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1

Proofreading process

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Smart Sensor and Active Packaging System Based on Bacterial Cellulose from *Acetobacter xylinum* Applied on Meat Product

Abstract.
Combining smart and active packaging serves a dual purpose of detecting and prolonging the shelf life of food. The purpose of this study was to determine the characteristics of smart and active packaging made from bacterial cellulose and its effect on packaged fresh beef. This research includes evaluate the properties of smart packaging sensor indicators and active packaging films, as well as their application to fresh beef storage at room temperature (28±2°C) for 24 hours. The results suggested that the BTB solution indicator (Bromothymol blue) with a pH of 2.75 on the smart packaging indicator displayed easy determined color change from orange to dark green, indicating a change in beef quality from fresh to rotten. Meanwhile, when the qualities of meat treated with active packaging with 10% and 15% garlic extract were tested, it decayed at the 16th hour, but meat treated with active packaging without the addition of garlic extract rotted at the 12th hour. Additionally, the indicator's color shift is linearly related to the TPC (Total Plate Count), TVBN (Total Volatile Basic Nitrogen), and pH of meat packaged using active packaging. Thus, the conclusion of this study is that BTB (Bromothymol blue) pH 2.75 solution can be used as a smart packaging indicator, allowing consumers to more easily assess the quality of packaged meat, and that the addition of 10-15% garlic extract to active packaging films as an antimicrobial agent can help delay packaged meat spoilage.

1. Introduction

Global beef consumption is predicted to rise as the world population and family incomes rise, particularly in the developing Asian countries (Dupont & Fiebelkorn 2020; González et al. 2020; OECD/FAO 2021). By 2030, worldwide meat consumption and availability are expected to increase by 14% and 5.9%, respectively, over the 2018-2020 period's average (OECD/FAO 2021). As a result, increases in meat consumption must be complemented by improvements in the quality of fresh meat produced. One aspect affecting the quality and characteristics of meat is the material and packaging technologies used (Abdurehman Musa 2019). Meat is a perishable item that spoils rapidly when stored above the optimum temperature range (below 0-40°F) (Beltrán, Roncalés & Bellés 2018; Franco, da Cunha & Bianchi 2021). However, the facts demonstrate that in traditional markets, the meat is displayed at room temperature without packaging which might accelerate microbial contamination and cause quality degradation to occurs faster. Even in supermarkets where meat has been maintained in cold temperatures, standard meat packing circumstances still make it difficult for consumers to subjectively determine the quality of meat. This demonstrates the need for additional functions in meat packaging to prevent quality degradation caused by microbes and to make it easier for consumers to determine the quality of packed meat (Dirpan et al. 2019). Conventional meat packaging can be designed to perform dual functions through the use of smart and active packaging systems.

Smart packaging is a term that refers to sensors in the form of indicators that monitor and provide information about the quality of the food contained within the packaging via color changes caused by chemical reactions between the indicators and the products of microbial metabolism or changes in the chemical composition of the food (Dobrucka & Cierpiszewski 2014). During storage, chemical components of meat degrade into volatile compounds as a result of microbial activity, increasing the value of Total Volatile Base Nitrogen (TVBN) (Bekhit et al. 2021; Ma et al. 2021). Accumulation of TVBN results in an increase in the pH of the packaging system, which is detected by the indicator, resulting in a visible color shift in the indicator (Pacquit et al. 2006; Ma et al. 2021). Smart packaging

allows easier monitoring of packed products through transportation and storage (Dobrucka dan Cierpiszewski, 2014), provides a more accurate estimate of product condition than conventional expired labels (Pacquit, Crowley & Diamond 2008). Several studies have demonstrated the widespread usage of color-based pH indicator solutions as smart indicators. Dirpan et al. developed bromophenol blue as a smart indicator dye for mangoes (Dirpan et al. 2018) and Hidayat et al. used two types of color indicators (phenol red and bromothymol blue) with determined concentrations on fresh meat packaging (Hidayat et al. 2019). Additionally, smart packaging indicators based on natural pigments are being developed, such as smart packaging films that include anthocyanin-loaded Lycium ruthenicum nanocomplexes into starch/polyvinyl alcohol mixtures (PVA) (Qin et al. 2021) as well as the development of anthocyanins from saffron petals immobilized on chitosan nanofiber and methyl cellulose matrix (Alizadeh-Sani et al. 2021).

While, Active packaging refers to the integration of particular additives into a packaging system with the purpose of extending shelf life, preserving product quality, and ensuring product safety. In terms of extending shelf life, antimicrobial agents are used as components of active packaging additives. Volatile bioactive compounds contained in active packaging are evaporated or diffused to the food surface, where they limit the growth of spoilage and pathogenic microbes (Yildirim et al. 2018; Wrona et al. 2021). This strategy is more effective than coating bioactive compounds on the food surface (Iriani dkk*,* 2014). The safest, cheapest, and most readily available antimicrobial agents for use in active packaging are essential oils. The production of antimicrobial alginate edible films by incorporating garlic essential oil has been carried out by Pranoto *et al. (*2005) that shows substantial inhibitory impact on *Staphylococcus aureus* and *Bacillus cereus* in meat*.* Antimicrobial activity has also been established for the use of essential oil from Plectranthus amboinicus in active packaging based on chitosan (Vishnu Priya, Vinitha & Meenakshi Sundaram 2021). Not only as separate systems, smart and active packaging can be merged into a single packaging system. Julyaningsih et al have combined smart packaging based on Methyl Red-Bromothymol Blue indicator with active packaging based on lemongrass oil as a component of tuna fish fillet packaging. (Julyaningsih, Latief & Dirpan 2020). In other studies, active and smart packaging based on starch, PVA, and betacyanins from various types of plants has been applied to shrimp (Yao et al. 2021).

In general, active packaging containing antimicrobial agents and smart packaging containing indicator solutions are immobilized on a polymer. In comparison to plant cellulose or synthetic polymers, bacterial cellulose fermented by *Acetobacter xylinum* has a unique nanofibrillar structure and superior physical properties, suggesting that it has the potential to act as the basis for developing smart and active packaging. (Cazón & Vázquez 2021; Xu et al. 2021). Bacterial cellulose has garnered interest as a component of active packaging due to its edibility, biodegradability, high water holding capacity, and potential as an antimicrobial agent carrier (Nguyen, Gidley & Dykes 2008).

The packaging function is advancing in the food business. As a means of advancing innovation, this research aims to maximize the potential of smart and active packaging by combining them in a packaging system based on a bacterial cellulose membrane biopolymer to enhance the quality of packaged meat and make it easier for consumers to form perceptions of meat freshness.

2. Method and Materials

2.1. Materials

The main ingredients used in the production of smart and active packaging systems are bacterial cellulose produced by Acetobacter xylinum which is fermented in natural media of coconut water. Beef tenderloin obtained from slaughterhouse Tamangapa Raya. Coconut water and garlic (Allium

sativum) were purchased from traditional market. Ammonium sulfate (ZA) food grade (CAS Number: 7783-20-2), yeast extract (Merck, CAS Number: 8013-01-2), acetic acid 96%, Acetobacter xylinum culture, 5% NaOH 1N, sucrose, bromothymol blue, alcohol, aquabides, aquades, Tashiro indicator (methyl red 0.1% and bromothymol blue 0.1% with a ratio of 2:1), Trichloroacetic Acid (TCA 7%) (Merck), Nutrient Agar (NA) (Merck), glycerol (Merck, CAS Number: 56-81-5), Carboxymethyl-cellulose (CMC) food grade (Foodchem, E466), corn starch.

2.2. Method

2.2.1. Determination of the Best Nitrogen Source in *Acetobacter xylinum* Fermentation Media

Acetobacter xylinum bacterial cellulose membrane production begins with defining the amount and type of the most suitable nitrogen source for *Acetobacter xylinum* growth media, which references to prior studies (Dirpan et al. 2019).

2.2.2. Purification of bacterial cellulose

Bacterial cellulose was removed from the fermentation medium and rinsed in running water before being soaked for two days with periodic water changes. Additionally, cellulose was soaked in 70% alcohol for 1 minute, heated to 100°C in distilled water for 20 minutes, and reheated in a 1N 5% NaOH solution at 100°C for 60 minutes to remove remaining bacterial cells and substrate attached to the cellulose layer. Then, rinse with running water and soak for 24 hours, changing the water periodically, until the pH reaches 7. The purified cellulose will appear transparent (Dirpan et al. 2019).

2.2.3. Production of Smart Packaging

2.2.3.1. Pembuatan larutan indikator

Bromothymol Blue (BTB) indicator solution was chosen for this study based on earlier research as the indicator with the most visually identifiable color change reaction (Dirpan et al. 2019). First the BTB solution 1% (b/v) in 95% ethanol was made. The pH of the bromothymol blue (BTB) solution was then decreased to 2.74 by adding 20% acetic acid. Following that, a closed container was used to hold the bromothymol blue (BTB) solution.

2.2.3.2. Production of Smart Packaging Indicator Label

The purified cellulose film was kept on a filter cloth for 24 hours to decrease the water content. Half-dried cellulose was cut into 1.5 cm × 4 cm strips and pushed flat against the pyrex glass surface. Cellulose was dried for 30 minutes at 70ºC. The BTB indicator solution was then absorbed into dry cellulose via centrifugation at 3000 rpm for 15 minutes. If the color indicator is successfully absorbed, the BTB will impart an orange hue to the cellulose. Following that, the cellulose was rinsed with distilled water to eliminate any unbound color indicators and then dried (Modified from Kuswandi and Maryska, 2013; Shukla *et al.,* 2015).

2.2.4. Production of active packaging film

2.2.4.1. Production of Garlic Extract (*Allium Sativum*) as active element

500g garlic peeled, washed under running water until clean, drained and then mashed. Minced garlic was extracted by maceration method by immersing finely ground garlic in 96% alcohol with a ratio of 1:4 (Garlic: Alcohol) for 4 days at a temperature of 3-5ºC and periodically homogenized using a water bath shaker. After that, the extract was filtered using

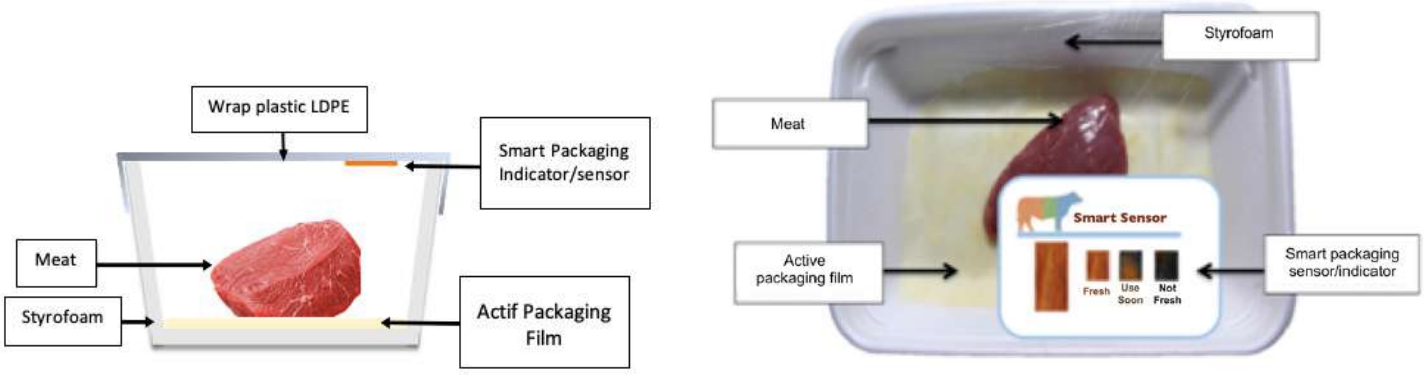
filter paper and then concentrated using a rotary evaporator at a speed of 50 rpm at 40°C to obtain a thick extract. (Rotty dan Tjitrosantoso, 2015; Shetty *et al*., 2013).

2.2.4.2. Production of active packaging film

Bacterial cellulose was crushed to form a cellulose slurry, and all pre-treatments took place at room temperature. Cellulose suspension was prepared using a 30% chitosan (w/w), 10% carboxymethyl-cellullose (CMC) (w/w), and 15% corn starch (w/w) of cellulose dry weight. The suspension was heated at 50°C for 60 minutes with a hot plate stirrer until thoroughly suspended. Thirty percent glycerol (w/w) was added at the 50th minute. Additionally, the garlic extract was added at quantities of 0% as a control, 5%, 10%, and 15% (v/v) immediately following the final heating step. 60g of suspension was then put onto a glass plate and dried for 2x24 hours at 37°C. Then, it is cooled to room temperature before being removed from the glass plate, then wrapped in aluminium foil to place in a desiccator Modified from Indrarti *et al.,* 2016; Iriani dkk*,* 2014).

2.2.5. Application of Smart and Active Packaging Indicators on Fresh Beef

Fresh beef tenderloin was collected from the Tamangapa Raya Makassar Slaughterhouse after 1 hour postmortem which was immediately placed in a special food box and put into a 38x29x30cm styrofoam box filled with ice crystals. The samples were promptly transported to the laboratory and sterilely processed into 200 g/pack pieces. The meat is packaged in a styrofoam tray (1.05 g/cm3) coated with active packaging film on a styrofoam base, and a smart packaging indicator label is attached to the LDPE plastic wrap film that covers the styrofoam container. For 24 hours, samples were maintained at room temperature (28±2°C) with normal light exposure.



a **b**

Figure 1. Smart and active packaging system (a) design, (b) directly applied on fresh meat.

2.2.6. Observation Parameter

2.2.6.1. Smart Packaging Indicator Color Measurement

The color of smart packaging indicators is quantitatively determined using a Chromameter Digital color meter (T-135). Meat packaged in a smart and active packaging system is placed on a flat surface with a black background with uniform lighting. The chromameter detector is placed on the surface of the smart packaging indicator, the start button is pressed until the measurement results are displayed on

the display. The measurement results are expressed in the Hunter's Lab Colorimetric System notation which is presented in 3 values, namely L* (Lightness), a* (Redness), and b* (Yellowness) (Modified from Yam & Papadakis 2004; Nurmawati 2011). Following that, the color of the smart packaging indicator is determined by calculating the °Hue value using the formula below.

$$^{\circ}Hue = tan^{-1}\frac{b}{a}$$

Explanation:

^{o}Hue = parameters for color range

a = is a red-green mixed color

b = is a red-green mixed color yellow-blue

2.2.6.2. Testing the Antimicrobial Activity of Active Packaging Films with Agar Diffusion Method

The antimicrobial activity of the active packaging film was determined. Each active packing film was cut into 5 mm circles (prepared in a sterile environment) and then placed on NA agar media with 0.1 ml of the Staphylococcus aureus test microorganism culture containing 10^6 CFU/ml. Petri dishes were incubated for 24 hours at 37°C. After incubation, the inhibitory zone was measured using a caliper (Padgett *et al.,* 1998).

2.2.6.3. pH Determination of Beef

The pH of beef was measured using a pH meter (Oakton pH 510). 5 g crushed meat was combined with 45 ml distilled water until homogenous. The pH meter's electrode is then immersed in the beef suspension until the pH value on the monitor remains constant.

2.2.6.4. TVBN (*Total Volatile Base Nitrogen*) Measurement Using Conway Dish Method

30 ml of 7% TCA solution was added to a 10g±0.1g meat sample and mixed before filtering. 1 ml boric acid solution was placed in the "inner chamber" of the Conway dish and then the lid of the cup was placed in a position almost covering the cup. The filtrate is placed into the left outer chamber. 1 ml saturated K_2CO_3 solution into the right outer chamber to avoid mixing the filtrate with K_2CO_3. Close the cup and rotate it to mix the two liquids in the outer chamber. The blank solution was prepared using the same process, but with 7 percent TCA instead of filtrate. Then stored 2 hours at 37°C. The boric acid solution with the blank and the filtrate sample was then titrated with 0.01 N HCl until it turned pink. The formula for determining TVBN is as follows (AOAC, 1995).

$$\text{TVBN content (mg/100g)} = \frac{(Vc\text{-}Vb)\text{x } N \text{ x } 14{,}007 \; x \; fp \; x \; 100}{W}$$

Explanation:

V_c = Volume of HCl solution use for sample titration

V_b = Volume of HCl solution use for blank titration

N = Normality of HCl solution

W = sample weight (g)

14.07 = Molecular Weight of Nitrogen

Fp = Dilution Factor

2.2.6.5. Total Plate Count (TPC)

The total number of microorganisms was determined using the total plate count method described in SNI 2332.3: 2015. One gram of the sample was added to the test tube containing 9 ml of physiological

solution until homogeneous, referred to as the 10^{-1} dilution, and the dilution is continued until 10^{-6}, at which point the diluted sample is inoculated on NA media in duplicate using the pour plate method. Following solidification of the media, the petri dishes containing the media and sample solution were incubated upside down at 30°C for 48 hours. Following that, the Total Plate Count (TPC) is calculated using the formula below (Badan Standar Nasional, 2015).

$$N = \frac{\sum C}{[(1 \times n_1) + (0.1 \times n_2)] \times (d)}$$

Explanation:

N = Total plate count (cfu/ml)
$\sum C$ = Number of colonies in all petri dishes counted
n_1 = Number of petri dishes on first dilution counted
n_2 = Number of petri dishes on second dilution counted
d = First dilution counted

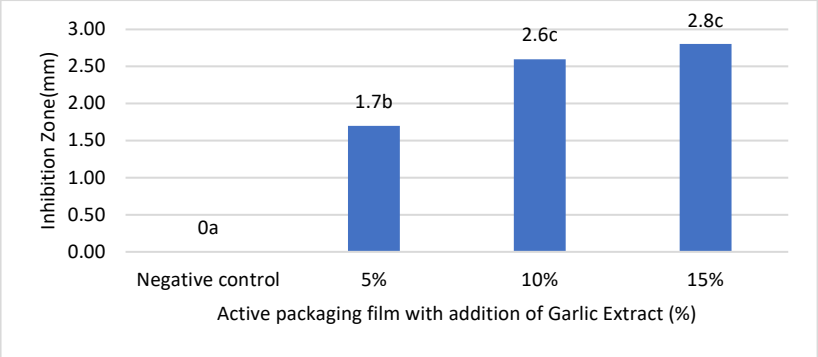
2.2.6.6. Data analysis

Parameters observed on smart packaging indicator, parameter antimicrobial activity of active packaging film, as well as parameters observed on beef including pH, TVBN, and TPC processed using analysis of variance (ANOVA) with three replications, difference between treatment determined using *duncan test*. While, correlation of smart packaging indicator color changing value and the effect of active packaging application in all parameters observed on meat decay presented in 1 graph using Sigma Plot 12 application. *Software* applied on data analysis were *Microsoft Excel* 2019, SPSS versi 19, and Sigma Plot 12.

3. Results and discussion

3.1. Antimicrobial Activity of Active Packaging Film against *Staphylococcus Aureus*

The antimicrobial activity test of the active packaging film is presented in **Figure 2**.



Note: Means value followed by different letter imply significant difference at 5% level (P<0.05)

Figure 2. Antimicrobial Activity of Active Packaging Film against *Staphylococcus Aureus*

The antimicrobial activity of the active packaging film against Staphylococcus aureus was assessed from the diameter of the inhibition zone formed. Figure 2 indicates that the negative control

did not generate an inhibitory zone, meanwhile, the higher the concentration of garlic extract added to the active paper, the greater the inhibition against bacteria, although the inhibition zone was not significantly different at 10% and 15% garlic extract concentrations. Figure 2 shows that the active packaging film with the addition of garlic extract has antimicrobial activity against gram-positive bacteria *Staphylococcus aureus* with a weak category as indicated by the diameter of the inhibition zone formed is less than 5mm (Morales et al., 2003). The diameter difference between the inhibitory zones is determined by the ability and rate of diffusion of antimicrobial compounds in the medium, the growth rate of microorganisms, their sensitivity to antimicrobial chemicals, and the viscosity and thickness of the medium.

Garlic extract's antibacterial effect is due to the chemical allicin, which is generated when garlic is damaged. When the fruit flesh of the garlic extract is damaged during the refining process, allicin compounds are rapidly generated due to the release of the enzyme alliinase, which reacts with non-protein amino acids, namely alliin. According to several studies, allicin is a type of garlic defense that is antimicrobial for both gram-positive and gram-negative bacteria by inhibiting RNA and lipid synthesis. This inhibits the production of amino acids and proteins, and the phospholipid bilayer of the bacterial cell wall, preventing growth and development. Allicin is highly permeable and easily penetrates bacterial cells across the cell membrane. The thiosulfinate S(=O)S group in allicin then binds to the sulfhydryl groups of bacteria, inhibiting the activation mechanism of bacterial proteinases(Dwivedi et al. 2019; Reiter et al. 2020). The findings of this investigation reveal that garlic extract has comparable antibacterial effects at concentrations of 10-15%.

3.2. Beef pH

pH measurements were performed to investigate the effect of employing active packaging material as a meat base in a packaging system during 24 hours of room temperature storage. The findings of the pH test of meat are shown in **Figure 3**.

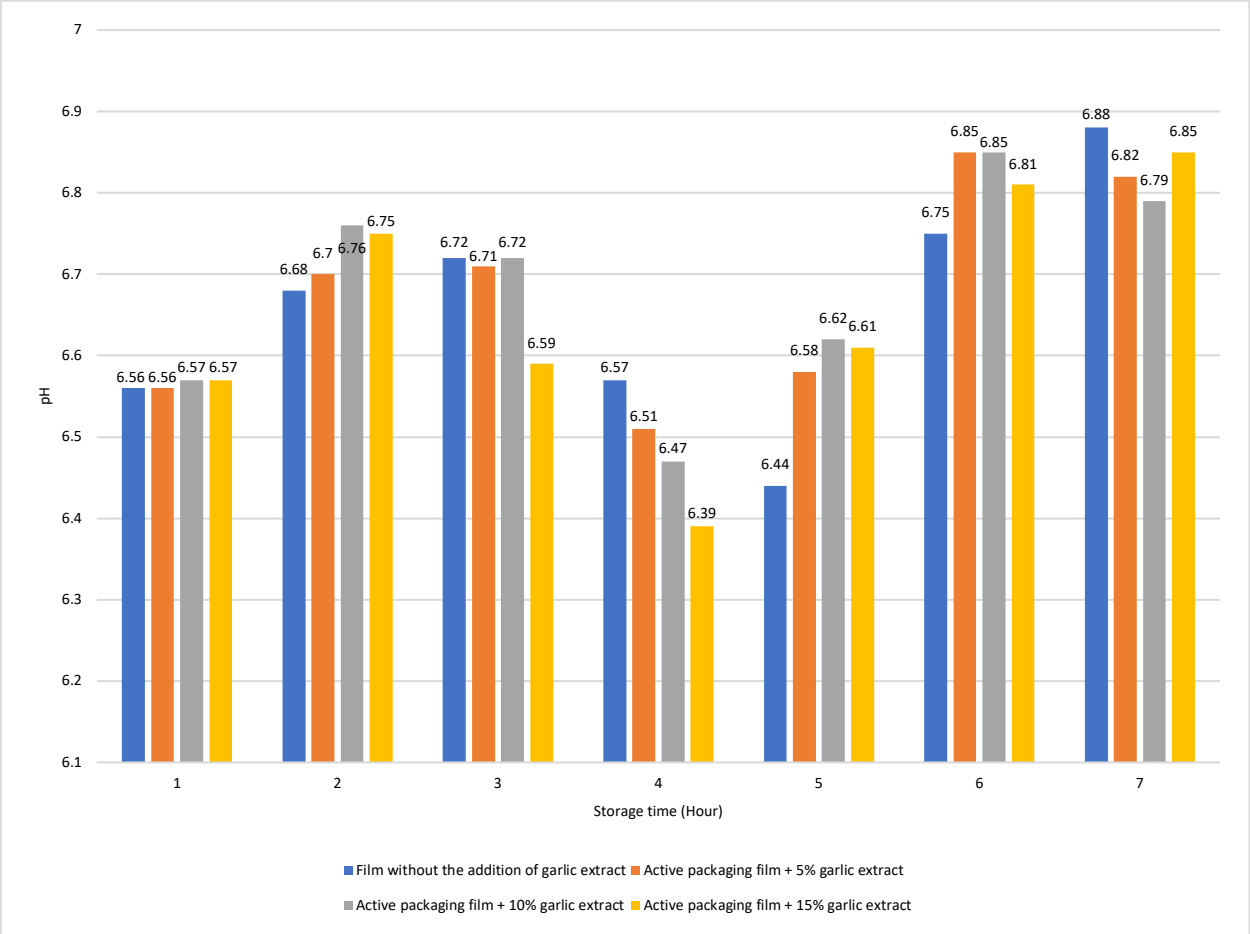


Figure 3. pH value of packaged meat stored for 24 hours in room temperature.

As illustrated in Figure 3, the initial pH value of the meat was 6.57, which was determined immediately following the slaughtering procedure and classified as normal. The pH value of meat fluctuates throughout storage; during the fourth hour of storage, the pH value of the meat ranges from 6.68 to 6.76, and during the sixth hour of storage, the pH value of the meat ranges from 6.59 to 6.72. Several studies have demonstrated that the pH of normal meat ranges between 5.4-5.8 at 6 hours postmortem (Weglarz 2010; Susanto 2014; Soeparno 2015). In this study, beef has a pH value that is classified as quite high in comparison to that confirmed in previous studies. This is presumably due to stress and a brief rest period prior to slaughter, which depletes glycogen reserves, allowing the anaerobic glycolysis process to occur quickly after slaughter and the lactic acid produced in the tissue to become depleted; as a result, the pH of the meat drops to an unsatisfactory level. Additionally, cow are believed to be fatigued before to slaughter, resulting in a depleted supply of ATP and a high enough temperature during slaughter to expedite the depletion of ATP, so expediting the rigor mortis process. The sharp rigor mortis causes the pH of the flesh to remain elevated and above normal. This is confirmed by (Sánchez-Macías et al. 2019; Moreno et al. 2020) that each variety of meat has a different glycogen content, resulting in a variation in glycolysis rates. The less glycogen in the meat, the slower the glycolysis process will be and the higher the final pH will be. However, muscle pH decreases can be influenced by internal elements such as species, muscle type, muscle glycogen, and livestock variability, as well as external factors such as environmental temperature, additional treatment before to slaughter, and pre-slaughter stress.

Additionally, Figure 3 demonstrates that after 20 hours of storage, the meat's pH value ranged between 6.75 and 6.85 and remained steady during the subsequent storage duration, which was classified as decayed. According to (Prache, Schreurs & Guillier 2021), The meat's pH continues to decline until the glycogen is depleted into lactic acid followed by the process of neutralization of alkaline compounds resulting from microbial metabolism, resulting in an increase in pH. If the pH reaches 6.8 or higher, protein decomposition will occur which ends in spoilage.

3.3. TVBN (*Total Volatile Base Nitrogen*) of Meat

TVBN values obtained in this study are presented in **Figure 4**.

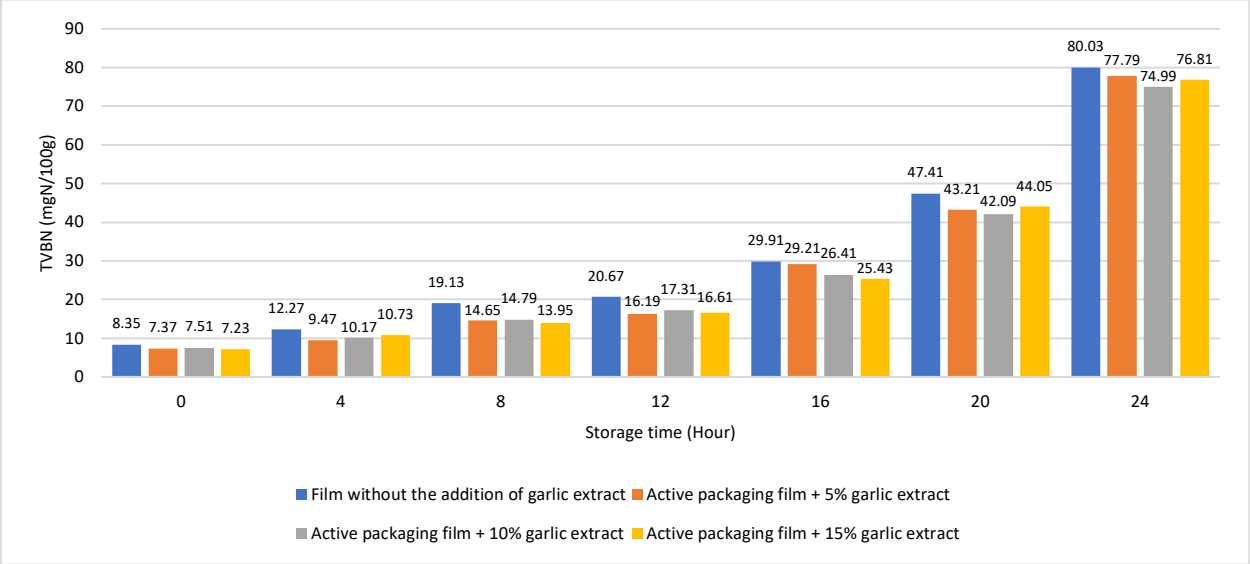


Figure 4. Total Volatile Basic Nitrogen (TVBN) Value of Packed Meat Stored for 24 Hours at Room Temperature.

Figure 4 illustrates that all meat sample at 0 hour storage had a TVBN value ranging from 7.23 to 8.35 mgN/100g and was therefore classified as fresh meat. After 12 hours of storage, meat that had not been treated with active packaging film had a TVBN value of 20.67 mg N/100g, indicating that it was rotten. Meanwhile, samples treated with active packaging film with the addition of 5%, 10%, and 15% garlic extract had TVBN values respectively, namely 16.19 mgN/100g, 17.31 mgN/100g, and 16.61 mgN/100g which were categorized as semi-fresh meat (stale meat) or the limit of meat is allowed to be consumed. Meanwhile, the TVBN value of all samples taken between the 16th and 24th hours of storage exceeded the TVBN value threshold for food-grade beef. This demonstrates the effectiveness of adding garlic extracts at concentrations of 5%, 10%, and 15% to active packaging film in reducing the amount of TVBN in fresh meat by 21.67 percent, 16.26 percent, and 19.64 percent, respectively, when compared to meat samples without active packaging film application. According to several theories, beef or livestock is considered fresh if the TVBN value is less than 15 mg/100g (National Standard of the People's Republic of China 2016), TVBN <10 mg N/100g (Farber 1965), as well as standard levels of TVBN fit for consumption in accordance with SNI 2354.8:2009 is 20-30 mg N/100g. This indicates that the TVBN threshold is inconsistent with a measure of meat freshness. Numerous TVB-N tests, on the other hand, are used to independently examine the quality of beef products.

In this study, the value of TVBN increased throughout storage (observed every four hours), indicating that the meat's quality continued to deteriorate due to the additional breakdown of protein into volatile base compounds. The increase in TVBN value is due to the activity of microorganisms that degrade proteins into simpler molecules that eventually undergo deamination to generate ammonia, which contributes to odor, as well as the synthesis of nitrogen-containing compounds that are volatile bases. Additionally, high temperatures can promote protein degradation, resulting in the formation of more alkaline components. According to (Bekhit et al. 2021), The increase in TVBN value is due to protein degradation by microorganisms, which results in the formation of foul-smelling chemicals such as ammonia (NH_3), basic skatole and indole compounds, mercaptans and H_2S, which are weak acids, and amines and cadaverin, which are strong bases.

This demonstrates that the addition of garlic extract to active packaging films can delay the spoiling of meat based on TVBN criteria when compared to untreated meat. This is most likely because garlic's active components prevent microbial development, hence lowering the synthesis of nitrogenous base compounds in meat caused by bacteria and autolytic enzymes during the rotting process. This is supported by (Al Hakim, Hartanto & Nurhrtadi 2016; Reiter et al. 2020), that garlic extract has the ability to block microbe-produced enzymes involved in the breakdown of proteins into volatile base chemicals.

3.4. TPC (*Total Plate Count)* of Microbes in Beef

The total number of bacteria in meat samples was analyzed to determine the effect of utilizing active packaging films on the microbiological quality of meat packaging systems. The findings of the TPC measurement on meat are shown in **Figure 5**.

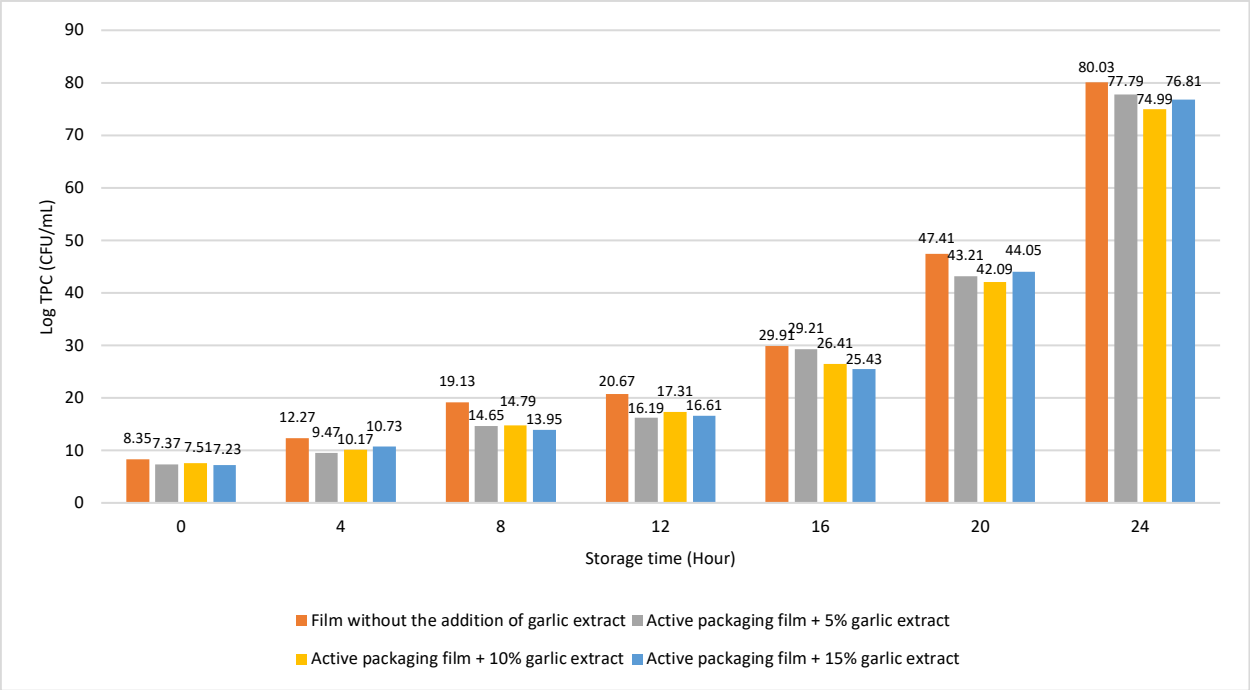


Figure 5. Total Plate Count (TPC) of Packed Meat Stored for 24 hours in Room Temperature.

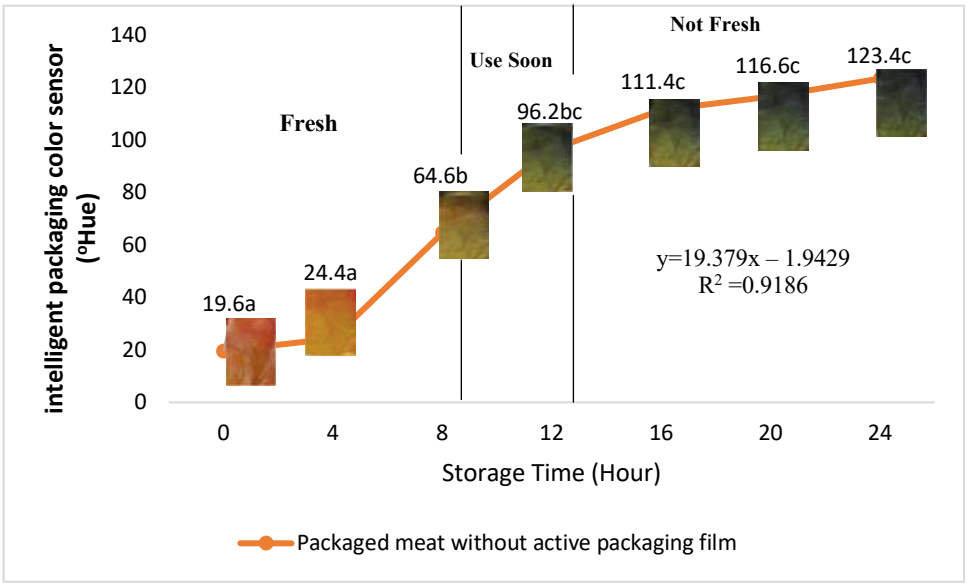
Figure 5 illustrates the initial TPC value for all samples at 0 hour storage, which is Log TPC 2.53±0.64 CFU/mL and classified as fresh meat based on microbiological quality. Throughout storage, the TPC value increases until it reaches the maximum number of meat microbes permitted by SNI 3932:2008

about Carcass and Beef Quality, which is $1x10^6$ CFU/ml or equivalent to Log TPC 6 CFU/ml. At 12 hours of storage, meat samples treated with active packaging film without addition of garlic extract (0%) and with addition of garlic extract (5%) were characterized as not fulfilling microbiological requirements, with Log TPC values of 7.65±0.39 CFU/ml and Log TPC 6.20±0.00 CFU/ml, respectively. Meanwhile, samples treated with active packaging film and 10% and 15% garlic extract were classed as not fulfilling microbiological requirements after 16 hours of storage, with Log TPC values of 7.47±0.26 CFU/ml and Log TPC 6.78±0.67 CFU/ml, respectively. This demonstrates that the use of active packaging films in combination with 10% and 15% garlic extract in the meat packaging system can inhibit microbial growth and extend the shelf life of meat by up to 4 hours. This is thought to be due to the active compound Allicin's ability to inhibit the growth of both gram-positive and gram-negative bacteria by destroying the S-H group (sulfhydryl group) bound to bacterial protein, which is a required group for bacterial cell division or a specific stimulator for cell multiplication. Allicin works by damaging RNA and DNA in bacteria, thereby inhibiting their growth and development in meat. This is in line with Deresse's (2010) statement that Allicin in garlic has the ability to suppress the growth of gram-positive and gram-negative bacteria by completely inhibiting bacterial RNA synthesis, as well as DNA and protein synthesis.

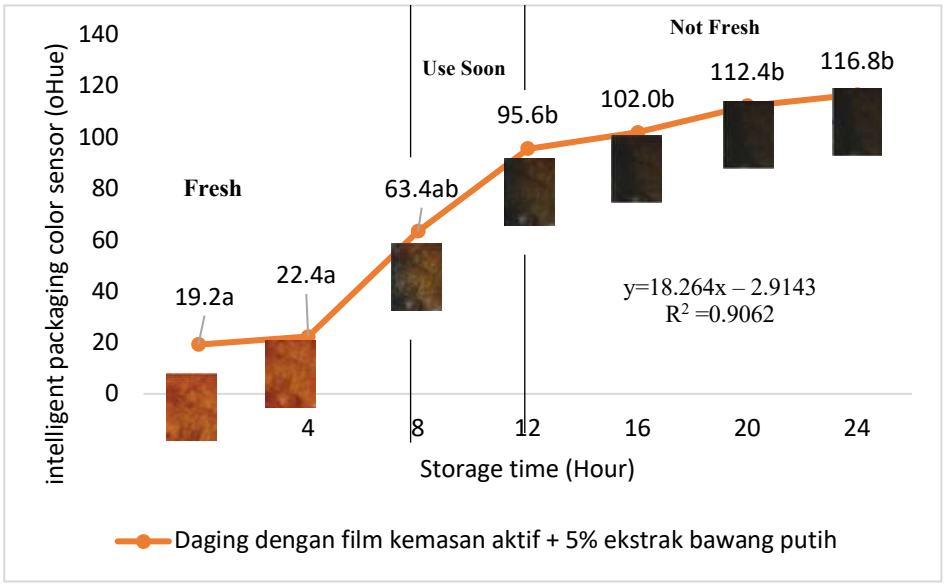
The TPC test results in Figure 5 indicate that the total microbial content of meat continues to increase during storage. This is because meat contains a high nutrient and water content, which provides an ideal environment for microorganism growth. Additionally, storage at room temperature can accelerate the growth of microorganisms. This is supported by Soeparno (2015), meat complies the requirements for microorganism growth because it contains a high proportion of water (68-75 percent), is rich in nitrogen-containing substances of varying complexity, contains a variety of fermentable carbohydrates, is rich in minerals and essential factors for microorganism growth, and has a pH suitable for microorganism growth (pH around 5.3-6.5). The findings of the variance analysis indicated that the storage duration of meat and the usage of active packaging film containing garlic extract had a highly significant effect on the TPC value of meat at the 1% (0.01) level.

3.5. Color Change of Smart Packaging BTB (Bromothymol Blue) pH 2.75 in Fresh Meat Packaged with Active Packaging

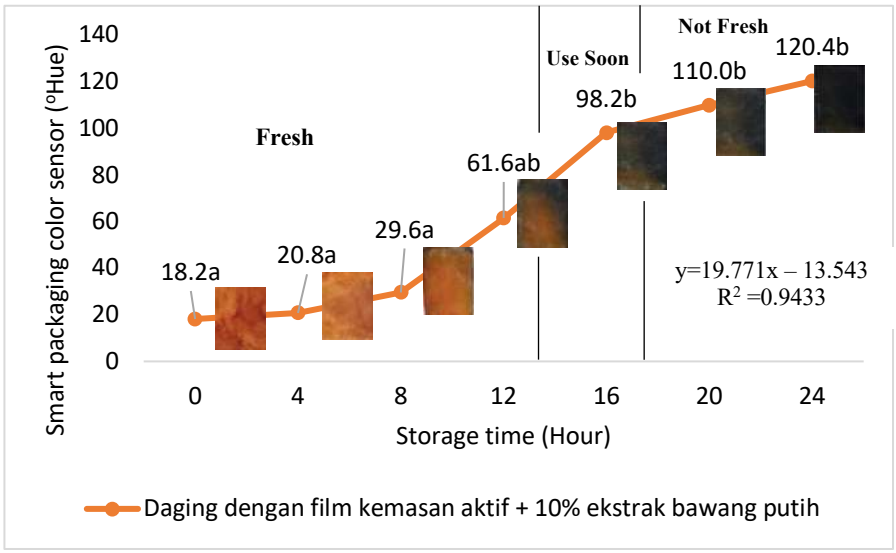
Based on the results of previous studies' sensitivity tests of the smart packaging indicator (Dirpan et al. 2019) The BTB (Bromothymol blue) pH 2.75 solution was determined to have the most readily visible color change reaction during sensitivity testing utilizing fresh beef packaged and maintained at room temperature for 24 hours. As a result, the smart packaging indicator BTB pH 2.75 was used again in this investigation to evaluate how the indicator's color value changes when paired with active packaging material in a fresh meat packaging system. The findings of the color reaction of the smart packaging indicator are shown in **Figure 6**.


intelligent packaging color sensor (ºHue)
140
120
100
80
60
40
20
0
Fresh
Use Soon
Not Fresh
19.6a
24.4a
64.6b
96.2bc
111.4c
116.6c
123.4c
y=19.379x – 1.9429
R² =0.9186
0
4
8
12
16
20
24
Storage Time (Hour)
Packaged meat without active packaging film

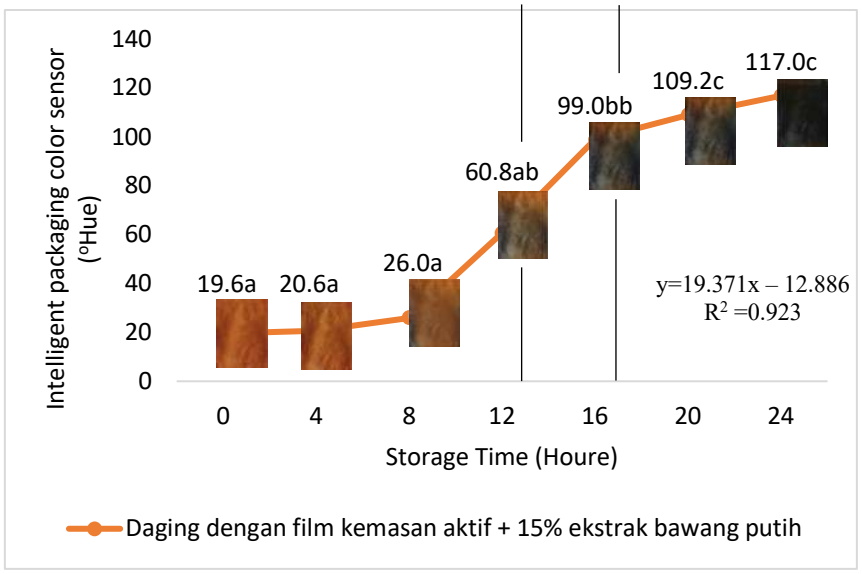
(a)


intelligent packaging color sensor (oHue)
140
120
100
80
60
40
20
0
Fresh
Use Soon
Not Fresh
19.2a
22.4a
63.4ab
95.6b
102.0b
112.4b
116.8b
y=18.264x – 2.9143
R² =0.9062
4
8
12
16
20
24
Storage time (Hour)
Daging dengan film kemasan aktif + 5% ekstrak bawang putih

(b)



c)



(d)

Figure 6. Color Change of Smart Packaging BTB (Bromothymol Blue) pH 2.75 Applied on Fresh Meat with Active Packaging Film 0% **(a)**, 5% **(b)**, 10% **(c)**, and 15% **(d) of** Garlic Extract.

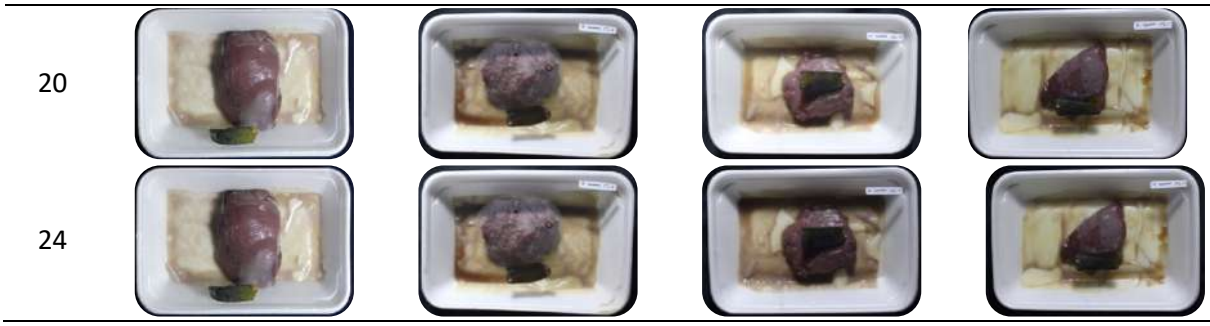
Figure 6 illustrates the three phases of the smart packaging indicator changing color in the fresh meat packaging system during storage, namely phase I orange indicates the meat is still fresh, phase II green with an orange gradation indicates the meat must be consumed immediately, and phase III dark green indicates the meat has been cooked. The indicator's color change from phase I (orange) to phase II (green with orange gradation) shows that the initial deterioration of the meat has occurred, as demonstrated by the indicator's color change. The indicator's color changes occurred due to the intereaction of alkaline volatile resulted by the due to enzyme activity, and the metabolism of microorganisms grows with storage duration. Microorganisms and enzymes degrade the nutritional content of meat, releasing volatile alkaline compounds as an early sign of spoilage. These compounds gradually accumulate in the packaging system, causing an increase in pH, which is detected by smart packaging indicators via gradual color changes. The color change of the smart packaging indicator BTB

(Bromothymol blue) pH 2.75 from orange to green induced by deprotonation, or the release of a proton from the smart packaging indicator dye (De Meyer *et al*. 2014).

According to Figure 6, meat packaged in active packaging film with no additives (0 percent) and a 5% garlic extract addition is declared fresh during the 0 to 8 hour storage period, must be consumed immediately during the 8 to 12 hour storage period, and rotted during the 12 to 24 hour storage period. This is consistent with the TPC test results, which indicated that the TPC value had above the acceptable threshold for microbial contaminants in meat, which was 1x106 or equivalent to 6 CFU/ml, after 12 hours. Meanwhile, beef packaged with active packaging film containing 10% and 15% garlic extract was considered fresh during the 0 to 12 hour storage period, must be consumed immediately during the 12 to 16 hour storage period, and was pronounced rotten during the 16 to 24 hour storage period. This is consistent with the TPC test results (Figure 5), which indicate that at the 16th hour, the TPC value surpassed the permissible level of microbiological contamination in beef. The statistical results indicate that the storage duration of fresh meat has a very significant effect on the Hue value, the indicator of color change in smart packaging. The visualization of the color change of the smart packaging indicator BTB (Bromothymol blue) pH 2.75 when used together with active packaging film to detect meat rot is presented in **Table 1**.

