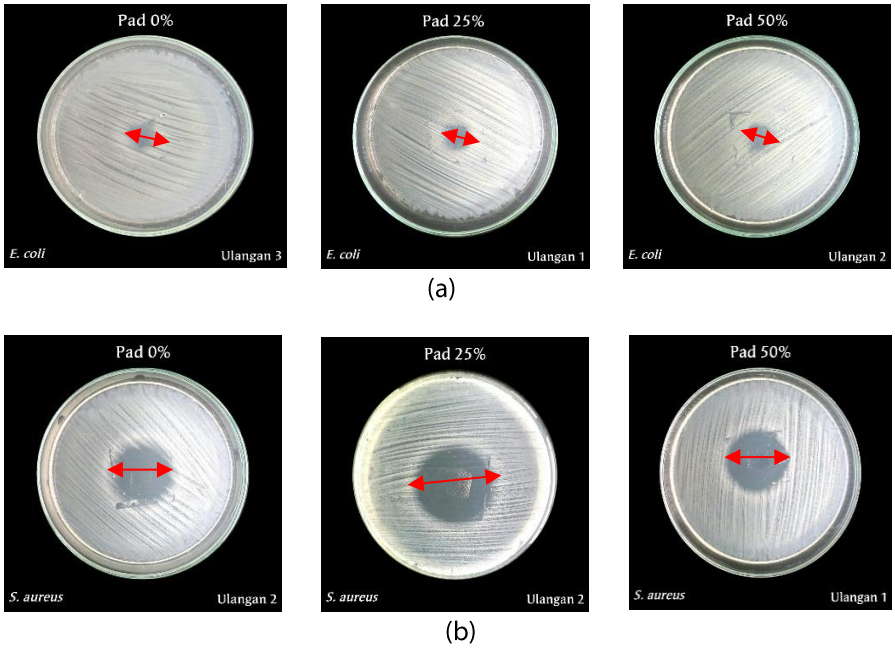

Figure 4. Inhibitory zones (red arrowheads) of active pads with SWA/WE ratios of 0, 2, and 4 (wt/vol) against (a) *E. coli* and (b) *S. aureus*

The active packaging (pad) was able to inhibit both gram-negative and gram-positive bacteria. The active packaging (pad) SWA/EE ratio of 2 (b/v) and 4 (b/v) was not effective in inhibiting *E. coli*, characterized by inhibition zones that were not significantly different from the SWA/EE ratio of 0 (b/v). This is because *E. coli* bacteria have cell walls and high lipid content (11-22%) as well as a multilayer cell wall structure consisting of lipoproteins, phospholipid outer membranes, and lipopolysaccharides, causing gram-negative bacterial cell walls to be difficult to penetrate by antibacterial substances compared to gram-positive bacteria (Anggraini et al., 2018).

The addition of SWA/EE effectively inhibited the growth of gram-positive bacteria. The antibacterial activity of the three concentrations of active packaging against *S. aureus* showed different inhibition zones

(p<0.05). The active packaging with SWA/EE ratio of 4 (b/v) had the largest inhibition zone (34.58 mm) when compared to the active packaging (pad) with SWA/EE ratio of 0 (b/v) and 2 (b/v) (Table 2). It is reported that ethanol is a broad-spectrum antimicrobial that is widely used for storage of food products. Ethanol can damage microbial cell walls, cell membranes, and induce coagulation of vital proteins in microbes, thus inhibiting the growth or killing spoilage microorganisms (Mu et al., 2017).

Table 2. Zone of inhibition of active packaging (pad) SWA/EE ratio 0 (b/v), 2 (b/v), and 4 (b/v) against *E. coli* and *S. aureus*

Active packaging *(pad)*	Diameter of inhibition zone (mm)	
	E. coli	*S. aureus*
SWA/EE 0 (b/v)	14,19 ± 2,02[a]	28,79 ± 0,61[a]
SWA/EE 2 (b/v)	13,36 ± 0,05[a]	31,84 ± 0,95[b]
SWA/EE 4 (b/v)	15,41 ± 0,85[a]	34,58 ± 0,42[c]
Kruskall wallis test	Statistic value = 3,822; df = 2; P = 0,148	Statistic value = 7,2; df = 2; P = 0,027

Notes: Numbers in the same column followed by different letters indicate significant differences (p < 0.05) using Tukey HSD further test.