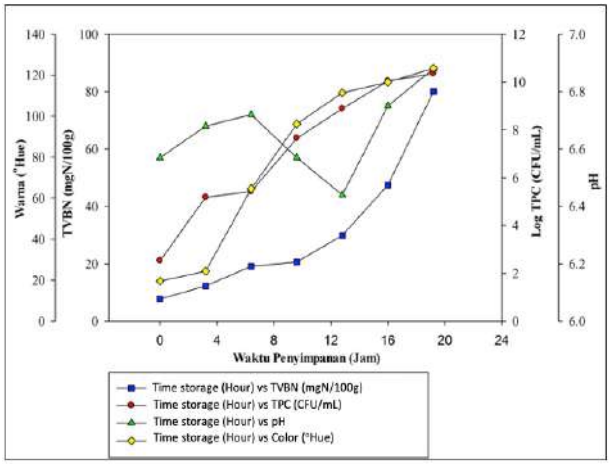
Tabel 1. Visualization of Color Change of Smart Packaging Indicator BTB (Bromothymol Blue) pH 2.75 when Used Together with Active Packaging Sheets in Fresh Meat Packaging Systems to Detect Rotten Meat.

Storage Time (Hour)	Active Packaging Sheets with the Addition of Garlic Extract			
	0%	5%	10%	15%
0				
4				
8				
12				
16				

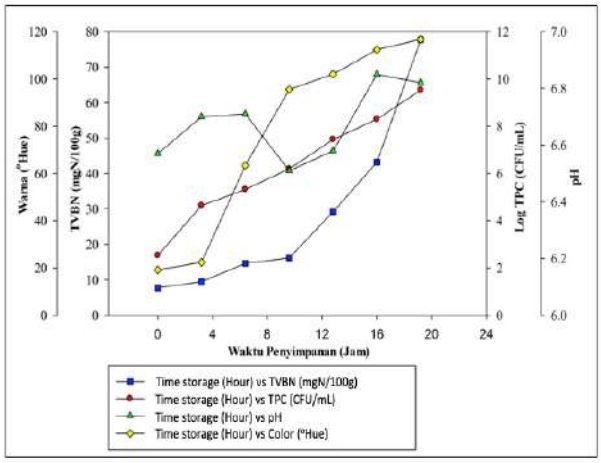


3.1. Correlation of Changes in Color Value Indicators of BTB Smart Packaging (Bromothymol Blue) pH 2.75 with the Effect of Active Packaging on All Meat Rotten Parameters

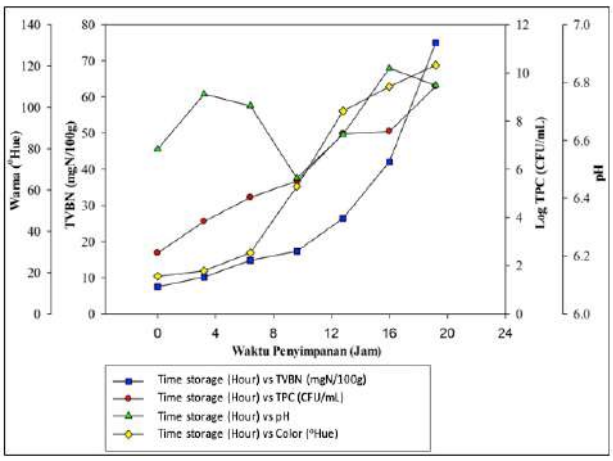
Correlations between changes in the color value of the smart packaging indicator and meat quality deterioration parameters (pH, TVBN, and TPC) should be performed to ascertain the relationship between the sensitivity of the smart packaging indicator for detecting meat rot and the effectiveness of active packaging films in slowing the process of meat spoilage.



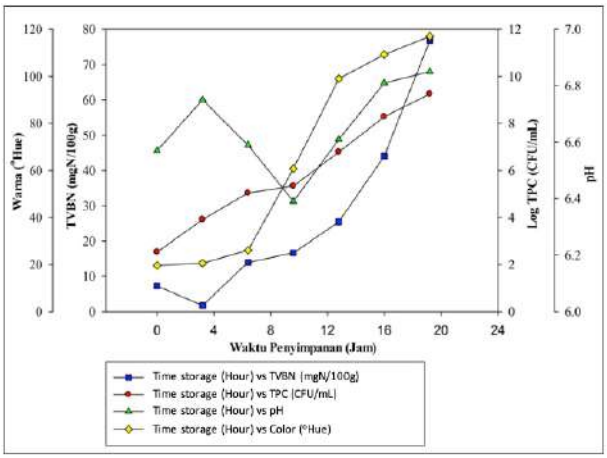
(a)



(b)



(c)



(d)

Figure 7. Correlation between Changes in the Color Value of the Smart Packaging Indicator BTB (Bromothymol Blue) pH 2.75 and the Effects of Active Packaging Film Application 0% **(a)** 5% **(b)** 10% **(c)** 15% (d) of garlic extract with all meat rotten parameters for 24 hours storage.

According to Figure 7, meat treated with active packaging film without adding garlic extract increased in TPC and TVBN values, which corresponded to an increase in the color value of the smart packaging indicator BTB (Bromothymol Blue) pH 2.75. Meanwhile, the pH of meat fluctuates, which is caused by changes in the pH value caused not only by the production of volatile base compounds caused by microorganism activity in meat during the storage process, but also by factors such as glycogen and lactic acid content in livestock prior to and after slaughter. Figure 7 demonstrates that meat treated with active packaging film without the addition of garlic extract and meat treated with garlic extract at a concentration of 5% was declared rotten and unfit for consumption after 12 hours of storage, based on the Log TPC values of 7.65±0.39 CFU/mL and 6.20±0.00 CFU/mL, respectively, and the TVBN values of 20.67±2.68 mgN/100g and 16.19±0.28 mgN/100g, respectively. Meanwhile, meat treated with active packaging film and 10% and 15% garlic extract was declared rotten and unfit for consumption after 16 hours of storage, based on the Log TPC values of 7.47±0.26 CFU/mL and 6.78±0.67 CFU/mL, respectively, and the TVBN values of 26.41±3.31mgN/100g and 25.43±4.89mgN/100g, respectively.

Figure 7 shows that the TPC value gives the same indication of the TVBN value and is in line with the change in color of the smart packaging indicator of meat treated with active packaging film without addition (0%) and the addition of 5% garlic extract was declared rotten at 12 hours of storage. the color of the indicator changes from orange (fresh) with a color value of 19.6°Hue and 19.2°Hue to green (rotten) with a color value of 96.2°Hue and 95.6oHue respectively. Meanwhile, meat that was treated with active packaging film with the addition of 10% and 15% garlic extract was declared rotten at the 16th hour of storage, indicated the color of the indicator changed from orange (fresh) with color values 18.2°Hue and 19.6°Hue, respectively to green (rotten) with color values of 98.2°Hue, and 99°Hue, respectively. According to Wiryawan dkk (2005), When garlic extract was added to active packaging, the value of TPC, TVBN, and pH of the meat increased more slowly, as did the color of the smart packaging indicator, compared to meat without active packaging.

The increase in TPC, TVBN, and pH values of meat is strongly influenced by its nutrient content and high water content, implying a high risk of microbial contamination. Additionally, storing meat at room temperature accelerates the growth of bacteria. In general, an increase in the number of microorganisms is followed by an increase in the production of a volatile base chemical called TVBN (Total Volatile Basic Nitrogen). The more microorganisms present and active, the more volatile base compounds are released, as indicated by the growing TVBN value, which also has an effect on the meat's pH value. The increase in TPC, TVBN, and pH values also correlates linearly with the increase in Hue value and color change of the smart packaging indicator, as the accumulated volatile base compounds raise the pH value of the packaging system, causing the smart packaging indicator to experience a color shift. This explanation in line with study conducted by Pacquit *et al.* (2006) that applies active packaging on Cod fish, it is stated that there is a linear correlation pattern between the increase in the TPC value of cod with changes in the color of the cellulose-acetate packaging film sensor.

4. Conclusion

It is concluded that using BTB (Bromothymol blue) pH 2.75 solution, the smart packaging indicator has a sensor color change that is easy to observe visually through 3 phases, namely phase I orange indicates the meat is still fresh, phase II green with orange gradation indicates meat must be consumed immediately, and phase III dark green indicates the meat has rotten and unfit for consumption. Meat treated with active packaging sheet treatment containing 10% and 15% garlic extract rotted after 16 hours, while meat treated with active packaging sheet treatment containing 0% and 5% garlic extract rotted after 12 hours. BTB (Bromothymol blue) pH 2.75 color change has linear and positive association with TPC, TVBN, and pH test parameters of meat packaged utilizing active packaging sheets.

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Application of a Smart Sensor and Active Packaging System Based on the Cellulose of *Acetobacter xylinum* to Meat Products

Abstract.

Combining smart and active packaging serves the dual purpose of detecting color changes in food that reflect changes in its quality and prolonging its shelf life. This study developed a smart and active packaging system made from the cellulose of *Acetobacter xylinum* and assessed its ability to detect changes in the quality of packaged fresh beef. The properties of the smart packaging's sensor and active packaging films were determined. The application of this system to fresh beef stored at room temperature (28±2 °C) for 24 h was tested. The color of the bromothymol blue (BTB) solution (pH 2.75) in the indicator of the smart packaging system changed from orange to dark green to indicate that beef quality changed from fresh to rotten. The meat treated with the active packaging with 10% and 15% garlic extract decayed on the 16th h. By contrast, the meat treated with the active packaging but without the garlic extracts rotted on the 12th h. The shift in the indicator's color was linearly related to the total plate count, total volatile basic nitrogen, and pH of the meat packaged using the active packaging system. Therefore, BTB solution (pH 2.75) can be used as a smart packaging indicator that will allow consumers to assess the quality of packaged meat easily. As an antimicrobial agent, the addition of 10%–15% garlic extract to the active packaging films can help delay the spoilage of packaged meat.

1. Introduction

Global beef consumption is predicted to rise as the world population and family income increase, particularly in developing Asian countries (Dupont & Fiebelkorn 2020; González et al. 2020; OECD/FAO 2021). By 2030, worldwide meat consumption and availability are expected to increase by 14% and 5.9%, respectively, over the average of the 2018–2020 period (OECD/FAO 2021). Thus, the expected increase in meat consumption must be complemented by improvements in the quality of fresh meat produced. One aspect affecting the quality and characteristics of meat is the material and packaging technologies used (Abdurehman Musa 2019). Meat is a perishable item that rapidly spoils when stored above the optimum temperature range (below 0–40 °F) (Beltrán, Roncalés & Bellés 2018; Franco, da Cunha & Bianchi 2021). However, in traditional markets, meat is displayed at room temperature without packaging, a practice that might accelerate microbial contamination and cause rapid quality degradation. Even in supermarkets where meat is maintained in cold temperatures, standard meat packaging still prevent consumers from subjectively determining the quality of meat. Thus, meat packaging must have additional functions that will prevent quality degradation due to microbial contamination and will help consumers to determine the quality of packaged meat easily (Dirpan et al. 2019). Conventional meat packaging can be designed to perform dual functions through smart and active packaging systems.

Smart packaging is a term that refers to sensors in the form of indicators that monitor and provide information on the quality of the food contained within the packaging via color changes caused by chemical reactions between the indicators and the products of microbial metabolism or changes in the chemical composition of the food (Dobrucka & Cierpiszewski 2014). During storage, the chemical components of meat degrade into volatile compounds because of microbial activity, thereby increasing the value of total volatile base nitrogen (TVBN) (Bekhit et al. 2021; Ma et al. 2021). Accumulation of TVBN increases the pH of the packaging system, which is detected by the indicator, resulting in a visible color shift in the indicator (Pacquit et al. 2006; Ma et al. 2021). Smart packaging allows easier monitoring of

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packed products during transportation and storage (Dobrucka dan Cierpiszewski, 2014). Moreover, it provides a more accurate estimate of product condition than conventional expiration labels (Pacquit, Crowley & Diamond 2008). Color-based pH indicator solutions are widely used as smart indicators. Dirpan et al. (2018) developed bromophenol blue as a smart indicator dye for mangoes. Hidayat et al. (2019) used two types of color indicators with predetermined concentrations, namely, phenol red and bromothymol blue, to assess the freshness of meat packaging. Smart packaging indicators based on natural pigments are being developed, such as smart packaging films that include anthocyanin-loaded *Lycium ruthenicum* nanocomplexes in starch/polyvinyl alcohol mixtures (PVA) (Qin et al. 2021), as well as anthocyanins from saffron petals immobilized in chitosan nanofibers and methyl cellulose matrix (Alizadeh-Sani et al. 2021).

Active packaging refers to the integration of particular additives into a packaging system for the purpose of extending the shelf life, preserving the quality, and ensuring the safety of food products. Antimicrobial agents are used as components of active packaging additives to extend product shelf life. The volatile bioactive compounds in active packaging evaporate or diffuse onto the food surface, where they limit the growth of pathogenic microbes and thus delay spoilage (Yildirim et al. 2018; Wrona et al. 2021). This strategy is more effective than coating bioactive compounds onto the food surface (Iriani 2014). The safest, cheapest, and most readily available antimicrobial agents for use in active packaging are essential oils. Pranoto et al. (2005) produced antimicrobial alginate edible films by incorporating the essential oils of garlic. They reported that these films substantially inhibited the growth of *Staphylococcus aureus* and *Bacillus cereus* in meat. Priya, Vinitha, and Sundaram (2021) utilized the essential oils of *Plectranthus amboinicus* in a chitosan-based active packaging to restrict antimicrobial activity. Smart and active packaging can be merged into a single packaging system. Julyaningsih, Latief, and Dirpan (2020) combined a smart packaging system based on methyl red–bromothymol blue (BTB) indicator with an active packaging system based on lemongrass oil as a component of tuna fish fillet packaging. Yao et al. (2021) developed an active and smart packaging system based on starch, PVA, and betacyanins from various types of plants for shrimp packaging.

In general, an active packaging that contains antimicrobial agents and a smart packaging that contains indicator solutions are immobilized in a polymer. Compared with plant cellulose or synthetic polymers, the bacterial cellulose fermented by *Acetobacter xylinum* has a unique nanofibrillar structure and superior physical properties, suggesting that it has the potential to serve as a basis for developing a smart and active packaging system (Cazón & Vázquez 2021; Xu et al. 2021). Bacterial cellulose has received interest as a component of active packaging owing to its edibility, biodegradability, high water-holding capacity, and great potential as an antimicrobial agent carrier (Nguyen, Gidley & Dykes 2008).

The development of packaging systems with additional functions is advancing. To promote this innovation, this study aimed to maximize the potential of smart and active packaging by combining them into a single packaging system based on a bacterial cellulose membrane biopolymer to enhance the quality of packaged meat and help consumers to determine meat freshness easily.

2. Method and Materials

2.1. Materials

The main ingredients used in the smart and active packaging system developed herein were the bacterial cellulose produced by *A. xylinum*, which was fermented in natural media of coconut water. Beef tenderloin

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was obtained from a slaughterhouse in Tamangapa Raya. Coconut water and garlic (*Allium sativum*) were purchased from a local market. Food-grade ammonium sulfate (CAS Number: 7783-20-2), yeast extract (Merck, CAS Number: 8013-01-2), 96% acetic acid, *A. xylinum* culture, 5% 1 N NaOH, sucrose, BTB, alcohol, aquabides, aquades, Tashiro's indicator (0.1% methyl red and 0.1% BTB at a ratio of 2:1), 7% trichloroacetic acid (TCA, Merck), nutrient Agar (NA, Merck), glycerol (Merck, CAS Number: 56-81-5), food-grade carboxymethyl–cellulose (CMC) (Foodchem, E466), and corn starch were used.

2.2. Method

2.2.1. Determination of the best nitrogen source from *A. xylinum* fermentation media

The production of *A. xylinum* bacterial cellulose membranes was started by determining the amount and type of the most suitable nitrogen source from *A. xylinum* growth media following the method of a previous study (Dirpan et al. 2019).

2.2.2. Purification of bacterial cellulose

Bacterial cellulose was removed from the fermentation medium, rinsed in running water, and then soaked for 2 days with periodic water changes. The cellulose was also soaked in 70% alcohol for 1 min, heated to 100 °C in distilled water for 20 min, and reheated in 1 N 5% NaOH solution at 100 °C for 60 min to remove the remaining bacterial cells and substrate attached to the cellulose layer. Afterward, the cellulose was rinsed with running water and soaked in periodically changed water for 24 h until pH reached 7. The purified cellulose appeared transparent (Dirpan et al. 2019).

2.2.3. Production of smart packaging

2.2.3.1. Preparation of the indicator solution

BTB indicator solution was chosen for this study because a previous work established this solution as the indicator with the most visually identifiable color change reaction (Dirpan et al. 2019). First, 1% BTB solution (b/v) was prepared in 95% ethanol. Then, the pH of the BTB solution was decreased to 2.74 by adding 20% acetic acid. Finally, the BTB solution was stored in a closed container.

2.2.3.2. Production of smart packaging indicator label

The purified cellulose film was kept in a filter cloth for 24 h to decrease its water content. Half-dried cellulose was cut into 1.5 cm × 4 cm strips and pushed flat against the surface of a Pyrex glass. The cellulose was dried for 30 min at 70 °C. The BTB indicator solution was then absorbed into a dry cellulose via centrifugation at 3000 rpm for 15 min. When the color indicator was successfully absorbed, the BTB indicator solution imparted an orange hue to the cellulose. Afterward, the cellulose was rinsed with distilled water to eliminate any unbound color indicators and then dried (Kuswandi and Maryska, 2013; Shukla et al. 2015).

2.2.4. Production of active packaging film

2.2.4.1. Production of garlic extract as active element

First, 500 g garlic was peeled, washed under running water until clean, drained, and then mashed. The minced garlic was extracted via the maceration method by immersing the finely ground

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garlic in 96% alcohol at a ratio of 1:4 (garlic:alcohol) for 4 days at 3 °C–5 °C and periodically homogenized using a water bath shaker. Afterward, the extract was filtered using a filter paper and then concentrated using a rotary evaporator at 50 rpm at 40 °C to obtain a thick extract (Rotty dan Tjitrosantoso 2015; Shetty et al, 2013).

2.2.4.2. Production of active packaging film

The bacterial cellulose was crushed to form a cellulose slurry, and all pretreatments were performed at room temperature. A cellulose suspension was prepared using 30% chitosan (w/w), 10% CMC (w/w), and 15% corn starch (w/w) of cellulose dry weight. The suspension was heated at 50 °C for 60 min with a hot plate stirrer until thoroughly suspended. At the 50th min, 30% glycerol (w/w) was added. Additionally, the garlic extract was added at quantities of 0% (as the control), 5%, 10%, and 15% (v/v) immediately after the final heating step. Subsequently, 60 g of the suspension was then placed onto a glass plate and dried for 48 h at 37 °C. Finally, the suspension was cooled to room temperature, removed from the glass plate, wrapped in aluminum foil, and placed in a desiccator (Indrarti et al, 2016; Iriani, 2014).

2.2.5. Application of the smart and active packaging indicators to fresh beef

Fresh beef tenderloin was collected from a slaughterhouse in Tamangapa Raya Makassar 1 h after the cow was slaughtered. It was immediately placed in a special food box and put into a 38 cm x 29 cm x 30 cm Styrofoam box filled with ice crystals. The samples were promptly transported to the laboratory and processed into 200 g/pack pieces under sterile conditions. The meat was packaged in a Styrofoam tray (1.05 g/cm^3) coated with the active packaging film on a Styrofoam base, and a smart packaging indicator label was attached to the LDPE plastic wrap film that covered the Styrofoam container. The samples were maintained at room temperature (28±2 °C) with normal light exposure for 24 h.

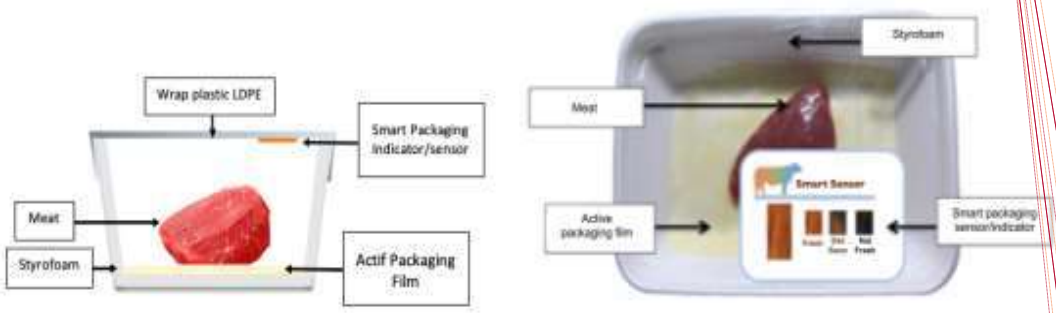

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Figure 1. (a) Design of the smart and active packaging system and its (b) application to fresh meat.

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2.2.6. Observation parameters

2.2.6.1. Measurement of the color of the smart packaging indicator

The color of the smart packaging indicators was quantitatively determined using a chromameter digital color meter (T-135). The meat packaged in the smart and active packaging system was placed on a flat surface with a black background with uniform lighting. The chromameter detector was placed on the surface of the smart packaging indicator. The start button was pressed until the measurement results were shown on the display. The measurement results were expressed according to the notation of the Hunter's Lab Colorimetric System, which is presented in three values, namely L* (lightness), a* (redness), and b* (yellowness) (Yam & Papadakis 2004; Nurmawati 2011). The color of the smart packaging indicator was determined by calculating the °Hue value by using the formula below:

$$°Hue = tan^{-1}\frac{b}{a}$$

where $°Hue$ represents the parameters for color range, a is a red-green mixed color, and b is a red-green mixed color yellow-blue.

2.2.6.2. Antimicrobial activity of the active packaging films

The antimicrobial activity of the active packaging films was determined via the agar diffusion method. Each active packaging film was cut into 5 mm circles in a sterile environment and then placed on NA agar media with 0.1 ml of the test microorganism culture (*Staphylococcus aureus*) containing 10^6 CFU/ml. Petri dishes were incubated for 24 h at 37 °C. After incubation, the inhibitory zone was measured using a caliper (Padgett et al, 1998).

2.2.6.3. Determination of pH of the beef samples

The pH of the beef samples was measured using a pH meter (Oakton pH 510). First, 5 g of crushed meat was combined with 45 ml of distilled water until the mixture became homogenous. The pH meter's electrode was then immersed in the beef suspension until the pH value on the monitor became constant.

2.2.6.4. Measurement of TVBN

First, 30 ml of 7% TCA solution was added to a meat sample (10±0.1 g) and mixed before filtering. The, 1 ml boric acid solution was placed in the "inner chamber" of the Conway dish. The lid of the cup was placed in such a way that it almost covered the cup. The filtrate was placed into the left outer chamber of the Conway dish. Afterward, 1 ml saturated K_2CO_3 solution was put into the right outer chamber to avoid mixing the filtrate with K_2CO_3. The cup was closed and rotated to mix the two liquids in the outer chamber. The blank solution was prepared following the same process but with 7% TCA instead of the filtrate. The solutions were stored at 37 °C for 2 h. The boric acid solution with the blank and filtrate samples was then titrated with 0.01 N HCl until it turned pink. TVBN was calculated as follows (AOAC, 1995):

$$\text{TVBN content (mg/100 g)} = \frac{(Vc\text{-}Vb)\text{x } N \text{ x } 14{,}007 \; x \; fp \; x \; 100}{W}$$

where V_c is the volume of the HCl solution used in sample titration, V_b is the volume of the HCl solution used in blank titration, N is the normality of the HCl solution, W is the sample's weight (g), 14.07

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is the molecular weight of nitrogen, and *Fp* is the dilution factor.

2.2.6.5. Total plate count

The total number of microorganisms was determined via the total plate count (TPC) method described in SNI 2332.3: 2015. First, 1 g of the sample was added to a test tube containing 9 ml of physiological solution until homogeneous (10^{-1} dilution). The dilution was continued until 10^{-6}, at which point the diluted sample was inoculated on NA media in duplicate via the pour plate technique. After the media solidified, the Petri dishes containing the media and the sample solution were incubated upside down at 30 °C for 48 h. Afterward, TPC was calculated using the formula below (Badan Standar Nasional, 2015):

$$N = \frac{\sum C}{[(1\times n_1)+(0.1\times n_2)]\times (d)^{t}}$$

where N is TPC (CFU/ml), $\sum C$ is the number of colonies counted in all Petri dishes, n_1 is the number of colonies counted in all Petri dishes at first dilution, n_2 is the number of colonies counted in all Petri dishes at second dilution, and d is the number of colonies counted in all Petri dishes at first dilution.

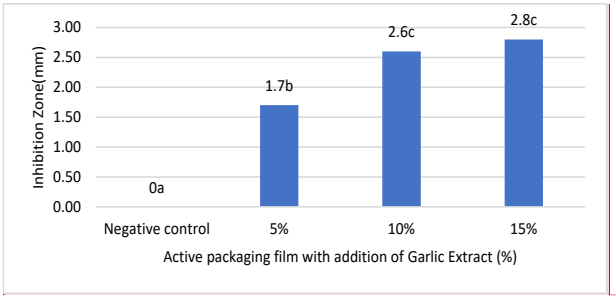
2.2.6.6. Data analysis

ANOVA was used to analyze the parameters of the smart packaging indicator, antimicrobial activity of the active packaging films, and quality of the beef samples, including pH, TVBN, and TPC with three replications. Differences between treatments were determined using Duncan's test. The correlations between the changes in the color of the smart packaging indicator and the effects of the active packaging on all parameters of meat spoilage were explored and presented in graphs by using the Sigma Plot 12 software. Data were analyzed using Microsoft Excel 2019, SPSS 19, and Sigma Plot 12.

3. Results and discussion

3.1. Antimicrobial activity of the active packaging films against *S. aureus*

The antimicrobial activity of the active packaging films is presented in **Figure 2**.



Note: Means followed by different letters imply significant differences at 5% level (P<0.05)

Figure 2. Antimicrobial activity of the active packaging films against *S. aureus*

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The antimicrobial activity of the active packaging films against *S. aureus* was assessed by measuring the diameter of the inhibition zone. As shown in Figure 2, the negative control did not generate an inhibitory zone. However, when high concentrations of the garlic extract were added to the active packaging films, the inhibitory activity against the bacteria was high, although the inhibition zone was not significantly different between 10% and 15% garlic extract. The active packaging films added with garlic extract exhibited antimicrobial activity against the Gram-positive bacteria *S. aureus*, but the activity could be considered weak because the diameter of the inhibition zone was less than 5 mm (Figure 2) (Morales et al., 2003). Differences in the diameter of inhibitory zones are influenced by the ability and rate of diffusion of antimicrobial compounds in the medium, the growth rate of microorganisms, and their sensitivity to antimicrobial chemicals, and the viscosity and thickness of the medium.

The antibacterial effects of garlic extract are due to allicin, which is generated when garlic is damaged. When the flesh of garlic is damaged during the refining process, allicin is rapidly generated because of the release of alliinase, which reacts with nonprotein amino acids, namely, alliin. Allicin is a part of the defense mechanism of garlic that exerts antimicrobial effects on both Gram-positive and Gram-negative bacteria by inhibiting RNA and lipid syntheses, which in turn inhibit the production of amino acids and proteins and the phospholipid bilayer of bacterial cell wall, thereby preventing bacterial growth and development. Allicin is highly permeable and can easily penetrate bacterial cells across the cell membrane. The thiosulfinate S(=O)S group in allicin then binds to the sulfhydryl groups of bacteria, thus inhibiting the activation mechanism of bacterial proteinases (Dwivedi et al. 2019; Reiter et al. 2020). This study demonstrated that 10%–15% garlic extract has comparable antibacterial effects.

3.2. pH of the beef samples

The pH of the beef samples was measured to investigate the effects of the active packaging films as the meat base in the packaging system. The beef samples were stored at room temperature for 24 h. The results of pH measurements are shown in **Figure 3**.

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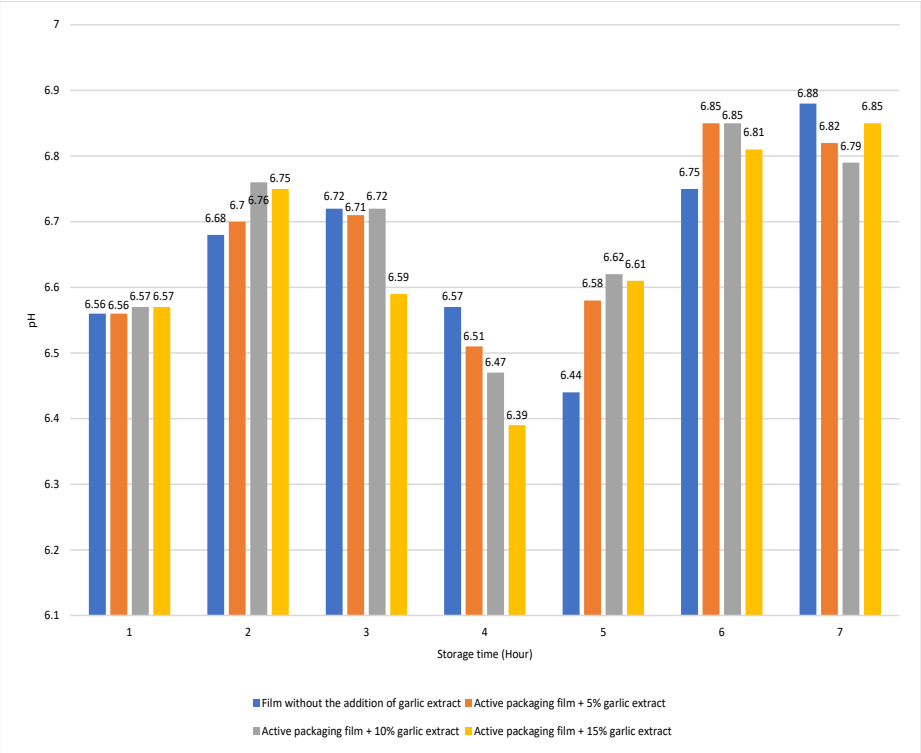

Figure 3. pH values of packaged meat sample stored at room temperature for 24 h.

The initial pH of the meat samples, which was immediately determined after the cow was slaughtered, was normal (6.57) (Figure 3). The pH fluctuated during the storage period. At the 4th hour of the storage period, the pH of the meat ranged from 6.68 to 6.76. At the 6th hour of the storage period, the pH of the meat ranged from 6.59 to 6.72. The normal pH of meat ranges from 5.4 to 5.8 at 6 h postmortem (Weglarz 2010; Susanto 2014; Soeparno 2015). In this study, the beef samples had a pH value that could be classified as quite high compared with that reported in previous studies. This result could be presumably attributed to the stress and the brief rest period that the cow experienced before it was slaughtered. These factors depleted the cow's glycogen reserves, allowing anaerobic glycolysis to occur quickly after it was slaughtered and depleting the lactic acid produced in its tissues. As a result, the pH of the meat dropped to an unsatisfactory level. Cows are thought to experience fatigue before they are slaughtered, thereby depleting the supply of ATP. Moreover, the sufficiently high temperature during slaughter accelerates the depletion of ATP, thus expediting the process of rigor mortis. The acceleration of rigor mortis causes the pH of the flesh to remain elevated and above normal, as confirmed by Sánchez-Macías et al. (2019) and Moreno et al. (2020), who reported that each variety of meat has a different glycogen content; thus, they have different glycolysis rates. The lower the content of glycogen in the meat is, the slower the glycolysis process will be and the higher the final pH will be. However, the decrease in pH in muscles can be influenced by internal factors, such as species, muscle type, muscle glycogen

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content, and livestock variability, as well as external factors, such as environmental temperature, additional treatment prior to slaughter, and pre-slaughter stress.

After 20 h of storage, the meat's pH value ranged from 6.75 and 6.85 and remained steady thereafter; at this point, the meat was classified as decayed (Figure 3). According to Prache, Schreurs, and Guillier (2021), the meat's pH continues to decline until glycogen is depleted into lactic acid and alkaline compounds are neutralized because of microbial metabolism, resulting in an increase in pH. If the pH reaches 6.8 or higher, protein decomposition will occur, resulting in spoilage.

3.3. TVBN of the meat samples

The TVBN values of the meat samples are presented in **Figure 4**.

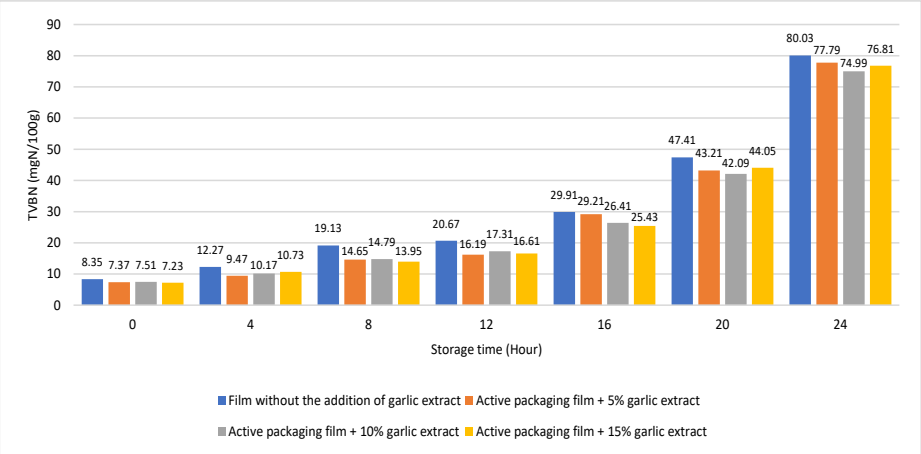

Figure 4. Total volatile basic nitrogen (TVBN) of the packed meat stored at room temperature for 24 h.

At 0 h, all meat samples had TVBN values ranging from 7.23 mgN/100 g to 8.35 mgN/100 g (Figure 4). Therefore, they were classified as fresh meat. After 12 h of storage, the meat samples that had not been treated with the active packaging films had a TVBN value of 20.67 mg N/100g, indicating that they were rotten. By comparison, the meat samples treated with the active packaging films and added with 5%, 10%, and 15% garlic extract had TVBN values of 16.19, 17.31, and 16.61 mgN/100 g, respectively. Thus, they were categorized as semi-fresh meat (stale meat) or could still be consumed. However, the TVBN values of all meat samples taken between the 16th and 24th h of storage exceeded the threshold for food-grade beef, demonstrating that adding 5%, 10%, and 15% garlic extract to the active packaging films effectively reduced the amount of TVBN in fresh meat by 21.67%, 16.26%, and 19.64%, respectively, compared with the meat samples not treated with the active packaging films. Beef or livestock is considered fresh if the TVBN value is less than 15 mg/100 g (National Standard of the People's Republic of China 2016) or TVBN is <10 mg N/100 g (Farber 1965). Moreover, SNI 2354.8:2009 states that the standard levels of TVBN fit for consumption is 20–30 mg N/100g. These differences in TVBN threshold indicates that it is an

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inconsistent measure of meat freshness. TVBN tests can be performed to examine the quality of beef products independently.

In this study, the values of TVBN increased throughout the storage period (observed every 4 h), indicating that the meat's quality continued to deteriorate owing to the breakdown of proteins into volatile base compounds. The increase in TVBN values was due to the activity of microorganisms that degraded proteins into simpler molecules, which eventually underwent deamination to generate ammonia, which contributed to the foul odor of the meat samples, as well as the synthesis of volatile nitrogen-containing compounds. High temperatures can promote protein degradation, resulting in the formation of more alkaline components. According to Bekhit et al. (2021), the increase in TVBN value is due to protein degradation by microorganisms that results in the formation of foul-smelling chemicals, such as ammonia (NH_3), basic skatole and indole compounds, mercaptans and H_2S (which are weak acids), and amines and cadaverin (which are strong bases).

The results demonstrated that the addition of garlic extract to the active packaging films delayed the spoiling of the meat samples, likely because the garlic's active components prevented microbial development, thereby lowering the synthesis of nitrogenous base compounds in the meat caused by bacteria and autolytic enzymes during the rotting process. This conjecture was supported by Al Hakim, Hartanto, and Nurhrtadi (2016) and Reiter et al. (2020), who reported that garlic extract has the ability to block microbe-produced enzymes involved in the breakdown of proteins into volatile base chemicals.

3.4. TPC of the microbes in the beef samples

The TPC of bacteria in the meat samples was determined to assess the utility of the active packaging films (**Figure 5**).

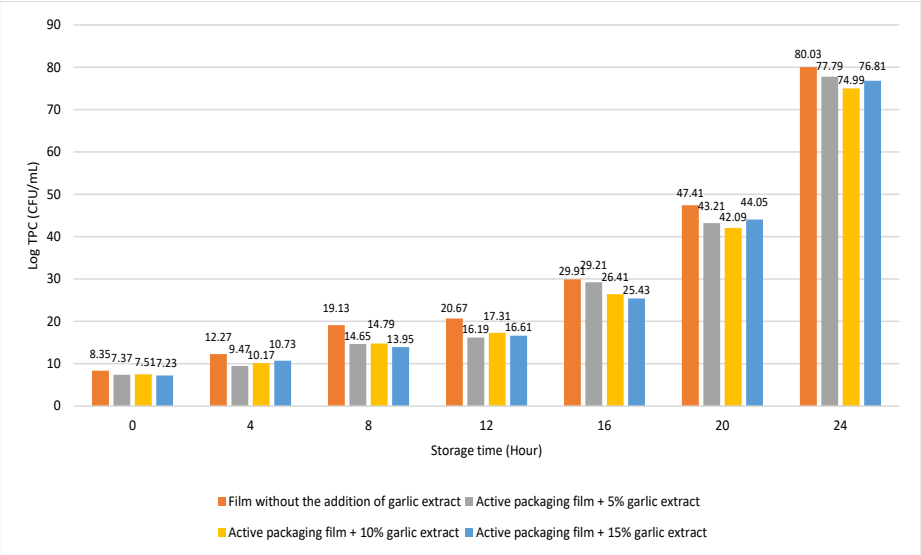

Figure 5. Total plate count (TPC) of packed meat stored at room temperature for 24 h.

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At 0 h of the storage period, the initial TPC value (Log TPC) of all meat samples was 2.53±0.64 CFU/ml (Figure 5). Thus, the meat samples were classified as fresh on the basis of microbiological quality. Throughout the storage period, the TPC value increased until it reached the maximum number of meat microbes permitted by SNI 3932:2008 on carcass and beef quality, which is 1×10^6 CFU/ml or equivalent to Log TPC 6 CFU/ml. At 12 h of storage, the meat samples treated with the active packaging films but without garlic extract (0%) and those added with 5% garlic extract did not fulfil the microbiological requirements as they had a Log TPC value of 7.65±0.39 and 6.20±0.00 CFU/ml, respectively. By comparison, the meat samples treated with the active packaging films and 10% and 15% garlic extract also did not fulfil the microbiological requirements after 16 h of storage as they has a Log TPC value of 7.47±0.26 and 6.78±0.67 CFU/ml, respectively. This result demonstrated that the active packaging films with 10% and 15% garlic extract in the meat packaging system can inhibit microbial growth and extend the shelf life of meat by up to 4 h because allicin can inhibit the growth of both Gram-positive and Gram-negative bacteria by destroying the sulfhydryl group bound to bacterial proteins. This process is important because the sulfhydryl group is required for bacterial cell division or acts as a specific stimulator for cell multiplication. Allicin damaged the RNA and DNA of bacteria and thus inhibits their growth and development in meat. Likewise, Deresse (2010) reported that allicin can suppress the growth of both Gram-positive and Gram-negative bacteria by completely inhibiting the syntheses of bacterial RNA, DNA, and proteins.

The total microbial content of the meat samples continued to increase during the entire storage period (Figure 5), because meat contains a high nutrient and water content, which provides an ideal environment for microorganism growth. Moreover, storage at room temperature can accelerate the growth of microorganisms. According to Soeparno (2015), meat has the ideal conditions for microorganism growth because it contains a high proportion of water (68%–75%), it is rich in nitrogen-containing substances of varying complexity, it contains various fermentable carbohydrates, it is rich in minerals and essential nutrients for microorganism growth, and it has a suitable pH for microorganism growth (pH 5.3–6.5). Variance analysis revealed that the duration of storage of the meat samples and the use of the active packaging films with garlic extract had a highly significant effect on the TPC value of the samples ($P>0.01$).

3.5. Changes in the color of the smart packaging BTB indicator solution as a measure of the freshness of the meat packaged with the active packaging films

Using fresh beef packaged and maintained at room temperature for 24 h, Dirpan et al. (2019) determined that BTB solution (pH 2.75) produces the most readily visible color changes to sensitivity tests. In this study, the BTB solution (pH 2.75), as the smart packaging indicator, was also utilized to evaluate changes in its color as a reflection of the freshness of the meat samples packed with the active packaging films (**Figure 6**).

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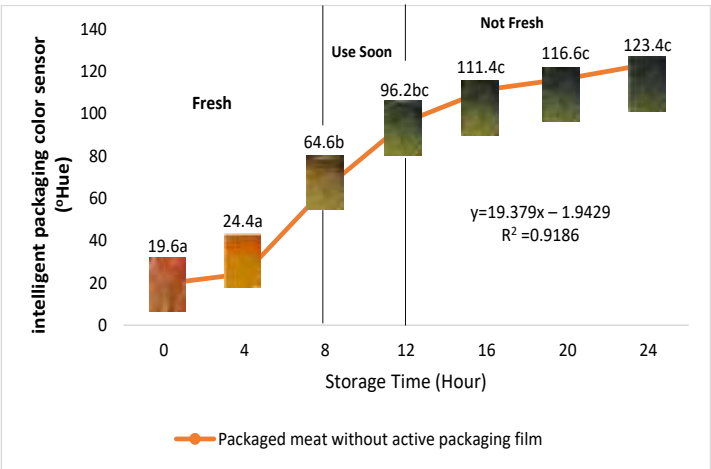
intelligent packaging color sensor (°Hue)
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64.6b
96.2bc
111.4c
116.6c
123.4c
y=19.379x – 1.9429
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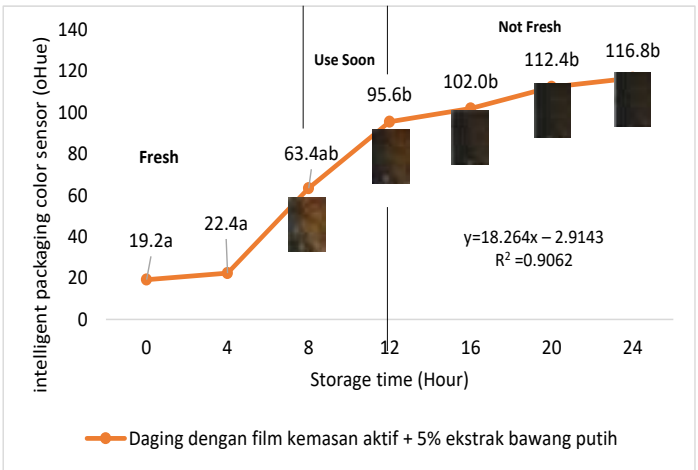
Storage Time (Hour)
Packaged meat without active packaging film

(a)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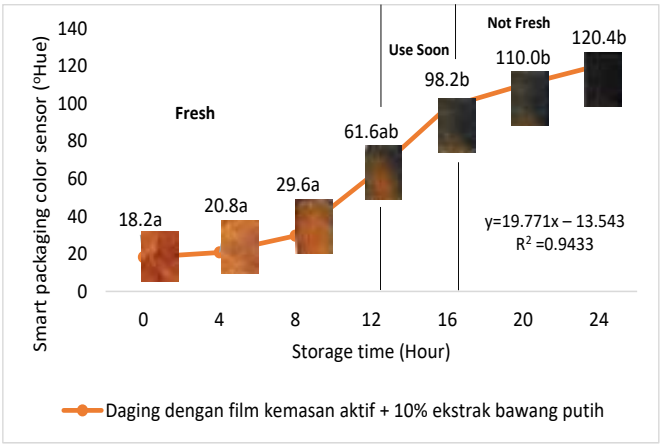
Storage time (Hour)
Daging dengan film kemasan aktif + 5% ekstrak bawang putih

(b)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120.4b
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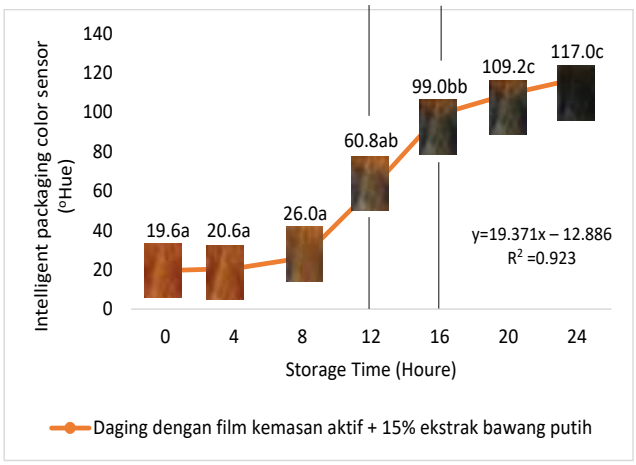
Daging dengan film kemasan aktif + 10% ekstrak bawang putih

c)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Storage Time (Houre)
Daging dengan film kemasan aktif + 15% ekstrak bawang putih

(d)

Figure 6. Changes in the color of the BTB solution (pH 2.75) as the smart packaging indicator reflecting the freshness of the meat samples packed with the active packaging films with 0% **(a)**, 5% **(b)**, 10% **(c)**, and 15% **(d)** of garlic extract.

During the entire storage period, the smart packaging indicator changed its color three times that corresponded to three phases of the meat samples' level of quality (Figure 6). In phase I, its color was orange, indicating that the meat samples were still fresh. In phase II, its color was green with an orange hue, suggesting that the meat samples should be consumed immediately. In phase III, its color was dark green, denoting that the meat samples were already spoiled. The change in the indicator's color from orange to green indicated that the quality of the meat samples had deteriorated. The changes in the indicator's color were due to the interactions of alkaline volatile compounds produced by enzyme activity, and the metabolism of the microorganisms present in the meat samples increased with storage time. The early sign of spoilage was indicated by the release of volatile alkaline compounds as the microorganisms and the enzymes degraded the nutritional content of the meat samples. These compounds gradually accumulated in the packaging system, causing an increase in pH, which was detected by the smart packaging indicator and displayed as gradual color changes. The change in color of the smart packaging indicator (BTB, pH 2.75) from orange to green was induced by deprotonation or the release of a proton from the smart packaging indicator dye (De Meyer *et al*. 2014).

The meat samples packaged with the active packaging films but with no (0%) and 5% garlic extract were still fresh from the start of the storage up to 8 h (Figure 6). However, they must be immediately consumed from the 8th h to the 12th h of the storage period. Thereafter (12–24 h of the storage period), they were already spoiled. This results was consistent with that of TPC tests, which showed that the TPC values were above the acceptable threshold for microbial contaminants (1×10^6 or equivalent to 6 CFU/ml) in meat after 12 h. By comparison, the meat samples packaged with the active packaging films containing 10% and 15% garlic extract were still considered fresh from the start of the storage period up to the 12th h. They must be immediately consumed when they had been in storage for 12–16 h. Finally, they were considered rotten when they had been in storage for 16–24 h. This result was also consistent with that of TPC tests (Figure 5), which indicated that at the 16th hour, the TPC value surpassed the permissible level of microbiological contamination in beef. Statistical analysis revealed that storage duration had a very significant effect on the Hue value, the indicator of color change in the smart packaging. The changes in the color of the smart packaging indicator (BTB solution, pH 2.75) when used together with the active packaging films to reflect the freshness of meat are presented in **Table 1**.

Table 1. Changes in the color of the smart packaging indicator (BTB solution, pH 2.75) when used together with the active packaging films to reflect the freshness of meat.

Storage Time (Hour)	Active Packaging Films Added with Garlic Extract			
	0%	5%	10%	15%

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3.6. Correlations between changes in the color of the smart packaging indicator and the effects of the active packaging films on the parameters of meat freshness

The correlations between changes in the color of the smart packaging indicator and parameters of meat quality deterioration (pH, TVBN, and TPC) were explored to ascertain the relationship between the sensitivity of the smart packaging indicator to meat freshness and the effectiveness of the active packaging films in slowing the process of meat spoilage.

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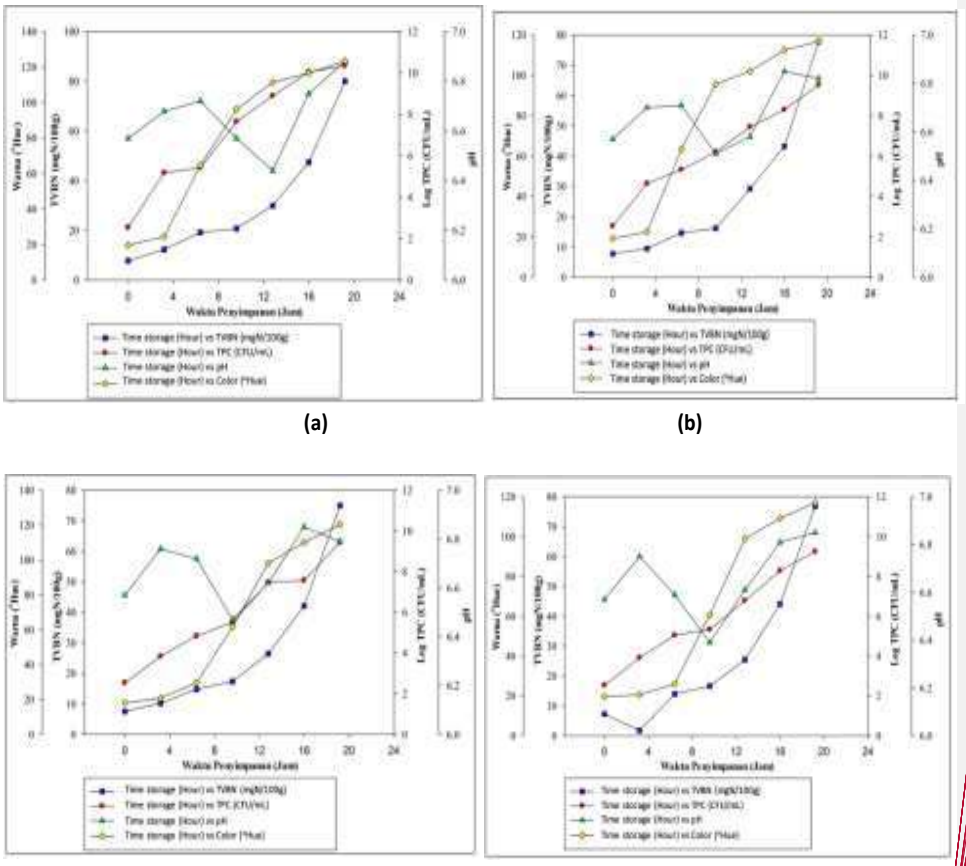

Figure 7. Correlations between changes in the color of the smart packaging indicator and the effects of the active packaging films with 0% **(a)**, 5% **(b)**, 10% **(c)**, and 15% **(d)** garlic extract on the parameters of quality deterioration of meat stored for 24 h.

The TPC and TVBN values of the meat samples treated with the active packaging films but without garlic extract increased, which was reflected by the change in the color of the smart packaging indicator (Figure 7). The pH of the meat samples fluctuated not only because of the production of volatile base compounds due to the activity of microorganisms in the samples during the storage period but also because of various factors, such as the contents of glycogen and lactic acid in the livestock prior to and after the slaughter. The meat samples treated with the active packaging films but without garlic extract and those treated with 5% garlic extract were rotten and unfit for consumption after 12 h of storage as their Log TPC value was 7.65±0.39 and 6.20±0.00 CFU/mL, respectively, and their TVBN value was 20.67±2.68 and 16.19±0.28 mgN/100g, respectively (Figure 7). By comparison, the meat samples treated with the active packaging films and 10% and 15% garlic extract were rotten and unfit for consumption after 16 h of storage as their Log TPC value was 7.47±0.26 and 6.78±0.67 CFU/mL, respectively, and their TVBN value was 26.41±3.31 and 25.43±4.89 mgN/100 g, respectively.

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The TPC values indicated the same level of quality deterioration as the TVBN values and reflected the changes in the color of the smart packaging indicator of the freshness of the meat samples treated with the active packaging films without (0%) and with 5% garlic extract; these samples were deemed rotten after 12 h of storage (Figure 7). The color of the indicator changed from orange (fresh) with a color value of 19.6°Hue and 19.2°Hue to green (rotten) with a color value of 96.2°Hue and 95.6°Hue respectively. By comparison, the meat samples treated with the active packaging films and added with 10% and 15% garlic extract were deemed rotten after 16 h of storage; the color of the indicator changed from orange (fresh) with color values of 18.2°Hue and 19.6°Hue, respectively, to green (rotten) with color values of 98.2°Hue and 99°Hue, respectively. Wiryawan (2005) observed that when garlic extract was added to the active packaging, the values of TPC and TVBN and the pH of the meat increased more slowly, as did the color of the smart packaging indicator, compared with those of the meat without the active packaging.

The increase in the values of TPC, TVBN, and pH of meat is strongly influenced by its high nutrient and water content, which is conducive to microbial contamination. Moreover, storing meat at room temperature accelerates bacterial growth. In general, an increase in the number of microorganisms is followed by an increase in the production of the volatile base chemical called TVBN. More volatile base compounds are released when more microorganisms are present and active, as indicated by the increase in TVBN value, which also has an effect on the meat's pH value. Furthermore, the increase in the values of TPC, TVBN, and pH linearly correlates with the increase in Hue value and color changes of the smart packaging indicator because the accumulated volatile base compounds raise the pH value of the packaging system, causing the smart packaging indicator to experience a color shift. This explanation was in agreement with that of Pacquit et al. (2006), who applied active packaging films to cod fish. They stated that the increase in the TPC value of cod fish has a linear correlation with changes in the color of the cellulose-acetate packaging film sensor.

4. Conclusion

As a smart packaging indicator, the BTB solution (pH 2.75) produce color changes that are easy to observe and reflect three phases of meat quality deterioration. In phase I, its color was orange, indicating that the meat was still fresh. In phase II, its color was green with an orange hue, suggesting that the meat must be consumed immediately. In phase III, its color was dark green, denoting that the meat was already rotten and unfit for consumption. The meat samples treated with the active packaging films and added with 10% and 15% garlic extract rotted after 16 h, whereas the meat samples treated with the active packaging films and added with 0% and 5% garlic extract rotted after only 12 h. The changes in the color of the BTB solution was linearly and positively associated with the values of TPC, TVBN, and pH of the meat samples packaged with the active packaging films.

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Application of a Smart Sensor and Active Packaging System Based on the Cellulose of *Acetobacter xylinum* to Meat Products

Abstract.
Combining smart and active packaging serves the dual purpose of detecting color changes in food that reflect changes in its quality and prolonging its shelf life. This study developed a smart and active packaging system made from the cellulose of *Acetobacter xylinum* and assessed its ability to detect changes in the quality of packaged fresh beef. The properties of the smart packaging's sensor and active packaging films were determined. The application of this system to fresh beef stored at room temperature (28±2 °C) for 24 h was tested. The color of the bromothymol blue (BTB) solution (pH 2.75) in the indicator of the smart packaging system changed from orange to dark green to indicate that beef quality changed from fresh to rotten. The meat treated with the active packaging with 10% and 15% garlic extract decayed on the 16th h. By contrast, the meat treated with the active packaging but without the garlic extracts rotted on the 12th h. The shift in the indicator's color was linearly related to the total plate count, total volatile basic nitrogen, and pH of the meat packaged using the active packaging system. Therefore, BTB solution (pH 2.75) can be used as a smart packaging indicator that will allow consumers to assess the quality of packaged meat easily. As an antimicrobial agent, the addition of 10%–15% garlic extract to the active packaging films can help delay the spoilage of packaged meat.

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1. Introduction

Global beef consumption is predicted to rise as the world population and family income increase, particularly in developing Asian countries (Dupont & Fiebelkorn 2020; González et al. 2020; OECD/FAO 2021). By 2030, worldwide meat consumption and availability are expected to increase by 14% and 5.9%, respectively, over the average of the 2018–2020 period (OECD/FAO 2021). Thus, the expected increase in meat consumption must be complemented by improvements in the quality of fresh meat produced. One aspect affecting the quality and characteristics of meat is the material and packaging technologies used (Abdurehman Musa 2019). Meat is a perishable item that rapidly spoils when stored above the optimum temperature range (below 0–40 °F) (Beltrán, Roncalés & Bellés 2018; Franco, da Cunha & Bianchi 2021). However, in traditional markets, meat is displayed at room temperature without packaging, a practice that might accelerate microbial contamination and cause rapid quality degradation. Even in supermarkets where meat is maintained in cold temperatures, standard meat packaging still prevent consumers from subjectively determining the quality of meat. Thus, meat packaging must have additional functions that will prevent quality degradation due to microbial contamination and will help consumers to determine the quality of packaged meat easily (Dirpan et al. 2019). Conventional meat packaging can be designed to perform dual functions through smart and active packaging systems.

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Smart packaging is a term that refers to sensors in the form of indicators that monitor and provide information on the quality of the food contained within the packaging via color changes caused by chemical reactions between the indicators and the products of microbial metabolism or changes in the chemical composition of the food (Dobrucka & Cierpiszewski 2014). During storage, the chemical components of meat degrade into volatile compounds because of microbial activity, thereby increasing the value of total volatile base nitrogen (TVBN) (Bekhit et al. 2021; Ma et al. 2021). Accumulation of TVBN increases the pH of the packaging system, which is detected by the indicator, resulting in a visible color shift in the indicator (Pacquit et al. 2006; Ma et al. 2021). Smart packaging allows easier monitoring of

packed products during transportation and storage (Dobrucka dan Cierpiszewski, 2014). Moreover, it provides a more accurate estimate of product condition than conventional expiration labels (Pacquit, Crowley & Diamond 2008). Color-based pH indicator solutions are widely used as smart indicators. Dirpan et al. (2018) developed bromophenol blue as a smart indicator dye for mangoes. Hidayat et al. (2019) used two types of color indicators with predetermined concentrations, namely, phenol red and bromothymol blue, to assess the freshness of meat packaging. Smart packaging indicators based on natural pigments are being developed, such as smart packaging films that include anthocyanin-loaded *Lycium ruthenicum* nanocomplexes in starch/polyvinyl alcohol mixtures (PVA) (Qin et al. 2021), as well as anthocyanins from saffron petals immobilized in chitosan nanofibers and methyl cellulose matrix (Alizadeh-Sani et al. 2021).

Active packaging refers to the integration of particular additives into a packaging system for the purpose of extending the shelf life, preserving the quality, and ensuring the safety of food products. Antimicrobial agents are used as components of active packaging additives to extend product shelf life. The volatile bioactive compounds in active packaging evaporate or diffuse onto the food surface, where they limit the growth of pathogenic microbes and thus delay spoilage (Yildirim et al. 2018; Wrona et al. 2021). This strategy is more effective than coating bioactive compounds onto the food surface (Iriani 2014). The safest, cheapest, and most readily available antimicrobial agents for use in active packaging are essential oils. Pranoto et al. *(*2005) produced antimicrobial alginate edible films by incorporating the essential oils of garlic. They reported that these films substantially inhibited the growth of *Staphylococcus aureus* and *Bacillus cereus* in meat. Priya, Vinitha, and Sundaram (2021) utilized the essential oils of *Plectranthus amboinicus* in a chitosan-based active packaging to restrict antimicrobial activity. Smart and active packaging can be merged into a single packaging system. Julyaningsih, Latief, and Dirpan (2020) combined a smart packaging system based on methyl red–bromothymol blue (BTB) indicator with an active packaging system based on lemongrass oil as a component of tuna fish fillet packaging. Yao et al. (2021) developed an active and smart packaging system based on starch, PVA, and betacyanins from various types of plants for shrimp packaging.

In general, an active packaging that contains antimicrobial agents and a smart packaging that contains indicator solutions are immobilized in a polymer. Compared with plant cellulose or synthetic polymers, the bacterial cellulose fermented by *Acetobacter xylinum* has a unique nanofibrillar structure and superior physical properties, suggesting that it has the potential to serve as a basis for developing a smart and active packaging system (Cazón & Vázquez 2021; Xu et al. 2021). Bacterial cellulose has received interest as a component of active packaging owing to its edibility, biodegradability, high water-holding capacity, and great potential as an antimicrobial agent carrier (Nguyen, Gidley & Dykes 2008).

The development of packaging systems with additional functions is advancing. To promote this innovation, this study aimed to maximize the potential of smart and active packaging by combining them into a single packaging system based on a bacterial cellulose membrane biopolymer to enhance the quality of packaged meat and help consumers to determine meat freshness easily.

2. Method and Materials

2.1. Materials

The main ingredients used in the smart and active packaging system developed herein were the bacterial cellulose produced by *A. xylinum*, which was fermented in natural media of coconut water. Beef tenderloin

was obtained from a slaughterhouse in Tamangapa Raya. Coconut water and garlic (*Allium sativum*) were purchased from a local market. Food-grade ammonium sulfate (CAS Number: 7783-20-2), yeast extract (Merck, CAS Number: 8013-01-2), 96% acetic acid, *A. xylinum* culture, 5% 1 N NaOH, sucrose, BTB, alcohol, aquabides, aquades, Tashiro's indicator (0.1% methyl red and 0.1% BTB at a ratio of 2:1), 7% trichloroacetic acid (TCA, Merck), nutrient Agar (NA, Merck), glycerol (Merck, CAS Number: 56-81-5), food-grade carboxymethyl–cellulose (CMC) (Foodchem, E466), and corn starch were used.

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Commented [199474]: Instead of merely listing these items, describe for what purpose they were used.

2.2. Method

2.2.1. Determination of the best nitrogen source from *A. xylinum* fermentation media

The production of *A. xylinum* bacterial cellulose membranes was started by determining the amount and type of the most suitable nitrogen source from *A. xylinum* growth media following the method of a previous study (Dirpan et al. 2019).

2.2.2. Purification of bacterial cellulose

Bacterial cellulose was removed from the fermentation medium, rinsed in running water, and then soaked for 2 days with periodic water changes. The cellulose was also soaked in 70% alcohol for 1 min, heated to 100 °C in distilled water for 20 min, and reheated in 1 N 5% NaOH solution at 100 °C for 60 min to remove the remaining bacterial cells and substrate attached to the cellulose layer. Afterward, the cellulose was rinsed with running water and soaked in periodically changed water for 24 h until pH reached 7. The purified cellulose appeared transparent (Dirpan et al. 2019).

2.2.3. Production of smart packaging

2.2.3.1. Preparation of the indicator solution

BTB indicator solution was chosen for this study because a previous work established this solution as the indicator with the most visually identifiable color change reaction (Dirpan et al. 2019). First, 1% BTB solution (b/v) was prepared in 95% ethanol. Then, the pH of the BTB solution was decreased to 2.74 by adding 20% acetic acid. Finally, the BTB solution was stored in a closed container.

2.2.3.2. Production of smart packaging indicator label

The purified cellulose film was kept in a filter cloth for 24 h to decrease its water content. Half-dried cellulose was cut into 1.5 cm × 4 cm strips and pushed flat against the surface of a Pyrex glass. The cellulose was dried for 30 min at 70 °C. The BTB indicator solution was then absorbed into a dry cellulose via centrifugation at 3000 rpm for 15 min. When the color indicator was successfully absorbed, the BTB indicator solution imparted an orange hue to the cellulose. Afterward, the cellulose was rinsed with distilled water to eliminate any unbound color indicators and then dried (Kuswandi and Maryska, 2013; Shukla et al. 2015).

2.2.4. Production of active packaging film

2.2.4.1. Production of garlic extract as active element

First, 500 g garlic was peeled, washed under running water until clean, drained, and then mashed. The, minced garlic was extracted via the maceration method by immersing the finely ground

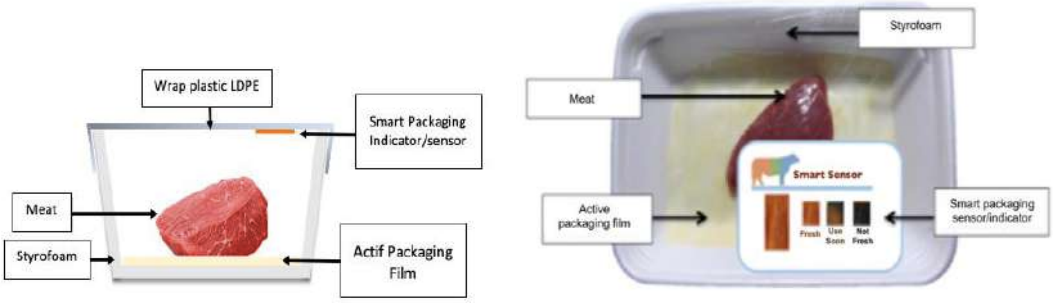
garlic in 96% alcohol at a ratio of 1:4 (garlic:alcohol) for 4 days at 3 °C–5 °C and periodically homogenized using a water bath shaker. Afterward, the extract was filtered using a filter paper and then concentrated using a rotary evaporator at 50 rpm at 40 °C to obtain a thick extract (Rotty dan Tjitrosantoso 2015; Shetty et al. 2013).

2.2.4.2. Production of active packaging film

The bacterial cellulose was crushed to form a cellulose slurry, and all pretreatments were performed at room temperature. A cellulose suspension was prepared using 30% chitosan (w/w), 10% CMC (w/w), and 15% corn starch (w/w) of cellulose dry weight. The suspension was heated at 50 °C for 60 min with a hot plate stirrer until thoroughly suspended. At the 50th min, 30% glycerol (w/w) was added. Additionally, the garlic extract was added at quantities of 0% (as the control), 5%, 10%, and 15% (v/v) immediately after the final heating step. Subsequently, 60 g of the suspension was then placed onto a glass plate and dried for 48 h at 37 °C. Finally, the suspension was cooled to room temperature, removed from the glass plate, wrapped in aluminum foil, and placed in a desiccator (Indrarti et al. 2016; Iriani 2014).

2.2.5. Application of the smart and active packaging indicators to fresh beef

Fresh beef tenderloin was collected from a slaughterhouse in Tamangapa Raya Makassar 1 h after the cow was slaughtered. It was immediately placed in a special food box and put into a 38 cm × 29 cm × 30 cm Styrofoam box filled with ice crystals. The samples were promptly transported to the laboratory and processed into 200 g/pack pieces under sterile conditions. The meat was packaged in a Styrofoam tray (1.05 g/cm^3) coated with the active packaging film on a Styrofoam base, and a smart packaging indicator label was attached to the LDPE plastic wrap film that covered the Styrofoam container. The samples were maintained at room temperature (28±2 °C) with normal light exposure for 24 h.



a **b**

Figure 1. (a) Design of the smart and active packaging system and its (b) application to fresh meat.

2.2.6. Observation parameters

2.2.6.1. Measurement of the color of the smart packaging indicator

The color of the smart packaging indicators was quantitatively determined using a chromameter digital color meter (T-135). The meat packaged in the smart and active packaging system was placed on a flat surface with a black background with uniform lighting. The chromameter detector was placed on the surface of the smart packaging indicator. The start button was pressed until the measurement results were shown on the display. The measurement results were expressed according to the notation of the Hunter's Lab Colorimetric System, which is presented in three values, namely L* (lightness), a* (redness), and b* (yellowness) (Yam & Papadakis 2004; Nurmawati 2011). The color of the smart packaging indicator was determined by calculating the °Hue value by using the formula below:

$$°Hue = tan^{-1}\frac{b}{a},$$

where $°Hue$ represents the parameters for color range, *a* is a red-green mixed color, and *b* is a red-green mixed color yellow-blue.

2.2.6.2. Antimicrobial activity of the active packaging films

The antimicrobial activity of the active packaging films was determined via the agar diffusion method. Each active packaging film was cut into 5 mm circles in a sterile environment and then placed on NA agar media with 0.1 ml of the test microorganism culture (*Staphylococcus aureus*) containing 10^6 CFU/ml. Petri dishes were incubated for 24 h at 37 °C. After incubation, the inhibitory zone was measured using a caliper (Padgett et al. 1998).

2.2.6.3. Determination of pH of the beef samples

The pH of the beef samples was measured using a pH meter (Oakton pH 510). First, 5 g of crushed meat was combined with 45 ml of distilled water until the mixture became homogenous. The pH meter's electrode was then immersed in the beef suspension until the pH value on the monitor became constant.

2.2.6.4. Measurement of TVBN

First, 30 ml of 7% TCA solution was added to a meat sample (10±0.1 g) and mixed before filtering. The, 1 ml boric acid solution was placed in the "inner chamber" of the Conway dish. The lid of the cup was placed in such a way that it almost covered the cup. The filtrate was placed into the left outer chamber of the Conway dish. Afterward, 1 ml saturated K_2CO_3 solution was put into the right outer chamber to avoid mixing the filtrate with K_2CO_3. The cup was closed and rotated to mix the two liquids in the outer chamber. The blank solution was prepared following the same process but with 7% TCA instead of the filtrate. The solutions were stored at 37 °C for 2 h. The boric acid solution with the blank and filtrate samples was then titrated with 0.01 N HCl until it turned pink. TVBN was calculated as follows (AOAC, 1995):

$$\text{TVBN content (mg/100 g)} = \frac{(Vc\text{-}Vb)\text{x } N \text{ x } 14{,}007\ x\ fp\ x\ 100}{W},$$

where V_c is the volume of the HCl solution used in sample titration, V_b is the volume of the HCl solution used in blank titration, *N* is the normality of the HCl solution, *W* is the sample's weight (g), 14.07

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is the molecular weight of nitrogen, and *Fp*is the dilution factor.

2.2.6.5. Total plate count

The total number of microorganisms was determined via the total plate count (TPC) method described in SNI 2332.3: 2015. First, 1 g of the sample was added to a test tube containing 9 ml of physiological solution until homogeneous (10^{-1} dilution). The dilution was continued until 10^{-6}, at which point the diluted sample was inoculated on NA media in duplicate via the pour plate technique. After the media solidified, the Petri dishes containing the media and the sample solution were incubated upside down at 30 °C for 48 h. Afterward, TPC was calculated using the formula below (Badan Standar Nasional, 2015):

$$N = \frac{\sum C}{[(1\times n_1)+(0.1\times n_2)]\times (d)}$$

where *N* is TPC (CFU/ml), $\sum C$ is the number of colonies counted in all Petri dishes, n_1 is the number of colonies counted in all Petri dishes at first dilution, n_2 is the number of colonies counted in all Petri dishes at second dilution, and d is the number of colonies counted in all Petri dishes at first dilution.

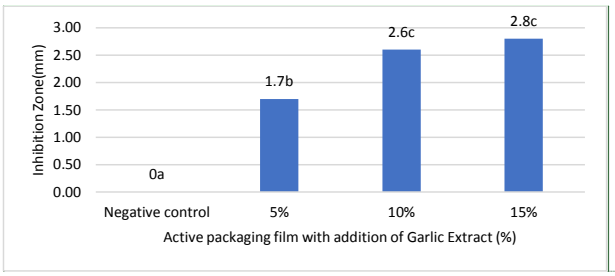
2.2.6.6. Data analysis

ANOVA was used to analyze the parameters of the smart packaging indicator, antimicrobial activity of the active packaging films, and quality of the beef samples, including pH, TVBN, and TPC with three replications. Differences between treatments were determined using Duncan's test. The correlations between the changes in the color of the smart packaging indicator and the effects of the active packaging on all parameters of meat spoilage were explored and presented in graphs by using the Sigma Plot 12 software. Data were analyzed using Microsoft Excel 2019, SPSS 19, and Sigma Plot 12.

3. Results and discussion

3.1. Antimicrobial activity of the active packaging films against *S. aureus*

The antimicrobial activity of the active packaging films is presented in **Figure 2**.



Note: Means followed by different letters imply significant differences at 5% level (P<0.05)

Figure 2. Antimicrobial activity of the active packaging films against *S. aureus*

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Commented [1994711]: Change the label of the x axis into "Active packaging films added with garlic extract (%)"

The antimicrobial activity of the active packaging films against *S. aureus* was assessed by measuring the diameter of the inhibition zone. As shown in Figure 2, the negative control did not generate an inhibitory zone. However, when high concentrations of the garlic extract were added to the active packaging films, the inhibitory activity against the bacteria was high, although the inhibition zone was not significantly different between 10% and 15% garlic extract. The active packaging films added with garlic extract exhibited antimicrobial activity against the Gram-positive bacteria *S. aureus*, but the activity could be considered weak because the diameter of the inhibition zone was less than 5 mm (Figure 2) (Morales et al., 2003). Differences in the diameter of inhibitory zones are influenced by the ability and rate of diffusion of antimicrobial compounds in the medium, the growth rate of microorganisms and their sensitivity to antimicrobial chemicals, and the viscosity and thickness of the medium.

The antibacterial effects of garlic extract are due to allicin, which is generated when garlic is damaged. When the flesh of garlic is damaged during the refining process, allicin is rapidly generated because of the release of alliinase, which reacts with nonprotein amino acids, namely, alliin. Allicin is a part of the defense mechanism of garlic that exerts antimicrobial effects on both Gram-positive and Gram-negative bacteria by inhibiting RNA and lipid syntheses, which in turn inhibit the production of amino acids and proteins and the phospholipid bilayer of bacterial cell wall, thereby preventing bacterial growth and development. Allicin is highly permeable and can easily penetrate bacterial cells across the cell membrane. The thiosulfinate S(=O)S group in allicin then binds to the sulfhydryl groups of bacteria, thus inhibiting the activation mechanism of bacterial proteinases (Dwivedi et al. 2019; Reiter et al. 2020). This study demonstrated that 10%–15% garlic extract has comparable antibacterial effects.

3.2. pH of the beef samples

The pH of the beef samples was measured to investigate the effects of the active packaging films as the meat base in the packaging system. The beef samples were stored at room temperature for 24 h. The results of pH measurements are shown in **Figure 3**.

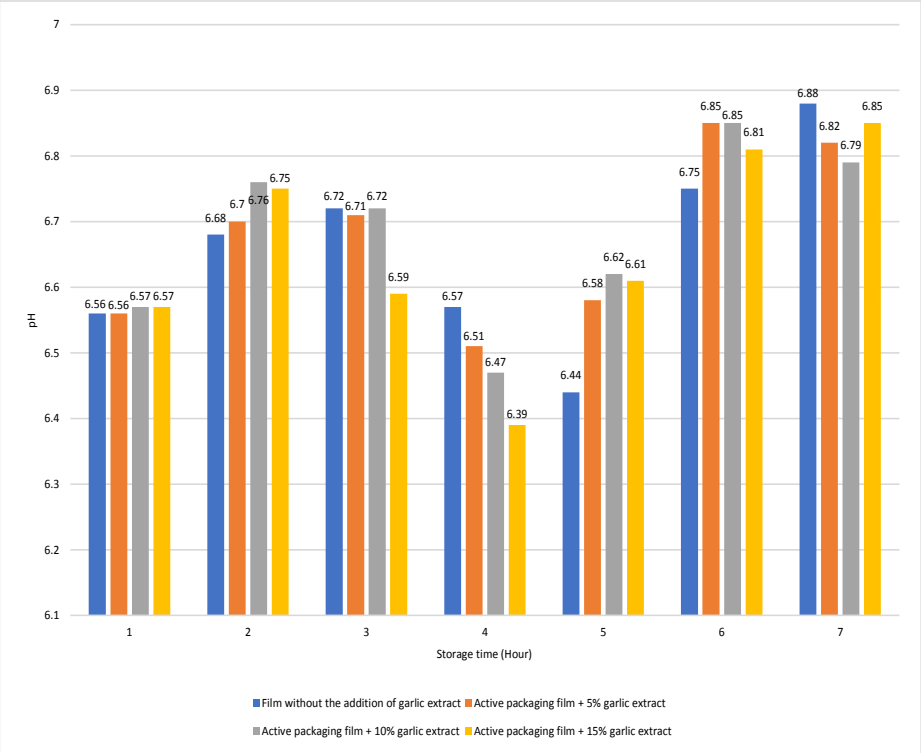

Figure 3. pH values of packaged meat sample stored at room temperature for 24 h.

The initial pH of the meat samples, which was immediately determined after the cow was slaughtered, was normal (6.57) (Figure 3). The pH fluctuated during the storage period. At the 4th hour of the storage period, the pH of the meat ranged from 6.68 to 6.76. At the 6th hour of the storage period, the pH of the meat ranged from 6.59 to 6.72. The normal pH of meat ranges from 5.4 to 5.8 at 6 h postmortem (Weglarz 2010; Susanto 2014; Soeparno 2015). In this study, the beef samples had a pH value that could be classified as quite high compared with that reported in previous studies. This result could be presumably attributed to the stress and the brief rest period that the cow experienced before it was slaughtered. These factors depleted the cow's glycogen reserves, allowing anaerobic glycolysis to occur quickly after it was slaughtered and depleting the lactic acid produced in its tissues. As a result, the pH of the meat dropped to an unsatisfactory level. Cows are thought to experience fatigue before they are slaughtered, thereby depleting the supply of ATP. Moreover, the sufficiently high temperature during slaughter accelerates the depletion of ATP, thus expediting the process of rigor mortis. The acceleration of rigor mortis causes the pH of the flesh to remain elevated and above normal, as confirmed by Sánchez-Macías et al. (2019) and Moreno et al. (2020), who reported that each variety of meat has a different glycogen content; thus, they have different glycolysis rates. The lower the content of glycogen in the meat is, the slower the glycolysis process will be and the higher the final pH will be. However, the decrease in pH in muscles can be influenced by internal factors, such as species, muscle type, muscle glycogen

content, and livestock variability, as well as external factors, such as environmental temperature, additional treatment prior to slaughter, and pre-slaughter stress.

After 20 h of storage, the meat's pH value ranged from 6.75 and 6.85 and remained steady thereafter; at this point, the meat was classified as decayed (Figure 3). According to Prache, Schreurs, and Guillier (2021), the meat's pH continues to decline until glycogen is depleted into lactic acid and alkaline compounds are neutralized because of microbial metabolism, resulting in an increase in pH. If the pH reaches 6.8 or higher, protein decomposition will occur, resulting in spoilage.

3.3. TVBN of the meat samples

The TVBN values of the meat samples are presented in **Figure 4**.

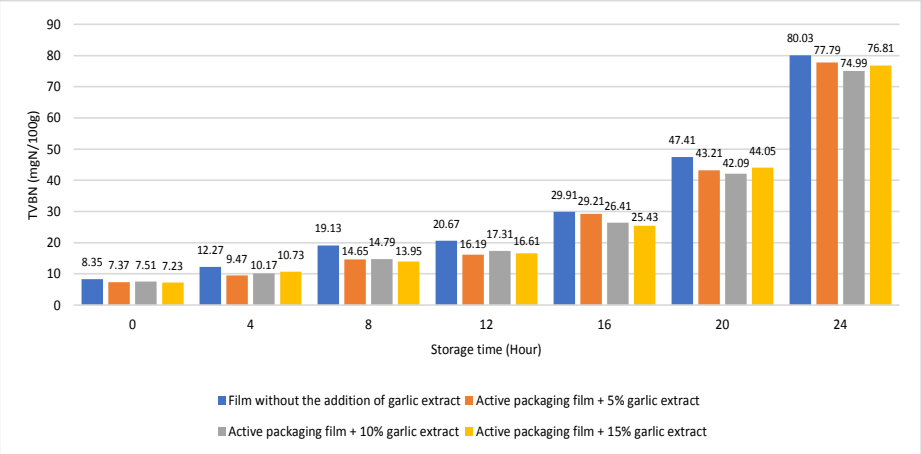

Figure 4. Total volatile basic nitrogen (TVBN) of the packed meat stored at room temperature for 24 h.

At 0 h, all meat samples had TVBN values ranging from 7.23 mgN/100 g to 8.35 mgN/100 g (Figure 4). Therefore, they were classified as fresh meat. After 12 h of storage, the meat samples that had not been treated with the active packaging films had a TVBN value of 20.67 mg N/100g, indicating that they were rotten. By comparison, the meat samples treated with the active packaging films and added with 5%, 10%, and 15% garlic extract had TVBN values of 16.19, 17.31, and 16.61 mgN/100 g, respectively. Thus, they were categorized as semi-fresh meat (stale meat) or could still be consumed. However, the TVBN values of all meat samples taken between the 16th and 24th h of storage exceeded the threshold for food-grade beef, demonstrating that adding 5%, 10%, and 15% garlic extract to the active packaging films effectively reduced the amount of TVBN in fresh meat by 21.67%, 16.26%, and 19.64%, respectively, compared with the meat samples not treated with the active packaging films. Beef or livestock is considered fresh if the TVBN value is less than 15 mg/100 g (National Standard of the People's Republic of China 2016) or TVBN is <10 mg N/100 g (Farber 1965). Moreover, SNI 2354.8:2009 states that the standard levels of TVBN fit for consumption is 20–30 mg N/100g. These differences in TVBN threshold indicates that it is an

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inconsistent measure of meat freshness. TVBN tests can be performed to examine the quality of beef products independently.

In this study, the values of TVBN increased throughout the storage period (observed every 4 h), indicating that the meat's quality continued to deteriorate owing to the breakdown of proteins into volatile base compounds. The increase in TVBN values was due to the activity of microorganisms that degraded proteins into simpler molecules, which eventually underwent deamination to generate ammonia, which contributed to the foul odor of the meat samples, as well as the synthesis of volatile nitrogen-containing compounds. High temperatures can promote protein degradation, resulting in the formation of more alkaline components. According to Bekhit et al. (2021), the increase in TVBN value is due to protein degradation by microorganisms that results in the formation of foul-smelling chemicals, such as ammonia (NH_3), basic skatole and indole compounds, mercaptans and H_2S (which are weak acids), and amines and cadaverin (which are strong bases).

The results demonstrated that the addition of garlic extract to the active packaging films delayed the spoiling of the meat samples likely because the garlic's active components prevented microbial development, thereby lowering the synthesis of nitrogenous base compounds in the meat caused by bacteria and autolytic enzymes during the rotting process. This conjecture was supported by Al Hakim, Hartanto, and Nurhrtadi (2016) and Reiter et al. (2020), who reported that garlic extract has the ability to block microbe-produced enzymes involved in the breakdown of proteins into volatile base chemicals.

3.4. TPC of the microbes in the beef samples

The TPC of bacteria in the meat samples was determined to assess the utility of the active packaging films (**Figure 5**).

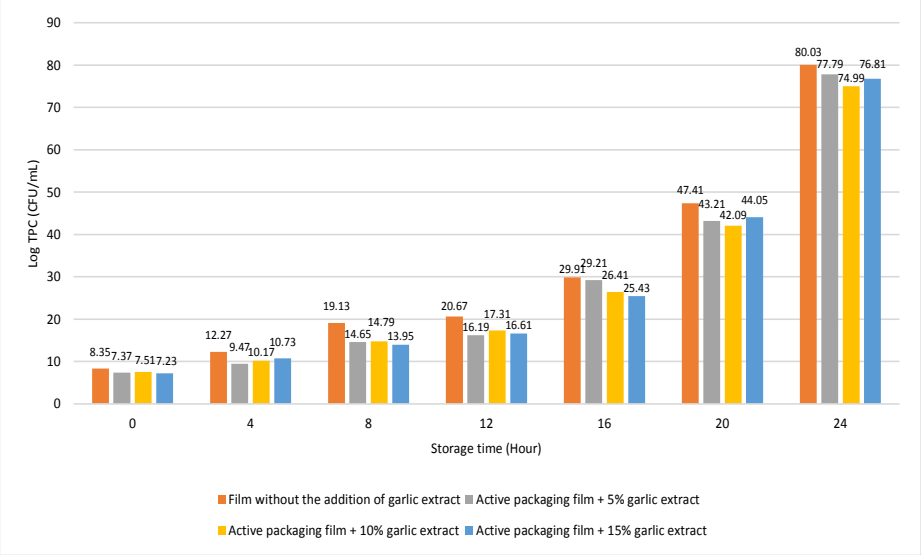


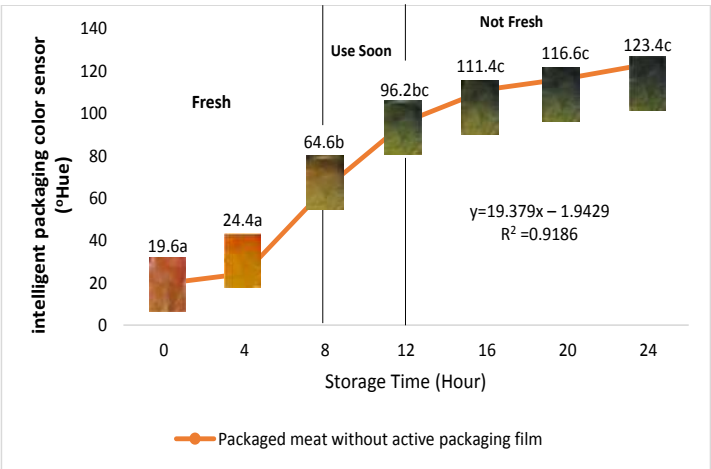
Figure 5. Total plate count (TPC) of packed meat stored at room temperature for 24 h.

At 0 h of the storage period, the initial TPC value (Log TPC) of all meat samples was 2.53±0.64 CFU/ml (Figure 5). Thus, the meat samples were classified as fresh on the basis of microbiological quality. Throughout the storage period, the TPC value increased until it reached the maximum number of meat microbes permitted by SNI 3932:2008 on carcass and beef quality, which is 1×10^6 CFU/ml or equivalent to Log TPC 6 CFU/ml. At 12 h of storage, the meat samples treated with the active packaging films but without garlic extract (0%) and those added with 5% garlic extract did not fulfil the microbiological requirements as they had a Log TPC value of 7.65±0.39 and 6.20±0.00 CFU/ml, respectively. By comparison, the meat samples treated with the active packaging films and 10% and 15% garlic extract also did not fulfil the microbiological requirements after 16 h of storage as they has a Log TPC value of 7.47±0.26 and 6.78±0.67 CFU/ml, respectively. This result demonstrated that the active packaging films with 10% and 15% garlic extract in the meat packaging system can inhibit microbial growth and extend the shelf life of meat by up to 4 h because allicin can inhibit the growth of both Gram-positive and Gram-negative bacteria by destroying the sulfhydryl group bound to bacterial proteins. This process is important because the sulfhydryl group is required for bacterial cell division or acts as a specific stimulator for cell multiplication. Allicin damaged the RNA and DNA of bacteria and thus inhibits their growth and development in meat. Likewise, Deresse (2010) reported that allicin can suppress the growth of both Gram-positive and Gram-negative bacteria by completely inhibiting the syntheses of bacterial RNA, DNA, and proteins.

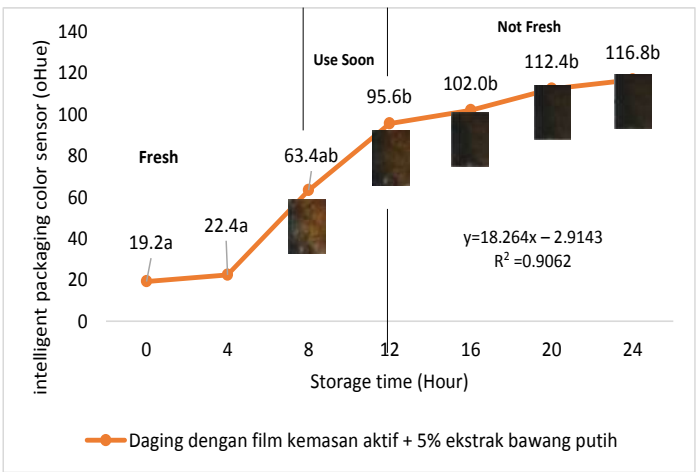
The total microbial content of the meat samples continued to increase during the entire storage period (Figure 5) because meat contains a high nutrient and water content, which provides an ideal environment for microorganism growth. Moreover, storage at room temperature can accelerate the growth of microorganisms. According to Soeparno (2015), meat has the ideal conditions for microorganism growth because it contains a high proportion of water (68%–75%), it is rich in nitrogen-containing substances of varying complexity, it contains various fermentable carbohydrates, it is rich in minerals and essential nutrients for microorganism growth, and it has a suitable pH for microorganism growth (pH 5.3–6.5). Variance analysis revealed that the duration of storage of the meat samples and the use of the active packaging films with garlic extract had a highly significant effect on the TPC value of the samples ($P>0.01$).

3.5. Changes in the color of the smart packaging BTB indicator solution as a measure of the freshness of the meat packaged with the active packaging films

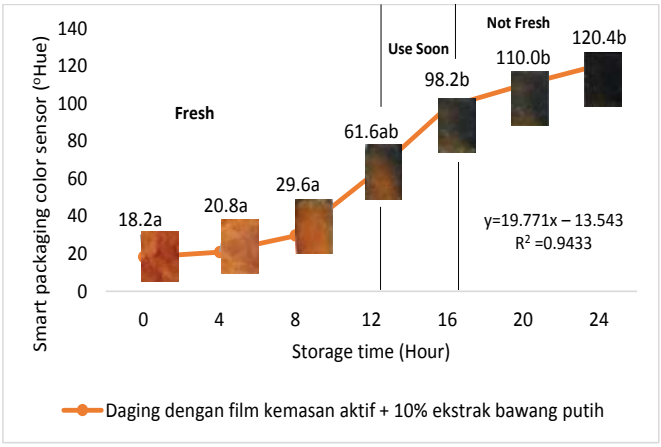
Using fresh beef packaged and maintained at room temperature for 24 h, Dirpan et al. (2019) determined that BTB solution (pH 2.75) produces the most readily visible color changes to sensitivity tests. In this study, the BTB solution (pH 2.75), as the smart packaging indicator, was also utilized to evaluate changes in its color as a reflection of the freshness of the meat samples packed with the active packaging films (**Figure 6**).


intelligent packaging color sensor (°Hue)
140
120
100
80
60
40
20
0
Fresh
Use Soon
Not Fresh
19.6a
24.4a
64.6b
96.2bc
111.4c
116.6c
123.4c
y=19.379x – 1.9429
R² =0.9186
0
4
8
12
16
20
24
Storage Time (Hour)
Packaged meat without active packaging film

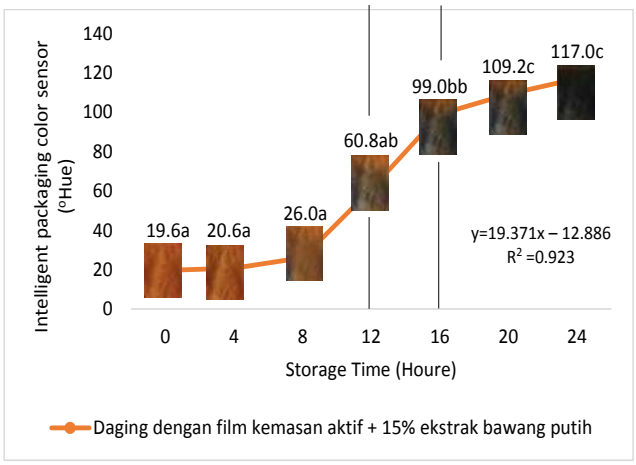
(a)


intelligent packaging color sensor (oHue)
140
120
100
80
60
40
20
0
Fresh
Use Soon
Not Fresh
19.2a
22.4a
63.4ab
95.6b
102.0b
112.4b
116.8b
y=18.264x – 2.9143
R² =0.9062
0
4
8
12
16
20
24
Storage time (Hour)
Daging dengan film kemasan aktif + 5% ekstrak bawang putih

(b)


Smart packaging color sensor (°Hue)
140
120
100
80
60
40
20
0
Fresh
Use Soon
Not Fresh
18.2a
20.8a
29.6a
61.6ab
98.2b
110.0b
120.4b
y=19.771x – 13.543
R² =0.9433
0
4
8
12
16
20
24
Storage time (Hour)
Daging dengan film kemasan aktif + 10% ekstrak bawang putih

c)


Intelligent packaging color sensor (°Hue)
140
120
100
80
60
40
20
0
19.6a
20.6a
26.0a
60.8ab
99.0bb
109.2c
117.0c
y=19.371x – 12.886
R² =0.923
0
4
8
12
16
20
24
Storage Time (Houre)
Daging dengan film kemasan aktif + 15% ekstrak bawang putih

(d)

Figure 6. Changes in the color of the BTB solution (pH 2.75) as the smart packaging indicator reflecting the freshness of the meat samples packed with the active packaging films with 0% **(a)**, 5% **(b)**, 10% **(c)**, and 15% **(d)** of garlic extract.

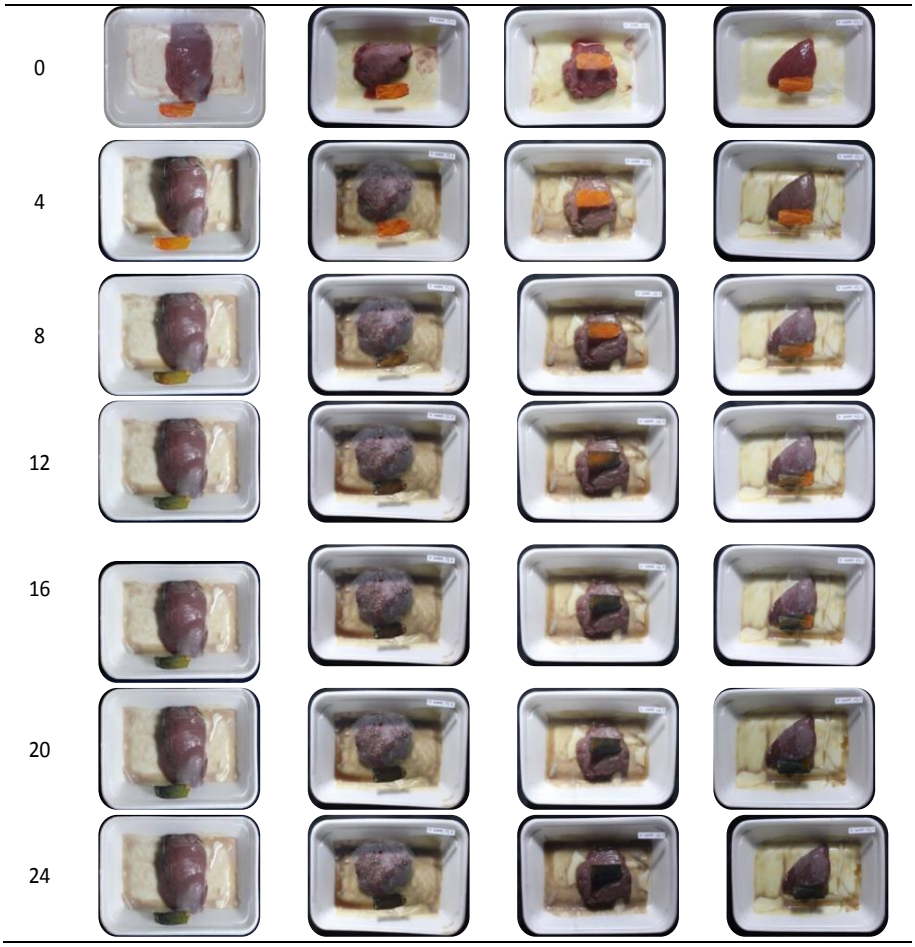
During the entire storage period, the smart packaging indicator changed its color three times that corresponded to three phases of the meat samples' level of quality (Figure 6). In phase I, its color was orange, indicating that the meat samples were still fresh. In phase II, its color was green with an orange hue, suggesting that the meat samples should be consumed immediately. In phase III, its color was dark green, denoting that the meat samples were already spoiled. The change in the indicator's color from orange to green indicated that the quality of the meat samples had deteriorated. The changes in the indicator's color were due to the interactions of alkaline volatile compounds produced by enzyme activity, and the metabolism of the microorganisms present in the meat samples increased with storage time. The early sign of spoilage was indicated by the release of volatile alkaline compounds as the microorganisms and the enzymes degraded the nutritional content of the meat samples. These compounds gradually accumulated in the packaging system, causing an increase in pH, which was detected by the smart packaging indicator and displayed as gradual color changes. The change in color of the smart packaging indicator (BTB, pH 2.75) from orange to green was induced by deprotonation or the release of a proton from the smart packaging indicator dye (De Meyer *et al*. 2014).

The meat samples packaged with the active packaging films but with no (0%) and 5% garlic extract were still fresh from the start of the storage up to 8 h (Figure 6). However, they must be immediately consumed from the 8th h to the 12th h of the storage period. Thereafter (12–24 h of the storage period), they were already spoiled. This results was consistent with that of TPC tests, which showed that the TPC values were above the acceptable threshold for microbial contaminants (1×10^6 or equivalent to 6 CFU/ml) in meat after 12 h. By comparison, the meat samples packaged with the active packaging films containing 10% and 15% garlic extract were still considered fresh from the start of the storage period up to the 12th h. They must be immediately consumed when they had been in storage for 12–16 h. Finally, they were considered rotten when they had been in storage for 16–24 h. This result was also consistent with that of TPC tests (Figure 5), which indicated that at the 16th hour, the TPC value surpassed the permissible level of microbiological contamination in beef. Statistical analysis revealed that storage duration had a very significant effect on the Hue value, the indicator of color change in the smart packaging. The changes in the color of the smart packaging indicator (BTB solution, pH 2.75) when used together with the active packaging films to reflect the freshness of meat are presented in **Table 1**.

Commented [1994713]: In scientific, technical, and medical fields, significant/significance/significantly should only be used when referring to a statistical threshold and with a p value. In the absence of a p value, use an alternative word.

Table 1. Changes in the color of the smart packaging indicator (BTB solution, pH 2.75) when used together with the active packaging films to reflect the freshness of meat.

Storage Time (Hour)	Active Packaging Films Added with Garlic Extract			
	0%	5%	10%	15%



3.6. Correlations between changes in the color of the smart packaging indicator and the effects of the active packaging films on the parameters of meat freshness

The correlations between changes in the color of the smart packaging indicator and parameters of meat quality deterioration (pH, TVBN, and TPC) were explored to ascertain the relationship between the sensitivity of the smart packaging indicator to meat freshness and the effectiveness of the active packaging films in slowing the process of meat spoilage.