Table 3. Classification of bacterial growth inhibition response (Davis & Stout, 1971)

Diameter of inhibition zone (mm)	Growth inhibition response
> 20	Very strong
11-20	Strong
5-10	Medium
< 5	Weak

Overall, based on the classification of bacterial growth inhibition response, the inhibition zone of SWA/EE active packaging against *E.coli* and *S. aureus* bacteria is classified as having a strong growth inhibition response (10-20 mm) and very strong (>20mm) (Table 3). The zone of inhibition of SWA/EE active packaging (pad) against gram-positive bacteria (*S. aureus*) is stronger when compared to gram-negative bacteria (*E. coli*).

Total plate count

Based on the results of ALT testing of bacterial growth in each treatment, it has not shown significant differences on day 1. Significant differences in bacterial growth can be seen on storage days 3, 5, and 7 (p<0,05). In that period, the non-pad treatment showed the highest total bacterial growth value, while the pad SWA/EE ratio of 4 (b/v) showed the lowest bacterial growth value on day 3 (1.52 log CFU/g) (Figure 5). This is in line with the fractional release results (Figure 2), which show that the SWA/EE ratio of 4 (b/v) has a low release speed, allowing the pad to inhibit bacterial growth for a longer period.

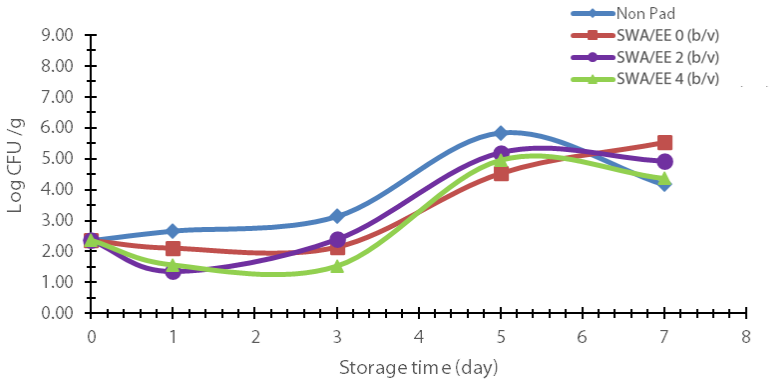

Figure 5. Graph of total bacterial growth of chicken using double active packaging during 0, 1, 3, 5, and 7 days of storage.

The results of research by Dirpan et al. (2023) reported that the combination of pad with lemongrass essential oil as active packaging can extend the shelf life of chicken meat for 3 days at chiller temperature (4°C). Another study also reported that active packaging (pad) placed under the meat was able to delay the growth of all microorganisms studied, only on day 3 ($p<0.05$) compared to the control group (Castrica et al., 2020). Antibacterial substances in active packaging can be released gradually to the food surface. These active substances can inhibit the growth of pathogenic and spoilage bacteria that are the main cause of food product damage, thereby reducing the rate of bacterial growth and extending shelf life. The optimum shelf life in some studies is around 3 days (Castrica et al., 2020; Dirpan et al., 2023).

Conclusion

The addition of SWA and EE affects the antibacterial activity and total bacterial growth. Based on the results obtained, the SWA/EE ratio of 4 (b/v) is a better ratio in ethanol release rate and water absorption transmission rate. The zone of inhibition of active packaging (pad) of SWA/EE ratio of 4 (b/v) against *E. coli* and *S. aureus* bacteria showed the greatest value of 15.41 mm and 34.58 mm. The total bacterial growth of the SWA/EE ratio of 4 (b/v) had the lowest bacterial growth value on day 3 of storage (1.52 log CFU/g). Further research needs to be done to determine the potential of SWA/EE pads in other fresh food products and to determine the shelf life.

Acknowledgements

Thanks to the RIIM and HITN funding programs of the National Research and Innovation Agency for funding this research.

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