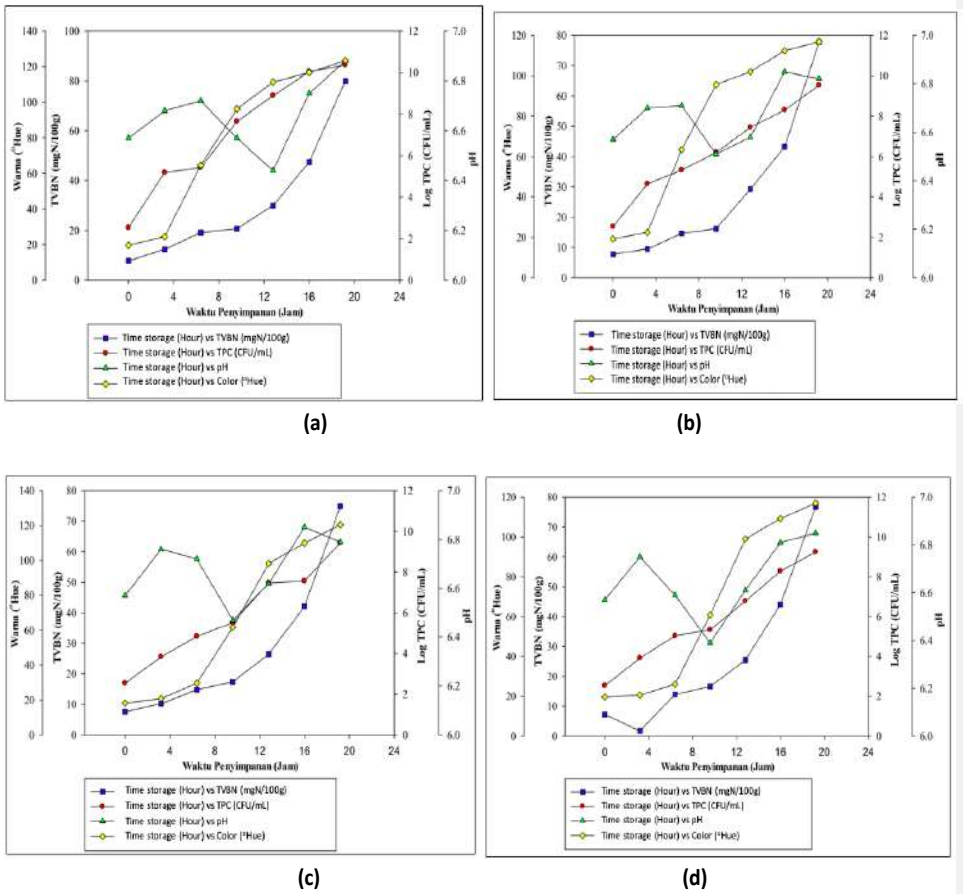


Figure 7. Correlations between changes in the color of the smart packaging indicator and the effects of the active packaging films with 0% **(a)**, 5% **(b)**, 10% **(c)**, and 15% **(d)** garlic extract on the parameters of quality deterioration of meat stored for 24 h.

The TPC and TVBN values of the meat samples treated with the active packaging films but without garlic extract increased, which was reflected by the change in the color of the smart packaging indicator (Figure 7). The pH of the meat samples fluctuated not only because of the production of volatile base compounds due to the activity of microorganisms in the samples during the storage period but also because of various factors, such as the contents of glycogen and lactic acid in the livestock prior to and after the slaughter. The meat samples treated with the active packaging films but without garlic extract and those treated with 5% garlic extract were rotten and unfit for consumption after 12 h of storage as their Log TPC value was 7.65±0.39 and 6.20±0.00 CFU/mL, respectively, and their TVBN value was 20.67±2.68 and 16.19±0.28 mgN/100g, respectively (Figure 7). By comparison, the meat samples treated with the active packaging films and 10% and 15% garlic extract were rotten and unfit for consumption after 16 h of storage as their Log TPC value was 7.47±0.26 and 6.78±0.67 CFU/mL, respectively, and their TVBN value was 26.41±3.31 and 25.43±4.89 mgN/100 g, respectively.

The TPC values indicated the same level of quality deterioration as the TVBN values and reflected the changes in the color of the smart packaging indicator of the freshness of the meat samples treated with the active packaging films without (0%) and with 5% garlic extract; these samples were deemed rotten after 12 h of storage (Figure 7). The color of the indicator changed from orange (fresh) with a color value of 19.6°Hue and 19.2°Hue to green (rotten) with a color value of 96.2°Hue and 95.6°Hue respectively. By comparison, the meat samples treated with the active packaging films and added with 10% and 15% garlic extract were deemed rotten after 16 h of storage; the color of the indicator changed from orange (fresh) with color values of 18.2°Hue and 19.6°Hue, respectively, to green (rotten) with color values of 98.2°Hue and 99°Hue, respectively. Wiryawan (2005) observed that when garlic extract was added to the active packaging, the values of TPC and TVBN and the pH of the meat increased more slowly, as did the color of the smart packaging indicator, compared with those of the meat without the active packaging.

The increase in the values of TPC, TVBN, and pH of meat is strongly influenced by its high nutrient and water content, which is conducive to microbial contamination. Moreover, storing meat at room temperature accelerates bacterial growth. In general, an increase in the number of microorganisms is followed by an increase in the production of the volatile base chemical called TVBN. More volatile base compounds are released when more microorganisms are present and active, as indicated by the increase in TVBN value, which also has an effect on the meat's pH value. Furthermore, the increase in the values of TPC, TVBN, and pH linearly correlates with the increase in Hue value and color changes of the smart packaging indicator because the accumulated volatile base compounds raise the pH value of the packaging system, causing the smart packaging indicator to experience a color shift. This explanation was in agreement with that of Pacquit et al. (2006), who applied active packaging films to cod fish. They stated that the increase in the TPC value of cod fish has a linear correlation with changes in the color of the cellulose-acetate packaging film sensor.

4. Conclusion

As a smart packaging indicator, the BTB solution (pH 2.75) produce color changes that are easy to observe and reflect three phases of meat quality deterioration. In phase I, its color was orange, indicating that the meat was still fresh. In phase II, its color was green with an orange hue, suggesting that the meat must be consumed immediately. In phase III, its color was dark green, denoting that the meat was already rotten and unfit for consumption. The meat samples treated with the active packaging films and added with 10% and 15% garlic extract rotted after 16 h, whereas the meat samples treated with the active packaging films and added with 0% and 5% garlic extract rotted after only 12 h. The changes in the color of the BTB solution was linearly and positively associated with the values of TPC, TVBN, and pH of the meat samples packaged with the active packaging films.

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Application of a Smart Sensor and Active Packaging System Based on the Bacterial Cellulose of *Acetobacter xylinum* to Meat Products

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Abstract: Combining smart and active packaging serves the dual purpose of detecting color changes in food that reflect changes in its quality and prolonging its shelf life. This study developed a smart and active packaging system made from the cellulose of Acetobacter xylinum and assessed its ability to detect changes in the quality of packaged fresh beef. The properties of the smart packaging's sensor and active packaging films were determined. The application of this system to fresh beef stored at room temperature (28±2 °C) for 24 h was tested. The color of the bromothymol blue (BTB) solution (pH 2.75) in the indicator of the smart packaging system changed from orange to dark green to indicate that beef quality changed from fresh to rotten. The meat treated with the active packaging with 10% and 15% garlic extract decayed on the 16th h. By contrast, the meat treated with the active packaging but without the garlic extracts rotted on the 12th h. The shift in the indicator's color was linearly related to the total plate count (TPC), total volatile basic nitrogen (TVBN) , and pH of the meat packaged using the active packaging system. Therefore, BTB solution (pH 2.75) can be used as a smart packaging indicator that will allow consumers to assess the quality of packaged meat easily. As an antimicrobial agent, the addition of 10%–15% garlic extract to the active packaging films can help delay the spoilage of packaged meat.

Keywords: Smart Sensor; Smart packaging; active packaging; Bacterial Cellulose; Meat

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1. Introduction

Global beef consumption is predicted to rise as the world population and family income increase, particularly in developing Asian countries [1–3]. By 2030, worldwide meat consumption and availability are expected to increase by 14% and 5.9%, respectively, over the average of the 2018–2020 period [3]. Thus, the expected increase in meat consumption must be complemented by improvements in the quality of fresh meat produced. One aspect affecting the quality and characteristics of meat is the material and packaging technologies used [4]. Meat is a perishable item that rapidly spoils when stored above the optimum temperature range (below 0–40 °F) [5,6]. However, in traditional markets, meat is displayed at room temperature without packaging, a practice that might accelerate microbial contamination and cause rapid quality degradation. Even in supermarkets where meat is maintained in cold temperatures, standard meat packaging still prevent consumers from subjectively determining the quality of meat. Thus, meat packaging must have additional functions that will prevent quality degradation due to microbial contamination and will help consumers to determine the quality of packaged meat easily [7]. Conventional meat packaging can be designed to perform dual functions through smart and active packaging systems.

Smart packaging is a term that refers to sensors in the form of indicators that monitor and provide information on the quality of the food contained within the packaging via

color changes caused by chemical reactions between the indicators and the products of microbial metabolism or changes in the chemical composition of the food [8,9]. During storage, the chemical components of meat degrade into volatile compounds because of microbial activity, thereby increasing the value of total volatile base nitrogen (TVBN) [10,11]. Accumulation of TVBN increases the pH of the packaging system, which is detected by the indicator, resulting in a visible color shift in the indicator [11,12]. Smart packaging allows easier monitoring of packed products during transportation and storage [7]. Moreover, it provides a more accurate estimate of product condition than conventional expiration labels [12]. Color-based pH indicator solutions are widely used as smart indicators. Dirpan et al. [13] developed bromophenol blue as a smart indicator dye for mangoes. Hidayat et al. [14] used two types of color indicators with predetermined concentrations, namely, phenol red and bromothymol blue, to assess the freshness of meat packaging. Smart packaging indicators based on natural pigments are being developed, such as smart packaging films that include anthocyanin-loaded Lycium ruthenicum nanocomplexes in starch/polyvinyl alcohol mixtures (PVA) [15], as well as anthocyanins from saffron petals immobilized in chitosan nanofibers and methyl cellulose matrix [16].

Active packaging refers to the integration of particular additives into a packaging system for the purpose of extending the shelf life, preserving the quality, and ensuring the safety of food products. Antimicrobial agents are used as components of active packaging additives to extend product shelf life. The volatile bioactive compounds in active packaging evaporate or diffuse onto the food surface, where they limit the growth of pathogenic microbes and thus delay spoilage [17,18]. This strategy is more effective than coating bioactive compounds onto the food surface [19]. The safest, cheapest, and most readily available antimicrobial agents for use in active packaging are essential oils. Pranoto et al. [20] produced antimicrobial alginate edible films by incorporating the essential oils of garlic. They reported that these films substantially inhibited the growth of Staphylococcus aureus and Bacillus cereus in meat. Vishnu et al. [21] utilized the essential oils of Plectranthus amboinicus in a chitosan-based active packaging to restrict antimicrobial activity. Smart and active packaging can be merged into a single packaging system. Julyaningsih et al. [22] combined a smart packaging system based on methyl red–bromothymol blue (BTB) indicator with an active packaging system based on lemongrass oil as a component of tuna fish fillet packaging. Yao et al. [23] developed an active and smart packaging system based on starch, PVA, and betacyanins from various types of plants for shrimp packaging.

In general, an active packaging that contains antimicrobial agents and a smart packaging that contains indicator solutions are immobilized in a polymer. Compared with plant cellulose or synthetic polymers, the bacterial cellulose fermented by Acetobacter xylinum has a unique nanofibrillar structure and superior physical properties, suggesting that it has the potential to serve as a basis for developing a smart and active packaging system [24,25]. Bacterial cellulose has received interest as a component of active packaging owing to its edibility, biodegradability, high water-holding capacity, and great potential as an antimicrobial agent carrier [26].

The development of packaging systems with additional functions is advancing. To promote this innovation, this study aimed to maximize the potential of smart and active packaging by combining them into a single packaging system based on a bacterial cellulose membrane biopolymer to enhance the quality of packaged meat and help consumers to determine meat freshness easily.

2. Materials and Methods

2.1. Materials

The main ingredients used in the smart and active packaging system developed herein were the bacterial cellulose produced by *A. xylinum*, which was fermented in natural media of coconut water. Beef tenderloin was obtained from a slaughterhouse in

Tamangapa Raya. Coconut water and garlic (Allium sativum) were purchased from a local market. Food-grade ammonium sulfate (CAS Number: 7783-20-2), yeast extract (Merck, CAS Number: 8013-01-2), 96% acetic acid (Brenntag Inc, CAS No: 64-19-7), A. xylinum culture, 5% 1 N NaOH (Brenntag Inc, CAS No: 1310-73-2), sucrose, Bromothymol blue (BTB, Merck, CAS No: 76-59-5), alcohol, aquabides, aquades, Tashiro's indicator (0.1% methyl red and 0.1% BTB at a ratio of 2:1), 7% trichloroacetic acid (TCA, Merck), nutrient Agar (NA, Merck), glycerol (Merck, CAS No: 56-81-5), food-grade carboxymethyl–cellulose (CMC) (Foodchem, E466), and corn starch were used.

2.2. Methods

2.2.1. Determination of the best nitrogen source from A. xylinum fermentation media

The production of *A. xylinum* bacterial cellulose membranes was started by determining the amount and type of the most suitable nitrogen source from A. xylinum growth media following the method of a previous study [7].

2.2.2. Purification of bacterial cellulose

Bacterial cellulose was removed from the fermentation medium, rinsed in running water, and then soaked for 2 days with periodic water changes. The cellulose was also soaked in 70% alcohol for 1 min, heated to 100 °C in distilled water for 20 min, and reheated in 1 N 5% NaOH solution at 100 °C for 60 min to remove the remaining bacterial cells and substrate attached to the cellulose layer. Afterward, the cellulose was rinsed with running water and soaked in periodically changed water for 24 h until pH reached 7. The purified cellulose appeared transparent [7].

2.2.3. Production of smart packaging

2.2.3.1. Preparation of the indicator solution

BTB indicator solution was chosen for this study because a previous work established this solution as the indicator with the most visually identifiable color change reaction [7]. First, 1% BTB solution (b/v) was prepared in 95% ethanol. Then, the pH of the BTB solution was decreased to 2.74 by adding 20% acetic acid. Finally, the BTB solution was stored in a closed container.

2.2.3.2. Production of smart packaging indicator label

The purified cellulose film was kept in a filter cloth for 24 h to decrease its water content. Half-dried cellulose was cut into 1.5 cm × 4 cm strips and pushed flat against the surface of a Pyrex glass. The cellulose was dried for 30 min at 70 °C. The BTB indicator solution was then absorbed into a dry cellulose via centrifugation at 3000 rpm for 15 min. When the color indicator was successfully absorbed, the BTB indicator solution imparted an orange hue to the cellulose. Afterward, the cellulose was rinsed with distilled water to eliminate any unbound color indicators and then dried [27,28].

2.2.4. Production of active packaging film

2.2.4.1. Production of garlic extract as active element

First, 500 g garlic was peeled, washed under running water until clean, drained, and then mashed. The, minced garlic was extracted via the maceration method by immersing the finely ground garlic in 96% alcohol at a ratio of 1:4 (garlic:alcohol) for 4 days at 3 °C–5 °C and periodically homogenized using a water bath shaker. Afterward, the extract was filtered using a filter paper and then concentrated using a rotary evaporator at 50 rpm at 40 °C to obtain a thick extract, modified [29].

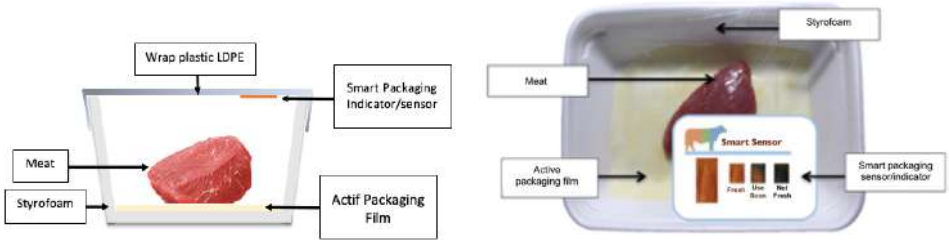
2.2.4.2. Production of active packaging film

The bacterial cellulose was crushed to form a cellulose slurry, and all pretreatments were performed at room temperature. A cellulose suspension was prepared using 30% chitosan (w/w), 10% CMC (w/w), and 15% corn starch (w/w) of cellulose dry weight. The

suspension was heated at 50 °C for 60 min with a hot plate stirrer until thoroughly suspended. At the 50th min, 30% glycerol (w/w) was added. Additionally, the garlic extract was added at quantities of 0% (as the control), 5%, 10%, and 15% (v/v) immediately after the final heating step. Subsequently, 60 g of the suspension was then placed onto a glass plate and dried for 48 h at 37 °C. Finally, the suspension was cooled to room temperature, removed from the glass plate, wrapped in aluminum foil, and placed in a desiccator [19,30].

2.2.5. Application of the smart and active packaging indicators to fresh beef

Fresh beef tenderloin was collected from a slaughterhouse in Tamangapa Raya Makassar 1 h after the cow was slaughtered. It was immediately placed in a special food box and put into a 38 cm × 29 cm × 30 cm Styrofoam box filled with ice crystals. The samples were promptly transported to the laboratory and processed into 200 g/pack pieces under sterile conditions. The meat was packaged in a Styrofoam tray (1.05 g/cm^3) coated with the active packaging film on a Styrofoam base, and a smart packaging indicator label was attached to the LDPE plastic wrap film that covered the Styrofoam container (**Figure 1)**. The samples were maintained at room temperature (28±2 °C) with normal light exposure for 24 h.



(a) **(b)**

Figure 1. (a) Design of the smart and active packaging system; **(b)** and its application to fresh meat.

2.2.6. Observation parameters

2.2.6.1. Measurement of the color of the smart packaging indicator

The color of the smart packaging indicators was quantitatively determined using a chromameter digital color meter (T-135). The meat packaged in the smart and active packaging system was placed on a flat surface with a black background with uniform lighting. The chromameter detector was placed on the surface of the smart packaging indicator. The start button was pressed until the measurement results were shown on the display. The measurement results were expressed according to the notation of the Hunter's Lab Colorimetric System, which is presented in three values, namely L* (lightness), a* (redness), and b* (yellowness) [31]. The color of the smart packaging indicator was determined by calculating the °Hue value by using the formula (1) below:

$$°\boldsymbol{Hue} = \boldsymbol{tan^{-1}}\frac{\boldsymbol{b}}{\boldsymbol{a}} \quad (1)$$

where °Hue represents the parameters for color range, a is a red-green mixed color, and b is a red-green mixed color yellow-blue.

2.2.6.2. Antimicrobial activity of the active packaging films

The antimicrobial activity of the active packaging films was determined via the agar diffusion method. Each active packaging film was cut into 5 mm circles in a sterile environment and then placed on NA agar media with 0.1 ml of the test microorganism culture (Staphylococcus aureus) containing 106 CFU/ml. Petri dishes were incubated for 24 h at 37 °C. After incubation, the inhibitory zone was measured using a caliper [32].

2.2.6.3. Determination of pH of the beef samples

The pH of the beef samples was measured using a pH meter (Oakton pH 510). First, 5 g of crushed meat was combined with 45 ml of distilled water until the mixture became homogenous. The pH meter's electrode was then immersed in the beef suspension until the pH value on the monitor became constant.

2.2.6.4. Measurement of TVBN

First, 30 ml of 7% TCA solution was added to a meat sample (10±0.1 g) and mixed before filtering. The, 1 ml boric acid solution was placed in the "inner chamber" of the Conway dish. The lid of the cup was placed in such a way that it almost covered the cup. The filtrate was placed into the left outer chamber of the Conway dish. Afterward, 1 ml saturated K2CO3 solution was put into the right outer chamber to avoid mixing the filtrate with K2CO3. The cup was closed and rotated to mix the two liquids in the outer chamber. The blank solution was prepared following the same process but with 7% TCA instead of the filtrate. The solutions were stored at 37 °C for 2 h. The boric acid solution with the blank and filtrate samples was then titrated with 0.01 N HCl until it turned pink. TVBN was calculated by formula (2) as follows [33]:

$$TVBN\ content\ \left(\frac{mg}{100g}\right) = \frac{(Vc-Vb)\ x\ 14.007\ x\ fp\ x\ 100}{W} \quad (2)$$

where Vc is the volume of the HCl solution used in sample titration, Vb is the volume of the HCl solution used in blank titration, N is the normality of the HCl solution, Wis the sample's weight (g), 14.07 is the molecular weight of nitrogen, and Fpis the dilution factor.

2.2.6.5. Total plate count

The total number of microorganisms was determined via the total plate count (TPC) method described in SNI 2332.3: 2015. First, 1 g of the sample was added to a test tube containing 9 ml of physiological solution until homogeneous (10^{-1} dilution). The dilution was continued until 10^{-6}, at which point the diluted sample was inoculated on NA media in duplicate via the pour plate technique. After the media solidified, the Petri dishes containing the media and the sample solution were incubated upside down at 30 °C for 48 h. Afterward, TPC was calculated using the formula (3) below [34]:

$$N = \frac{\sum C}{[(1\times n_1)+(0.1\times n_2)]\times (d)} \quad (3)$$

where N is TPC (CFU/ml), ∑C is the number of colonies counted in all Petri dishes, n_1 is the number of colonies counted in all Petri dishes at first dilution, n_2 is the number of colonies counted in all Petri dishes at second dilution, and d is the number of colonies counted in all Petri dishes at first dilution.

2.2.6.6. Data analysis

ANOVA was used to analyze the parameters of the smart packaging indicator, antimicrobial activity of the active packaging films, and quality of the beef samples, including pH, TVBN, and TPC with three replications. Differences between treatments were determined using Duncan's test. The correlations between the changes in the color of the smart packaging indicator and the effects of the active packaging on all parameters of meat spoilage were explored and presented in graphs by using the Sigma Plot 12 software. Data were analyzed using Microsoft Excel 2019, SPSS 19, and Sigma Plot 12.

3. Results and Discussion

3.1. Antimicrobial activity of the active packaging films against Staphylococcus aureus

The antimicrobial activity of the active packaging films is presented in **Figure 2.**

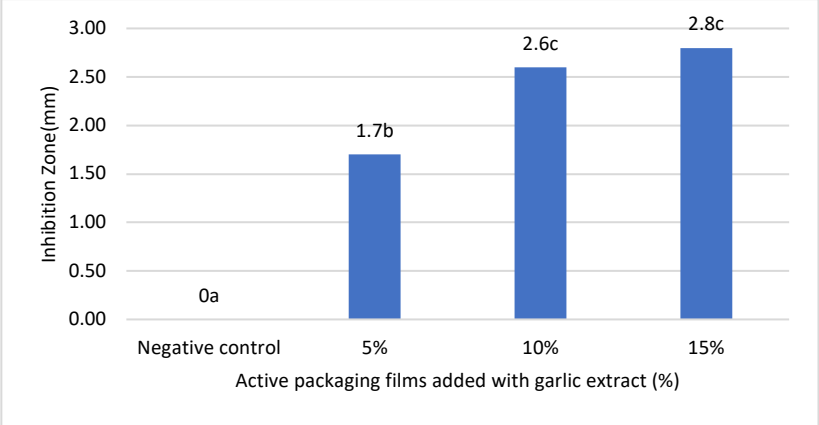


Figure 2. Antimicrobial activity of the active packaging films against *S. aureus*.

The antimicrobial activity of the active packaging films against *S. aureus* was assessed by measuring the diameter of the inhibition zone. As shown in **Figure 2**, the negative control did not generate an inhibitory zone. However, when high concentrations of the garlic extract were added to the active packaging films, the inhibitory activity against the bacteria was high, although the inhibition zone was not significantly different between 10% and 15% garlic extract. The active packaging films added with garlic extract exhibited antimicrobial activity against the Gram-positive bacteria S. aureus, but the activity could be considered weak because the diameter of the inhibition zone was less than 5 mm **(Figure 2)** [35]. Differences in the diameter of inhibitory zones are influenced by the ability and rate of diffusion of antimicrobial compounds in the medium, the growth rate of microorganisms and their sensitivity to antimicrobial chemicals, and the viscosity and thickness of the medium.

The antibacterial effects of garlic extract are due to allicin, which is generated when garlic is damaged. When the flesh of garlic is damaged during the refining process, allicin is rapidly generated because of the release of alliinase, which reacts with nonprotein amino acids, namely, alliin. Allicin is a part of the defense mechanism of garlic that exerts antimicrobial effects on both Gram-positive and Gram-negative bacteria by inhibiting RNA and lipid syntheses, which in turn inhibit the production of amino acids and proteins and the phospholipid bilayer of bacterial cell wall, thereby preventing bacterial growth and development. Allicin is highly permeable and can easily penetrate bacterial cells across the cell membrane. The thiosulfinate S(=O)S group in allicin then binds to the sulfhydryl groups of bacteria, thus inhibiting the activation mechanism of bacterial proteinases [36,37]. This study demonstrated that 10%–15% garlic extract has comparable antibacterial effects

3.2. pH of the beef samples

The pH of the beef samples was measured to investigate the effects of the active packaging films as the meat base in the packaging system. The beef samples were stored at room temperature for 24 h. The results of pH measurements are shown in **Figure 3.**

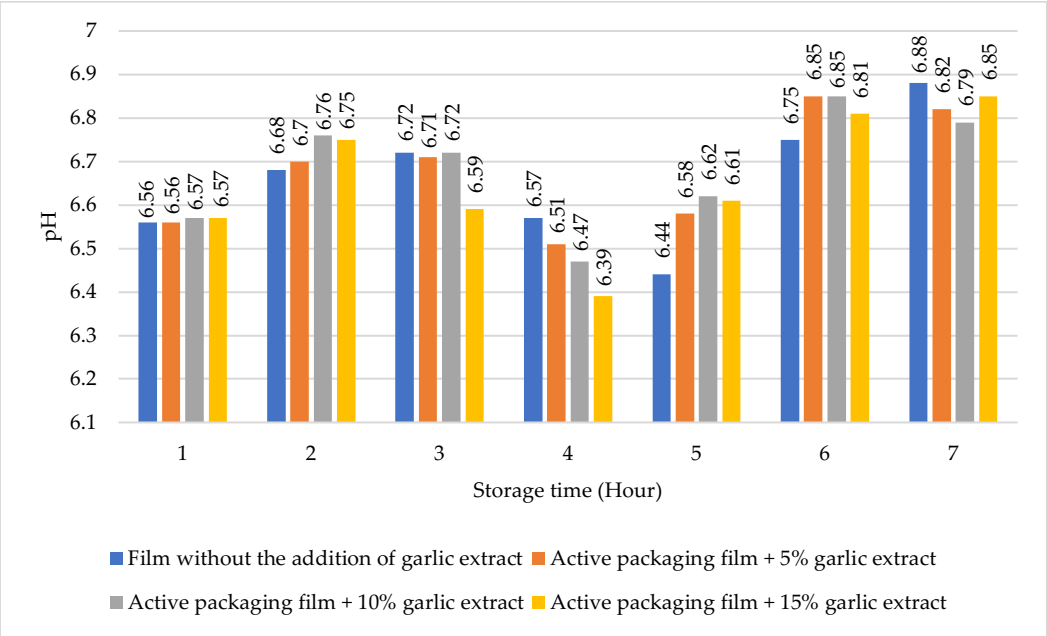


Figure 3. pH values of packaged meat sample stored at room temperature for 24 h.

The initial pH of the meat samples, which was immediately determined after the cow was slaughtered, was normal (6.57) **(Figure 3)**. The pH fluctuated during the storage period. At the 4th hour of the storage period, the pH of the meat ranged from 6.68 to 6.76. At the 6th hour of the storage period, the pH of the meat ranged from 6.59 to 6.72. The normal pH of meat ranges from 5.4 to 5.8 at 6 h postmortem [38–40]. In this study, the beef samples had a pH value that could be classified as quite high compared with that reported in previous studies. This result could be presumably attributed to the stress and the brief rest period that the cow experienced before it was slaughtered. These factors depleted the cow's glycogen reserves, allowing anaerobic glycolysis to occur quickly after it was slaughtered and depleting the lactic acid produced in its tissues. As a result, the pH of the meat dropped to an unsatisfactory level. Cows are thought to experience fatigue before they are slaughtered, thereby depleting the supply of ATP. Moreover, the sufficiently high temperature during slaughter accelerates the depletion of ATP, thus expediting the process of rigor mortis. The acceleration of rigor mortis causes the pH of the flesh to remain elevated and above normal, as confirmed by Sánchez-Macías et al. [41] and Moreno et al. [42], who reported that each variety of meat has a different glycogen content; thus, they have different glycolysis rates. The lower the content of glycogen in the meat is, the slower the glycolysis process will be and the higher the final pH will be. However, the decrease in pH in muscles can be influenced by internal factors, such as species, muscle type, muscle glycogen content, and livestock variability, as well as external factors, such as environmental temperature, additional treatment prior to slaughter, and pre-slaughter stress.

After 20 h of storage, the meat's pH value ranged from 6.75 and 6.85 and remained steady thereafter; at this point, the meat was classified as decayed **(Figure 3)**. According to Prache et al. [43], the meat's pH continues to decline until glycogen is depleted into lactic acid and alkaline compounds are neutralized because of microbial metabolism, resulting in an increase in pH. If the pH reaches 6.8 or higher, protein decomposition will occur, resulting in spoilage

3.3. TVBN of the meat samples

The TVBN values of the meat samples are presented in **Figure 4**.

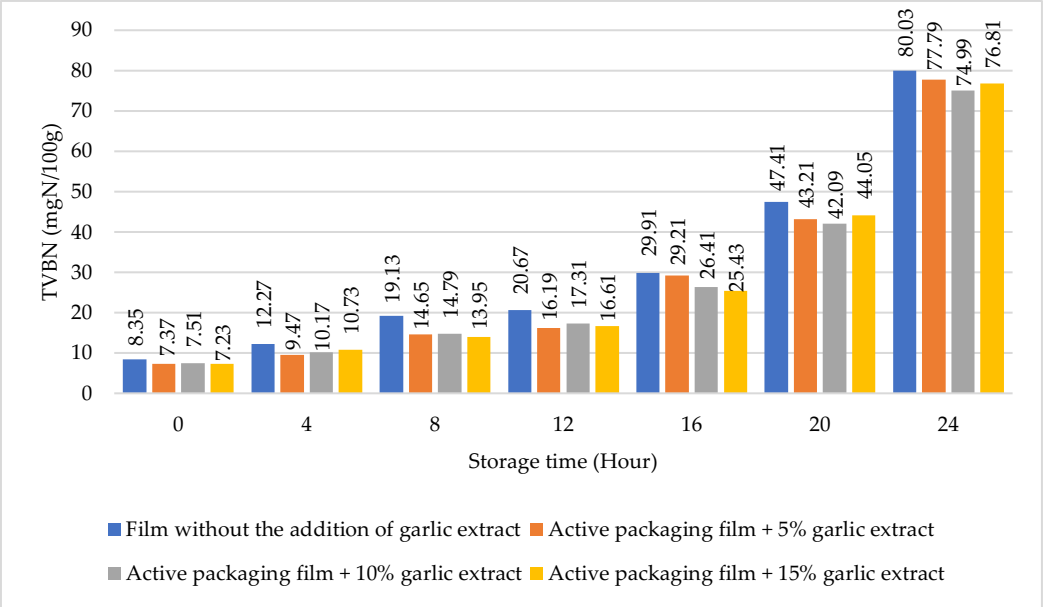


Figure 4. Total volatile basic nitrogen (TVBN) of the packed meat stored at room temperature for 24 h.

At 0 h, all meat samples had TVBN values ranging from 7.23 mgN/100 g to 8.35 mgN/100 g **(Figure 4)**. Therefore, they were classified as fresh meat. After 12 h of storage, the meat samples that had not been treated with the active packaging films had a TVBN value of 20.67 mg N/100g, indicating that they were rotten. By comparison, the meat samples treated with the active packaging films and added with 5%, 10%, and 15% garlic extract had TVBN values of 16.19, 17.31, and 16.61 mgN/100 g, respectively. Thus, they were categorized as semi-fresh meat (stale meat) or could still be consumed. However, the TVBN values of all meat samples taken between the 16th and 24th h of storage exceeded the threshold for food-grade beef, demonstrating that adding 5%, 10%, and 15% garlic extract to the active packaging films effectively reduced the amount of TVBN in fresh meat by 21.67%, 16.26%, and 19.64%, respectively, compared with the meat samples not treated with the active packaging films. Beef or livestock is considered fresh if the TVBN value is less than 15 mg/100 g [44] or TVBN is <10 mg N/100 g [45]. Moreover, SNI 2354.8:2009 by National Standardization Agency of Indonesia (BSN) [46] states that the standard levels of TVBN fit for consumption is 20–30 mg N/100g. These differences in TVBN threshold indicates that it is an inconsistent measure of meat freshness. TVBN tests can be performed to examine the quality of beef products independently.

In this study, the values of TVBN increased throughout the storage period (observed every 4 h), indicating that the meat's quality continued to deteriorate owing to the breakdown of proteins into volatile base compounds. The increase in TVBN values was due to the activity of microorganisms that degraded proteins into simpler molecules, which eventually underwent deamination to generate ammonia, which contributed to the foul odor of the meat samples, as well as the synthesis of volatile nitrogen-containing compounds. High temperatures can promote protein degradation, resulting in the formation of more alkaline components. According to Bekhit et al. [10], the increase in TVBN value is due to protein degradation by microorganisms that results in the formation of foul-smelling chemicals, such as ammonia (NH_3), basic skatole and indole compounds, mercaptans and H_2S (which are weak acids), and amines and cadaverin (which are strong bases).

The results demonstrated that the addition of garlic extract to the active packaging films delayed the spoiling of the meat samples likely because the garlic's active components prevented microbial development, thereby lowering the synthesis of nitrogenous base compounds in the meat caused by bacteria and autolytic enzymes during the rotting

process. This conjecture was supported by Al Hakim et al. [47] and Reiter et al. [37], who reported that garlic extract has the ability to block microbe-produced enzymes involved in the breakdown of proteins into volatile base chemicals.

3.4. TPC of the microbes in the beef samples

The TPC of bacteria in the meat samples was determined to assess the utility of the active packaging films **(Figure 5)**.

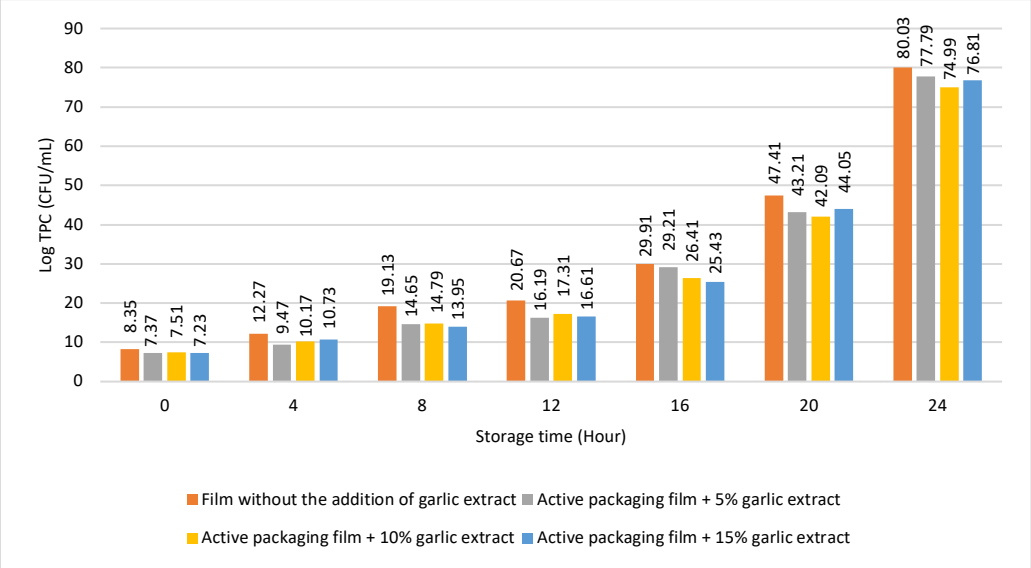


Figure 5. Total plate count (TPC) of packed meat stored at room temperature for 24 h.

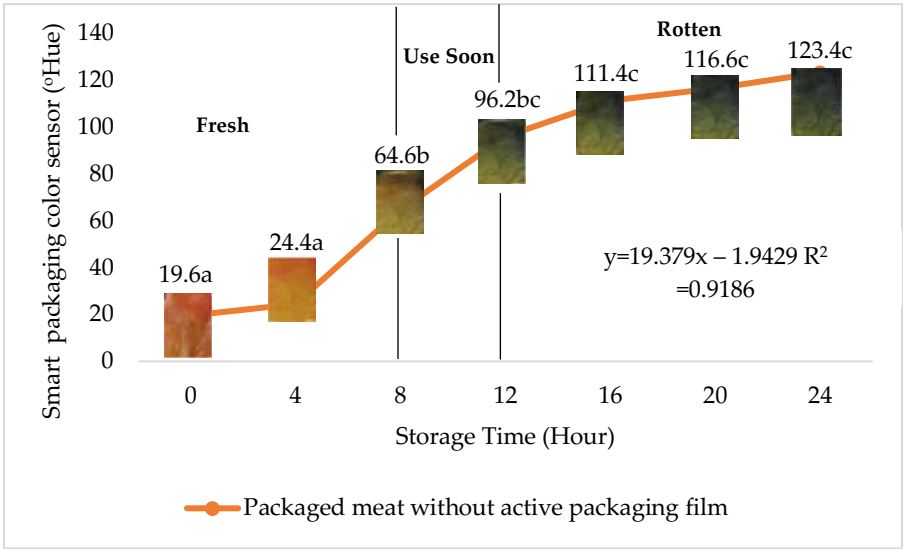
At 0 h of the storage period, the initial TPC value (Log TPC) of all meat samples was 2.53±0.64 CFU/ml **(Figure 5)**. Thus, the meat samples were classified as fresh on the basis of microbiological quality. Throughout the storage period, the TPC value increased until it reached the maximum number of meat microbes permitted by SNI 3932:2008 on carcass and beef quality, which is 1×106 CFU/ml or equivalent to Log TPC 6 CFU/ml. At 12 h of storage, the meat samples treated with the active packaging films but without garlic extract (0%) and those added with 5% garlic extract did not fulfil the microbiological requirements as they had a Log TPC value of 7.65±0.39 and 6.20±0.00 CFU/ml, respectively. By comparison, the meat samples treated with the active packaging films and 10% and 15% garlic extract also did not fulfil the microbiological requirements after 16 h of storage as they has a Log TPC value of 7.47±0.26 and 6.78±0.67 CFU/ml, respectively. This result demonstrated that the active packaging films with 10% and 15% garlic extract in the meat packaging system can inhibit microbial growth and extend the shelf life of meat by up to 4 h because allicin can inhibit the growth of both Gram-positive and Gram-negative bacteria by destroying the sulfhydryl group bound to bacterial proteins. This process is important because the sulfhydryl group is required for bacterial cell division or acts as a specific stimulator for cell multiplication. Allicin damaged the RNA and DNA of bacteria and thus inhibits their growth and development in meat. Likewise, Deresse [48] reported that allicin can suppress the growth of both Gram-positive and Gram-negative bacteria by completely inhibiting the syntheses of bacterial RNA, DNA, and proteins.

The total microbial content of the meat samples continued to increase during the entire storage period **(Figure 5)** because meat contains a high nutrient and water content, which provides an ideal environment for microorganism growth. Moreover, storage at room temperature can accelerate the growth of microorganisms. According to Soeparno [40], meat has the ideal conditions for microorganism growth because it contains a high proportion of water (68%–75%), it is rich in nitrogen-containing substances of varying complexity, it contains various fermentable carbohydrates, it is rich in minerals and

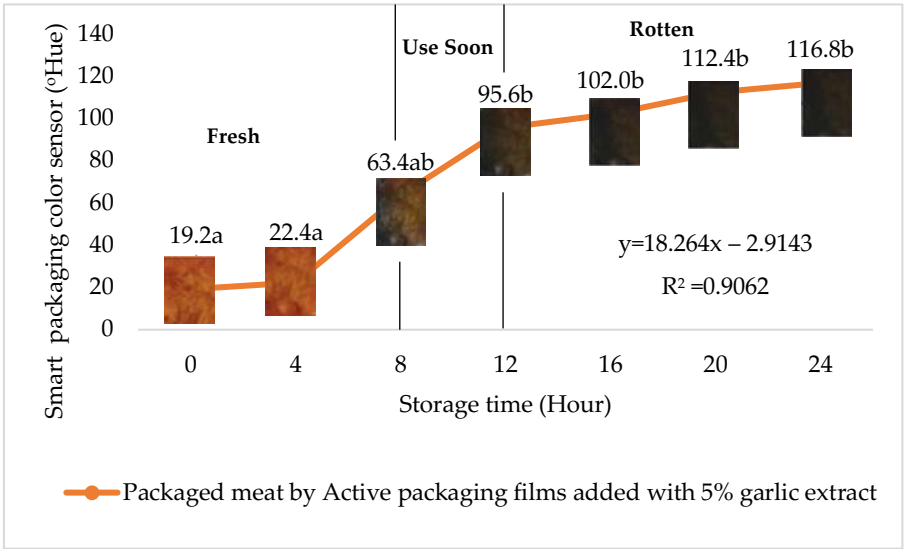
essential nutrients for microorganism growth, and it has a suitable pH for microorganism growth (pH 5.3–6.5). Variance analysis revealed that the duration of storage of the meat samples and the use of the active packaging films with garlic extract had a highly significant effect on the TPC value of the samples (P>0.01).

3.5. Changes in the color of the smart packaging BTB indicator solution as a measure of the freshness of the meat packaged with the active packaging films

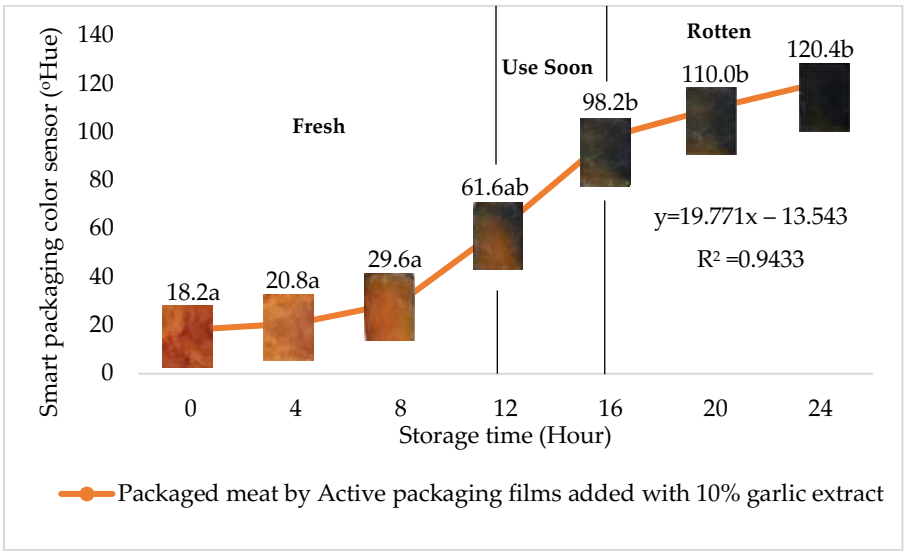
Using fresh beef packaged and maintained at room temperature for 24 h, Dirpan et al. [7] determined that BTB solution (pH 2.75) produces the most readily visible color changes to sensitivity tests. In this study, the BTB solution (pH 2.75), as the smart packaging indicator, was also utilized to evaluate changes in its color as a reflection of the freshness of the meat samples packed with the active packaging films **(Figure 6)**.



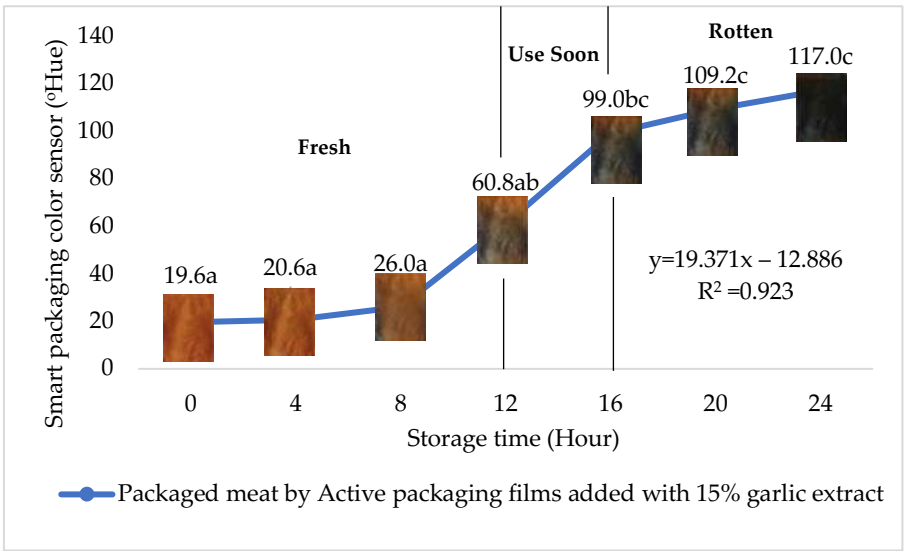
(a)



(b)



(c)



(d)

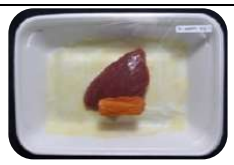
Figure 6. Changes in the color of the BTB solution (pH 2.75) as the smart packaging indicator reflecting the freshness of the meat samples packed with the active packaging films with **(a)** 0%; **(b)** 5%; **(c)** 10%; and **(d)** 15% of garlic extract.

During the entire storage period, the smart packaging indicator changed its color three times that corresponded to three phases of the meat samples' level of quality **(Figure 6)**. In phase I, its color was orange, indicating that the meat samples were still fresh. In phase II, its color was green with an orange hue, suggesting that the meat samples should be consumed immediately. In phase III, its color was dark green, denoting that the meat samples were already spoiled. The change in the indicator's color from orange to green indicated that the quality of the meat samples had deteriorated. The changes in the indicator's color were due to the interactions of alkaline volatile compounds produced by enzyme activity, and the metabolism of the microorganisms present in the meat samples increased with storage time. The early sign of spoilage was indicated by the release of volatile alkaline compounds as the microorganisms and the enzymes degraded the nutritional content of the meat samples. These compounds gradually accumulated in the packaging system, causing an increase in pH, which was detected by the smart packaging indicator and displayed as gradual color changes. The change in color of the smart

packaging indicator (BTB, pH 2.75) from orange to green was induced by deprotonation or the release of a proton from the smart packaging indicator dye [49].

The meat samples packaged with the active packaging films but with no (0%) and 5% garlic extract were still fresh from the start of the storage up to 8 h **(Figure 6)**. However, they must be immediately consumed from the 8th h to the 12th h of the storage period. Thereafter (12–24 h of the storage period), they were already spoiled. This results was consistent with that of TPC tests, which showed that the TPC values were above the acceptable threshold for microbial contaminants (1×106 or equivalent to 6 CFU/ml) in meat after 12 h. By comparison, the meat samples packaged with the active packaging films containing 10% and 15% garlic extract were still considered fresh from the start of the storage period up to the 12th h. They must be immediately consumed when they had been in storage for 12–16 h. Finally, they were considered rotten when they had been in storage for 16–24 h. This result was also consistent with that of TPC tests **(Figure 5)**, which indicated that at the 16th hour, the TPC value surpassed the permissible level of microbiological contamination in beef. Statistical analysis revealed that storage duration had a very significant effect on the Hue value, the indicator of color change in the smart packaging. The changes in the color of the smart packaging indicator (BTB solution, pH 2.75) when used together with the active packaging films to reflect the freshness of meat are presented in **Table 1**.

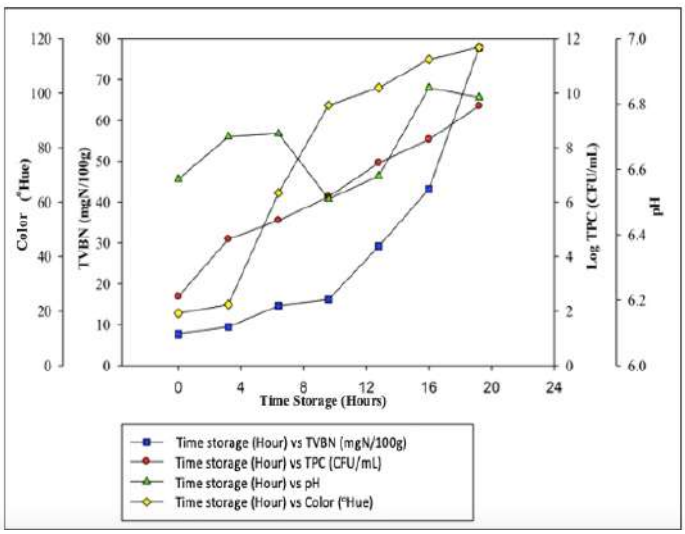
Table 1. Changes in the color of the smart packaging indicator (BTB solution, pH 2.75) when used together with the active packaging films to reflect the freshness of meat.

Storage Time (Hour)	Active Packaging Films Added with Garlic Extract			
	0%	5%	10%	15%
0				
4				
8				
12				
16				
20				

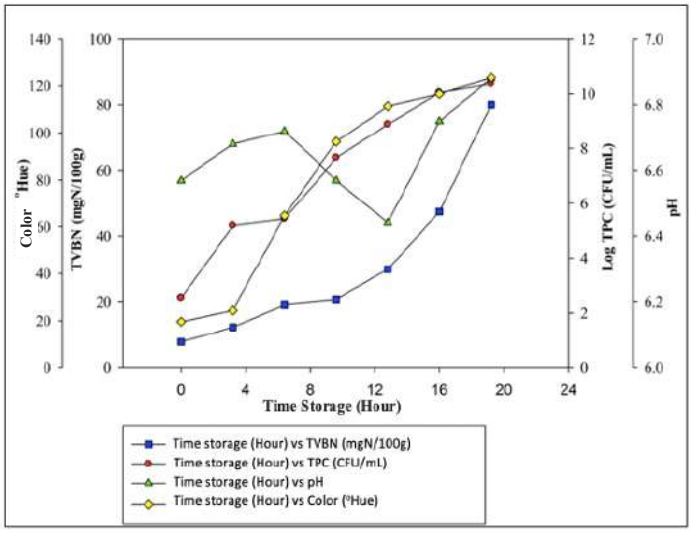
24				

3.6. Correlations between changes in the color of the smart packaging indicator and the effects of the active packaging films on the parameters of meat freshness

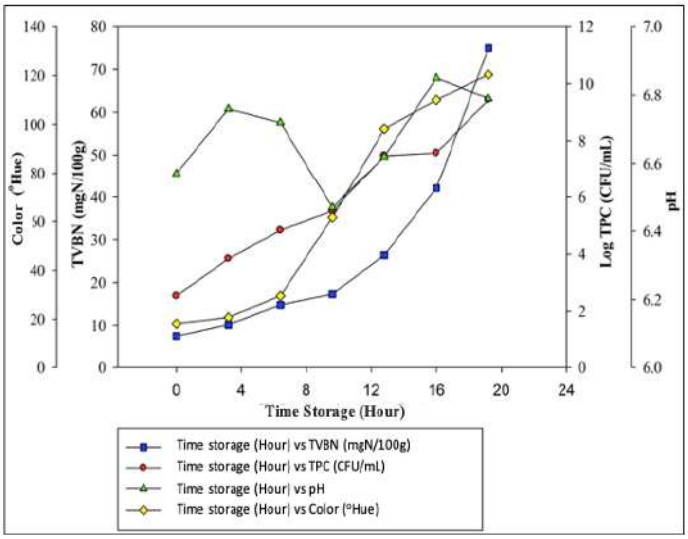
The correlations between changes in the color of the smart packaging indicator and parameters of meat quality deterioration (pH, TVBN, and TPC) were explored to ascertain the relationship between the sensitivity of the smart packaging indicator to meat freshness and the effectiveness of the active packaging films in slowing the process of meat spoilage.



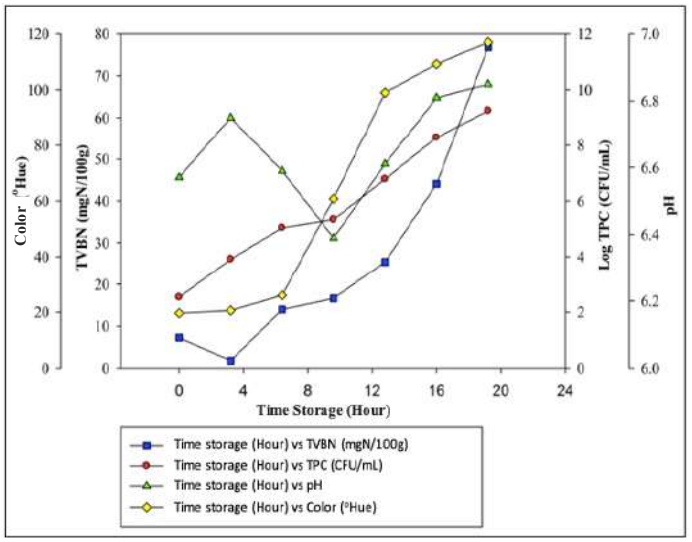
(a)



(b)



(c)



(d)

Figure 7. Correlations between changes in the color of the smart packaging indicator and the effects of the active packaging films with **(a)** 0%; **(b)** 5%; **(c)** 10%; and **(d)** 15% garlic extract on the parameters of quality deterioration of meat stored for 24 h.

The TPC and TVBN values of the meat samples treated with the active packaging films but without garlic extract increased, which was reflected by the change in the color of the smart packaging indicator **(Figure 7)**. The pH of the meat samples fluctuated not

only because of the production of volatile base compounds due to the activity of microorganisms in the samples during the storage period but also because of various factors, such as the contents of glycogen and lactic acid in the livestock prior to and after the slaughter. The meat samples treated with the active packaging films but without garlic extract and those treated with 5% garlic extract were rotten and unfit for consumption after 12 h of storage as their Log TPC value was 7.65±0.39 and 6.20±0.00 CFU/mL, respectively, and their TVBN value was 20.67±2.68 and 16.19±0.28 mgN/100g, respectively **(Figure 7)**. By comparison, the meat samples treated with the active packaging films and 10% and 15% garlic extract were rotten and unfit for consumption after 16 h of storage as their Log TPC value was 7.47±0.26 and 6.78±0.67 CFU/mL, respectively, and their TVBN value was 26.41±3.31 and 25.43±4.89 mgN/100 g, respectively.

The TPC values indicated the same level of quality deterioration as the TVBN values and reflected the changes in the color of the smart packaging indicator of the freshness of the meat samples treated with the active packaging films without (0%) and with 5% garlic extract; these samples were deemed rotten after 12 h of storage **(Figure 7)**. The color of the indicator changed from orange (fresh) with a color value of 19.6°Hue and 19.2°Hue to green (rotten) with a color value of 96.2°Hue and 95.6°Hue respectively. By comparison, the meat samples treated with the active packaging films and added with 10% and 15% garlic extract were deemed rotten after 16 h of storage; the color of the indicator changed from orange (fresh) with color values of 18.2°Hue and 19.6°Hue, respectively, to green (rotten) with color values of 98.2°Hue and 99°Hue, respectively. Wiryawan [50] observed that when garlic extract was added to the active packaging, the values of TPC and TVBN and the pH of the meat increased more slowly, as did the color of the smart packaging indicator, compared with those of the meat without the active packaging.

The increase in the values of TPC, TVBN, and pH of meat is strongly influenced by its high nutrient and water content, which is conducive to microbial contamination. Moreover, storing meat at room temperature accelerates bacterial growth. In general, an increase in the number of microorganisms is followed by an increase in the production of the volatile base chemical called TVBN. More volatile base compounds are released when more microorganisms are present and active, as indicated by the increase in TVBN value, which also has an effect on the meat's pH value. Furthermore, the increase in the values of TPC, TVBN, and pH linearly correlates with the increase in Hue value and color changes of the smart packaging indicator because the accumulated volatile base compounds raise the pH value of the packaging system, causing the smart packaging indicator to experience a color shift. This explanation was in agreement with that of Pacquit et al. [12], who applied active packaging films to cod fish. They stated that the increase in the TPC value of cod fish has a linear correlation with changes in the color of the cellulose-acetate packaging film sensor.

4. Conclusions

As a smart packaging indicator, the BTB solution (pH 2.75) produce color changes that are easy to observe and reflect three phases of meat quality deterioration. In phase I, its color was orange, indicating that the meat was still fresh. In phase II, its color was green with an orange hue, suggesting that the meat must be consumed immediately. In phase III, its color was dark green, denoting that the meat was already rotten and unfit for consumption. The meat samples treated with the active packaging films and added with 10% and 15% garlic extract rotted after 16 h, whereas the meat samples treated with the active packaging films and added with 0% and 5% garlic extract rotted after only 12 h. The changes in the color of the BTB solution was linearly and positively associated with the values of TPC, TVBN, and pH of the meat samples packaged with the active packaging films.

Author Contributions: Conceptualization, A.D; methodology, A.D. and I.K; software, A.D. and I.K; validation, A.D. and M.D.; formal analysis, A.D. and I.K.; investigation, I.K.; resources, A.D.; data curation, A.D. and I.K.; writing—original draft preparation, M.D. and I.K.; writing—review and editing, A.D., M.D., and I.K.; visualization, I.K.; supervision, Z.; project administration, A.D. and Z. All authors have read and agreed to the published version of the manuscript.

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2

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Journal name: Sensors
Manuscript ID: sensors-1516382
Type of manuscript: Article
Title: Application of a Smart Sensor and Active Packaging System Based on the Bacterial Cellulose of Acetobacter xylinum to Meat Products
Authors: Andi Dirpan *, Muspirah Djalal, Irma Kamaruddin
Received: 3 December 2021
E-mails: dirpan@unhas.ac.id, muspirah_djalal@agri.unhas.ac.id, irmakama9@gmail.com
Submitted to section: Biosensors, https://www.mdpi.com/journal/sensors/sections/biosensors
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Dear Dr. Dirpan,

Thank you again for your manuscript submission:

Manuscript ID: sensors-1516382
Type of manuscript: Article
Title: Application of a Smart Sensor and Active Packaging System Based on the Bacterial Cellulose of Acetobacter xylinum to Meat Products
Authors: Andi Dirpan *, Muspirah Djalal, Irma Kamaruddin
Received: 3 December 2021
E-mails: dirpan@unhas.ac.id, muspirah_djalal@agri.unhas.ac.id, irmakama9@gmail.com
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Do not hesitate to contact us if you have any questions regarding the revision of your manuscript. We look forward to hearing from you soon.

Kind regards,
Vickie Wang
Assistant Editor, MDPI
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Assistant Editor, MDPI
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Dear Dr. Dirpan,

Thank you very much for resubmitting the modified version of the following manuscript:

Manuscript ID: sensors-1516382
Type of manuscript: Article
Title: Application of a Smart Sensor and Active Packaging System Based on the Bacterial Cellulose of Acetobacter xylinum to Meat Products
Authors: Andi Dirpan *, Muspirah Djalal, Irma Kamaruddin
Received: 3 December 2021
E-mails: dirpan@unhas.ac.id, muspirah_djalal@agri.unhas.ac.id, irmakama9@gmail.com
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Thank you very much for providing the revised version of your paper:

Manuscript ID: sensors-1516382
Type of manuscript: Article
Title: Application of a Smart Sensor and Active Packaging System Based on the Bacterial Cellulose of Acetobacter xylinum to Meat Products
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E-mails: dirpan@unhas.ac.id, muspirah_djalal@agri.unhas.ac.id, irmakama9@gmail.com
Submitted to section: Biosensors, https://www.mdpi.com/journal/sensors/sections/biosensors
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We will continue processing your paper and will keep you informed about the status of your submission.

Kind regards,
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Revised version received
b. Revisions and Amends

sensors

Reviewer 1 : Round 1

Journal	Sensors (ISSN 1424-8220)
Manuscript ID	sensors-1516382
Type	Article
Title	Application of an Intelligent Sensor and Active Packaging System Based on the Bacterial Cellulose of Acetobacter Xylinum to Meat Products
Authors	Andi Dirpan * , Muspirah Djalal , Irma Kamaruddin
Section	Biosensors
Special Issue	Smart Materials and Technology for Biological and Medical Sensor Applications

Abstract

Combining smart and active packaging serves the dual purpose of detecting color changes in food that reflect changes in its quality and prolonging its shelf life. This study developed a smart and active packaging system made from the cellulose of Acetobacter xylinum and assessed its ability to detect changes in the quality of packaged fresh beef. The properties of the smart packaging's sensor and active packaging films were determined. The application of this system to fresh beef stored at room temperature (28±2 °C) for 24 h was tested. The color of the bromothymol blue (BTB) solution (pH 2.75) in the indicator of the smart packaging system changed from orange to dark green to indicate that beef quality changed from fresh to rotten. The meat treated with the active packaging with 10% and 15% garlic extract decayed on the 16th h. By contrast, the meat treated with the active packaging but without the garlic extracts rotted on the 12th h. The shift in the indicator's color was linearly related to the total plate count (TPC), total volatile basic nitrogen (TVBN) , and pH of the meat packaged using the active packaging system. Therefore, BTB solution (pH 2.75) can be used as a smart packaging indicator that will allow consumers to assess the quality of packaged meat easily. As an antimicrobial agent, the addition of 10%–15% garlic extract to the active packaging films can help delay the spoilage of packaged meat.

Review Report Form

English language and style
( ) Extensive editing of English language and style required
( ) Moderate English changes required
(x) English language and style are fine/minor spell check required
( ) I don't feel qualified to judge about the English language and style

	Yes	Can be improved	Must be improved	Not applicable
Does the introduction provide sufficient background and include all relevant references?	( )	(x)	( )	( )
Is the research design appropriate?	(x)	( )	( )	( )
Are the methods adequately described?	( )	(x)	( )	( )
Are the results clearly presented?	( )	( )	(x)	( )
Are the conclusions supported by the results?	( )	( )	(x)	( )

Comments and Suggestions for Authors

The experiments performed are really interesting. However, there is a lack of discussion and interpretation of the results in general.

Mayor revisions:

- Line 80 onwards. Authors refer to the benefits and potential use of bacterial cellulose compared to other plant cellulose. However, the films developed in this assay are not bacterial cellulose pure but use a 55% of other sources….Instead of highlighting the potential of this matrix, they should talk about the benefits of the composed polymers in food packaging.

- Described briefly the method employed for the determination of the best nitrogen source from reference [7]. Why do authors carry out that determination?

- The production of smart packaging in section 2.2.3.2. is inaccurate. Do some changes.
  - Line 126 What was the water content after the drying of the cellulose?
  - What was the amount of BTB added?
  - When do you know the indicator has been successfully absorbed? Does the film have to absorb all the BTB added?
  - What was the water content of the final film?

- The production of smart packaging in section 2.2.4.2. is inaccurate. Do some changes.

- How do you crush the cellulose? Why do you use other polysaccharide sources instead of just bacterial cellulose? The references have to be included at the beginning of the paragraph, mentioning if there or not have been modifications.

- “The bacterial cellulose was crushed to form a cellulose slurry, and all pretreatments were performed at room temperature” Please rewrite this sentence, the first part does not fit with the second part of the sentence.

- Section 2.2.3.2. Include the description of the change of color of the indicator, as can be seen in figure 1b. Substitute the indicator of that figure by the one in the experiment, as can be seen in table 1.

- Section 2.2.6.1. The section says “Measurement of the color of the smart packaging indicator” but then describes the color measurement of the meat and active packaging. Make the appropriate changes.

- Section 2.2.6.2., 2.2.6.3., 2.2.6.4. and 2.2.6.5. Have replicates been done? Include this information in each section.

- Section 2.2.6.4. Please, do some correction in the methodology. What is the total volume of the Conway dish? What is the volume of extract added? The sentence of lines 194-195 seems wrong. Does the reaction have any indicator? If reference 33 describes this procedure, please include it at the beginning of the paragraph.

- Section 2.2.6.5.. Include the volume of the sample used in the inoculation. n1 has the same description as d parameter. Make the appropriate changes.

- Figure 2. Include error bars. If the authors have the picture of the assays, it will be helpful to include them next to the graphic. Include p-value in the name of the figure

- Lines 239-242. This statement does not contribute to explaining the results. Eliminate it.

- Lines 257-258. Eliminate the storage information since it is specified in materials and methods section.

- Figures 3, 4 and 5. Figure 3. Include the error bars and the standard deviation in the text. The authors say in 2.2.3. section that these results have been studied statistically. Are those results significantly different? Include the agglomeration of the results and the p-value. If not, you can’t conclude the active packaging is having an effect as authors conclude in the different sections. Include the units of the storage time as h. Renamed the samples of the figure. Named the sample “Film without the addition of garlic extract” as control film. Explain it in the material and method section. Add just the % of garlic extract in the legend.

- Section 3.2. Lack of discussion.
  - Why pH was only measured at 7 h of storage since the experiment was carried out until 24 h? These results should be included since the figure expressed the storage for 24 h. Are the x-axis units correct?
  - Line 284: Are this measure done at 20 h or 24 h? If it is ok, the graphic should be named accordingly.
  - On line 264 authors say that the pH of the meat is normal (6.57) but in line 267 they say it is quite high. Please do the appropriate changes in this section. The description of the information in 269 to 283 is correct, but should be reorganized to a better comprehension. summarize the information as it does not provide any discussion of the data.
  - Line 284: which are those results? Please refer in the text the pH values to the type of samples for a better understanding. Have been comparable results been reported?
- Section 3.3. Lack of discussion.

- Renamed the samples on the figure. Named the sample “Film without the addition of garlic extract” as the control film. Explain it in the material and method section.
- Add just the % of garlic extract in the legend. Consider this change also in the text to make the lecture more fluid.

- Line 303. Is this classification reported elsewhere?

- Line 307. Which data do authors get these conclusions?

- Lines 314-324. summarize the information as it does not provide any discussion of the data.

- Section 3.4. Lack of discussion. According to the information exposed by the authors about the meet quality, (line 344), all samples present a very poor quality from the beginning of the experiment, so the results about the efficiency or not of the active packaging is not valid. The extending of shelf life on 4 h is not significant.
  - Line 352-359 is repetitive.

- Section 3.5. Lack of discussion
  - Line 417. This information is not correct since with 0% extract at 8h the samples are significantly different.
  - Line 421. The TPC values of data from the time 0 of the experiment exceed the 6 UFC/mL, so this data are not consistent.
  - There is something unclear in the results. In figure 6a, the value 64.4 is significantly different from 19.6 and 24.4. However, similar values are statistically the same in the rest of the figures. Are there any replicates of the measurements?

- Section 3.6. Lack of discussion. The aim of performing this correlation is not clear. The information included in this section is mainly the results of the previous analysis and does not contribute to the discussion of this section. Which variable has a better correlation with the colour changes? The correlation model is not described.

- Conclusion section: Total lack of conclusions. The statement described has been already commented on the previous section. Does the active packaging have an effect on preservation? Does the smart packaging have any effect on monitoring freshness? Is has any potential solution to the problems described in the introduction?

Minor revisions:

- Acetobacter xylinum must be written in italics. Please change it in the abstract and lines 81, 99 and 108. This correction has to be applied also on line 58, 70, 71, 97, 183, 237.
- Line 34. Change to ºC
- Line 96: substitute obtained by purchase.
- Unify the section 2.2.1 and 2.2.2. as production of bacterial cellulose from A. xylinum.
- Unify the section 2.2.3.1. and 2.2.3.2 as production of smart packaging
- Unify the section 2.2.4.1 and 2.2.4.2. as production of active packaging film
- Line 136, erase the ,

- Line 136…it was extracted *via* maceration, instead of maceration method. In that section, if reference [29] is describing the same procedure, you must include it at the beginning of the section.
- Line 153. Eliminate the information already included in line 96.
- Line 154. Rewrite the sentence.
- Line 154 Describe the sterile conditions.
- Line 171. Eliminate this information.
- Line 177 and 178: Substitute a and b by *a** and *b**. Include also in italics the CIELAB parameters on line 173.
- Line 178: the red-green color is not applicable to *b** Make the changes.
- Line 181. Include the reference [32] at the end of the sentence.
- Line 183. Correct the superscript.
- Line 187. Substitute the term “combined” by introduced, or extracted.
- Line 192: eliminate the , and the “
- Line 195 and 196: Correct the subscript.
- Line 203. Substitute Wis by W is and Fpis by Fp is. Change Fp by DF from dilution factor.
- Line 204. Substitute 14.07 by 14.007 as it is in the formula (2)
- Line 206: substitute number by amount.
- Line 218. Renamed the section
- Line 235: Substitute “was high”, by “increased”. Include at the end of the sentence “showing comparable antibacterial effects”, and eliminate this information at the end of the following paragraph, since is repetitive.

Submission Date 03 December 2021
Date of this review 19 Dec 2021 21:45:34

MATRICES OF AMENDMENTS FOR REVIEWER 1

Mayor revision

Comments and Suggestions for Authors and **Author’s responds**
1. Line 80 onwards. Authors refer to the benefits and potential use of bacterial cellulose compared to other plant cellulose. However, the films developed in this assay are not bacterial cellulose pure but use a 55% of other sources. Instead of highlighting the potential of this matrix, they should talk about the benefits of the composed polymers in food packaging. *Thank you for your input; we greatly appreciate it.* *We updated the section in accordance with your instructions. The basic thing to note is that we produce two types of packaging films: smart packaging films made entirely of bacterial cellulose and active packaging films made by combining bacterial cellulose with other ingredients (garlic extract as an antimicrobial agent; other polysaccharide ingredients) as well as glycerol to create a more elastic and firm film sheet).* 2. Described briefly the method employed for the determination of the best nitrogen source from reference [7]. Why do authors carry out that determination?

Thanks for the comment, we refer to reference [7] because the reference is the result of our research and publications that have been done previously. In this study, we used the results of that research as a formulation to create bacterial cellulose with good physical properties to be used as the basic material for making smart and active packaging

3. The production of smart packaging in section 2.2.3.2. is inaccurate. Do some changes.
   a. Line 126 What was the water content after the drying of the cellulose?
      Thank you for your suggestions, and thank you for reminding us to include it in our writing.
      The moisture content of dried bacterial cellulose ranges between 4-6%, this refers to the water content of paper in general.

   b. What was the amount of BTB added?
      The comment is very much appreciated, and thank you for reminding us to include it in our writing. We used 35ml BTB. Each 1 sheet of intelligent packaging film 1.5 cm × 4 cm was put into a 50ml centrifuge tube containing 35ml BTB, then centrifuged it at 3000 rpm for 15 min. Not all of the BTB indicator solution will be adsorbed into the smart packaging film, the absorption parameter is adjusted according to the absorption ability of the smart packaging film for every 15 minutes.

   c. When do you know the indicator has been successfully absorbed? Does the film have to absorb all the BTB added?
      Thank you for your feedback.
      Because not all of the BTB indicator solution is adsorbed into the smart packaging film, the absorption parameter is adjusted every 15 minutes based on the absorption ability of the smart packaging film. When the smart packaging film has an orange color that is visually uniform for each film, it indicates absorption success, and we determined this by measuring the color (^{o}Hue).

   d. What was the water content of the final film?
      Thank you for your comments. Unfortunately, we did not measure the film's final moisture content; the final parameter when drying the film is when the smart packaging film no longer leaves stains on the object's surface.

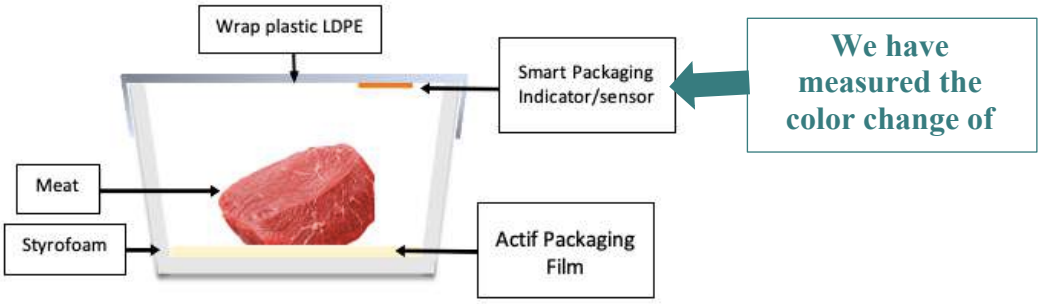
4. The production of smart packaging in section 2.2.4.2. is inaccurate. Do some changes.
   a. How do you crush the cellulose? Why do you use other polysaccharide sources instead of just bacterial cellulose? The references have to be included at the beginning of the paragraph, mentioning if there or not have been modifications.
      Thank you for your input. Using a food blender, we blend bacterial cellulose. The main point to emphasize is that we use 100 percent bacterial cellulose in the production of smart packaging films (without going through crushing). Meanwhile, crushing is done in the production of active packaging films so that the "garlic extract" can mix with the active packaging films. The use of other polysaccharides and materials is intended to re-glue bacterial cellulose, resulting in a flexible and sturdy film..

   b. “The bacterial cellulose was crushed to form a cellulose slurry, and all pretreatments were performed at room temperature” Please rewrite this sentence, the first part does not fit with the second part of the sentence.
      Thank you for your feedback. We have made the necessary changes in accordance with your instructions.

5. Section 2.2.3.2. Include the description of the change of color of the indicator, as can be seen in figure 1b. Substitute the indicator of that figure by the one in the experiment, as can be seen in table 1.

Thank you for your feedback. We have made the necessary changes in accordance with your instructions.

6. Section 2.2.6.1. The section says “Measurement of the color of the smart packaging indicator” but then describes the color measurement of the meat and active packaging. Make the appropriate changes.
   Thank you for your feedback. Please accept my apologies for the ambiguous sentence. In actuality, we measure the color change of smart packaging used in meat packaging.
   As is shown below:



7. Section 2.2.6.2., 2.2.6.3., 2.2.6.4. and 2.2.6.5. Have replicates been done? Include this information in each section.
   Thank you for your comment. We have made the necessary changes in accordance with your instructions.

8. Section 2.2.6.4. Please, do some correction in the methodology. What is the total volume of the Conway dish? What is the volume of extract added? The sentence of lines 194-195 seems wrong. Does the reaction have any indicator? If reference 33 describes this procedure, please include it at the beginning of the paragraph.
   Thank you for your comment. We have made the necessary changes in accordance with your instructions. In this study, we used a Conway dish with an outer diameter of 10 cm and an inner diameter of 5 cm, 1 ml of filtrate was used, and added with indicators of methyl red and bromothymol blue (2: 1).

9. Section 2.2.6.5.. Include the volume of the sample used in the inoculation. n1 has the same description as d parameter. Make the appropriate changes.
   Thank you for your comment. We apologize for our mistake. We have made the necessary changes in accordance with your instructions

10. Figure 2. Include error bars. If the authors have the picture of the assays, it will be helpful to include them next to the graphic. Include p-value in the name of the figure.
    Thank you for your feedback. We fixed it in accordance with your instructions. We value your input and understand your concerns about the use of data. Unfortunately, we do not have high-quality photo assays to include.

11. Lines 239-242. This statement does not contribute to explaining the results. Eliminate it.
    Thank you for your comment. We have made the necessary changes in accordance with your instructions.

12. Lines 257-258. Eliminate the storage information since it is specified in materials and methods section.

Thanks for the comment. We appreciate your comment. However, we do not discuss storage in this section.

13. Figures 3, 4 and 5. Figure 3. Include the error bars and the standard deviation in the text. The authors say in 2.2.3. section that these results have been studied statistically. Are those results significantly different? Include the agglomeration of the results and the p-value. If not, you can't conclude the active packaging is having an effect as authors conclude in the different sections. Include the units of the storage time as h. Renamed the samples of the figure. Named the sample "Film without the addition of garlic extract" as control film. Explain it in the material and method section. Add just the % of garlic extract in the legend.
    Thanks for your comprehensive views on the content, which made us to write our analyzes more clarified. we have added statistical test notation to our data. We apologize in advance that we need to change the figure into table to make it more convenience for reader, in this way all the data and statistical analysis can be presented in detail. We hope you understand and we hope changing this is one of best decision we made. And, we have designated "film without the addition of garlic extract" as the control film in our method.

14. Section 3.2. Lack of discussion.
    a. Why pH was only measured at 7 h of storage since the experiment was carried out until 24 h? These results should be included since the figure expressed the storage for 24 h. Are the x-axis units correct?
    Thank you for your feedback. We are grateful for your attention to the human error that we made. True, the experiment lasted for 24 hours. We apologize in advance that we need to change the figure into table to make it more convenience for reader, in this way all the data and statistical analysis can be presented in detail. We hope you understand and we hope changing this is one of best decision we made.

    b. Line 284: Are this measure done at 20 h or 24 h? If it is ok, the graphic should be named accordingly.
    Thanks for the comments. The pH measurement was completed at 24 hours of storage. There is insufficient writing in our explanation, so it does not appear to match the data presented on the graph. We've fixed it.

    c. On line 264 authors say that the pH of the meat is normal (6.57) but in line 267 they say it is quite high. Please do the appropriate changes in this section. The description of the information in 269 to 283 is correct, but should be reorganized to a better comprehension. summarize the information as it does not provide any discussion of the data.
    We appreciate your comment. Actually, at pH 6.57 is the pH at 0 hours of storage (categorized as normal). Looks like we made a typo. On your instructions, we have fixed it.

    d. Line 284: which are those results? Please refer in the text the pH values to the type of samples for a better understanding. Have been comparable results been reported?
    Thank you for your comment. We have made the necessary changes.

15. Section 3.3. Lack of discussion.
    a. Renamed the samples on the figure. Named the sample "Film without the addition of garlic extract" as the control film. Explain it in the material and method section.

Thank you for your comment. We have made the necessary changes in accordance with your instructions.

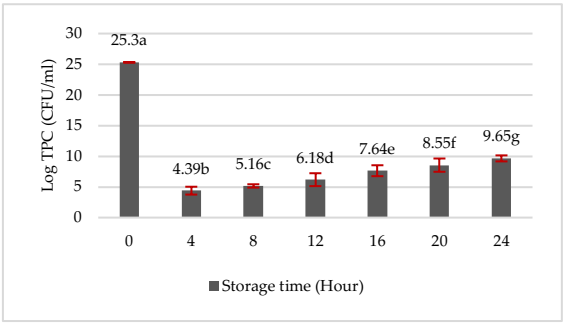
16. Add just the % of garlic extract in the legend. Consider this change also in the text to make the lecture more fluid.
    We appreciate your input and we have made the necessary changes in accordance with your instructions.

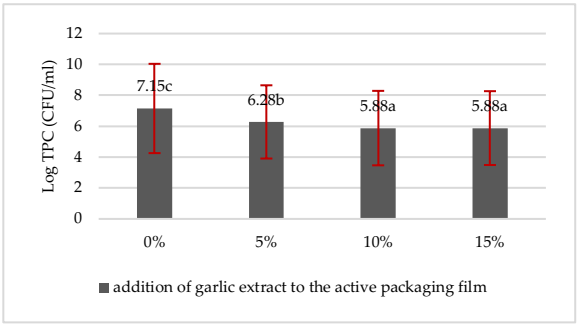
17. Line 303. Is this classification reported elsewhere?
    Thank you for your comment. The classification of fresh or rotten meat based on its TVBN value has been widely reported. For example, in the text we include several reports on the classification, namely "Beef or livestock is considered fresh if the TVBN value is less than 15 mg/100 g [44] or TVBN is <10 mg N/100 g [45]. Moreover, SNI 2354.8:2009 by National Standardization Agency of Indonesia (BSN) [46] states that the standard levels of TVBN fit for consumption is 20–30 mg N/100g.". Also, we have never had our data published in other journals.

18. Line 307. Which data do authors get these conclusions?
    Thank you for your input. We highly appreciate your attention to the data we have. The data is derived from the calculation of the increase in the value of TVBN (final value (categorized as rotten, 16 hours of storage) – initial (0 hours of storage) / beginning (0 hours of storage) x 100%. However, after noticing an error in writing the value, we corrected it.

19. Lines 314-324. summarize the information as it does not provide any discussion of the data.
    Thank you for your feedback. We fixed it in accordance with your instructions. We decided to remove annotations that had nothing to do with the data.

20. Section 3.4. Lack of discussion. According to the information exposed by the authors about the meet quality, (line 344), all samples present a very poor quality from the beginning of the experiment, so the results about the efficiency or not of the active packaging is not valid. The extending of shelf life on 4 h is not significant.
    Thank you for your concern in our data. Regrettably, we apologize for entering the incorrect graph in this section. The graph is data from TVBN values. Actually, our TPC chart is as follows.





Storage time (Hour)	Addition of garlic extract to the active packaging film				Average
	0%	5%	10%	15%	
0	2.53 ± 0.64	2.53 ± 0.64	2.53 ± 0.64	2.53 ± 0.64	2.53 ± 0.00[a]
4	5.18 ± 0.20	4.64 ± 0.16	3.83 ± 0.30	3.91 ± 0.10	4.39 ± 0.64[b]
8	5.43 ± 0.21	5.33 ± 0.07	4.83 ± 0.40	5.04 ± 0.06	5.16 ± 0.27[c]
12	7.65 ± 0.39	6.20 ± 0.00	5.51 ± 0.10	5.34 ± 0.08	6.18 ± 1.05[d]
16	8.89 ± 0.67	7.44 ± 0.03	7.47 ± 0.26	6.78 ± 0.67	7.64 ± 0.89[e]
20	10.04 ± 0.58	8.30 ± 1.35	7.57 ± 0.60	8.28 ± 0.06	8.55 ± 1.08[f]
24	10.36 ± 0.15	9.53 ± 0.39	9.44 ± 0.68	9.25 ± 0.03	9.65 ± 0.49[g]
Average	7.15 ± 2.89[c]	6.28 ± 2.37[b]	5.88 ± 2.41[a]	5.88 ± 2.39[a]	

a. Line 352-359 is repetitive.
Thank you for your feedback. We fixed it in accordance with your instructions.

21. Section 3.5. Lack of discussion

c. Line 417. This information is not correct since with 0% extract at 8h the samples are significantly different.
Thank you for your feedback. We highly appreciate your attention to the data we have. It is true that at 0-4 hours it was significantly different from the 8th hour (0% garlic extract). While the use of 5%, 10%, 15% garlic extract at 0-4 hours did not appear to be significantly different from storage at 8 hours. This shows that the use of 5%, 10%, 15% garlic extract can inhibit the acceleration of the increase in TPC. I hope you are satisfied and understand our answer.

d. Line 421. The TPC values of data from the time 0 of the experiment exceed the 6 UFC/mL, so this data are not consistent.
Thank you for your thoughtful comments and attention to our writing. We sincerely apologize for our mistake. In fact, we attached the incorrect TPC figure; we pinned the TVBN figure to Figure 5, which should have been a TPC figure. We corrected the error and placed the appropriate and correct data as a result of your input. We hope you are satisfied with our response. we have replaced with correct data and based on our tpc data, the meat we have used is fresh. We apologize in advance that we need to change the figure into table to make it more convenience for reader, in this way all the data and statistical analysis can be presented in detail. We hope you understand and we hope changing this is one of best decision we made.

e. There is something unclear in the results. In figure 6a, the value 64.4 is significantly different from 19.6 and 24.4. However, similar values are statistically the same in the rest of the figures. Are there any replicates of the measurements?
Sorry for your inconvenience. The color measurement was repeated 5 times with the same sample. In figure 6a, it is true that the value of 64.4 ^{o}Hue at the 8th hour is significantly different from the values of 19.6 and 24.4 oHue at the 0th and 4th hour storage. We hope you are satisfied with our answers and we are happy to accept if there are still revisions for the sake of our writing. Here we attach the Duncan test results for 0% garlic extract.

indicator_color_(0% Garlic Ex) HUE

Duncan[a,b]

Time storage (Hour)	N	Subset 1	Subset 2	Subset 3
0	5	23.0000		
4	5	24.4000		
8	5		64.6000	
12	5		96.2000	96.2000
16	5			111.4000
20	5			116.6000
24	5			123.4000
Sig.		.938	.088	.176

Means for groups in homogeneous subsets are displayed.

22. Section 3.6. Lack of discussion. The aim of performing this correlation is not clear. The information included in this section is mainly the results of the previous analysis and does not contribute to the discussion of this section. Which variable has a better correlation with the colour changes? The correlation model is not described.
Thank you for your feedback. Based on the analysis of all data, we can conclude that the change in color of the smart packaging indicator, as indicated by an increase in the Hue value, corresponds to an increase in the value of the meat deterioration parameters (TPC and TVBN). We have concluded this discussion, and I hope you are satisfied with the results.

23. Conclusion section: Total lack of conclusions. The statement described has been already commented on the previous section. Does the active packaging have an effect on preservation? Does the smart packaging have any effect on monitoring freshness? Is has any potential solution to the problems described in the introduction?
Thank you for your feedback. Based on your guidance to us in revising this paper, we have updated this conclusion. We hope it meets your expectations.

Minor revision

Comments and Suggestions for Authors and **Author's responds**
Acetobacter xylinum must be written in italics. Please change it in the abstract and lines 81, 99 and 108. This correction has to be applied also on line 58, 70, 71, 97, 183, 237. *Thank you for your feedback. We have made the necessary changes in accordance with your instructions.* Line 34. Change to ºC *Thanks for the comments. We've fixed it.*

Line 96: substitute obtained by purchase.
Thanks for the comments. We've fixed it.

Unify the section 2.2.1 and 2.2.2. as production of bacterial cellulose from A. xylinum.
Thank you for your feedback. We have made the necessary changes in accordance with your instructions

Unify the section 2.2.3.1. and 2.2.3.2 as production of smart packaging
Thank you for your feedback. We have made the necessary changes in accordance with your instructions

Unify the section 2.2.4.1 and 2.2.4.2. as production of active packaging film
Thank you for your feedback. We have made the necessary changes in accordance with your instructions

Line 136, erase the ,
Thanks for the comments. We've fixed it.

Line 136…it was extracted *via* maceration, instead of maceration method. In that section, if reference [29] is describing the same procedure, you must include it at the beginning of the section.
Thanks for the comments. We've fixed it.

Line 153. Eliminate the information already included in line 96.
Thank you for your feedback. We fixed it in accordance with your instructions

Line 154. Rewrite the sentence.
Thank you for your feedback. We fixed it in accordance with your instructions

Line 154 Describe the sterile conditions.
Thank you for your feedback. We fixed it in accordance with your instructions

Line 171. Eliminate this information.
Thanks for the comments. We've fixed it.

Line 177 and 178: Substitute a and b by *a** and *b**. Include also in italics the CIELAB parameters on line 173.
Thanks for the comments. We've fixed it.

Line 178: the red-green color is not applicable to *b** Make the changes.
Thanks for the comments. We've fixed it.

Line 181. Include the reference [32] at the end of the sentence.
Thanks for the comments. We've fixed it.

Line 183. Correct the superscript.
Thanks for the comments. We've fixed it.

Line 187. Substitute the term “combined” by introduced, or extracted.
Thanks for the comments. We've fixed it.

Line 192: eliminate the , and the “
Thanks for the comments. We've fixed it.

Line 195 and 196: Correct the subscript.
Thank you for your feedback. We fixed it in accordance with your instructions.

Line 203. Substitute Wis by W is and Fpis by Fp is. Change Fp by DF from dilution factor.
Thank you for your feedback. We fixed it in accordance with your instructions.

Line 204. Substitute 14.07 by 14.007 as it is in the formula (2)
Thank you for your feedback. We fixed it in accordance with your instructions.

Line 206: substitute number by amount.
Thank you for your feedback. We fixed it in accordance with your instructions.

Line 218. Renamed the section
Thank you for your feedback. We fixed it in accordance with your instructions.

Line 235: Substitute “was high”, by “increased”. Include at the end of the sentence “showing comparable antibacterial effects”, and eliminate this information at the end of the following paragraph, since is repetitive.
Thank you for your feedback. We fixed it in accordance with your instructions.

sensors

Reviewer 2 : Round 1

Journal	Sensors (ISSN 1424-8220)
Manuscript ID	sensors-1516382
Type	Article
Title	Application of an Intelligent Sensor and Active Packaging System Based on the Bacterial Cellulose of Acetobacter Xylinum to Meat Products
Authors	Andi Dirpan * , Muspirah Djalal , Irma Kamaruddin
Section	Biosensors
Special Issue	Smart Materials and Technology for Biological and Medical Sensor Applications

Abstract

Combining smart and active packaging serves the dual purpose of detecting color changes in food that reflect changes in its quality and prolonging its shelf life. This study developed a smart and active packaging system made from the cellulose of Acetobacter xylinum and assessed its ability to detect changes in the quality of packaged fresh beef. The properties of the smart packaging's sensor and active packaging films were determined. The application of this system to fresh beef stored at room temperature (28±2 °C) for 24 h was tested. The color of the bromothymol blue (BTB) solution (pH 2.75) in the indicator of the smart packaging system changed from orange to dark green to indicate that beef quality changed from fresh to rotten. The meat treated with the active packaging with 10% and 15% garlic extract decayed on the 16th h. By contrast, the meat treated with the active packaging but without the garlic extracts rotted on the 12th h. The shift in the indicator's color was linearly related to the total plate count (TPC), total volatile basic nitrogen (TVBN) , and pH of the meat packaged using the active packaging system. Therefore, BTB solution (pH 2.75) can be used as a smart packaging indicator that will allow consumers to assess the quality of packaged meat easily. As an antimicrobial agent, the addition of 10%–15% garlic extract to the active packaging films can help delay the spoilage of packaged meat.

Review Report Form

English language and style

( ) Extensive editing of English language and style required
( ) Moderate English changes required
( ) English language and style are fine/minor spell check required
(x) I don't feel qualified to judge about the English language and style

	Yes	Can be improved	Must be improved	Not applicable
Does the introduction provide sufficient background and include all relevant references?	( )	( )	(x)	( )
Is the research design appropriate?	( )	( )	(x)	( )
Are the methods adequately described?	( )	( )	(x)	( )
Are the results clearly presented?	( )	( )	(x)	( )
Are the conclusions supported by the results?	( )	( )	(x)	( )

Comments and Suggestions for Authors

The paper « Application of a Smart Sensor and Active Packaging System Based on the Bacterial Cellulose of Acetobacter xylinum to Meat Products" by Dirpan et al., presents application of bacterial cellulose for active and smart packaging.

- The novelty of the paper is not clear. Is it the utilization of th eBTB solution?
- This is not the first paper presenting the application of cellulose of Acetobacter xylinum for food packaging, nor the first paper on garlic aa an antimicrobial, nor the first paper presenting a combination of smart (intelligent) and active packaging.
- The aim off the study should be clearly presented in the last paragraph of Introduction.
- "Smart" packaging should be changed to "intelligent" packaging. Check recent reviews on smart packaging (Omerovic et al., Compr. Rev. Food Sci FooD Sec, 2021; or Nikolic et al., Trends Food Sci Tech, 2021)
- Bacterial names should be in Italic throught the whole manuscript
- The number of titles should be reduced in Materials and Methods. No need to have so many sob-titles
- What was the negative control in Fig. 2? What diameter was obtained with cellulose alone?
- How many times experiment in Fig 2 was done? Statistic should be added.
- Why there is a decrease at 4h in Fig 3? Statistics should be added in Fig 3
- It looks like there is no difference in Fig 4, all values are similar at a given time. How many time this experiment was performed?
- Idem for Fig 5
- Fig6 (a to d) should be presented in a better way to enable visualization of all results on the same page.
- The whole manuscript should be rewritten in a more concise way. Actual form is not focused on the message

Submission Date 03 December 2021
Date of this review 17 Dec 2021 15:41:37

MATRICES OF AMENDMENTS FOR REVIEWER 1

Comments and Suggestions for Authors and **Author's responds**
1. The novelty of the paper is not clear. Is it the utilization of the BTB solution? *Thank you for the comment. We highly respect your comment and understand your concerns about the novelty. Our novelty is to create smart and active packaging from the same main ingredient, namely bacterial cellulose which we produce ourselves, and we combine smart and active packaging or combine them together in one packaging system. Furthermore, smart and active packaging are typically studied separately by other researchers, including us in previous studies. Then, using our new innovation, we combined the two into a single packaging system made of the same main ingredient, bacterial cellulose. Furthermore, the method we use is a modified and low-cost method that, hopefully, can be applied commercially. Meanwhile, we must acknowledge that BTB is not a novel material, as there have been numerous smart packaging studies comparing BTB to other dye solutions in the production of smart packaging. We hope this answer satisfies you.* 2. This is not the first paper presenting the application of cellulose of Acetobacter xylinum for food packaging, nor the first paper on garlic aa an antimicrobial, nor the first paper presenting a combination of smart (intelligent) and active packaging. *Thanks for the comment and sorry for any inconvenience. We acknowledge that research on bacterial cellulose for food packaging, garlic extract as an antimicrobial, or combining smart and active packaging is not the first research in this world. In today's modern era, researchers have been given convenience by the studies that have been found in the last few decades; thus, as a form of novelty, we update existing research by broadening its scope so that it can be applied in practice. As a result, we create smart and active packaging from the same main ingredient, bacterial cellulose, where the smart indicator in smart packaging uses BTB and microbial active compounds in active packaging use garlic extract, and then we combine them all as a single packaging system for meat. We hope you are pleased with our response.* 3. The aim off the study should be clearly presented in the last paragraph of Introduction. *Thanks for your comment. Actually, we stated the goal in the last paragraph of our introduction "this study aimed to maximize the potential of smart and active packaging by combining them into a single packaging system based on a bacterial cellulose membrane biopolymer to enhance the quality of packaged meat and help consumers to determine meat freshness easily."* 4. "Smart" packaging should be changed to "intelligent" packaging. Check recent reviews on smart packaging (Omerovic et al., Compr. Rev. Food Sci FooD Sec, 2021; or Nikolic et al., Trends Food Sci Tech, 2021) *Thank you for your help. We have corrected it in accordance with your instructions.* 5. Bacterial names should be in Italic throught the whole manuscript. *Thanks for your comment. We have corrected it.* 6. The number of titles should be reduced in Materials and Methods. No need to have so many sob-titles *We appreciate your input, and we have fixed it.* 7. What was the negative control in Fig. 2? What diameter was obtained with cellulose alone?

Thank you for your feedback. On fig. 2, our negative control is a cellulose film without the addition of garlic extract, and no inhibition zone was formed. Each film has a consistent diameter of 5mm.

8. How many times experiment in Fig 2 was done? Statistic should be added.
   Thank you for your feedback. The experiment was carried out three times. Statistics have been added.

9. Why there is a decrease at 4h in Fig 3? Statistics should be added in Fig 3.
   Thank you for your feedback. After the animal dies, the blood flow that supplies oxygen to the muscles stops causing an anaerobic glycolysis process to occur. During anaerobic glycolysis, glycogen conversion occurs in the muscles to lactic acid which accumulates in the tissues, causing the pH of the meat to decrease (4th hour storage), during anaerobically glycolysis, the decrease in pH continues until the glycogen is converted to lactic acid followed by the neutralization of alkaline compounds resulting from the metabolism of microorganisms, so that the pH of the meat rises again (storage hours 16-24). Also, we have added statistics. We apologize in advance that we need to change the figure into table to make it more convenience for reader, in this way all the data and statistical analysis can be presented in detail. We hope you understand and we hope changing this is one of best decision we made.

10. It looks like there is no difference in Fig 4, all values are similar at a given time. How many time this experiment was performed?
    Thank you for your responses. The TVBN value of the control sample was higher than that of the active packaging film sample. The process was repeated three times. We have added statistics to make the appearance of the figure more understandable. We apologize in advance that we need to change the figure into table to make it more convenience for reader, in this way all the data and statistical analysis can be presented in detail. We hope you understand and we hope changing this is one of best decision we made.

11. Idem for Fig 5
    We appreciate your comments and apologize for the technical error.
    Actually, the figure we pinned on Figure 5 was a mistake; we pinned the TVBN figure on Figure 5 instead of the TPC figure. We have corrected this error and attached the appropriate and correct data for your correction. We apologize in advance that we need to change the figure into table to make it more convenience for reader, in this way all the data and statistical analysis can be presented in detail. We hope you understand and we hope changing this is one of best decision we made. We hope you are satisfied with our response.

12. Fig6 (a to d) should be presented in a better way to enable visualization of all results on the same page.
    Thank you for your feedback. Your suggestion is very important to us, and we have addressed it.

13. The whole manuscript should be rewritten in a more concise way. Actual form is not focused on the message
    Thank you for your thoughtful comments and suggestions.
    We have updated several sections to make it easier for readers to understand, thanks to your attention to the improvement of our writing.

4
Revised version received
c. Revised version with highlights

sensors

MDPI

Article

Application of a Intelligent Sensor and Active Packaging System Based on the Bacterial Cellulose of *Acetobacter xylinum* to Meat Products

Andi Dirpan*, Muspirah Djalal and Irma Kamaruddin

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Abstract: Combining intelligent and active packaging serves the dual purpose of detecting color changes in food that reflect changes in its quality and prolonging its shelf life. This study developed a intelligent and active packaging system made from the cellulose of *Acetobacter xylinum* and assessed its ability to detect changes in the quality of packaged fresh beef. The properties of the intelligent packaging's sensor and active packaging films were determined. The application of this system to fresh beef stored at room temperature (28±2 °C) for 24 h was tested. The color of the bromothymol blue (BTB) solution (pH 2.75) in the indicator of the intelligent packaging system changed from orange to dark green to indicate that beef quality changed from fresh to rotten. The meat treated with the active packaging with 10% and 15% garlic extract decayed on the 16th h. By contrast, the meat treated with the active packaging but without the garlic extracts rotted on the 12th h. The shift in the indicator's color was linearly related to the total plate count (TPC), total volatile basic nitrogen (TVBN) , and pH of the meat packaged using the active packaging system. Therefore, BTB solution (pH 2.75) can be used as a intelligent packaging indicator that will allow consumers to assess the quality of packaged meat easily. As an antimicrobial agent, the addition of 10%–15% garlic extract to the active packaging films can help delay the spoilage of packaged meat.

Keywords: Intelligent Sensor; Intelligent packaging; active packaging; Bacterial Cellulose; Meat

Citation: Lastname, F.; Lastname, F.; Lastname, F. Title. *Sensors* **2021**, *21*, x. https://doi.org/10.3390/xxxxx

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1. Introduction

Global beef consumption is predicted to rise as the world population and family income increase, particularly in developing Asian countries [1–3]. By 2030, worldwide meat consumption and availability are expected to increase by 14% and 5.9%, respectively, over the average of the 2018–2020 period [3]. Thus, the expected increase in meat consumption must be complemented by improvements in the quality of fresh meat produced. One aspect affecting the quality and characteristics of meat is the material and packaging technologies used [4]. Meat is a perishable item that rapidly spoils when stored above the optimum temperature range (below -17–4 °C) [5,6]. However, in traditional markets, meat is displayed at room temperature without packaging, a practice that might accelerate microbial contamination and cause rapid quality degradation. Even in supermarkets where meat is maintained in cold temperatures, standard meat packaging still prevent consumers from subjectively determining the quality of meat. Thus, meat packaging must have additional functions that will prevent quality degradation due to microbial contamination and will help consumers to determine the quality of packaged meat easily [7]. Conventional meat packaging can be designed to perform dual functions through intelligent and active packaging systems.

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Intelligent packaging is a term that refers to sensors in the form of indicators that monitor and provide information on the quality of the food contained within the packaging via color changes caused by chemical reactions between the indicators and the products of microbial metabolism or changes in the chemical composition of the food [8,9]. During storage, the chemical components of meat degrade into volatile compounds because of microbial activity, thereby increasing the value of total volatile base nitrogen (TVBN) [10,11]. Accumulation of TVBN increases the pH of the packaging system, which is detected by the indicator, resulting in a visible color shift in the indicator [11,12]. Intelligent packaging allows easier monitoring of packed products during transportation and storage [7]. Moreover, it provides a more accurate estimate of product condition than conventional expiration labels [12]. Color-based pH indicator solutions are widely used as intelligent indicators. Dirpan et al. [13] developed bromophenol blue as a intelligent indicator dye for mangoes. Hidayat et al. [14] used two types of color indicators with predetermined concentrations, namely, phenol red and bromothymol blue, to assess the freshness of meat packaging. Intelligent packaging indicators based on natural pigments are being developed, such as intelligent packaging films that include anthocyanin-loaded Lycium ruthenicum nanocomplexes in starch/polyvinyl alcohol mixtures (PVA) [15], as well as anthocyanins from saffron petals immobilized in chitosan nanofibers and methyl cellulose matrix [16].

Active packaging refers to the integration of particular additives into a packaging system for the purpose of extending the shelf life, preserving the quality, and ensuring the safety of food products. Antimicrobial agents are used as components of active packaging additives to extend product shelf life. The volatile bioactive compounds in active packaging evaporate or diffuse onto the food surface, where they limit the growth of pathogenic microbes and thus delay spoilage [17,18]. This strategy is more effective than coating bioactive compounds onto the food surface [19]. The safest, cheapest, and most readily available antimicrobial agents for use in active packaging are essential oils. Pranoto et al. [20] produced antimicrobial alginate edible films by incorporating the essential oils of garlic. They reported that these films substantially inhibited the growth of Staphylococcus aureus and Bacillus cereus in meat. Vishnu et al. [21] utilized the essential oils of Plectranthus amboinicus in a chitosan-based active packaging to restrict antimicrobial activity.

Intelligent and active packaging can be merged into a single packaging system. Julyaningsih et al. [22] combined a intelligent packaging system based on methyl red–bromothymol blue (BTB) indicator with an active packaging system based on lemongrass oil as a component of tuna fish fillet packaging. Yao et al. [23] developed an active and intelligent packaging system based on starch, PVA, and betacyanins from various types of plants for shrimp packaging. In general, an active packaging that contains antimicrobial agents and a intelligent packaging that contains indicator solutions are immobilized in a polymer. Compared with synthetic polymers or plant cellulose, the bacterial cellulose fermented by *Acetobacter xylinum* has a unique nanofibrillar structure and superior physical properties, suggesting that it has the potential to serve as a basis for developing a intelligent and active packaging system [24,25]. Bacterial cellulose has received interest as a component of active packaging owing to its biodegradability, high water-holding capacity so that it can be employed entirely as a polymer for immobilizing color solutions in intelligent packaging indicators [26]. Besides, bacterial cellulose possess great potential as an antimicrobial agent carrier in order for it to be utilized as an ingredient in the production of active packaging films [27].

The development of packaging systems with additional functions is advancing. To promote this innovation, this study aimed to maximize the potential of intelligent and active packaging by combining them into a single packaging system based on a bacterial cellulose membrane biopolymer to enhance the quality of packaged meat and help consumers to determine meat freshness easily.

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2. Materials and Methods

2.1. Materials

The main ingredients used in the intelligent and active packaging system developed herein were the bacterial cellulose produced by *A. xylinum*, which was fermented in natural media of coconut water. Beef tenderloin was purchase from a slaughterhouse in Tamangapa Raya. Coconut water and garlic (*Allium sativum*) were purchased from a local market. Food-grade ammonium sulfate (CAS Number: 7783-20-2), yeast extract (Merck, CAS Number: 8013-01-2), 96% acetic acid (Brenntag Inc, CAS No: 64-19-7), *A. xylinum* culture, 5% 1 N NaOH (Brenntag Inc, CAS No: 1310-73-2), sucrose, Bromothymol blue (BTB, Merck, CAS No: 76-59-5), alcohol, aquabides, aquades, Tashiro's indicator (0.1% methyl red and 0.1% BTB at a ratio of 2:1), 7% trichloroacetic acid (TCA, Merck), nutrient Agar (NA, Merck), glycerol (Merck, CAS No: 56-81-5), food-grade carboxymethyl–cellulose (CMC) (Foodchem, E466), and corn starch were used.

2.2. Methods

2.2.1. production of bacterial cellulose from A. xylinum

Based on our previous research Dirpan et al. [7], 5% (w/v) of food grade Ammonium Sulfate is the best source of Nitrogen in ***Acetobacter xylinum*** growth media to produce optimal bacterial cellulose membranes. Determination of the composition and type of Nitrogen source, Then, **purification of bacterial cellulose** by removed from the fermentation medium, rinsed in running water, and then soaked for 2 days with periodic water changes. The cellulose was also soaked in 70% alcohol for 1 min, heated to 100 °C in distilled water for 20 min, and reheated in 1 N 5% NaOH solution at 100 °C for 60 min to remove the remaining bacterial cells and substrate attached to the cellulose layer. Afterward, the cellulose was rinsed with running water and soaked in periodically changed water for 24 h until pH reached 7. The purified cellulose appeared transparent [7].

2.2.2. *Production of intelligent packaging*

First, preparation of the indicator solution. BTB indicator solution was chosen for this study because a previous work established this solution as the indicator with the most visually identifiable color change reaction [7]. First, 1% BTB solution (b/v) was prepared in 95% ethanol. Then, the pH of the BTB solution was decreased to 2.74 by adding 20% acetic acid. Finally, the BTB solution was stored in a closed container. *Second*, p**roduction of** intelligent **packaging indicator label**. The purified cellulose film was kept in a filter cloth for 24 h to decrease its water content. Half-dried cellulose was cut into 1.5 cm × 4 cm strips and pushed flat against the surface of a Pyrex glass. The cellulose was dried for 30 min at 70 °C until the moisture content reaches 6 percent. 35ml of The BTB indicator solution was then absorbed into a dry cellulose via centrifugation at 3000 rpm for 15 min. When the color indicator was successfully absorbed, the BTB indicator solution imparted an orange hue to the cellulose. Afterward, the cellulose was rinsed with distilled water to eliminate any unbound color indicators and then dried [26,28].

2.2.3. *production of active packaging film*

First,production of garlic extract as active element. The method applied in this research referred to [29] with a slight modification. garlic 500 g was peeled, washed under running water until clean, drained, and then mashed. The minced garlic was extracted via the maceration method by immersing the finely ground garlic in 96% alcohol at a ratio of 1:4 (garlic:alcohol) for 4 days at 3-5 °C and periodically homogenized using a water bath shaker. Afterward, the extract was filtered using a filter paper and then concentrated using a rotary evaporator at 50 rpm at 40 °C to obtain a thick extract. Second, p**roduction of active packaging film**. The method used referred to [19,30] with a slight modification. The bacterial cellulose was crushed to form a cellulose pulp. A cellulose suspension was prepared using 30% chitosan (w/w), 10% CMC (w/w), and 15% corn starch (w/w) of cellulose dry

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weight. The suspension was heated at 50 °C for 60 min with a hot plate stirrer until thoroughly suspended. At the 50th min, 30% glycerol (w/w) was added. Additionally, the garlic extract was added at quantities of 0% (as the control), 5%, 10%, and 15% (v/v) immediately after the final heating step. Subsequently, 60 g of the suspension was then placed onto a glass plate and dried for 48 h at 37 °C. Finally, the suspension was cooled to room temperature, removed from the glass plate, wrapped in aluminum foil, and placed in a desiccator.

2.2.4. Application of the intelligent and active packaging indicators to fresh beef

Fresh beef tenderloin was collected from a slaughterhouse in Tamangapa Raya Makassar 1 h after the cow was slaughtered. It was immediately placed in a special food box and put into a 38 cm × 29 cm × 30 cm Styrofoam box filled with ice crystals. The samples were promptly transported to the laboratory and processed into 200 g/pack pieces under sterile conditions (Meat containers must be sterilized in an autoclave at 121 °C, 1 atm for 15 minutes before use). The meat was packaged in a Styrofoam tray (1.05 g/cm³) coated with the active packaging film on a Styrofoam base, and a intelligent packaging indicator label was attached to the LDPE plastic wrap film that covered the Styrofoam container (**Figure 1**). The samples were maintained at room temperature (28±2 °C) with normal light exposure for 24 h.

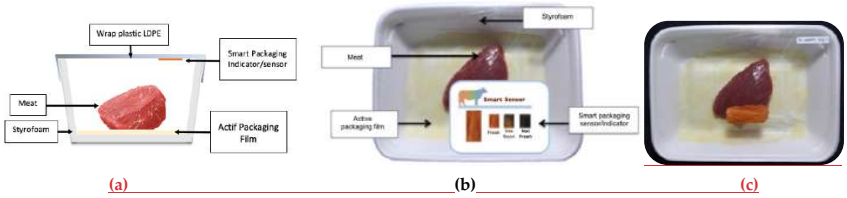

Figure 1. (a) Design of the intelligent and active packaging system; **(b)** prototype of the intelligent packaging ; **(c)** and its application to fresh meat.

During the entire storage period, the intellignet packaging label changed its color three times that corresponded to three phases of the meat samples' level of quality (**Figure 1b**). In phase I, its color was orange, indicating that the meat samples were still fresh. In phase II, its color was green with an orange hue, suggesting that the meat samples should be consumed immediately (Use soon). In phase III, its color was dark green, denoting that the meat samples were already spoiled (Not Fresh).

2.2.5. Observation parameters

2.2.5.1. Measurement of Intelligent Packaging Indicator Color Response on Meat

The color of the intelligent packaging indicators was quantitatively determined using a chromameter digital color meter (T-135). Intelligent and active packaging system containing meat is placed on a flat black background with homogeneous lighting. The chromameter detector was placed on the surface of the intelligent packaging indicator. The measurement results were expressed according to the notation of the Hunter's Lab Colorimetric System, which is presented in three values, namely L^* (lightness), a^* (redness), and b^* (yellowness) [31]. The color of the intelligent packaging indicator was determined by calculating the °Hue value by using the formula (1) below:

$$°\boldsymbol{Hue} = \boldsymbol{tan^{-1}}\frac{\boldsymbol{b^*}}{\boldsymbol{a^*}} \quad (1)$$

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where °Hue represents the parameters for color range, a^* is a red-green mixed color, and b^* is a yellow-blue mixed color.

2.2.5.2. Antimicrobial activity of the active packaging films

The antimicrobial activity of the active packaging films was determined via the agar diffusion method. Each active packaging film was cut into 5 mm circles in a sterile environment and then placed on NA agar media with 0.1 ml of the test microorganism culture (*Staphylococcus aureus*) containing 106 CFU/ml. Petri dishes were incubated for 24 h at 37 °C. After incubation, the inhibitory zone was measured using a caliper, this measurement was carried out with three replications [32].

2.2.5.3. Determination of pH of the beef samples

The pH of the beef samples was measured using a pH meter (Oakton pH 510). First, 5 g of crushed meat was introduced with 45 ml of distilled water until the mixture became homogenous. The pH meter's electrode was then immersed in the beef suspension until the pH value on the monitor became constant. This measurement was carried out with three replications.

2.2.5.4. Measurement of TVBN

Method applied in this research referred to AOAC [33]. A Conway cup with an outer diameter of 10 cm and an inner diameter of 5 cm was utilized in this study. First, 30 ml of 7% TCA solution was added to a meat sample (10±0.1 g) and mixed before filtering. 1 ml boric acid solution was placed in the "inner chamber" of the Conway dish. The lid of the cup was placed in such a way that it almost covered the cup. The 1ml filtrate was placed into the outer chamber of the Conway dish. Afterward, 1 ml saturated K_2CO_3 solution was put into the outer chamber. The cup was closed and rotated to mix the two liquids in the outer chamber. The blank solution was prepared following the same process but with 7% TCA instead of the filtrate. The solutions were stored at 37 °C for 2 h. Then, 2 drops of methyl red and bromothymol blue (2:1) were added to the inner conway cup and then titrated with 0.01 N HCl until a pink hue was formed. TVBN was calculated by formula (2) as follows:

$$\textbf{TVBN content}\ \left(\frac{mg}{100g}\right) = \frac{(Vc-Vb)\ x\ 14.007\ x\ df\ x\ 100}{W} \quad (2)$$

where Vc is the volume of the HCl solution used in sample titration, Vb is the volume of the HCl solution used in blank titration, N is the normality of the HCl solution, W is the sample's weight (g), 14.007 is the molecular weight of nitrogen, and df is the dilution factor. This measurement was carried out with three replications.

2.2.5.5. Measurement of Total plate count

The total amount of microorganisms was determined via the total plate count (TPC) method described in SNI 2332.3: 2015. First, 1 g of the sample was added to a test tube containing 9 ml of physiological solution until homogeneous (10^{-1} dilution). The dilution was continued until 10^{-6}, at which point 1ml of the diluted sample was inoculated on NA media in duplicate via the pour plate technique. After the media solidified, the Petri dishes containing the media and the sample solution were incubated upside down at 30 °C for 48 h. Afterward, TPC was calculated using the formula (3) below [34]:

$$N = \frac{\sum C}{[(1\times n_1)+(0.1\times n_2)]\times (d)} \quad (3)$$

where N is TPC (CFU/ml), ∑C is the number of colonies counted in all Petri dishes, n_1 is the number of colonies counted in all Petri dishes at first dilution, n_2 is the number of

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colonies counted in all Petri dishes at second dilution, and d is the dilution factor corresponding to the first dilution.

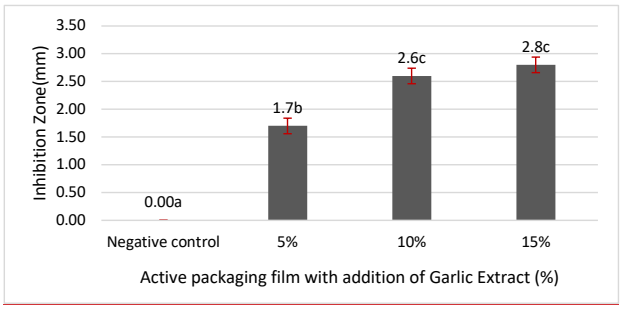
2.2.6. Data analysis

ANOVA was used to analyze the parameters of the intelligent packaging indicator, antimicrobial activity of the active packaging films, and quality of the beef samples, including pH, TVBN, and TPC. Differences between treatments were determined using Duncan's test. The correlations between the changes in the color of the intelligent packaging indicator and the effects of the active packaging on all parameters of meat spoilage were explored and presented in graphs by using the Sigma Plot 12 software. Data were analyzed using Microsoft Excel 2019, SPSS 19, and Sigma Plot 12.

3. Results and Discussion

3.1. Antimicrobial activity of the active packaging films against Staphylococcus aureus

The antimicrobial activity of the active packaging films is presented in **Figure 2.**



The mean value followed by different letters showed a significant difference based on the Duncan's test at the 5% level ($P < 0.05$).

Figure 2. Antimicrobial activity of the active packaging films against *S. aureus*.

The antimicrobial activity of the active packaging films against *S. aureus* was assessed by measuring the diameter of the inhibition zone. As shown in **Figure 2**, the negative control did not generate an inhibitory zone. However, when high concentrations of the garlic extract were added to the active packaging films, the inhibitory activity against the bacteria increased, although the inhibition zone was not significantly different between 10% and 15% garlic extract. This study demonstrated that 10%–15% garlic extract has antibacterial effects. According to Maroles *et al.* [35], differences in the diameter of inhibitory zones are influenced by the ability and rate of diffusion of antimicrobial compounds in the medium, the growth rate of microorganisms and their sensitivity to antimicrobial chemicals, and the viscosity and thickness of the medium.

The antibacterial effects of garlic extract are due to allicin, which is generated when garlic is damaged. When the flesh of garlic is damaged during the refining process, allicin is rapidly generated because of the release of alliinase, which reacts with nonprotein amino acids, namely, alliin. Allicin is a part of the defense mechanism of garlic that exerts antimicrobial effects on both Gram-positive and Gram-negative bacteria by inhibiting RNA and lipid syntheses, which in turn inhibit the production of amino acids and proteins and the phospholipid bilayer of bacterial cell wall, thereby preventing bacterial growth and development. Allicin is highly permeable and can easily penetrate bacterial cells across the cell membrane. The thiosulfinate S(=O)S group in allicin then binds to the

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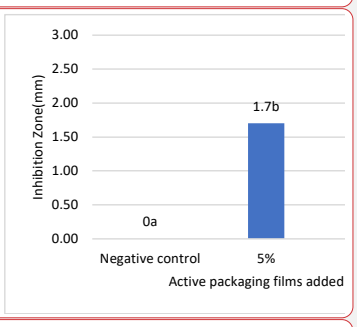

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sulfhydryl groups of bacteria, thus inhibiting the activation mechanism of bacterial proteinases [36,37].

3.2. pH of the beef samples

The pH of the beef samples was measured to investigate the effects of the active packaging films as the meat base in the packaging system. The beef samples were stored at room temperature for 24 h. The results of pH measurements are shown in **Table 1**.

Table 1. pH values of packaged meat sample stored at room temperature for 24 h.

Storage time (Hour)	Addition of garlic extract to the active packaging film				Average
	0%	5%	10%	15%	
0	6.56 ± 0.08	6.56 ± 0.08	6.57 ± 0.08	6.57 ± 0.08	6.56 ± 0.00[a]
4	6.68 ± 0.04	6.70 ± 0.06	6.76 ± 0.10	6.75 ± 0.06	6.72 ± 0.04[d]
8	6.72 ± 0.05	6.71 ± 0.07	6.72 ± 0.05	6.59 ± 0.20	6.68 ± 0.06[d]
12	6.57 ± 0.11	6.51 ± 0.03	6.47 ± 0.04	6.39 ± 0.02	6.48 ± 0.08[b]
16	6.44 ± 0.23	6.58 ± 0.06	6.62 ± 0.08	6.61 ± 0.11	6.56 ± 0.08[c]
20	6.75 ± 0.05	6.85 ± 0.01	6.85 ± 0.02	6.81 ± 0.02	6.81 ± 0.05[e]
24	6.88 ± 0.11	6.82 ± 0.11	6.79 ±0.10	6.8 5 ± 0.03	6.83 ± 0.04[e]
Average	6.66 ± 0.15[a]	6.68 ± 0.13[a]	6.68 ± 0.13[a]	6.65 ± 0.16[a]	

The mean value followed by different letters showed a significant difference based on the Duncan's test at the 5% level ($P < 0.05$).

A statistical test of the storage time showed a significant difference to the pH value ($p=0.000<0.005$). However, the statistical test results of the active packaging ($p=0.654>0.005$) and interaction between active packaging and storage time ($p=0.179>0.005$), on the other hand, did not show a significant effect on the pH value (**Table 1**). The initial pH of the meat samples, which was immediately determined after the cow was slaughtered, was normal (6.57) (**Table 1**). The pH fluctuated during the storage period, but the trend graph has shown a decrease in pH at the 12 h then the pH increased at the 16 h to 24 h storage. After the animal dies, the blood flow that supplies oxygen to the muscles stops causing an anaerobic glycolysis process to occur. During anaerobic glycolysis, glycogen conversion occurs in the muscles to lactic acid which accumulates in the tissues, causing the pH of the meat to decrease (4 h storage), during anaerobically glycolysis, the decrease in pH continues until the glycogen is converted to lactic acid followed by the neutralization of alkaline compounds resulting from the metabolism of microorganisms, so that the pH of the meat rises again (16-24 h storage).

According to Sánchez-Macías et al. [41] and Moreno et al. [42], reported that the lower the content of glycogen in the meat is, the slower the glycolysis process will be and the higher the final pH will be. However, the decrease in pH in muscles can be influenced by internal factors, such as species, muscle type, muscle glycogen content, and livestock variability, as well as external factors, such as environmental temperature, additional treatment prior to slaughter, and pre-slaughter stress.

After 20 h of storage, the meat's pH value ranged from 6.75 and 6.85 and remained steady up to 24 hour of storage; at this point, the meat was classified as decayed (**Table 1**). According to Prache et al. [43], the meat's pH continues to decline until glycogen is depleted into lactic acid and alkaline compounds are neutralized because of microbial metabolism, resulting in an increase in pH. If the pH reaches 6.8 or higher, protein decomposition will occur, resulting in spoilage

3.3. TVBN of the meat samples

The TVBN values of the meat samples are presented in **Table 2**.

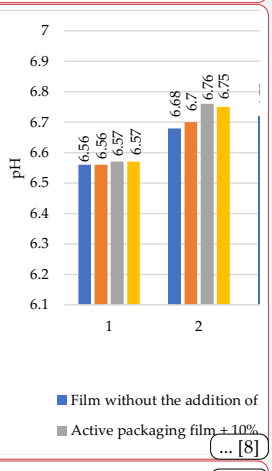

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Table 2. Total volatile basic nitrogen (TVBN) of the packed meat stored at room temperature for 24 h. Average Result of Meat's TVBN value.

Storage time (Hour)	Addition of garlic extract to the active packaging film				Average
	0%	5%	10%	15%	
0	8.35 ± 0.96	7.37 ± 0.56	7.51 ± 0.54	7.23 ± 1.24	7.62 ± 0.50[a]
4	12.27 ±0.54	9.47 ± 2.80	10.17 ± 2.17	10.73 ± 1.17	10.66 ± 1.19[b]
8	19.13 ± 2.07	14.65 ± 0.72	14.79 ± 1.40	13.95 ± 0.96	15.63 ± 2.36[c]
12	20.67 ± 2.68	16.19 ± 0.28	17.31 ± 1.73	16.61 ± 1.21	17.70 ± 2.04[c]
16	29.91 ± 3.78	29.21 ± 5.57	26.41 ± 3.31	25.43 ± 4.89	27.74 ± 2.16[d]
20	47.41 ± 3.17	43.21 ± 1.19	42.09 ± 1.19	44.05 ± 0.79	44.19 ± 2.29[e]
24	80.03 ± 8.65	77.79 ± 3.11	74.99 ± 5.63	76.81 ± 8.26	77.42 ± 2.12[f]
Average	31.12 ± 25.13[b]	28.27 ± 25.16[a]	27.61 ± 23.93 [a]	27.83 ± 24.84[a]	

The mean value followed by different letters showed a significant difference based on the Duncan's test at the 5% level ($P < 0.05$).

A statistical test revealed a highly significant difference between the active packaging (0.004<0.005) and storage time (0.000<0.005) on the TVBN value. However, the statistical test results of the interaction between active packaging and storage time (0.986>0.005), on the other hand, did not show a significant effect on the TVBN value **(Table 2)**. At 0 h, all meat samples had TVBN values ranging from 7.23 mgN/100 g to 8.35 mgN/100 g **(Table 2)**. Therefore, they were classified as fresh meat. After 12 h of storage, the meat samples that had not been treated with the active packaging films had a TVBN value of 20.67 mg N/100g, indicating that they were rotten. By comparison, the meat samples treated with the active packaging films and added with 5%, 10%, and 15% garlic extract had TVBN values of 16.19, 17.31, and 16.61 mgN/100 g, respectively. Thus, they were categorized as semi-fresh meat (stale meat) or could still be consumed.

However, the TVBN values of all meat samples taken between the 16th and 24th h of storage exceeded the threshold for food-grade beef, demonstrating that adding 5%, 10%, and 15% garlic extract to the active packaging films effectively reduced the amount of TVBN, indicated by the increase in TVBN values respectively 29.21percent, 16.41percent, and 25.3percent which showed the higher the concentration of garlic extract in the active packaging, the less the TVBN production increase. On the other hand, meat samples that were not treated active packaging film treatment had a 29.91 percent rise in TVBN value. Beef or livestock is considered fresh if the TVBN value is less than 15 mg/100 g [44] or TVBN is <10 mg N/100 g [45]. Moreover, SNI 2354.8:2009 by National Standardization Agency of Indonesia (BSN) [46] states that the standard levels of TVBN fit for consumption is 20–30 mg N/100g.

In this study, the values of TVBN increased throughout the storage period (observed every 4 h), indicating that the meat's quality continued to deteriorate owing to the breakdown of proteins into volatile base compounds. According to Bekhit et al. [10], the increase in TVBN value is due to protein degradation by microorganisms that results in the formation of foul-smelling chemicals, such as ammonia (NH_3), basic skatole and indole compounds, mercaptans and H_2S (which are weak acids), and amines and cadaverin (which are strong bases). The results demonstrated that the addition of garlic extract to the active packaging films delayed the spoiling of the meat samples likely because the garlic's active components prevented microbial development, thereby lowering the synthesis of nitrogenous base compounds in the meat caused by bacteria and autolytic enzymes during the rotting process. This conjecture was supported by Al Hakim et al. [47]

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and Reiter et al. [37], who reported that garlic extract has the ability to block microbe-produced enzymes involved in the breakdown of proteins into volatile base chemicals.

3.4. TPC of the microbes in the beef samples

The TPC of bacteria in the meat samples was determined to assess the utility of the active packaging films **(Table 3)**.

Table 3. Total plate count (TPC) of packed meat stored at room temperature for 24 h.

Storage time (Hour)	Addition of garlic extract to the active packaging film				Average
	0%	5%	10%	15%	
0	2.53 ± 0.64	2.53 ± 0.64	2.53 ± 0.64	2.53 ± 0.64	2.53 ± 0.00^{a}
4	5.18 ± 0.20	4.64 ± 0.16	3.83 ± 0.30	3.91 ± 0.10	4.39 ± 0.64^{b}
8	5.43 ± 0.21	5.33 ± 0.07	4.83 ± 0.40	5.04 ± 0.06	5.16 ± 0.27^{c}
12	7.65 ± 0.39	6.20 ± 0.00	5.51 ± 0.10	5.34 ± 0.08	6.18 ± 1.05^{d}
16	8.89 ± 0.67	7.44 ± 0.03	7.47 ± 0.26	6.78 ± 0.67	7.64 ± 0.89^{e}
20	10.04 ± 0.58	8.30 ± 1.35	7.57 ± 0.60	8.28 ± 0.06	8.55 ± 1.08^{f}
24	10.36 ± 0.15	9.53 ± 0.39	9.44 ± 0.68	9.25 ± 0.03	9.65 ± 0.49^{g}
Average	7.15 ± 2.89^{c}	6.28 ± 2.37^{b}	5.88 ± 2.41^{a}	5.88 ± 2.39^{a}	

The mean value followed by different letters showed a significant difference based on the Duncan's test at the 5% level ($P < 0.05$).

A statistical test revealed a highly significant difference between the active packaging (0.000<0.005) and storage time (0.000<0.005) on the Tpc value. However, the statistical test results of the interaction between active packaging and storage time (0.09>0.005), on the other hand, did not show a significant effect on the TPC value **(Table 3)**. At 0 h of the storage period, the initial TPC value (Log TPC) of all meat samples was 2.53±0.64 CFU/ml **(Table 3)**. Thus, the meat samples were classified as fresh on the basis of microbiological quality. Throughout the storage period, the TPC value increased until it reached the maximum number of meat microbes permitted by SNI 3932:2008 on carcass and beef quality, which is 1×106 CFU/ml or equivalent to Log TPC 6 CFU/ml. At 12 h of storage, the meat samples treated with the active packaging films but without garlic extract (0%) and those added with 5% garlic extract did not fulfil the microbiological requirements as they had a Log TPC value of 7.65±0.39 and 6.20±0.00 CFU/ml, respectively. By comparison, the meat samples treated with the active packaging films and 10% and 15% garlic extract also did not fulfil the microbiological requirements after 16 h of storage as they has a Log TPC value of 7.47±0.26 and 6.78±0.67 CFU/ml, respectively. This result demonstrated that the active packaging films with 10% and 15% garlic extract in the meat packaging system can inhibit microbial growth and extend the shelf life of meat by up to 4 h because allicin can inhibit the growth of both Gram-positive and Gram-negative bacteria by destroying the sulfhydryl group bound to bacterial proteins. This process is important because the sulfhydryl group is required for bacterial cell division or acts as a specific stimulator for cell multiplication. Allicin damaged the RNA and DNA of bacteria and thus inhibits their growth and development in meat. Likewise, Deresse [48] reported that allicin can suppress the growth of both Gram-positive and Gram-negative bacteria by completely inhibiting the syntheses of bacterial RNA, DNA, and proteins.

The total microbial content of the meat samples continued to increase during the entire storage period **(Table 3)** because meat contains a high nutrient and water content, which provides an ideal environment for microorganism growth. Moreover, storage at room temperature can accelerate the growth of microorganisms. According to Soeparno [40], meat has the ideal conditions for microorganism growth because it contains a high

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proportion of water (68%–75%), it is rich in nitrogen-containing substances of varying complexity, it contains various fermentable carbohydrates, it is rich in minerals and essential nutrients for microorganism growth, and it has a suitable pH for microorganism growth (pH 5.3–6.5). Variance analysis revealed that the duration of storage of the meat samples and the use of the active packaging films with garlic extract had a highly significant effect on the TPC value of the samples (P>0.01).

3.5. Changes in the color of the intelligent packaging BTB indicator solution as a measure of the freshness of the meat packaged with the active packaging films

Using fresh beef packaged and maintained at room temperature for 24 h, Dirpan et al. [7] determined that BTB solution (pH 2.75) produces the most readily visible color changes to sensitivity tests. In this study, the BTB solution (pH 2.75), as the intelligent packaging indicator, was also utilized to evaluate changes in its color as a reflection of the freshness of the meat samples packed with the active packaging films **(Figure 3)**.

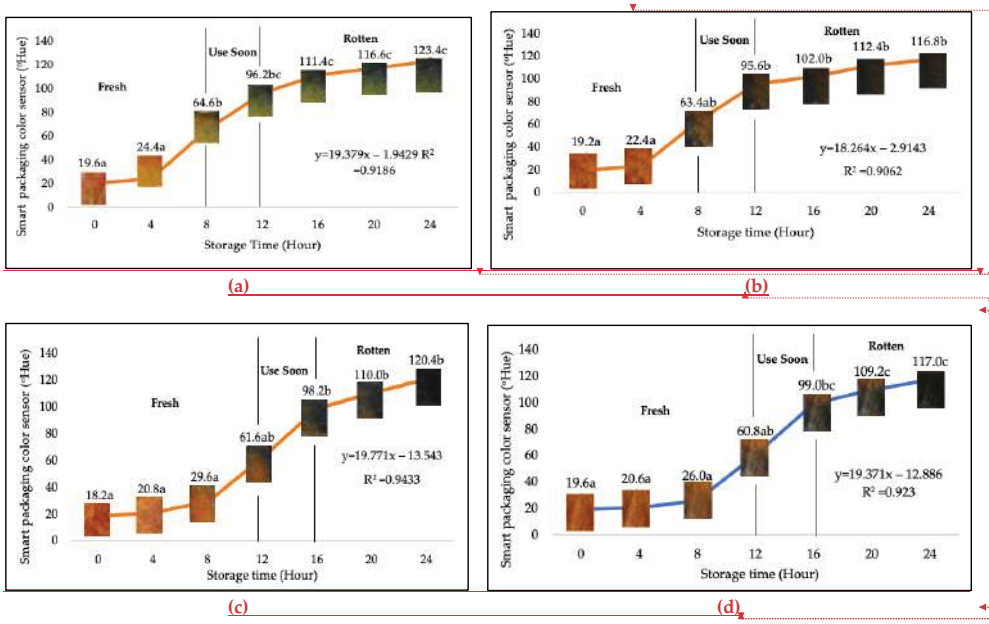

Figure 3. Changes in the color of the BTB solution (pH 2.75) as the intelligent packaging indicator reflecting the freshness of the meat samples packed with the active packaging films with **(a)** 0%; **(b)** 5%; **(c)** 10%; and **(d)** 15% of garlic extract.

During the entire storage period, the intelligent packaging indicator changed its color three times that corresponded to three phases of the meat samples' level of quality **(Figure 3)**. In phase I, its color was orange, indicating that the meat samples were still fresh. In phase II, its color was green with an orange hue, suggesting that the meat samples should be consumed immediately. In phase III, its color was dark green, denoting that the meat samples were already spoiled. The change in the indicator's color from orange to green indicated that the quality of the meat samples had deteriorated. The changes in the indica-

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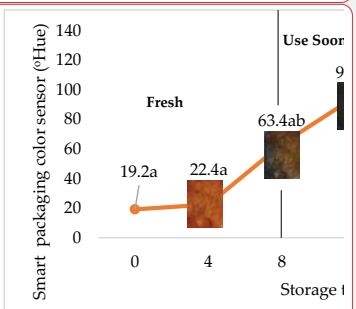

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tor's color were due to the interactions of alkaline volatile compounds produced by enzyme activity, and the metabolism of the microorganisms present in the meat samples increased with storage time. The early sign of spoilage was indicated by the release of volatile alkaline compounds as the microorganisms and the enzymes degraded the nutritional content of the meat samples. These compounds gradually accumulated in the packaging system, causing an increase in pH, which was detected by the intelligent packaging indicator and displayed as gradual color changes. The change in color of the intelligent packaging indicator (BTB, pH 2.75) from orange to green was induced by deprotonation or the release of a proton from the intelligent packaging indicator dye [49].

The meat samples packaged with the active packaging films but with no (0%) and 5% garlic extract were still fresh from the start of the storage up to 8 h **(Figure 3)**. However, they must be immediately consumed from the 8th h to the 12th h of the storage period. Thereafter (12–24 h of the storage period), they were already spoiled. This results was consistent with that of TPC tests, which showed that the TPC values were above the acceptable threshold for microbial contaminants (1×106 or equivalent to 6 CFU/ml) in meat after 12 h. By comparison, the meat samples packaged with the active packaging films containing 10% and 15% garlic extract were still considered fresh from the start of the storage period up to the 12th h. They must be immediately consumed when they had been in storage for 12–16 h. Finally, they were considered rotten when they had been in storage for 16–24 h. This result was also consistent with that of TPC tests (**Table 3**), which indicated that at the 16th hour, the TPC value surpassed the permissible level of microbiological contamination in beef. Statistical analysis revealed that storage duration had a very significant effect on the Hue value, the indicator of color change in the intelligent packaging. The changes in the color of the intelligent packaging indicator (BTB solution, pH 2.75) when used together with the active packaging films to reflect the freshness of meat are presented in **Table 4**.

Table 4. Changes in the color of the intelligent packaging indicator (BTB solution, pH 2.75) when used together with the active packaging films to reflect the freshness of meat.

Storage Time (Hour)	Active Packaging Films Added with Garlic Extract			
	0%	5%	10%	15%
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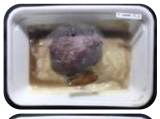
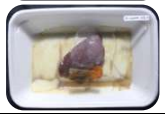
8				
12				

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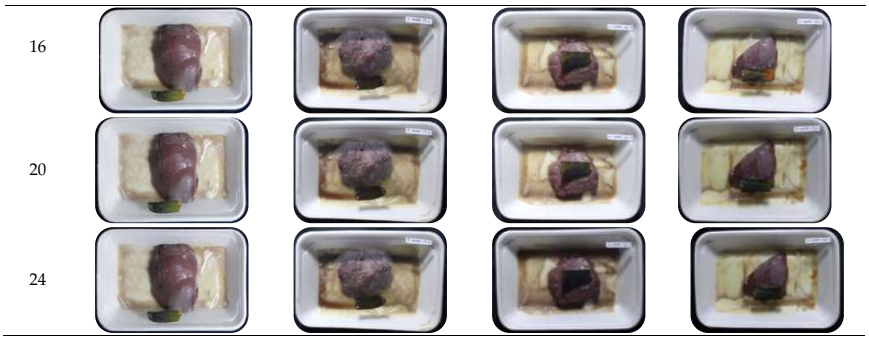
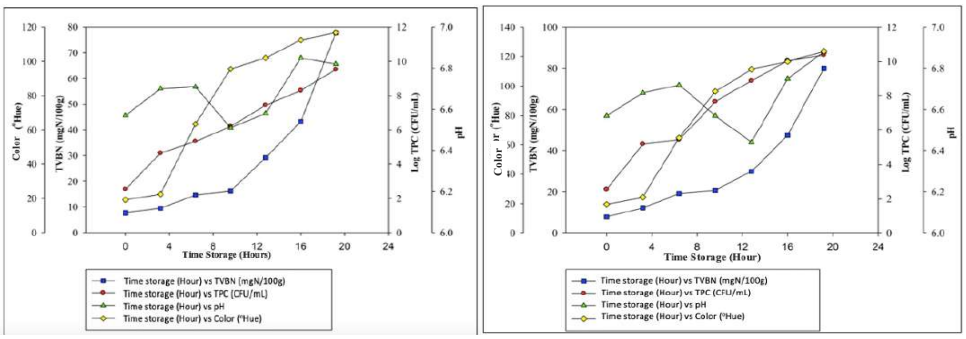


3.6. Correlations between changes in the color of the intelligent packaging indicator and the effects of the active packaging films on the parameters of meat freshness

The correlations between changes in the color of the intelligent packaging indicator and parameters of meat quality deterioration (pH, TVBN, and TPC) were explored to ascertain the relationship between the sensitivity of the intelligent packaging indicator to meat freshness and the effectiveness of the active packaging films in slowing the process of meat spoilage.



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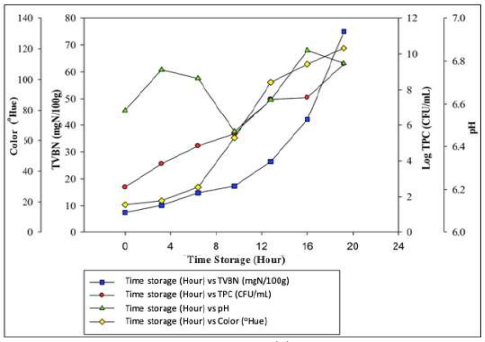

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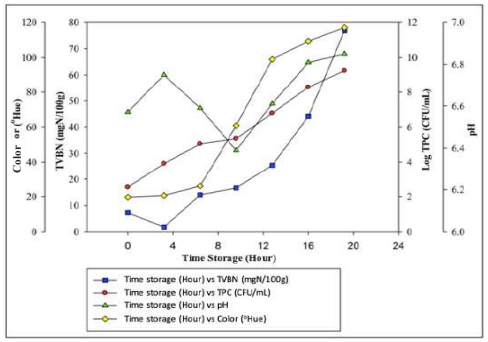

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Figure 4. Correlations between changes in the color of the intelligent packaging indicator and the effects of the active packaging films with **(a)** 0%; **(b)** 5%; **(c)** 10%; and **(d)** 15% garlic extract on the parameters of quality deterioration of meat stored for 24 h.

Based on **Figure 4**, it is known that the color change of the intelligent packaging indicator which is indicated by an increase in the Hue value is in line with the increase in all values of the meat deterioration parameter. The meat samples treated with the active packaging films but without garlic extract and those treated with 5% garlic extract were rotten and unfit for consumption after 12 h of storage as their Log TPC value was 7.65±0.39 and 6.20±0.00 CFU/mL, respectively, and their TVBN value was 20.67±2.68 and 16.19±0.28 mgN/100g, respectively **(Figure 4)**. By comparison, the meat samples treated with the active packaging films and 10% and 15% garlic extract were rotten and unfit for consumption after 16 h of storage as their Log TPC value was 7.47±0.26 and 6.78±0.67 CFU/mL, respectively, and their TVBN value was 26.41±3.31 and 25.43±4.89 mgN/100 g, respectively.

Meat that was treated with active packaging film without addition (0 percent) and with the addition of 5% garlic extract experienced a change in indicator color from orange (fresh) with a color value of 19.6°Hue and 19.20°Hue, respectively to green (rotten) with color values 96.2°Hue and 95.6°Hue, respectively. Meanwhile, the meat that was treated with active packaging film with the addition of 10 percent and 15 percent garlic extract experienced a change in indicator color from orange (fresh) with color values 18.2°Hue and 19.6°Hue, respectively, to green (rotten) with color values 98.2°Hue, and 99°Hue, respectively. Wiryawan [50] observed that when garlic extract was added to the active packaging, the values of TPC and TVBN and the pH of the meat increased more slowly, as did the color of the intelligent packaging indicator, compared with those of the meat without the active packaging.

Furthermore, the increase in the values of TPC and TVBN linearly correlates with the increase in Hue value and color changes of the intelligent packaging indicator because the accumulated volatile base compounds raise the pH value of the packaging system, causing the intelligent packaging indicator to experience a color shift. This explanation was in agreement with that of Pacquit et al. [12], who applied active packaging films to cod fish. They stated that the increase in the TPC value of cod fish has a linear correlation with changes in the color of the cellulose-acetate packaging film sensor.

On the other hand, the pH of the sample fluctuated making it difficult to determine the level of quality degradation in meat. However, the interpretation of the TPC and TVBN values, on the other hand, is clear enough to represent a decrease in meat quality which is correlated with an increase in the color value of changes in the intelligent packaging indicator.

4. Conclusions

The paper concludes that intelligent packaging indicators using BTB (Bromothymol blue) pH 2.75 solution can be used as an indicator to identify a decline in the quality of packaged meat. The indicator's color changes is easy to observe visually, namely the orange indicator indicating that the meat is still fresh and the dark green indicator indicating that the meat has rotted and is unfit for consumption. On the other hand, the use of active packaging can extend the shelf life of meat by 4 hours longer. Meat with active packaging sheet treatment without addition (0 percent) and addition of 5 percent garlic extract rotted at 12 hours of storage. Meanwhile, meat treated with active packaging sheets containing 10 percent and 15 percent garlic extract rotted after the 16th hour of storage. This demonstrates that intelligent and active packaging, which are typically studied separately, have the potential to be combined and researched together using the same basic ingre-dient, namely bacterial cellulose.

Author Contributions: Conceptualization, A.D; methodology, A.D. and I.K; software, A.D. and I.K; validation, A.D. and M.D.; formal analysis, A.D. and I.K.; investigation, I.K.; resources, A.D.; data

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Second revision : Accepted with minor revision (31-12-2021)

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Dear Dr. Dirpan,

Thank you again for your manuscript submission:

Manuscript ID: sensors-1516382
Type of manuscript: Article
Title: Application of a Smart Sensor and Active Packaging System Based on the Bacterial Cellulose of Acetobacter xylinum to Meat Products
Authors: Andi Dirpan *, Muspirah Djalal, Irma Kamaruddin
Received: 3 December 2021
E-mails: dirpan@unhas.ac.id, muspirah_djalal@agri.unhas.ac.id, irmakama9@gmail.com
Submitted to section: Biosensors, https://www.mdpi.com/journal/sensors/sections/biosensors
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Please do not hesitate to contact us if you have any questions regarding the revision of your manuscript or if you need more time. We look forward to hearing from you soon.

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Second revision submitted

a. Email from publisher : Manuscript resubmitted and revision received (3-1-2022)

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Cc: Muspirah Djalal <muspirah_djalal@agri.unhas.ac.id>, Irma Kamaruddin <irmakama9@gmail.com>

Dear Dr. Dirpan,

Thank you very much for resubmitting the modified version of the following manuscript:

Manuscript ID: sensors-1516382
Type of manuscript: Article
Title: Application of a Smart Sensor and Active Packaging System Based on the Bacterial Cellulose of Acetobacter xylinum to Meat Products
Authors: Andi Dirpan *, Muspirah Djalal, Irma Kamaruddin
Received: 3 December 2021
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Dear Dr. Dirpan,

Thank you very much for providing the revised version of your paper:

Manuscript ID: sensors-1516382
Type of manuscript: Article
Title: Application of a Smart Sensor and Active Packaging System Based on the Bacterial Cellulose of Acetobacter xylinum to Meat Products
Authors: Andi Dirpan *, Muspirah Djalal, Irma Kamaruddin
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We will continue processing your paper and will keep you informed about the status of your submission.

Kind regards,
Vickie Wang
Assistant Editor, MDPI
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Assistant Editor, MDPI
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6

Second revision submitted

b. Revisions and amends

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Reviewer 1 : Round 2

Journal	Sensors (ISSN 1424-8220)
Manuscript ID	sensors-1516382
Type	Article
Title	Application of an Intelligent Sensor and Active Packaging System Based on the Bacterial Cellulose of Acetobacter Xylinum to Meat Products
Authors	Andi Dirpan * , Muspirah Djalal , Irma Kamaruddin
Section	Biosensors
Special Issue	Smart Materials and Technology for Biological and Medical Sensor Applications

Abstract

Combining smart and active packaging serves the dual purpose of detecting color changes in food that reflect changes in its quality and prolonging its shelf life. This study developed a smart and active packaging system made from the cellulose of Acetobacter xylinum and assessed its ability to detect changes in the quality of packaged fresh beef. The properties of the smart packaging's sensor and active packaging films were determined. The application of this system to fresh beef stored at room temperature (28±2 °C) for 24 h was tested. The color of the bromothymol blue (BTB) solution (pH 2.75) in the indicator of the smart packaging system changed from orange to dark green to indicate that beef quality changed from fresh to rotten. The meat treated with the active packaging with 10% and 15% garlic extract decayed on the 16th h. By contrast, the meat treated with the active packaging but without the garlic extracts rotted on the 12th h. The shift in the indicator's color was linearly related to the total plate count (TPC), total volatile basic nitrogen (TVBN) , and pH of the meat packaged using the active packaging system. Therefore, BTB solution (pH 2.75) can be used as a smart packaging indicator that will allow consumers to assess the quality of packaged meat easily. As an antimicrobial agent, the addition of 10%–15% garlic extract to the active packaging films can help delay the spoilage of packaged meat.

Review Report Form

English language and style

( ) Extensive editing of English language and style required

( ) Moderate English changes required

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( ) I don't feel qualified to judge about the English language and style

	Yes	Can be improved	Must be improved	Not applicable
Does the introduction provide sufficient background and include all relevant references?	(x)	( )	( )	( )
Is the research design appropriate?	(x)	( )	( )	( )
Are the methods adequately described?	(x)	( )	( )	( )
Are the results clearly presented?	(x)	( )	( )	( )
Are the conclusions supported by the results?	(x)	( )	( )	( )

Comments and Suggestions for Authors

I want to thank the authors for their great effort in improving the manuscript. Now, just some minor changes have to be done.

- Figure 1a. Substitute Actif by Active
- Lines 120, 132 y 151. Please, ensure the linkage of the sentences of different paragraphs has grammatically sense. In the revision of the manuscript, they still appear in different paragraphs.
- Line 159. Before referring to a method optimized by someone, as in this case in reference [29], including the name of the researchers before the enumeration. Do this change along the manuscript
- Line 143. Include 6% instead of 6 percent. Do it also in lines 397 onwards and 585 onwards, and the conclusion section.
- Line 175: There is some information to clarify in this sentence. The sterilization was just done to the container alone or with the meat inside? As it is written, it seems that the meet was sterilized before the experiment, but it does not fit with the aim of the study that is the effectiveness of the packaging on fresh meat. Do the appropriate changes.
- Line 535: Correct the superscript
- Line 576: Replace “The meat samples treated with the active packaging films but without garlic extract” by “ the meat samples packaged with the control film”.
- Substitute replications by replicates along the manuscript
- Line 225: The method
- Table 1. The change of the graphic to the table clarifies the information. However, the inclusion of the Average values sums up too much information. It would offer more information to show the differences at each time among the different samples, and evaluate if there are differences among the different % at each time.
- The paragraph included after the table also needs more information. What conclusions arise from the statistical analysis? What is the importance of finding no statistical differences in some cases and do it in other ones? At the end of that section (line 365), mention where that point has been achieved on each sample. Are there differences?
- The value of the 15% at 25 h has extra space.
- Substitute P-value by p-value (p in italics)
- Employ the same statistical analysis for Tables 2 and 3
- It should be replaced “Storage time (Hour)” by “Storage time (h)” in all graphics and Tables. Include the units log UFC/mL in Table 3.

- Line 624. Include in the sentence a final conclusion. This good correlation demonstrates the accuracy of the film formulation in the monitoring of meat freshness, which is the aim of using smart packaging.
- Line 631 to 633. Eliminate this sentence since it is repetitive. Substitute by “On the other hand, the use of active packaging can extend the shelf life of meat by 4 hours longer when using high concentrations of garlic extract.” These conclusions are obtained from considering the good correlation among the overall decay parameters and the Hue value of the intelligent film, and this has to be highlighted in the conclusion section.

Submission Date 03 December 2021
Date of this review 30 Dec 2021 16:46:34

MATRICES OF AMENDMENTS FOR REVIEWER 1

Comments and Suggestions for Authors and **Author’s responds**
1. Figure 1a. Substitute Actif by Active *Thank you for your response and we greatly appreciate it. We have fixed it.*
2. Lines 120, 132 y 151. Please, ensure the linkage of the sentences of different paragraphs has grammatically sense. In the revision of the manuscript, they still appear in different paragraphs. *Thank you for your concern for our entire paper. actually we have made it in one paragraph.*
3. Line 159. Before referring to a method optimized by someone, as in this case in reference [29], including the name of the researchers before the enumeration. Do this change along the manuscript. *Thank you for your comments and attention to our paper. We have written the name of the researchers before the enumeration.*
4. Line 143. Include 6% instead of 6 percent. Do it also in lines 397 onwards and 585 onwards, and the conclusion section. *Thank you for your feedback. We have made the necessary changes in accordance with your instructions. We have changed “percent” to “%” throughout our paper.*
5. Line 175: There is some information to clarify in this sentence. The sterilization was just done to the container alone or with the meat inside? As it is written, it seems that the meet was sterilized before the experiment, but it does not fit with the aim of the study that is the effectiveness of the packaging on fresh meat. Do the appropriate changes. *Thank you for your response. Sterilization is only carried out in containers, while fresh meat does not go through sterilization or other handling (we only do the cutting process to uniform the size of the meat in the package). Actually, the meaning of "...and processed into 200 g/pack pieces **under sterile conditions"** is that all tools used in meat handling such as meat storage containers, knives for cutting, cutting boards, the laboratory environment, and researchers working kept sterile during sample preparation to avoid excessive contamination of the meat, so that the effectiveness of active packaging can be clearly observed.* *As for the consideration to avoid misunderstanding the reader, we decided to remove the word **"under sterile conditions"** in the sentence. I hope this decision right.*
6. Line 535: Correct the superscript

Thank you for your response. we have fixed the superscript according to your instructions.

7. Line 576: Replace “The meat samples treated with the active packaging films but without garlic extract” by “ the meat samples packaged with the control film”.
   Thank you for your feedback. We have made the necessary changes in accordance with your instructions.

8. Substitute replications by replicates along the manuscript
   Thank you for your feedback. We have made the necessary changes in accordance with your instructions.

9. Line 225: The method
   Thank you for your response and we greatly appreciate it. We have fixed it.

10. Table 1. The change of the graphic to the table clarifies the information. However, the inclusion of the Average values sums up too much information. It would offer more information to show the differences at each time among the different samples, and evaluate if there are differences among the different % at each time.
    Thank you for your comments and concern for our data. we have removed the averages that show no significant difference and we have added some explanation regarding our data.

11. The paragraph included after the table also needs more information. What conclusions arise from the statistical analysis? What is the importance of finding no statistical differences in some cases and do it in other ones? At the end of that section (line 365), mention where that point has been achieved on each sample. Are there differences?
    Thank you for your response and we greatly appreciate it. we have added some explanation regarding our data. we hope it meets your standards

12. The value of the 15% at 25 h has extra space.
    Thank you for your response and we greatly appreciate it. We have fixed it.

13. Substitute P-value by p-value (p in italics)
    Thank you for your response and we greatly appreciate it. We have fixed it.

14. Employ the same statistical analysis for Tables 2 and 3
    Thank you for your response and we greatly appreciate it. We have fixed it.

15. It should be replaced “Storage time (Hour)” by “Storage time (h)” in all graphics and Tables. Include the units log UFC/mL in Table 3.
    Thank you for your response and we greatly appreciate it. We have made the necessary changes in accordance with your instructions.

16. Line 624. Include in the sentence a final conclusion. This good correlation demonstrates the accuracy of the film formulation in the monitoring of meat freshness, which is the aim of using smart packaging.
    Thank you for your response and we greatly appreciate it. We have made the necessary changes in accordance with your instructions.

17. Line 631 to 633. Eliminate this sentence since it is repetitive. Substitute by “On the other hand, the use of active packaging can extend the shelf life of meat by 4 hours longer when using high concentrations of garlic extract.” These conclusions are obtained from considering the good correlation among the overall decay parameters

and the Hue value of the intelligent film, and this has to be highlighted in the conclusion section.

Thank you for your great advice which means a lot to us. your suggestions make the conclusion easier to understand. We have added your suggestions to our conclusion.

Reviewer 2 : Round 2

Journal	Sensors (ISSN 1424-8220)
Manuscript ID	sensors-1516382
Type	Article
Title	Application of an Intelligent Sensor and Active Packaging System Based on the Bacterial Cellulose of Acetobacter Xylinum to Meat Products
Authors	Andi Dirpan * , Muspirah Djalal , Irma Kamaruddin
Section	Biosensors
Special Issue	Smart Materials and Technology for Biological and Medical Sensor Applications
Abstract	Combining smart and active packaging serves the dual purpose of detecting color changes in food that reflect changes in its quality and prolonging its shelf life. This study developed a smart and active packaging system made from the cellulose of Acetobacter xylinum and assessed its ability to detect changes in the quality of packaged fresh beef. The properties of the smart packaging's sensor and active packaging films were determined. The application of this system to fresh beef stored at room temperature (28±2 °C) for 24 h was tested. The color of the bromothymol blue (BTB) solution (pH 2.75) in the indicator of the smart packaging system changed from orange to dark green to indicate that beef quality changed from fresh to rotten. The meat treated with the active packaging with 10% and 15% garlic extract decayed on the 16th h. By contrast, the meat treated with the active packaging but without the garlic extracts rotted on the 12th h. The shift in the indicator's color was linearly related to the total plate count (TPC), total volatile basic nitrogen (TVBN) , and pH of the meat packaged using the active packaging system. Therefore, BTB solution (pH 2.75) can be used as a smart packaging indicator that will allow consumers to assess the quality of packaged meat easily. As an antimicrobial agent, the addition of 10%–15% garlic extract to the active packaging films can help delay the spoilage of packaged meat.

Review Report Form

English language and style

( ) Extensive editing of English language and style required

( ) Moderate English changes required

( ) English language and style are fine/minor spell check required

(x) I don't feel qualified to judge about the English language and style

	Yes	Can be improved	Must be improved	Not applicable
Does the introduction provide sufficient background and include all relevant references?	( )	(x)	( )	( )
Is the research design appropriate?	( )	(x)	( )	( )
Are the methods adequately described?	( )	(x)	( )	( )
Are the results clearly presented?	( )	(x)	( )	( )
Are the conclusions supported by the results?	( )	(x)	( )	( )

Comments and Suggestions for Authors The manuscript has been improved.

Submission Date 03 December 2021
Date of this review 29 Dec 2021 13:41:16

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Second revision submitted

c. Revised version with highlights

sensors

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Article

Application of a Intelligent Sensor and Active Packaging System Based on the Bacterial Cellulose of *Acetobacter xylinum* to Meat Products

Andi Dirpan*, Muspirah Djalal and Irma Kamaruddin

Department of Agricultural Technology, Hasanuddin University, Makassar 90245, Indonesia; muspirah_djalal@agri.unhas.ac.id (M.D.); irmakama9@gmail.com (I.R.)

* Correspondence: dirpan@unhas.ac.id

Abstract: Combining intelligent and active packaging serves the dual purpose of detecting color changes in food that reflect changes in its quality and prolonging its shelf life. This study developed a intelligent and active packaging system made from the cellulose of *Acetobacter xylinum* and assessed its ability to detect changes in the quality of packaged fresh beef. The properties of the intelligent packaging's sensor and active packaging films were determined. The application of this system to fresh beef stored at room temperature (28±2 °C) for 24 h was tested. The color of the bromothymol blue (BTB) solution (pH 2.75) in the indicator of the intelligent packaging system changed from orange to dark green to indicate that beef quality changed from fresh to rotten. The meat treated with the active packaging with 10% and 15% garlic extract decayed on the 16th h. By contrast, the meat treated with the active packaging but without the garlic extracts rotted on the 12th h. The shift in the indicator's color was linearly related to the total plate count (TPC), total volatile basic nitrogen (TVBN) , and pH of the meat packaged using the active packaging system. Therefore, BTB solution (pH 2.75) can be used as a intelligent packaging indicator that will allow consumers to assess the quality of packaged meat easily. As an antimicrobial agent, the addition of 10%–15% garlic extract to the active packaging films can help delay the spoilage of packaged meat.

Keywords: Intelligent Sensor; Intelligent packaging; active packaging; Bacterial Cellulose; Meat

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1. Introduction

Global beef consumption is predicted to rise as the world population and family income increase, particularly in developing Asian countries [1–3]. By 2030, worldwide meat consumption and availability are expected to increase by 14% and 5.9%, respectively, over the average of the 2018–2020 period [3]. Thus, the expected increase in meat consumption must be complemented by improvements in the quality of fresh meat produced. One aspect affecting the quality and characteristics of meat is the material and packaging technologies used [4]. Meat is a perishable item that rapidly spoils when stored above the optimum temperature range (below -17–4 °C) [5,6]. However, in traditional markets, meat is displayed at room temperature without packaging, a practice that might accelerate microbial contamination and cause rapid quality degradation. Even in supermarkets where meat is maintained in cold temperatures, standard meat packaging still prevent consumers from subjectively determining the quality of meat. Thus, meat packaging must have additional functions that will prevent quality degradation due to microbial contamination and will help consumers to determine the quality of packaged meat easily [7]. Conventional meat packaging can be designed to perform dual functions through intelligent and active packaging systems.

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Intelligent packaging is a term that refers to sensors in the form of indicators that monitor and provide information on the quality of the food contained within the packaging via color changes caused by chemical reactions between the indicators and the products of microbial metabolism or changes in the chemical composition of the food [8,9]. During storage, the chemical components of meat degrade into volatile compounds because of microbial activity, thereby increasing the value of total volatile base nitrogen (TVBN) [10,11]. Accumulation of TVBN increases the pH of the packaging system, which is detected by the indicator, resulting in a visible color shift in the indicator [11,12]. Intelligent packaging allows easier monitoring of packed products during transportation and storage [7]. Moreover, it provides a more accurate estimate of product condition than conventional expiration labels [12]. Color-based pH indicator solutions are widely used as intelligent indicators. Dirpan et al. [13] developed bromophenol blue as a intelligent indicator dye for mangoes. Hidayat et al. [14] used two types of color indicators with predetermined concentrations, namely, phenol red and bromothymol blue, to assess the freshness of meat packaging. Intelligent packaging indicators based on natural pigments are being developed, such as intelligent packaging films that include anthocyanin-loaded Lycium ruthenicum nanocomplexes in starch/polyvinyl alcohol mixtures (PVA) [15], as well as anthocyanins from saffron petals immobilized in chitosan nanofibers and methyl cellulose matrix [16].

Active packaging refers to the integration of particular additives into a packaging system for the purpose of extending the shelf life, preserving the quality, and ensuring the safety of food products. Antimicrobial agents are used as components of active packaging additives to extend product shelf life. The volatile bioactive compounds in active packaging evaporate or diffuse onto the food surface, where they limit the growth of pathogenic microbes and thus delay spoilage [17,18]. This strategy is more effective than coating bioactive compounds onto the food surface [19]. The safest, cheapest, and most readily available antimicrobial agents for use in active packaging are essential oils. Pranoto et al. [20] produced antimicrobial alginate edible films by incorporating the essential oils of garlic. They reported that these films substantially inhibited the growth of Staphylococcus aureus and Bacillus cereus in meat. Vishnu et al. [21] utilized the essential oils of Plectranthus amboinicus in a chitosan-based active packaging to restrict antimicrobial activity.

Intelligent and active packaging can be merged into a single packaging system. Julyaningsih et al. [22] combined a intelligent packaging system based on methyl red–bromothymol blue (BTB) indicator with an active packaging system based on lemongrass oil as a component of tuna fish fillet packaging. Yao et al. [23] developed an active and intelligent packaging system based on starch, PVA, and betacyanins from various types of plants for shrimp packaging. In general, an active packaging that contains antimicrobial agents and a intelligent packaging that contains indicator solutions are immobilized in a polymer. Compared with synthetic polymers or plant cellulose, the bacterial cellulose fermented by *Acetobacter xylinum* has a unique nanofibrillar structure and superior physical properties, suggesting that it has the potential to serve as a basis for developing a intelligent and active packaging system [24,25]. Bacterial cellulose has received interest as a component of active packaging owing to its biodegradability, high water-holding capacity so that it can be employed entirely as a polymer for immobilizing color solutions in intelligent packaging indicators [26]. Besides, bacterial cellulose possess great potential as an antimicrobial agent carrier in order for it to be utilized as an ingredient in the production of active packaging films [27].

The development of packaging systems with additional functions is advancing. To promote this innovation, this study aimed to maximize the potential of intelligent and active packaging by combining them into a single packaging system based on a bacterial cellulose membrane biopolymer to enhance the quality of packaged meat and help consumers to determine meat freshness easily.

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2. Materials and Methods

2.1. Materials

The main ingredients used in the intelligent and active packaging system developed herein were the bacterial cellulose produced by *A. xylinum*, which was fermented in natural media of coconut water. Beef tenderloin was purchase from a slaughterhouse in Tamangapa Raya. Coconut water and garlic (*Allium sativum*) were purchased from a local market. Food-grade ammonium sulfate (CAS Number: 7783-20-2), yeast extract (Merck, CAS Number: 8013-01-2), 96% acetic acid (Brenntag Inc, CAS No: 64-19-7), *A. xylinum* culture, 5% 1 N NaOH (Brenntag Inc, CAS No: 1310-73-2), sucrose, Bromothymol blue (BTB, Merck, CAS No: 76-59-5), alcohol, aquabides, aquades, Tashiro's indicator (0.1% methyl red and 0.1% BTB at a ratio of 2:1), 7% trichloroacetic acid (TCA, Merck), nutrient Agar (NA, Merck), glycerol (Merck, CAS No: 56-81-5), food-grade carboxymethyl–cellulose (CMC) (Foodchem, E466), and corn starch were used.

2.2. Methods

2.2.1. production of bacterial cellulose from A. xylinum

Based on our previous research Dirpan et al. [7], 5% (w/v) of food grade Ammonium Sulfate is the best source of Nitrogen in ***Acetobacter xylinum*** growth media to produce optimal bacterial cellulose membranes. Determination of the composition and type of Nitrogen source. Then, **purification of bacterial cellulose** by removed from the fermentation medium, rinsed in running water, and then soaked for 2 days with periodic water changes. The cellulose was also soaked in 70% alcohol for 1 min, heated to 100 °C in distilled water for 20 min, and reheated in 1 N 5% NaOH solution at 100 °C for 60 min to remove the remaining bacterial cells and substrate attached to the cellulose layer. Afterward, the cellulose was rinsed with running water and soaked in periodically changed water for 24 h until pH reached 7. The purified cellulose appeared transparent [7].

2.2.2. Production of intelligent packaging

First, preparation of the indicator solution. BTB indicator solution was chosen for this study because a previous work established this solution as the indicator with the most visually identifiable color change reaction [7]. First, 1% BTB solution (b/v) was prepared in 95% ethanol. Then, the pH of the BTB solution was decreased to 2.74 by adding 20% acetic acid. Finally, the BTB solution was stored in a closed container. Second, **production of intelligent packaging indicator label.** The purified cellulose film was kept in a filter cloth for 24 h to decrease its water content. Half-dried cellulose was cut into 1.5 cm × 4 cm strips and pushed flat against the surface of a Pyrex glass. The cellulose was dried for 30 min at 70 °C until the moisture content reaches 6%. 35 mL of The BTB indicator solution was then absorbed into a dry cellulose via centrifugation at 3000 rpm for 15 min. When the color indicator was successfully absorbed, the BTB indicator solution imparted an orange hue to the cellulose. Afterward, the cellulose was rinsed with distilled water to eliminate any unbound color indicators and then dried [26,28].

2.2.3. production of active packaging film

First, production of garlic extract as active element. The method applied in this research referred to Yolanda et al. [29] with a slight modification. garlic 500 g was peeled, washed under running water until clean, drained, and then mashed. The minced garlic was extracted via the maceration method by immersing the finely ground garlic in 96% alcohol at a ratio of 1:4 (garlic:alcohol) for 4 days at 3–5 °C and periodically homogenized using a water bath shaker. Afterward, the extract was filtered using a filter paper and then concentrated using a rotary evaporator at 50 rpm at 40 °C to obtain a thick extract. **Second, production of active packaging film.** The method used referred to Iriani et al. and Indrarti et al. [19,30] with a slight modification. The bacterial cellulose was crushed to form a cellulose pulp. A cellulose suspension was prepared using 30% chitosan (w/w), 10% CMC

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(w/w), and 15% corn starch (w/w) of cellulose dry weight. The suspension was heated at 50 °C for 60 min with a hot plate stirrer until thoroughly suspended. At the 50th min, 30% glycerol (w/w) was added. Additionally, the garlic extract was added at quantities of 0% (as the control), 5%, 10%, and 15% (v/v) immediately after the final heating step. Subsequently, 60 g of the suspension was then placed onto a glass plate and dried for 48 h at 37 °C. Finally, the suspension was cooled to room temperature, removed from the glass plate, wrapped in aluminum foil, and placed in a desiccator.

2.2.4. Application of the intelligent and active packaging indicators to fresh beef

Fresh beef tenderloin was collected from a slaughterhouse in Tamangapa Raya Makassar 1 h after the cow was slaughtered. It was immediately placed in a special food box and put into a 38 cm × 29 cm × 30 cm Styrofoam box filled with ice crystals. The samples were promptly transported to the laboratory and processed into 200 g/pack pieces. The meat was packaged in a Styrofoam tray (1.05 g/cm³) coated with the active packaging film on a Styrofoam base, and a intelligent packaging indicator label was attached to the LDPE plastic wrap film that covered the Styrofoam container (**Figure 1**). The samples were maintained at room temperature (28±2 °C) with normal light exposure for 24 h.

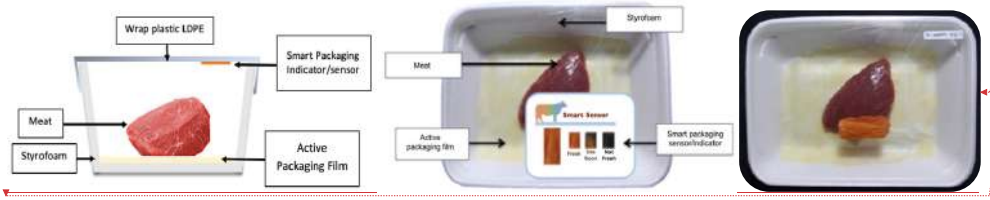

Figure 1. (a) Design of the intelligent and active packaging system; **(b)** prototype of the intelligent packaging ; **(c)** and its application to fresh meat.

During the entire storage period, the intellignet packaging label changed its color three times that corresponded to three phases of the meat samples' level of quality (**Figure 1b**). In phase I, its color was orange, indicating that the meat samples were still fresh. In phase II, its color was green with an orange hue, suggesting that the meat samples should be consumed immediately (Use soon). In phase III, its color was dark green, denoting that the meat samples were already spoiled (Not Fresh).

2.2.5. Observation parameters

2.2.5.1. Measurement of Intelligent Packaging Indicator Color Response on Meat

The color of the intelligent packaging indicators was quantitatively determined using a chromameter digital color meter (T-135). Intelligent and active packaging system containing meat is placed on a flat black background with homogeneous lighting. The chromameter detector was placed on the surface of the intelligent packaging indicator. The measurement results were expressed according to the notation of the Hunter's Lab Colorimetric System, which is presented in three values, namely L^* (lightness), a^* (redness), and b^* (yellowness) [31]. The color of the intelligent packaging indicator was determined by calculating the °Hue value by using the formula (1) below:

$$°Hue = tan^{-1}\frac{b^*}{a^*} \quad (1)$$

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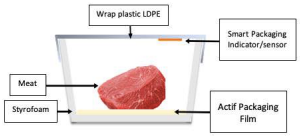

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where ◦Hue represents the parameters for color range, a^* is a red-green mixed color, and b^* is a yellow-blue mixed color.

2.2.5.2. Antimicrobial activity of the active packaging films

The antimicrobial activity of the active packaging films was determined via the agar diffusion method. Each active packaging film was cut into 5 mm circles in a sterile environment and then placed on NA agar media with 0.1 mL of the test microorganism culture (*Staphylococcus aureus*) containing 10^6 CFU/mL. Petri dishes were incubated for 24 h at 37 ◦C. After incubation, the inhibitory zone was measured using a caliper, this measurement was carried out with three replicates [32].

2.2.5.3. Determination of pH of the beef samples

The pH of the beef samples was measured using a pH meter (Oakton pH 510). First, 5 g of crushed meat was introduced with 45 mL of distilled water until the mixture became homogenous. The pH meter's electrode was then immersed in the beef suspension until the pH value on the monitor became constant. This measurement was carried out with three replicates.

2.2.5.4. Measurement of TVBN

The method applied in this research referred to AOAC [33]. A Conway cup with an outer diameter of 10 cm and an inner diameter of 5 cm was utilized in this study. First, 30 mL of 7% TCA solution was added to a meat sample (10±0.1 g) and mixed before filtering. 1 mL boric acid solution was placed in the "inner chamber" of the Conway dish. The lid of the cup was placed in such a way that it almost covered the cup. The 1 mL filtrate was placed into the outer chamber of the Conway dish. Afterward, 1 mL saturated K_2CO_3 solution was put into the outer chamber. The cup was closed and rotated to mix the two liquids in the outer chamber. The blank solution was prepared following the same process but with 7% TCA instead of the filtrate. The solutions were stored at 37 ◦C for 2 h. Then, 2 drops of methyl red and bromothymol blue (2:1) were added to the inner conway cup and then titrated with 0.01 N HCl until a pink hue was formed. TVBN was calculated by formula (2) as follows:

$$\boldsymbol{TVBN\ content}\left(\frac{\boldsymbol{mg}}{\boldsymbol{100g}}\right) = \frac{(\boldsymbol{Vc-Vb})\ \boldsymbol{x}\ \boldsymbol{14.007}\ \boldsymbol{x}\ \boldsymbol{df}\ \boldsymbol{x}\ \boldsymbol{100}}{\boldsymbol{W}} \quad (2)$$

where Vc is the volume of the HCl solution used in sample titration, Vb is the volume of the HCl solution used in blank titration, N is the normality of the HCl solution, W is the sample's weight (g), 14.007 is the molecular weight of nitrogen, and df is the dilution factor. This measurement was carried out with three replicates.

2.2.5.5. Measurement of Total plate count

The total amount of microorganisms was determined via the total plate count (TPC) method described in SNI 2332.3: 2015. First, 1 g of the sample was added to a test tube containing 9 mL of physiological solution until homogeneous (10^{-1} dilution). The dilution was continued until 10^{-6}, at which point 1 mL of the diluted sample was inoculated on NA media in duplicate via the pour plate technique. After the media solidified, the Petri dishes containing the media and the sample solution were incubated upside down at 30 ◦C for 48 h. Afterward, TPC was calculated using the formula (3) below [34]:

$$\boldsymbol{N} = \frac{\sum \boldsymbol{C}}{[(\boldsymbol{1\times n_1})+(\boldsymbol{0.1\times n_2})]\times(\boldsymbol{d})} \quad (3)$$

where N is TPC (CFU/ml), ∑C is the number of colonies counted in all Petri dishes, n_1 is the number of colonies counted in all Petri dishes at first dilution, n_2 is the number of

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colonies counted in all Petri dishes at second dilution, and d is the dilution factor corresponding to the first dilution.

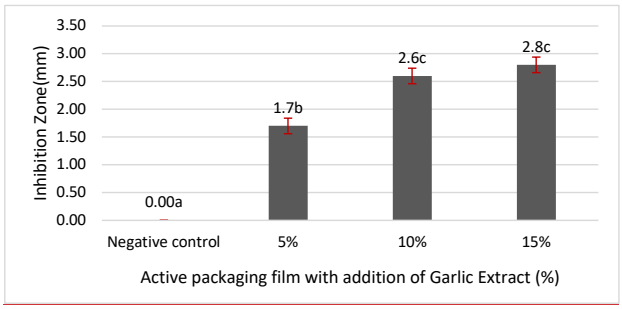
2.2.6. Data analysis

ANOVA was used to analyze the parameters of the intelligent packaging indicator, antimicrobial activity of the active packaging films, and quality of the beef samples, including pH, TVBN, and TPC. Differences between treatments were determined using Duncan's test. The correlations between the changes in the color of the intelligent packaging indicator and the effects of the active packaging on all parameters of meat spoilage were explored and presented in graphs by using the Sigma Plot 12 software. Data were analyzed using Microsoft Excel 2019, SPSS 19, and Sigma Plot 12.

3. Results and Discussion

3.1. Antimicrobial activity of the active packaging films against Staphylococcus aureus

The antimicrobial activity of the active packaging films is presented in **Figure 2.**



The mean value followed by different letters showed a significant difference based on the Duncan's test at the 5% level (p-value < 0.05).

Figure 2. Antimicrobial activity of the active packaging films against *S. aureus*.

The antimicrobial activity of the active packaging films against *S. aureus* was assessed by measuring the diameter of the inhibition zone. As shown in **Figure 2**, the negative control did not generate an inhibitory zone. However, when high concentrations of the garlic extract were added to the active packaging films, the inhibitory activity against the bacteria increased, although the inhibition zone was not significantly different between 10% and 15% garlic extract. This study demonstrated that 10%–15% garlic extract has antibacterial effects. According to Maroles *et al.* [35], differences in the diameter of inhibitory zones are influenced by the ability and rate of diffusion of antimicrobial compounds in the medium, the growth rate of microorganisms and their sensitivity to antimicrobial chemicals, and the viscosity and thickness of the medium.

The antibacterial effects of garlic extract are due to allicin, which is generated when garlic is damaged. When the flesh of garlic is damaged during the refining process, allicin is rapidly generated because of the release of alliinase, which reacts with nonprotein amino acids, namely, alliin. Allicin is a part of the defense mechanism of garlic that exerts antimicrobial effects on both Gram-positive and Gram-negative bacteria by inhibiting RNA and lipid syntheses, which in turn inhibit the production of amino acids and proteins and the phospholipid bilayer of bacterial cell wall, thereby preventing bacterial growth and development. Allicin is highly permeable and can easily penetrate bacterial cells across the cell membrane. The thiosulfinate S(=O)S group in allicin then binds to the

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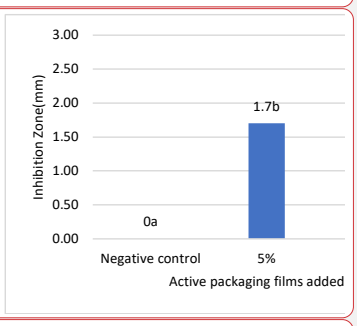

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sulfhydryl groups of bacteria, thus inhibiting the activation mechanism of bacterial proteinases [36,37].

3.2. pH of the beef samples

The pH of the beef samples was measured to investigate the effects of the active packaging films as the meat base in the packaging system. The beef samples were stored at room temperature for 24 h. The results of pH measurements are shown in Table 1.

Table 1. pH values of packaged meat sample stored at room temperature for 24 h.

Storage time (h)	Addition of garlic extract to the active packaging film				Average
	0%	5%	10%	15%	
0	6.56 ± 0.08	6.56 ± 0.08	6.57 ± 0.08	6.57 ± 0.08	6.56 ± 0.00[a]
4	6.68 ± 0.04	6.70 ± 0.06	6.76 ± 0.10	6.75 ± 0.06	6.72 ± 0.04[d]
8	6.72 ± 0.05	6.71 ± 0.07	6.72 ± 0.05	6.59 ± 0.20	6.68 ± 0.06[d]
12	6.57 ± 0.11	6.51 ± 0.03	6.47 ± 0.04	6.39 ± 0.02	6.48 ± 0.08[b]
16	6.44 ± 0.23	6.58 ± 0.06	6.62 ± 0.08	6.61 ± 0.11	6.56 ± 0.08[c]
20	6.75 ± 0.05	6.85 ± 0.01	6.85 ± 0.02	6.81 ± 0.02	6.81 ± 0.05[e]
24	6.88 ± 0.11	6.82 ± 0.11	6.79 ±0.10	6.8 5 ± 0.03	6.83 ± 0.04[e]

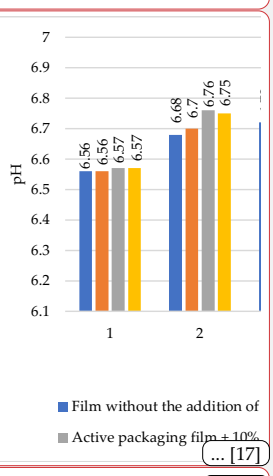
The mean value followed by different letters showed a significant difference based on the Duncan's test at the 5% level (p-value < 0.05).

A statistical test of the storage time showed a significant difference to the pH value (0.000<0.005). However, the statistical test results of the active packaging (0.654>0.005) and interaction between active packaging and storage time (0.179>0.005), on the other hand, did not show a significant effect on the pH value (**Table 1**). One of the characteristics that contribute to meat quality reduction is pH. However, pH cannot be used as the single indicator of meat rot. The pH value is used to confirm the results of other meat deterioration parameters such as TPC or TVBN. According to statistics, active packaging had no significant influence on the pH of the meat and the change in pH seemed to fluctuated, but the data still indicated a rise in pH at each increase in time.

The initial pH of the meat samples, which was immediately determined after the cow was slaughtered, was normal (6.57) (**Table 1**). The pH fluctuated during the storage period, but the trend graph has shown a decrease in pH at the 12 h then the pH increased at the 16 h to 24 h storage. After the animal dies, the blood flow that supplies oxygen to the muscles stops causing an anaerobic glycolysis process to occur. During anaerobic glycolysis, glycogen conversion occurs in the muscles to lactic acid which accumulates in the tissues, causing the pH of the meat to decrease (4 h storage), during anaerobically glycolysis, the decrease in pH continues until the glycogen is converted to lactic acid followed by the neutralization of alkaline compounds resulting from the metabolism of microorganisms, so that the pH of the meat rises again (16-24 h storage).

According to Sánchez-Macías et al. [38] and Moreno et al. [39], reported that the lower the content of glycogen in the meat is, the slower the glycolysis process will be and the higher the final pH will be. However, the decrease in pH in muscles can be influenced by internal factors, such as species, muscle type, muscle glycogen content, and livestock variability, as well as external factors, such as environmental temperature, additional treatment prior to slaughter, and pre-slaughter stress.

After 20 h of storage, the meat's pH value ranged from 6.75 and 6.85 and remained steady up to 24 hour of storage; at this point, the meat was classified as decayed (**Table 1**). According to Prache et al. [40], the meat's pH continues to decline until glycogen is depleted into lactic acid and alkaline compounds are neutralized because of microbial metabolism, resulting in an increase in pH. If the pH reaches 6.8 or higher, protein decomposition will occur, resulting in spoilage.



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3.3. TVBN of the meat samples

The TVBN values of the meat samples are presented in **Table 2**.

Table 2. Total volatile basic nitrogen (TVBN) of the packed meat stored at room temperature for 24 h. Average Result of Meat's TVBN value.

Storage time (h)	Addition of garlic extract to the active packaging film				
	0%	5%	10%	15%	Average
	mgN/100 g				
0	8.35 ± 0.96	7.37 ± 0.56	7.51 ± 0.54	7.23 ± 1.24	7.62 ± 0.50[a]
4	12.27 ±0.54	9.47 ± 2.80	10.17 ± 2.17	10.73 ± 1.17	10.66 ± 1.19[b]
8	19.13 ± 2.07	14.65 ± 0.72	14.79 ± 1.40	13.95 ± 0.96	15.63 ± 2.36[c]
12	20.67 ± 2.68	16.19 ± 0.28	17.31 ± 1.73	16.61 ± 1.21	17.70 ± 2.04[c]
16	29.91 ± 3.78	29.21 ± 5.57	26.41 ± 3.31	25.43 ± 4.89	27.74 ± 2.16[d]
20	47.41 ± 3.17	43.21 ± 1.19	42.09 ± 1.19	44.05 ± 0.79	44.19 ± 2.29[e]
24	80.03 ± 8.65	77.79 ± 3.11	74.99 ± 5.63	76.81 ± 8.26	77.42 ± 2.12[f]
Average	31.12 ± 25.13[b]	28.27 ± 25.16[a]	27.61 ± 23.93 [a]	27.83 ± 24.84[a]	

The mean value followed by different letters showed a significant difference based on the Duncan's test at the 5% level (p-value< 0.05).

A statistical test revealed a highly significant difference between the active packaging (0.004<0.005) and storage time (0.000<0.005) on the TVBN value. However, the statistical test results of the interaction between active packaging and storage time (0.986>0.005), on the other hand, did not show a significant effect on the TVBN value **(Table 2)**. At 0 h, all meat samples had TVBN values ranging from 7.23 mgN/100 g to 8.35 mgN/100 g **(Table 2)**. Therefore, they were classified as fresh meat. After 12 h of storage, the meat samples that had not been treated with the active packaging films had a TVBN value of 20.67 mg N/100g, indicating that they were rotten. By comparison, the meat samples treated with the active packaging films and added with 5%, 10%, and 15% garlic extract had TVBN values of 16.19, 17.31, and 16.61 mgN/100 g, respectively. Thus, they were categorized as semi-fresh meat (stale meat) or could still be consumed.

However, the TVBN values of all meat samples taken between the 16th and 24th h of storage exceeded the threshold for food-grade beef, demonstrating that adding 5%, 10%, and 15% garlic extract to the active packaging films effectively reduced the amount of TVBN, indicated by the increase in TVBN values respectively 29.21%, 16.41%, and 25.3%, which showed the higher the concentration of garlic extract in the active packaging, the less the TVBN production increase. On the other hand, meat samples that were not treated active packaging film treatment had a 29.91 % rise in TVBN value. Beef or livestock is considered fresh if the TVBN value is less than 15 mg/100 g [41] or TVBN is <10 mg N/100 g [42]. Moreover, SNI 2354.8:2009 by National Standardization Agency of Indonesia (BSN) [43] states that the standard levels of TVBN fit for consumption is 20–30 mg N/100g.

In this study, the values of TVBN increased throughout the storage period (observed every 4 h), indicating that the meat's quality continued to deteriorate owing to the breakdown of proteins into volatile base compounds. According to Bekhit et al. [10], the increase in TVBN value is due to protein degradation by microorganisms that results in the formation of foul-smelling chemicals, such as ammonia (NH_3), basic skatole and indole compounds, mercaptans and H_2S (which are weak acids), and amines and cadaverin (which are strong bases). The results demonstrated that the addition of garlic extract to the active packaging films delayed the spoiling of the meat samples likely because the garlic's active components prevented microbial development, thereby lowering the synthesis of nitrogenous base compounds in the meat caused by bacteria and autolytic enzymes during the rotting process. This conjecture was supported by Al Hakim et al. [44]

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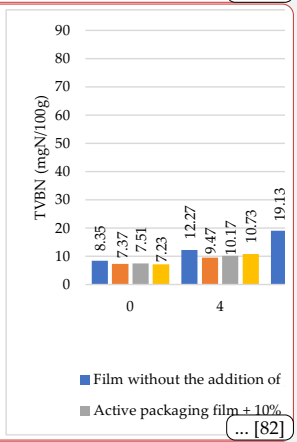

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and Reiter et al. [37], who reported that garlic extract has the ability to block microbe-produced enzymes involved in the breakdown of proteins into volatile base chemicals.

3.4. TPC of the microbes in the beef samples

The TPC of bacteria in the meat samples was determined to assess the utility of the active packaging films **(Table 3)**.

Table 3. Total plate count (TPC) of packed meat stored at room temperature for 24 h.

Storage time (h)	Addition of garlic extract to the active packaging film				Average
	0%	5%	10%	15%	
	log UFC/mL				
0	2.53 ± 0.64	2.53 ± 0.64	2.53 ± 0.64	2.53 ± 0.64	2.53 ± 0.00[a]
4	5.18 ± 0.20	4.64 ± 0.16	3.83 ± 0.30	3.91 ± 0.10	4.39 ± 0.64[b]
8	5.43 ± 0.21	5.33 ± 0.07	4.83 ± 0.40	5.04 ± 0.06	5.16 ± 0.27[c]
12	7.65 ± 0.39	6.20 ± 0.00	5.51 ± 0.10	5.34 ± 0.08	6.18 ± 1.05[d]
16	8.89 ± 0.67	7.44 ± 0.03	7.47 ± 0.26	6.78 ± 0.67	7.64 ± 0.89[e]
20	10.04 ± 0.58	8.30 ± 1.35	7.57 ± 0.60	8.28 ± 0.06	8.55 ± 1.08[f]
24	10.36 ± 0.15	9.53 ± 0.39	9.44 ± 0.68	9.25 ± 0.03	9.65 ± 0.49[g]
Average	7.15 ± 2.89[c]	6.28 ± 2.37[b]	5.88 ± 2.41[a]	5.88 ± 2.39[a]	

The mean value followed by different letters showed a significant difference based on the Duncan's test at the 5% level (p-value < 0.05).

A statistical test revealed a highly significant difference between the active packaging (0.000<0.005) and storage time (0.000<0.005) on the Tpc value. However, the statistical test results of the interaction between active packaging and storage time (0.09>0.005), on the other hand, did not show a significant effect on the TPC value **(Table 3)**. At 0 h of the storage period, the initial TPC value (Log TPC) of all meat samples was 2.53±0.64 CFU/mL **(Table 3)**. Thus, the meat samples were classified as fresh on the basis of microbiological quality. Throughout the storage period, the TPC value increased until it reached the maximum number of meat microbes permitted by SNI 3932:2008 on carcass and beef quality, which is 1×10^6 CFU/mL or equivalent to Log TPC 6 CFU/mL. At 12 h of storage, the meat samples packaged with the control film (0%) and those added with 5% garlic extract did not fulfil the microbiological requirements as they had a Log TPC value of 7.65±0.39 and 6.20±0.00 CFU/mL, respectively. By comparison, the meat samples treated with the active packaging films and 10% and 15% garlic extract also did not fulfil the microbiological requirements after 16 h of storage as they has a Log TPC value of 7.47±0.26 and 6.78±0.67 CFU/mL, respectively. This result demonstrated that the active packaging films with 10% and 15% garlic extract in the meat packaging system can inhibit microbial growth and extend the shelf life of meat by up to 4 h because allicin can inhibit the growth of both Gram-positive and Gram-negative bacteria by destroying the sulfhydryl group bound to bacterial proteins. This process is important because the sulfhydryl group is required for bacterial cell division or acts as a specific stimulator for cell multiplication. Allicin damaged the RNA and DNA of bacteria and thus inhibits their growth and development in meat. Likewise, Deresse [45] reported that allicin can suppress the growth of both Gram-positive and Gram-negative bacteria by completely inhibiting the syntheses of bacterial RNA, DNA, and proteins.

The total microbial content of the meat samples continued to increase during the entire storage period **(Table 3)** because meat contains a high nutrient and water content, which provides an ideal environment for microorganism growth. Moreover, storage at room temperature can accelerate the growth of microorganisms. According to Soeparno [46], meat has the ideal conditions for microorganism growth because it contains a high proportion of water (68%–75%), it is rich in nitrogen-containing substances of varying

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complexity, it contains various fermentable carbohydrates, it is rich in minerals and essential nutrients for microorganism growth, and it has a suitable pH for microorganism growth (pH 5.3–6.5). Variance analysis revealed that the duration of storage of the meat samples and the use of the active packaging films with garlic extract had a highly significant effect on the TPC value of the samples (P>0.01).

3.5. Changes in the color of the intelligent packaging BTB indicator solution as a measure of the freshness of the meat packaged with the active packaging films

Using fresh beef packaged and maintained at room temperature for 24 h, Dirpan et al. [7] determined that BTB solution (pH 2.75) produces the most readily visible color changes to sensitivity tests. In this study, the BTB solution (pH 2.75), as the intelligent packaging indicator, was also utilized to evaluate changes in its color as a reflection of the freshness of the meat samples packed with the active packaging films **(Figure 3)**.

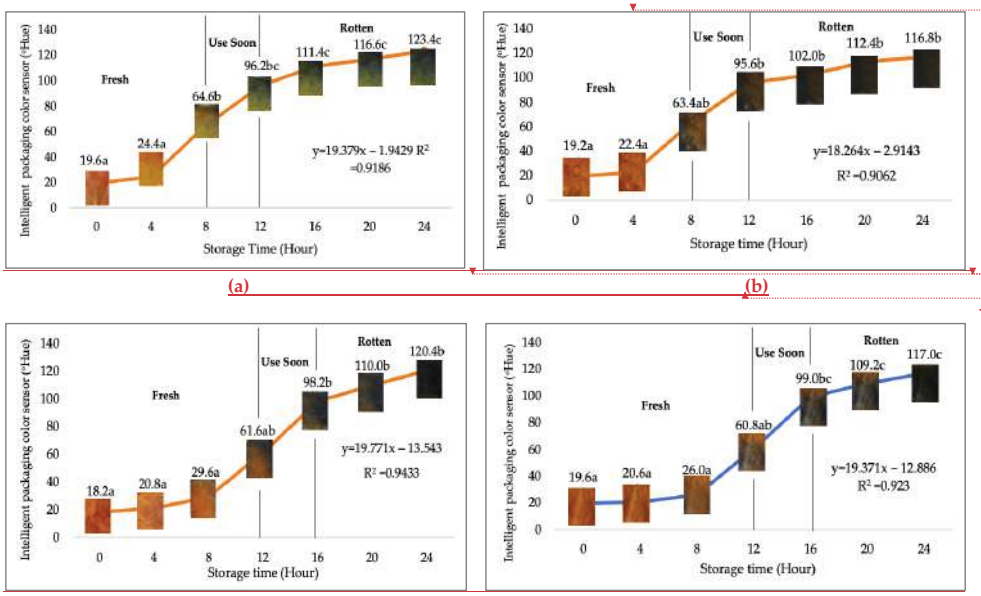

Figure 3. Changes in the color of the BTB solution (pH 2.75) as the intelligent packaging indicator reflecting the freshness of the meat samples packed with the active packaging films with **(a)** 0%; **(b)** 5%; **(c)** 10%; and **(d)** 15% of garlic extract.

During the entire storage period, the intelligent packaging indicator changed its color three times that corresponded to three phases of the meat samples' level of quality **(Figure 3)**. In phase I, its color was orange, indicating that the meat samples were still fresh. In phase II, its color was green with an orange hue, suggesting that the meat samples should be consumed immediately. In phase III, its color was dark green, denoting that the meat samples were already spoiled. The change in the indicator's color from orange to green indicated that the quality of the meat samples had deteriorated. The changes in the indicator's color were due to the interactions of alkaline volatile compounds produced by enzyme activity, and the metabolism of the microorganisms present in the meat samples

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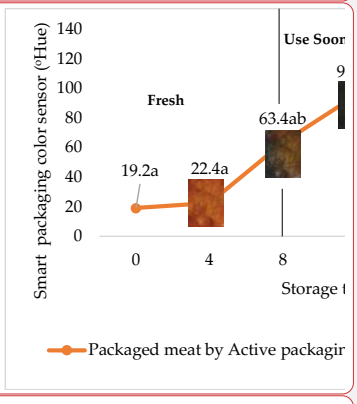

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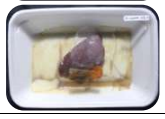
increased with storage time. The early sign of spoilage was indicated by the release of volatile alkaline compounds as the microorganisms and the enzymes degraded the nutritional content of the meat samples. These compounds gradually accumulated in the packaging system, causing an increase in pH, which was detected by the intelligent packaging indicator and displayed as gradual color changes. The change in color of the intelligent packaging indicator (BTB, pH 2.75) from orange to green was induced by deprotonation or the release of a proton from the intelligent packaging indicator dye [47].

The meat samples packaged with the active packaging films but with no (0%) and 5% garlic extract were still fresh from the start of the storage up to 8 h **(Figure 3)**. However, they must be immediately consumed from the 8th h to the 12th h of the storage period. Thereafter (12–24 h of the storage period), they were already spoiled. This results was consistent with that of TPC tests, which showed that the TPC values were above the acceptable threshold for microbial contaminants (1×10^6 or equivalent to 6 CFU/mL) in meat after 12 h. By comparison, the meat samples packaged with the active packaging films containing 10% and 15% garlic extract were still considered fresh from the start of the storage period up to the 12th h. They must be immediately consumed when they had been in storage for 12–16 h. Finally, they were considered rotten when they had been in storage for 16–24 h. This result was also consistent with that of TPC tests **(Table 3)**, which indicated that at the 16th hour, the TPC value surpassed the permissible level of microbiological contamination in beef. Statistical analysis revealed that storage duration had a very significant effect on the Hue value, the indicator of color change in the intelligent packaging. The changes in the color of the intelligent packaging indicator (BTB solution, pH 2.75) when used together with the active packaging films to reflect the freshness of meat are presented in **Table 4**.

Table 4. Changes in the color of the intelligent packaging indicator (BTB solution, pH 2.75) when used together with the active packaging films to reflect the freshness of meat.

Storage Time (h)	Active Packaging Films Added with Garlic Extract			
	0%	5%	10%	15%
0				
4				
8				
12				

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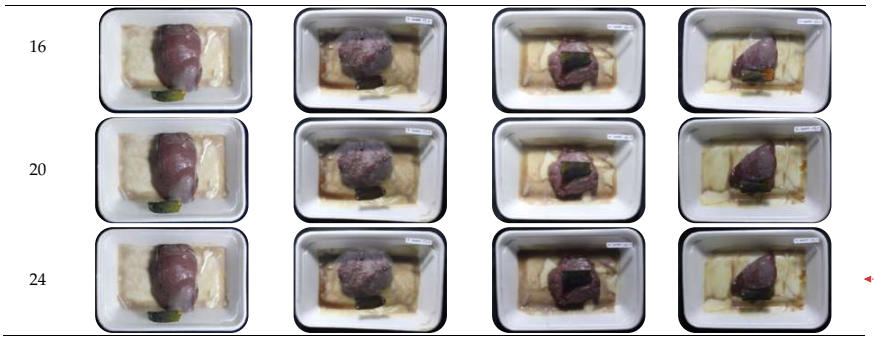

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3.6. Correlations between changes in the color of the intelligent packaging indicator and the effects of the active packaging films on the parameters of meat freshness

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The correlations between changes in the color of the intelligent packaging indicator and parameters of meat quality deterioration (pH, TVBN, and TPC) were explored to ascertain the relationship between the sensitivity of the intelligent packaging indicator to meat freshness and the effectiveness of the active packaging films in slowing the process of meat spoilage.

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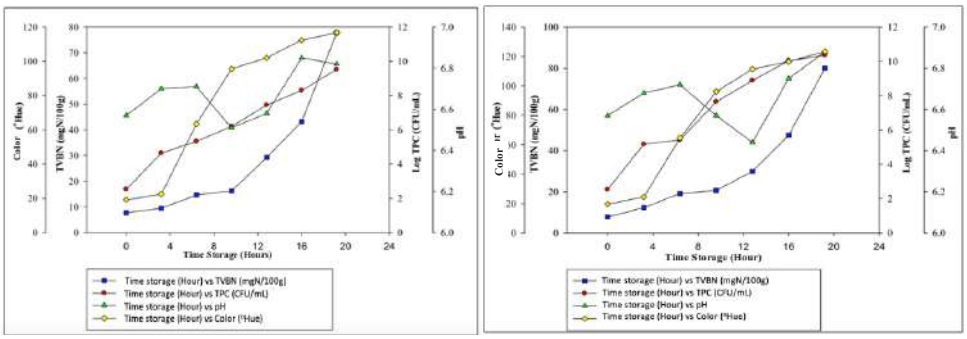

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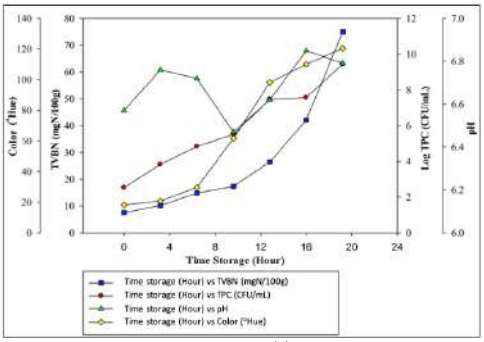

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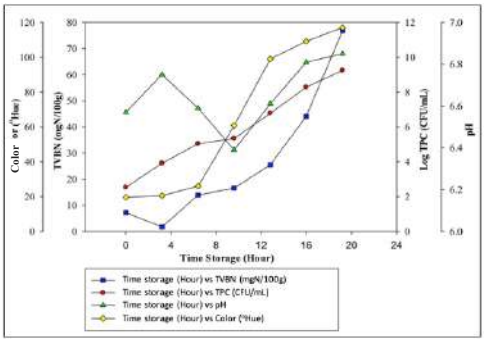

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Figure 4. Correlations between changes in the color of the intelligent packaging indicator and the effects of the active packaging films with **(a)** 0%; **(b)** 5%; **(c)** 10%; and **(d)** 15% garlic extract on the parameters of quality deterioration of meat stored for 24 h.

Based on **Figure 4**, it is known that the color change of the intelligent packaging indicator which is indicated by an increase in the Hue value is in line with the increase in all values of the meat deterioration parameter, the meat samples packaged with the control film and those treated with 5% garlic extract were rotten and unfit for consumption after 12 h of storage as their Log TPC value was 7.65±0.39 and 6.20±0.00 CFU/mL, respectively, and their TVBN value was 20.67±2.68 and 16.19±0.28 mgN/100g, respectively **(Figure 4)**. By comparison, the meat samples treated with the active packaging films and 10% and 15% garlic extract were rotten and unfit for consumption after 16 h of storage as their Log TPC value was 7.47±0.26 and 6.78±0.67 CFU/mL, respectively, and their TVBN value was 26.41±3.31 and 25.43±4.89 mgN/100 g, respectively.

Meat that was treated with active packaging film without addition (0 %) and with the addition of 5% garlic extract experienced a change in indicator color from orange (fresh) with a color value of 19.6°Hue and 19.20°Hue, respectively to green (rotten) with color values 96.2°Hue and 95.6°Hue, respectively. Meanwhile, the meat that was treated with active packaging film with the addition of 10 % and 15 % garlic extract experienced a change in indicator color from orange (fresh) with color values 18.2°Hue and 19.6°Hue, respectively, to green (rotten) with color values 98.2°Hue, and 99°Hue, respectively. Wiryawan [48] observed that when garlic extract was added to the active packaging, the values of TPC and TVBN and the pH of the meat increased more slowly, as did the color of the intelligent packaging indicator, compared with those of the meat without the active packaging.

Furthermore, the increase in the values of TPC and TVBN linearly correlates with the increase in Hue value and color changes of the intelligent packaging indicator because the accumulated volatile base compounds raise the pH value of the packaging system, causing the intelligent packaging indicator to experience a color shift. This explanation was in agreement with that of Pacquit et al. [12], who applied active packaging films to cod fish. They stated that the increase in the TPC value of cod fish has a linear correlation with changes in the color of the cellulose-acetate packaging film sensor.

On the other hand, the pH of the sample fluctuated making it difficult to determine the level of quality degradation in meat. However, the interpretation of the TPC and TVBN values, on the other hand, is clear enough to represent a decrease in meat quality which is correlated with an increase in the color value of changes in the intelligent packaging indicator. This good correlation demonstrates the accuracy of the film formulation in the monitoring of meat freshness, which is the aim of using intelligent packaging.

4. Conclusions

The paper concludes that intelligent packaging indicators using BTB (Bromothymol blue) pH 2.75 solution can be used as an indicator to identify a decline in the quality of packaged meat. The indicator's color changes is easy to observe visually, namely the orange indicator indicating that the meat is still fresh and the dark green indicator indicating that the meat has rotted and is unfit for consumption. On the other hand, the use of active packaging can extend the shelf life of meat by 4 hours longer when using high concentrations of garlic extract. This demonstrates that intelligent and active packaging, which are typically studied separately, have the potential to be combined and researched together using the same basic ingredient, namely bacterial cellulose.

Author Contributions: Conceptualization, A.D; methodology, A.D. and I.K; software, A.D. and I.K; validation, A.D. and M.D.; formal analysis, A.D. and I.K.; investigation, I.K.; resources, A.D.; data curation, A.D. and I.K.; writing—original draft preparation, M.D. and I.K.; writing—review and editing, A.D., M.D., and I.K.; visualization, I.K.; supervision, A.D; project administration, A.D. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Available data are presented in the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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Application of an Intelligent Sensor and Active Packaging System Based on the Bacterial Cellulose of *Acetobacter Xylinum* to Meat Products

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Department of Agricultural Technology, Hasanuddin University, Makassar 90245, Indonesia; muspirah_djalal@agri.unhas.ac.id (M.D.); irmakama9@gmail.com (I.K.)
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Abstract: Combining intelligent and active packaging serves the dual purpose of detecting color changes in food that reflect changes in its quality and prolonging its shelf life. This study developed an intelligent and active packaging system made from the cellulose of *Acetobacter xylinum* and assessed its ability to detect changes in the quality and to increase shelf-life of packaged fresh beef. The properties of the intelligent packaging's sensor and active packaging films were determined. The application of this system to fresh beef stored at room temperature (28 ± 2 °C) for 24 h was tested. The color of the bromothymol blue (BTB) solution (pH 2.75) in the indicator of the intelligent packaging system changed from orange to dark green to indicate that beef quality changed from fresh to rotten. The meat treated with the active packaging with 10% and 15% garlic extract decayed on the 16th h. In contrast, the meat treated with the active packaging without the garlic extracts rotted on the 12th h. The shift in the indicator's color was linearly related to the total plate count (TPC), total volatile basic nitrogen (TVBN), and pH of the meat packaged using the active packaging system. Therefore, BTB solution (pH 2.75) can be used as an intelligent packaging indicator that will allow consumers to assess the quality of packaged meat easily. As an antimicrobial agent, the addition of 10–15% garlic extract to the active packaging films can help delay the spoilage of packaged beef.

Keywords: smart sensor; smart packaging; meat shelf-life; food quality

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1. Introduction

Global beef consumption is predicted to rise as the world population and family income increase, particularly in developing Asian countries [1–3]. By 2030, worldwide meat consumption and availability are expected to increase by 14% and 5.9%, respectively, over the average of the 2018–2020 period [3]. Thus, the expected increase in meat consumption must be complemented by improvements in the quality of fresh meat produced. One aspect affecting the quality and characteristics of meat is the material and packaging technologies used [4]. Meat is a perishable item that rapidly spoils when stored above the optimum temperature range (below −17 to 4 °C) [5,6]. However, in traditional markets, meat is displayed at room temperature without packaging, a practice that might accelerate microbial contamination and cause rapid quality degradation. Even in supermarkets where meat is maintained in cold temperatures, standard meat packaging still prevents consumers from subjectively determining the quality of meat. Thus, meat packaging must have additional functions that will prevent quality degradation due to microbial contamination and will help consumers to determine the quality of packaged meat easily [7]. Conventional meat packaging can be designed to perform dual functions through intelligent and active packaging systems.

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Intelligent packaging is a term that refers to sensors in the form of indicators that monitor and provide information on the quality of the food contained within the packaging via color changes caused by chemical reactions between the indicators and the products of microbial metabolism or changes in the chemical composition of the food [8,9]. During storage, the chemical components of meat degrade into volatile compounds because of microbial activity, thereby increasing the value of total volatile base nitrogen (TVBN) [10,11]. Accumulation of TVBN increases the pH of the packaging system, which is detected by the indicator, resulting in a visible color shift in the indicator [11,12]. Intelligent packaging allows easier monitoring of packed products during transportation and storage [7]. Moreover, it provides a more accurate estimate of product condition than conventional expiration labels [12]. Color-based pH indicator solutions are widely used as intelligent indicators. Dirpan et al. [13] developed bromophenol blue as an intelligent indicator dye for mangoes. Hidayat et al. [14] used two types of color indicators with predetermined concentrations, namely, phenol red and bromothymol blue, to assess the freshness of meat packaging. Intelligent packaging indicators based on natural pigments are being developed, such as intelligent packaging films that include anthocyanin-loaded Lycium ruthenicum nanocomplexes in starch/polyvinyl alcohol mixtures (PVA) [15], as well as anthocyanins from saffron petals immobilized in chitosan nanofibers and methyl cellulose matrix [16].

Active packaging refers to the integration of particular additives into a packaging system for the purpose of extending the shelf life, preserving the quality, and ensuring the safety of food products. Antimicrobial agents are used as components of active packaging additives to extend product shelf life. The volatile bioactive compounds in active packaging evaporate or diffuse onto the food surface, where they limit the growth of microbes and thus delay spoilage [17,18]. This strategy is more effective than coating bioactive compounds onto the food surface [19]. The safest, cheapest, and most readily available antimicrobial agents for use in active packaging are essential oils. Pranoto et al. [20] produced antimicrobial alginate edible films by incorporating the essential oils of garlic. They reported that these films substantially inhibited the growth of *Staphylococcus aureus* and *Bacillus cereus* in meat. Vishnu et al. [21] utilized the essential oils of *Plectranthus amboinicus* in a chitosan-based active packaging to restrict antimicrobial activity.

Intelligent and active packaging can be merged into a single packaging system. Julyaningsih et al. [22] combined an intelligent packaging system based on methyl red and bromothymol blue (BTB) indicator with an active packaging system based on lemongrass oil as a component of tuna fish fillet packaging. Yao et al. [23] developed an active and intelligent packaging system based on starch, PVA, and betacyanins from various types of plants for shrimp packaging. In general, an active packaging that contains antimicrobial agents and an intelligent packaging that contains indicator solutions are immobilized in a polymer. Compared with synthetic polymers or plant cellulose, the bacterial cellulose fermented by *Acetobacter xylinum* has a unique nanofibrillar structure and superior physical properties, suggesting that it has the potential to serve as a basis for developing an intelligent and active packaging system [24,25]. Bacterial cellulose has received interest as a component of active packaging owing to its biodegradability, high water-holding capacity so that it can be employed entirely as a polymer for immobilizing color solutions in intelligent packaging indicators [26]. Moreover, bacterial cellulose possess great potential as an antimicrobial agent carrier in order for it to be utilized as an ingredient in the production of active packaging films [27].

The development of packaging systems with additional functions is advancing. To promote this innovation, this study aimed to maximize the potential of intelligent and active packaging by combining them into a single packaging system based on a bacterial cellulose membrane biopolymer to enhance the quality of packaged meat and help consumers to determine meat freshness easily.

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2. Materials and Methods

2.1. Materials

The main ingredients used in the intelligent and active packaging system developed herein were the bacterial cellulose produced by *A. xylinum*, which was fermented in natural media of coconut water. Beef tenderloin was purchased from a slaughterhouse in Tamangapa Raya. Coconut water and garlic (*Allium sativum*) were purchased from a local market. Food-grade ammonium sulfate (Lianyungang Zhonghong Chemical Co., Ltd., Jiangsu, China, CAS No: 7783-20-2), yeast extract (Merck, Darmstadt, Germany, CAS No: 8013-01-2), 96% acetic acid (Brenntag Inc, Essen, Germany, CAS No: 64-19-7), *A. xylinum* culture, 5% 1 N NaOH (Brenntag Inc, CAS No: 1310-73-2), sucrose, Bromothymol Blue (BTB) (Merck, Darmstadt, Germany, CAS No: 76-59-5), alcohol (Sd Fine Chem Limited, Chennai, Tamil Nadu, India), aquabides, aquades (Rofa Laboratorium Centre, Bandung, Indonesia), Tashiro's indicator (0.1% Methyl Red [Merck, Darmstadt, Germany, Cas No: 493-52-7] and 0.1% BTB at a ratio of 2:1), 7% trichloroacetic acid (TCA) (Merck, Darmstadt, Germany,), Nutrient Agar (NA) (Merck, Darmstadt, Germany,), glycerol (Merck, Darmstadt, Germany, CAS No: 56-81-5), food-grade carboxymethyl–cellulose (CMC) (Foodchem, Shanghai, China, E466), and corn starch were used.

2.2. Methods

2.2.1. Production of Bacterial Cellulose from *A. xylinum*

Based on our previous research Dirpan et al. [7], 5% (*w*/*v*) of food grade Ammonium Sulfate is the best source of Nitrogen in *Acetobacter xylinum* growth media to produce optimal bacterial cellulose membranes. Determination of the composition and type of Nitrogen source. Then, purification of bacterial cellulose was done by removal from the fermentation medium, rinsed in running water, and then soaked for 2 days with periodic water changes. The cellulose was also soaked in 70% alcohol for 1 min, heated to 100 °C in distilled water for 20 min, and reheated in 1 N 5% NaOH solution at 100 °C for 60 min to remove the remaining bacterial cells and substrate attached to the cellulose layer. Afterward, the cellulose was rinsed with running water and soaked in periodically changed water for 24 h until pH reached 7. The purified cellulose appeared transparent [7].

2.2.2. Production of Intelligent Packaging

First, preparation of the indicator solution. BTB indicator solution was chosen for this study because a previous work established this solution as the indicator with the most visually identifiable color change reaction [7]. First, 1% BTB solution (*b*/*v*) was prepared in 95% ethanol. Then, the pH of the BTB solution was decreased to 2.74 by adding 20% acetic acid. Finally, the BTB solution was stored in a closed container. Second, production of intelligent packaging indicator label. The purified cellulose film was kept in a filter cloth for 24 h to decrease its water content. Half-dried cellulose was cut into 1.5 cm × 4 cm strips and pushed flat against the surface of a Pyrex glass. The cellulose was dried for 30 min at 70 °C until the moisture content reaches 6%. A total of 35 mL of I BTB indicator solution was then absorbed into a dry cellulose via centrifugation at 3000 rpm for 15 min. When the color indicator was successfully absorbed, the BTB indicator solution imparted an orange hue to the cellulose. Afterward, the cellulose was rinsed with distilled water to eliminate any unbound color indicators and then dried [26,28].

2.2.3. Production of Active Packaging Film

First, the production of garlic extract as an active element. The method applied in this research referred to Yolanda et al. [29] with a slight modification. A total of 500 g of garlic was peeled, washed under running water until clean, drained, and then mashed. The minced garlic was extracted via the maceration method by immersing the finely ground garlic in 96% alcohol at a ratio of 1:4 (garlic: alcohol) for 4 days at 3–5 °C and periodically homogenized using a water bath shaker. Afterward, the extract was filtered using a filter

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paper and then concentrated using a rotary evaporator at 50 rpm at 40 °C to obtain a thick extract. Second, production of active packaging film. The method used referred to Iriani et al. and Indrarti et al. [19,30] with a slight modification. The bacterial cellulose was crushed to form a cellulose pulp. A cellulose suspension was prepared using 30% chitosan (*w*/*w*), 10% CMC (*w*/*w*), and 15% corn starch (*w*/*w*) of cellulose dry weight. The suspension was heated at 50 °C for 60 min with a hot plate stirrer until thoroughly suspended. At the 50th min, 30% glycerol (*w*/*w*) was added. Additionally, the garlic extract was added at quantities of 0% (as the control), 5%, 10%, and 15% (*v*/*v*) immediately after the final heating step. Subsequently, 60 g of the suspension was then placed onto a glass plate and dried for 48 h at 37 °C. Finally, the suspension was cooled to room temperature, removed from the glass plate, wrapped in aluminum foil, and placed in a desiccator.

2.2.4. Application of the Intelligent and Active Packaging Indicators to Fresh Beef

Fresh beef tenderloin was collected from a slaughterhouse in Tamangapa Raya Makassar 1 h after the cow was slaughtered. It was immediately placed in a special food box and put into a 38 cm × 29 cm × 30 cm Styrofoam box filled with ice crystals. The samples were promptly transported to the laboratory and processed into 200 g/pack pieces. The meat was packaged in a Styrofoam tray (1.05 g/cm^3) coated with the active packaging film on a Styrofoam base, and an intelligent packaging indicator label was attached to the LDPE plastic wrap film that covered the Styrofoam container (Figure 1c). The samples were maintained at room temperature (28 ± 2 °C) with normal light exposure for 24 h.

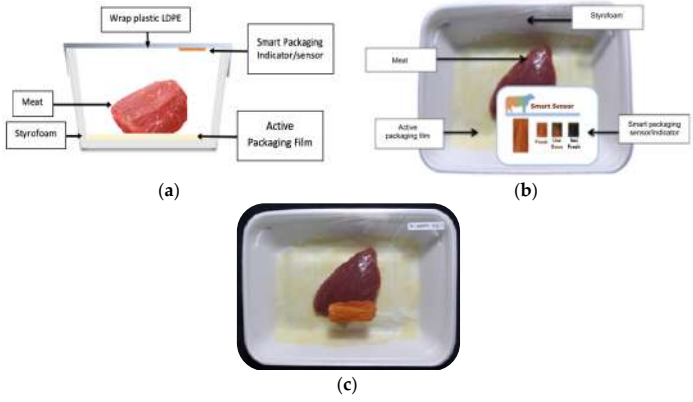

Figure 1. (**a**) Design of the intelligent and active packaging system; (**b**) prototype of the intelligent packaging ; (**c**) and its application on fresh beef.

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During the entire storage period, the intelligent packaging label changed its color three times that corresponded to three phases of the meat samples' level of quality (Figure 1b). In phase I, its color was orange, indicating that the meat samples were still fresh. In phase II, its color was green with an orange hue, suggesting that the meat samples should be consumed immediately (Use soon). In phase III, its color was dark green, denoting that the meat samples were already spoiled (Not Fresh).

2.2.5. Observation Parameters

Measurement of Intelligent Packaging Indicator Color Response on Meat

The color of the intelligent packaging indicators was quantitatively determined using a chromameter digital color meter (T-135). Intelligent and active packaging system containing meat is placed on a flat black background with homogeneous lighting. The

chromameter detector was placed on the surface of the intelligent packaging indicator. The measurement results were expressed according to the notation of the Hunter's Lab Colorimetric System, which is presented in three values, namely L* (lightness), a* (redness), and b* (yellowness) [31]. The color of the intelligent packaging indicator was determined by calculating the Hue value by using the formula (1) below:

$$\text{Hue (degrees)} = \tan^{-1}\frac{b^*}{a^*} \quad (1)$$

where Hue (360 degrees in a circle) represents the parameters for color range, a* is a red-green mixed color, and b* is a yellow-blue mixed color.

Antimicrobial Activity of the Active Packaging Films

The antimicrobial activity of the active packaging films was determined via the agar diffusion method. Each active packaging film was cut into 5 mm circles in a sterile environment and then placed on NA agar media with 0.1 mL of the test microorganism culture (*Staphylococcus aureus*) containing 10^6 CFU/mL. Petri dishes were incubated for 24 h at 37 °C. After incubation, the inhibitory zone was measured using a caliper, this measurement was carried out with three replicates [32].

Determination of pH of the Beef Samples

The pH of the beef samples was measured using a pH meter (Oakton pH 510). First, 5 g of crushed meat was introduced with 45 mL of distilled water until the mixture became homogenous. The pH meter's electrode was then immersed in the beef suspension until the pH value on the monitor became constant. This measurement was carried out with three replicates.

Measurement of TVBN

The method applied in this research referred to AOAC [33]. A Conway cup with an outer diameter of 10 cm and an inner diameter of 5 cm was utilized in this study. First, 30 mL of 7% TCA solution was added to a meat sample (10 ± 0.1 g) and mixed before filtering. A total of 1 mL boric acid solution was placed in the "inner chamber" of the Conway dish. The lid of the cup was placed in such a way that it almost covered the cup. The 1 mL filtrate was placed into the outer chamber of the Conway dish. Afterward, 1 mL saturated K_2CO_3 solution was put into the outer chamber. The cup was closed and rotated to mix the two liquids in the outer chamber. The blank solution was prepared following the same process but with 7% TCA instead of the filtrate. The solutions were stored at 37 °C for 2 h. Then, 2 drops of methyl red and bromothymol blue (2:1) were added to the inner Conway cup and then titrated with 0.01 N HCl until a pink hue was formed. TVBN was calculated by formula (2) as follows:

$$\text{TVBN content } \left(\frac{\text{mg}}{100\text{g}}\right) = \frac{(Vc - Vb) \times 14.007 \times df \times 100}{W} \quad (2)$$

where Vc is the volume of the HCl solution used in sample titration, Vb is the volume of the HCl solution used in blank titration, N is the normality of the HCl solution, W is the sample's weight (g), 14.007 is the molecular weight of nitrogen, and df is the dilution factor. This measurement was carried out with three replicates.

Measurement of Total Plate Count

The total amount of microorganisms was determined via the total plate count (TPC) method described in Indonesian National Standard (SNI) 2332.3: 2015. First, 1 g of the sample was added to a test tube containing 9 mL of physiological solution until homogeneous (10^{-1} dilution). The dilution was continued until 10^{-6}, at which point 1 mL of the diluted sample was inoculated on NA media in duplicate via the pour plate technique. After the media solidified, the Petri dishes containing the media and the sample solution

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were incubated upside down at 30 °C for 48 h. Afterward, TPC was calculated using the formula (3) below [34]:

$$N = \frac{\sum C}{[(1 \times n_1) + (0.1 \times n_2)] \times (d)} \quad (3)$$

where N is TPC (CFU/mL), $\sum C$ is the number of colonies counted in all Petri dishes, n_1 is the number of colonies counted in all Petri dishes at first dilution, n_2 is the number of colonies counted in all Petri dishes at second dilution, and d is the dilution factor corresponding to the first dilution.

2.2.6. Data Analysis

ANOVA was used to analyze the parameters of the intelligent packaging indicator, antimicrobial activity of the active packaging films, and quality of the beef samples, including pH, TVBN, and TPC. Differences between treatments were determined using Duncan's test. The correlations between the changes in the color of the intelligent packaging indicator and the effects of the active packaging on all parameters of meat spoilage were explored and presented in graphs by using the Sigma Plot 12 software. Data were analyzed using Microsoft Excel 2019, SPSS 19, and Sigma Plot 12.

3. Results and Discussion

3.1. Antimicrobial Activity of the Active Packaging Films against Staphylococcus Aureus

The antimicrobial activity of the active packaging films is presented in Figure 2.

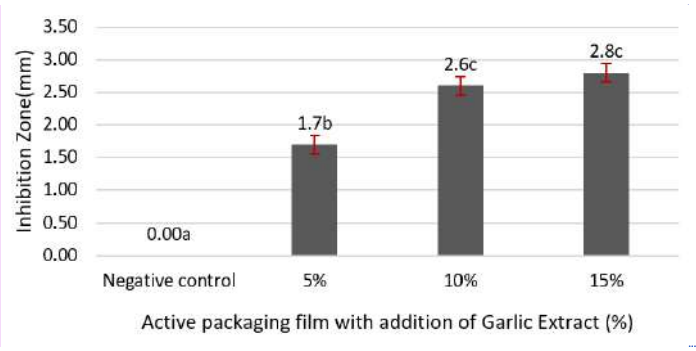

Figure 2. Antimicrobial activity of the active packaging films against *S. aureus*. The mean value followed by different letters showed a significant difference based on the Duncan's test at the 5% level (p-value < 0.05).

The antimicrobial activity of the active packaging films against *S. aureus* was assessed by measuring the diameter of the inhibition zone. As shown in Figure 2, the negative control did not generate an inhibitory zone. However, when high concentrations of the garlic extract were added to the active packaging films, the inhibitory activity against the bacteria increased, although the inhibition zone was not significantly different between 10% and 15% garlic extract. This study demonstrated that 10%–15% garlic extract has antibacterial effects. According to Maroles et al. [35], differences in the diameter of inhibitory zones are influenced by the ability and rate of diffusion of antimicrobial compounds in the medium, the growth rate of microorganisms and their sensitivity to antimicrobial chemicals, and the viscosity and thickness of the medium.

The antibacterial effects of garlic extract are due to allicin, which is generated when garlic is damaged. When the flesh of garlic is damaged during the refining process, allicin is rapidly generated because of the release of alliinase, which reacts with nonprotein

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amino acids, namely, alliin. Allicin is a part of the defense mechanism of garlic that exerts antimicrobial effects on both Gram-positive and Gram-negative bacteria by inhibiting RNA and lipid syntheses, which in turn inhibit the production of amino acids and proteins and the phospholipid bilayer of bacterial cell wall, thereby preventing bacterial growth and development. Allicin is highly permeable and can easily penetrate bacterial cells across the cell membrane. The thiosulfinate S(=O)S group in allicin then binds to the sulfhydryl groups of bacteria, thus inhibiting the activation mechanism of bacterial proteinases [36,37].

3.2. pH of the Beef Samples

The pH of the beef samples was measured to investigate the effects of the active packaging films as the meat base in the packaging system. The beef samples were stored at room temperature for 24 h. The results of pH measurements are shown in Table 1.

Table 1. pH values of packaged meat sample stored at room temperature for 24 h.

Storage Time (h)	Addition of Garlic Extract to the Active Packaging Film				Average
	0%	5%	10%	15%	
0	6.56 ± 0.08	6.56 ± 0.08	6.57 ± 0.08	6.57 ± 0.08	6.56 ± 0.00 [a]
4	6.68 ± 0.04	6.70 ± 0.06	6.76 ± 0.10	6.75 ± 0.06	6.72 ± 0.04 [d]
8	6.72 ± 0.05	6.71 ± 0.07	6.72 ± 0.05	6.59 ± 0.20	6.68 ± 0.06 [d]
12	6.57 ± 0.11	6.51 ± 0.03	6.47 ± 0.04	6.39 ± 0.02	6.48 ± 0.08 [b]
16	6.44 ± 0.23	6.58 ± 0.06	6.62 ± 0.08	6.61 ± 0.11	6.56 ± 0.08 [c]
20	6.75 ± 0.05	6.85 ± 0.01	6.85 ± 0.02	6.81 ± 0.02	6.81 ± 0.05 [e]
24	6.88 ± 0.11	6.82 ± 0.11	6.79 ± 0.10	6.8 5 ± 0.03	6.83 ± 0.04 [e]

The mean value followed by different letters showed a significant difference based on the Duncan's test at the 5% level (p-value < 0.05).

A statistical test of the storage time showed a significant difference to the pH value (0.000 < 0.005). However, the statistical test results of the active packaging (0.654 > 0.005) and interaction between active packaging and storage time (0.179 > 0.005), on the other hand, did not show a significant effect on the pH value (Table 1). One of the characteristics that contribute to meat quality reduction is pH. However, pH cannot be used as the single indicator of meat rot. The pH value is used to confirm the results of other meat deterioration parameters such as TPC or TVBN. According to statistics, active packaging had no significant influence on the pH of the meat and the change in pH seemed to fluctuated, but the data still indicated a rise in pH at each increase in time.

The initial pH of the meat samples, which was immediately determined after the cow was slaughtered, was normal (6.57) (Table 1). The pH fluctuated during the storage period, but the trend graph has shown a decrease in pH at 12 h then the pH increased at the 16 h to 24 h storage. After the animal dies, the blood flow that supplies oxygen to the muscles stops causing an anaerobic glycolysis process to occur. During anaerobic glycolysis, glycogen conversion occurs in the muscles to lactic acid which accumulates in the tissues, causing the pH of the meat to decrease (4 h storage), during anaerobically glycolysis, the decrease in pH continues until the glycogen is converted to lactic acid followed by the neutralization of alkaline compounds resulting from the metabolism of microorganisms, so that the pH of the meat rises again (16–24 h storage).

According to Sánchez-Macías et al. [38] and Moreno et al. [39], reported that the lower the content of glycogen in the meat is, the slower the glycolysis process will be and the higher the final pH will be. However, the decrease in pH in muscles can be influenced by internal factors, such as species, muscle type, muscle glycogen content, and livestock variability, as well as external factors, such as environmental temperature, additional treatment prior to slaughter, and pre-slaughter stress.

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After 20 h of storage, the meat's pH value ranged from 6.75 and 6.85 and remained steady up to 24 h of storage; at this point, the meat was classified as decayed (Table 1). According to Prache et al. [40], the meat's pH continues to decline until glycogen is depleted into lactic acid and alkaline compounds are neutralized because of microbial metabolism, resulting in an increase in pH. If the pH reaches 6.8 or higher, protein decomposition will occur, resulting in spoilage.

3.3. TVBN of the Meat Samples

The TVBN values of the meat samples are presented in Table 2.

Table 2. Total volatile basic nitrogen (TVBN) of the packed meat stored at room temperature for 24 h. Average Result of Meat's TVBN value.

Storage Time (h)	Addition of Garlic Extract to the Active Packaging Film				Average
	0%	5%	10%	15%	
	mgN/100 g				
0	8.35 ± 0.96	7.37 ± 0.56	7.51 ± 0.54	7.23 ± 1.24	7.62 ± 0.50 [a]
4	12.27 ± 0.54	9.47 ± 2.80	10.17 ± 2.17	10.73 ± 1.17	10.66 ± 1.19 [b]
8	19.13 ± 2.07	14.65 ± 0.72	14.79 ± 1.40	13.95 ± 0.96	15.63 ± 2.36 [c]
12	20.67 ± 2.68	16.19 ± 0.28	17.31 ± 1.73	16.61 ± 1.21	17.70 ± 2.04 [c]
16	29.91 ± 3.78	29.21 ± 5.57	26.41 ± 3.31	25.43 ± 4.89	27.74 ± 2.16 [d]
20	47.41 ± 3.17	43.21 ± 1.19	42.09 ± 1.19	44.05 ± 0.79	44.19 ± 2.29 [e]
24	80.03 ± 8.65	77.79 ± 3.11	74.99 ± 5.63	76.81 ± 8.26	77.42 ± 2.12 [f]
Average	31.12 ± 25.13 [b]	28.27 ± 25.16 [a]	27.61 ± 23.93 [a]	27.83 ± 24.84 [a]	

The mean value followed by different letters showed a significant difference based on the Duncan's test at the 5% level (p-value < 0.05).

A statistical test revealed a highly significant difference between the active packaging (0.004 < 0.005) and storage time (0.000 < 0.005) on the TVBN value. However, the statistical test results of the interaction between active packaging and storage time (0.986 > 0.005), on the other hand, did not show a significant effect on the TVBN value (Table 2). At 0 h, all meat samples had TVBN values ranging from 7.23 mgN/100 g to 8.35 mgN/100 g (Table 2). Therefore, they were classified as fresh meat. After 12 h of storage, the meat samples that had not been treated with the active packaging films had a TVBN value of 20.67 mg N/100g, indicating that they were rotten. By comparison, the meat samples treated with the active packaging films and added with 5%, 10%, and 15% garlic extract had TVBN values of 16.19, 17.31, and 16.61 mgN/100 g, respectively. Thus, they were categorized as semi-fresh meat (stale meat) or could still be consumed. However, the TVBN values of all meat samples taken between the 16th to 24th h of storage exceeded the threshold for food-grade beef, demonstrating that adding 5%, 10%, and 15% garlic extract to the active packaging films effectively reduced the amount of TVBN. On the other hand, meat samples that were not treated active packaging film had a significant increase in TVBN value at 12 hour storage. Beef or livestock is considered fresh if the TVBN value is less than 15 mg/100 g [41] or TVBN is <10 mg N/100 g [42]. Moreover, SNI 2354.8:2009 states that the standard levels of TVBN fit for consumption is 20–30 mg N/100 g [43].

In this study, the values of TVBN increased throughout the storage period (observed every 4 h), indicating that the meat's quality continued to deteriorate owing to the breakdown of proteins into volatile base compounds. According to Bekhit et al. [10], the increase in TVBN value is due to protein degradation by microorganisms that results in the formation of foul-smelling chemicals, such as ammonia (NH_3), basic skatole and indole compounds, mercaptans and H_2S (which are weak acids), and amines and cadaverin (which are strong bases). The results demonstrated that the addition of garlic extract to the active packaging films delayed the spoiling of the meat samples likely because the garlic's active components prevented microbial development, thereby lowering the

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synthesis of nitrogenous base compounds in the meat caused by bacteria and autolytic enzymes during the rotting process. This conjecture was supported by Al Hakim et al. [44] and Reiter et al. [37], who reported that garlic extract has the ability to block microbe-produced enzymes involved in the breakdown of proteins into volatile base chemicals.

3.4. TPC of the Microbes in the Beef Samples

The TPC of bacteria in the meat samples was determined to assess the utility of the active packaging films (Table 3).

Table 3. Total plate count (TPC) of packed meat stored at room temperature for 24 h.

Storage Time (h)	Addition of Garlic Extract to the Active Packaging Film				Average
	0%	5%	10%	15%	
	log CFU/mL				
0	2.53 ± 0.64	2.53 ± 0.64	2.53 ± 0.64	2.53 ± 0.64	2.53 ± 0.00 [a]
4	5.18 ± 0.20	4.64 ± 0.16	3.83 ± 0.30	3.91 ± 0.10	4.39 ± 0.64 [b]
8	5.43 ± 0.21	5.33 ± 0.07	4.83 ± 0.40	5.04 ± 0.06	5.16 ± 0.27 [c]
12	7.65 ± 0.39	6.20 ± 0.00	5.51 ± 0.10	5.34 ± 0.08	6.18 ± 1.05 [d]
16	8.89 ± 0.67	7.44 ± 0.03	7.47 ± 0.26	6.78 ± 0.67	7.64 ± 0.89 [e]
20	10.04 ± 0.58	8.30 ± 1.35	7.57 ± 0.60	8.28 ± 0.06	8.55 ± 1.08 [f]
24	10.36 ± 0.15	9.53 ± 0.39	9.44 ± 0.68	9.25 ± 0.03	9.65 ± 0.49 [g]
Average	7.15 ± 2.89 [c]	6.28 ± 2.37 [b]	5.88 ± 2.41[a]	5.88 ± 2.39 [a]	

The mean value followed by different letters showed a significant difference based on the Duncan's test at the 5% level (*p*-value < 0.05).

A statistical test revealed a highly significant difference between the active packaging (0.000 < 0.005) and storage time (0.000 < 0.005) on the TPC value. However, the statistical test results of the interaction between active packaging and storage time (0.09 > 0.005), on the other hand, did not show a significant effect on the TPC value (Table 3). At 0 h of the storage period, the initial TPC value (Log TPC) of all meat samples was 2.53 ± 0.64 CFU/mL (Table 3). Thus, the meat samples were classified as fresh on the basis of microbiological quality. Throughout the storage period, the TPC value increased until it reached the maximum number of meat microbes permitted by SNI 3932:2008 on carcass and beef quality, which is 1×10^6 CFU/mL or equivalent to Log TPC 6 CFU/mL. At 12 h of storage, the meat samples packaged with the control film (0%) and those added with 5% garlic extract did not fulfil the microbiological requirements as they had a Log TPC value of 7.65 ± 0.39 and 6.20 ± 0.00 CFU/mL, respectively. By comparison, the meat samples treated with the active packaging films and 10% and 15% garlic extract did not fulfil the microbiological requirements after 16 h of storage as they have a Log TPC value of 7.47 ± 0.26 and 6.78 ± 0.67 CFU/mL, respectively. This result demonstrated that the active packaging films with 10% and 15% garlic extract in the meat packaging system can inhibit microbial growth and extend the shelf life of meat by up to 4 h because allicin can inhibit the growth of both Gram-positive and Gram-negative bacteria by destroying the sulfhydryl group bound to bacterial proteins. This process is important because the sulfhydryl group is required for bacterial cell division or acts as a specific stimulator for cell multiplication. Allicin damaged the RNA and DNA of bacteria and thus inhibits their growth and development in meat. Likewise, Deresse [45] reported that allicin can suppress the growth of both Gram-positive and Gram-negative bacteria by completely inhibiting the syntheses of bacterial RNA, DNA, and proteins.

The total microbial content of the meat samples continued to increase during the entire storage period (Table 3) because meat contains a high nutrient and water content, which provides an ideal environment for microorganism growth. Moreover, storage at room temperature can accelerate the growth of microorganisms. According to Soeparno [46], meat has the ideal conditions for microorganism growth because it contains a high

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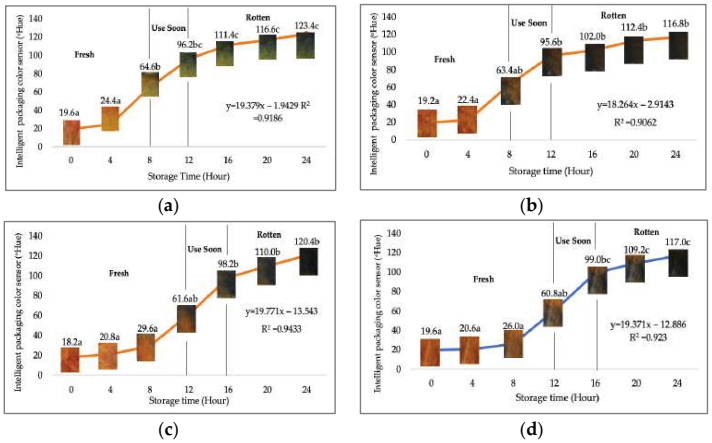
proportion of water (68–75%), it is rich in nitrogen-containing substances of varying complexity, it contains various fermentable carbohydrates, it is rich in minerals and essential nutrients for microorganism growth, and it has a suitable pH for microorganism growth (pH 5.3–6.5).

3.5. Changes in the Color of the Intelligent Packaging BTB Indicator Solution as a Measure of the Freshness of the Meat Packaged with the Active Packaging Films

Using fresh beef packaged and maintained at room temperature for 24 h, Dirpan et al. [7] determined that BTB solution (pH 2.75) produces the most readily visible color changes to sensitivity tests. In this study, the BTB solution (pH 2.75), as the intelligent packaging indicator, was also utilized to evaluate changes in its color as a reflection of the freshness of the meat samples packed with the active packaging films (Figure 3).



The mean value followed by different letters showed a significant difference based on the Duncan's test at the 5% level (p-value < 0.05).

Figure 3. Changes in the color of the BTB solution (pH 2.75) as the intelligent packaging indicator reflecting the freshness of the meat samples packed with the active packaging films with (**a**) 0%; (**b**) 5%; (**c**) 10%; and (**d**) 15% of garlic extract.

During the entire storage period, the intelligent packaging indicator changed in three different color that corresponded to three phases of the meat samples' level of quality (Figure 3). In phase I, its color was orange, indicating that the meat samples were still fresh. In phase II, its color was green with an orange hue, suggesting that the meat samples should be consumed immediately. In phase III, its color was dark green, denoting that the meat samples were already spoiled. The change in the indicator's color from orange to green indicated that the quality of the meat samples had deteriorated. The changes in the indicator's color were due to the interactions of alkaline volatile compounds produced by enzyme activity, and the metabolism of the microorganisms present in the meat samples increased with storage time. The early sign of spoilage was indicated by the release of volatile alkaline compounds as the microorganisms and the enzymes degraded the nutritional content of the meat samples. These compounds gradually accumulated in the packaging system, causing an increase in pH, which was detected by the intelligent packaging indicator and displayed as gradual color changes. The change in color of the intelligent packaging indicator (BTB, pH 2.75) from orange to green was induced by deprotonation or the release of a proton from the intelligent packaging indicator dye [47].

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The meat samples packaged with the active packaging films without garlic extract (0%) and 5% garlic extract were still fresh from the start of the storage up to 8 h (Figure 3). However, they must be immediately consumed from the 8th h to the 12th h of the storage period. Thereafter (12–24 h of the storage period), they were already spoiled. This result was consistent with that of TPC tests, which showed that the TPC values were above the acceptable threshold for microbial contaminants (1 × 10^{6} or equivalent to 6 CFU/mL) in meat after 12 h. In comparison, the meat samples packaged with the active packaging films containing 10% and 15% garlic extract were still considered fresh from the start of the storage period up to the 12th h. They must be immediately consumed when they had been in storage for 12–16 h. Finally, they were considered rotten when they had been in storage for 16–24 h. This result was also consistent with that of TPC tests (Table 3), which indicated that at the 16th h, the TPC value surpassed the permissible level of microbiological contamination in beef. Statistical analysis revealed that storage duration had a very significant effect on the Hue value, the indicator of color change in the intelligent packaging. The changes in the color of the intelligent packaging indicator (BTB solution, pH 2.75) when used together with the active packaging films to reflect the freshness of meat are presented in Table 4.

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Table 4. Changes in the color of the intelligent packaging indicator (BTB solution, pH 2.75) when used together with the active packaging films to reflect the freshness of meat.

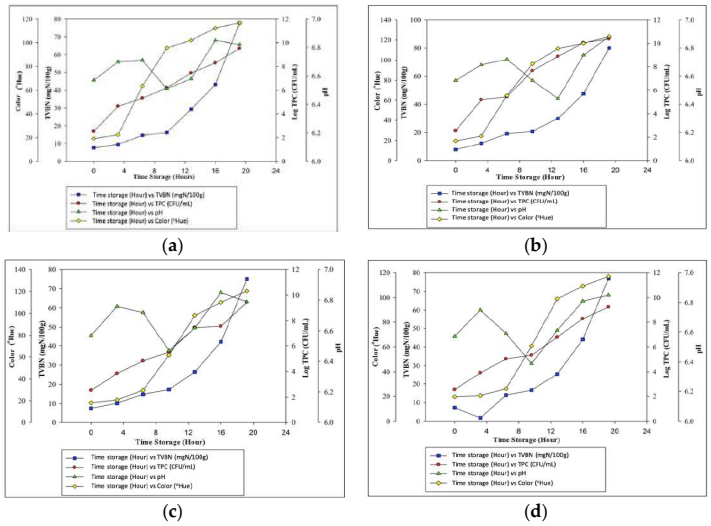
Storage Time (h)	Active Packaging Films Added with Garlic Extract			
	0%	5%	10%	15%
0				
4				
8				
12				
16				
20				

24				

3.6. Correlations between Changes in the Color of the Intelligent Packaging Indicator and the Effects of the Active Packaging Films on the Parameters of Meat Freshness

The correlations between changes in the color of the intelligent packaging indicator and parameters of meat quality deterioration (pH, TVBN, and TPC) were explored to ascertain the relationship between the sensitivity of the intelligent packaging indicator to meat freshness and the effectiveness of the active packaging films in slowing the process of meat spoilage.

Based on Figure 4, it is known that the color change of the intelligent packaging indicator which is indicated by an increase in the Hue value is in line with the increase in all values of the meat deterioration parameter. The meat samples packaged with the control film and those treated with 5% garlic extract were rotten and unfit for consumption after 12 h of storage as their Log TPC value was 7.65 ± 0.39 and 6.20 ± 0.00 CFU/mL, respectively, and their TVBN value was 20.67 ± 2.68 and 16.19 ± 0.28 mgN/100g, respectively (Figure 4). In comparison, the meat samples treated with the active packaging films and 10% and 15% garlic extract were rotten and unfit for consumption after 16 h of storage as their Log TPC value was 7.47 ± 0.26 and 6.78 ± 0.67 CFU/mL, respectively, and their TVBN value was 26.41 ± 3.31 and 25.43 ± 4.89 mgN/100 g, respectively.



(**a**) (**b**) (**c**) (**d**)

Figure 4. Correlations between changes in the color of the intelligent packaging indicator and the effects of the active packaging films with (**a**) 0%; (**b**) 5%; (**c**) 10%; and (**d**) 15% garlic extract on the parameters of quality deterioration of meat stored for 24 h.

Meat that was treated with active packaging film without addition (0%) and with the addition of 5% garlic extract experienced a change in indicator color from orange (fresh) with a Hue value of 19.6 ° and 19.20 °, respectively to green (rotten) with Hue values 96.2 ° and 95.6 °, respectively. Meanwhile, the meat that was treated with active packaging film

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with the addition of 10% and 15% garlic extract experienced a change in indicator color from orange (fresh) with Hue values 18.2 ° and 19.6 °, respectively, to green (rotten) with Hue values 98.2 ° and 99 °, respectively. Wiryawan [48] observed that when garlic extract was added to the active packaging, the values of TPC and TVBN and the pH of the meat increased more slowly, as did the color of the intelligent packaging indicator, compared with those of the meat without the active packaging.

Furthermore, the increase in the values of TPC and TVBN linearly correlates with the increase in Hue value and color changes of the intelligent packaging indicator because the accumulated volatile base compounds raise the pH value of the packaging system, causing the intelligent packaging indicator to experience a color shift. This explanation was in agreement with that of Pacquit et al. [12], who applied active packaging films to cod fish. They stated that the increase in the TPC value of cod fish has a linear correlation with changes in the color of the cellulose-acetate packaging film sensor.

On the other hand, the pH of the sample fluctuated making it difficult to determine the level of quality degradation in meat. However, the interpretation of the TPC and TVBN values, on the other hand, is clear enough to represent a decrease in meat quality which is correlated with an increase in the Hue value of changes in the intelligent packaging indicator. This good correlation demonstrates the accuracy of the film formulation in the monitoring of meat freshness, which is the aim of using intelligent packaging.

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4. Conclusions

The paper concludes that intelligent packaging indicators using BTB (Bromothymol blue) pH 2.75 solution can be used as an indicator to identify a decline in the quality of packaged meat. The indicator's color changes are easy to observe visually, namely the orange indicator indicating that the meat is still fresh and the dark green indicator indicating that the meat has rotted and is unfit for consumption. On the other hand, the use of active packaging can extend the shelf life of meat by 4 h longer when using high concentrations of garlic extract. This demonstrates that intelligent and active packaging, which are typically studied separately, have the potential to be combined and researched together using the same basic ingredient, namely bacterial cellulose.

Author Contributions: Conceptualization, A.D; methodology, A.D. and I.K; software, A.D. and I.K; validation, A.D. and M.D.; formal analysis, A.D. and I.K.; investigation, I.K.; resources, A.D.; data curation, A.D. and I.K.; writing—original draft preparation, M.D. and I.K.; writing—review and editing, A.D., M.D., and I.K.; visualization, I.K.; supervision, A.D; project administration, A.D. All authors have read and agreed to the published version of the manuscript.

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Abstract

Combining intelligent and active packaging serves the dual purpose of detecting color changes in food that reflect changes in its quality and prolonging its shelf life. This study developed an intelligent and active packaging system made from the cellulose of *Acetobacter xylinum* and assessed its ability to detect changes in the quality and to increase shelf-life of packaged fresh beef. The properties of the intelligent packaging's sensor and active packaging films were determined. The application of this system to fresh beef stored at room temperature (28 ± 2 °C) for 24 h was tested. The color of the bromothymol blue (BTB) solution (pH 2.75) in the indicator of the intelligent packaging system changed from orange to dark green to indicate that beef quality changed from fresh to rotten. The meat treated with the active packaging with 10% and 15% garlic extract decayed on the 16th h. In contrast, the meat treated with the active packaging without the garlic extracts rotted on the 12th h. The shift in the indicator's color was linearly related to the total plate count (TPC), total volatile basic nitrogen (TVBN), and pH of the meat packaged using the active packaging system. Therefore, BTB solution (pH 2.75) can be used as an intelligent packaging indicator that will allow consumers to assess the quality of packaged meat easily. As an antimicrobial agent, the addition of 10–15% garlic extract to the active packaging films can help delay the spoilage of packaged beef.

Keywords

smart sensor; smart packaging; meat shelf-life; food quality

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Application of an Intelligent Sensor and Active Packaging System Based on the Bacterial Cellulose of *Acetobacter xylinum* to Meat Products

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1. Introduction

Global beef consumption is predicted to rise as the world population and family income increase, particularly in developing Asian countries [1–3]. By 2030, worldwide meat consumption and availability are expected to increase by 14% and 5.9%, respectively, over the average of the 2018–2020 period [3]. Thus, the expected increase in meat consumption must be complemented by improvements in the quality of fresh meat produced. One aspect affecting the quality and characteristics of meat is the material and packaging technologies used [4]. Meat is a perishable item that rapidly spoils when stored above the optimum temperature range (below −17 to 4 °C) [5,6]. However, in traditional markets, meat is displayed at room temperature without packaging, a practice that might accelerate microbial contamination and cause rapid quality degradation. Even in supermarkets where meat is maintained in cold temperatures, standard meat packaging still prevents consumers from subjectively determining the quality of meat. Thus, meat packaging must have additional functions that will prevent quality degradation due to microbial contamination and will help consumers to determine the quality of packaged meat easily [7]. Conventional meat packaging can be designed to perform dual functions through intelligent and active packaging systems.

Intelligent packaging is a term that refers to sensors in the form of indicators that monitor and provide information on the quality of the food contained within the packaging via color changes caused by chemical reactions between the indicators and the products

of microbial metabolism or changes in the chemical composition of the food [8,9]. During storage, the chemical components of meat degrade into volatile compounds because of microbial activity, thereby increasing the value of total volatile base nitrogen (TVBN) [10,11]. Accumulation of TVBN increases the pH of the packaging system, which is detected by the indicator, resulting in a visible color shift in the indicator [11,12]. Intelligent packaging allows easier monitoring of packed products during transportation and storage [7]. Moreover, it provides a more accurate estimate of product condition than conventional expiration labels [12]. Color-based pH indicator solutions are widely used as intelligent indicators. Dirpan et al. [13] developed bromophenol blue as an intelligent indicator dye for mangoes. Hidayat et al. [14] used two types of color indicators with predetermined concentrations, namely, phenol red and bromothymol blue, to assess the freshness of meat packaging. Intelligent packaging indicators based on natural pigments are being developed, such as intelligent packaging films that include anthocyanin-loaded Lycium ruthenicum nanocomplexes in starch/polyvinyl alcohol mixtures (PVA) [15], as well as anthocyanins from saffron petals immobilized in chitosan nanofibers and methyl cellulose matrix [16].

Active packaging refers to the integration of particular additives into a packaging system for the purpose of extending the shelf life, preserving the quality, and ensuring the safety of food products. Antimicrobial agents are used as components of active packaging additives to extend product shelf life. The volatile bioactive compounds in active packaging evaporate or diffuse onto the food surface, where they limit the growth of microbes and thus delay spoilage [17,18]. This strategy is more effective than coating bioactive compounds onto the food surface [19]. The safest, cheapest, and most readily available antimicrobial agents for use in active packaging are essential oils. Pranoto et al. [20] produced antimicrobial alginate edible films by incorporating the essential oils of garlic. They reported that these films substantially inhibited the growth of *Staphylococcus aureus* and *Bacillus cereus* in meat. Vishnu et al. [21] utilized the essential oils of *Plectranthus amboinicus* in a chitosan-based active packaging to restrict antimicrobial activity.

Intelligent and active packaging can be merged into a single packaging system. Julyaningsih et al. [22] combined an intelligent packaging system based on methyl red and bromothymol blue (BTB) indicator with an active packaging system based on lemongrass oil as a component of tuna fish fillet packaging. Yao et al. [23] developed an active and intelligent packaging system based on starch, PVA, and betacyanins from various types of plants for shrimp packaging. In general, an active packaging that contains antimicrobial agents and an intelligent packaging that contains indicator solutions are immobilized in a polymer. Compared with synthetic polymers or plant cellulose, the bacterial cellulose fermented by *Acetobacter xylinum* has a unique nanofibrillar structure and superior physical properties, suggesting that it has the potential to serve as a basis for developing an intelligent and active packaging system [24,25]. Bacterial cellulose has received interest as a component of active packaging owing to its biodegradability, high water-holding capacity so that it can be employed entirely as a polymer for immobilizing color solutions in intelligent packaging indicators [26]. Moreover, bacterial cellulose possess great potential as an antimicrobial agent carrier in order for it to be utilized as an ingredient in the production of active packaging films [27].

The development of packaging systems with additional functions is advancing. To promote this innovation, this study aimed to maximize the potential of intelligent and active packaging by combining them into a single packaging system based on a bacterial cellulose membrane biopolymer to enhance the quality of packaged meat and help consumers to determine meat freshness easily.

2. Materials and Methods

2.1. Materials

The main ingredients used in the intelligent and active packaging system developed herein were the bacterial cellulose produced by *A. xylinum*, which was fermented in natural media of coconut water. Beef tenderloin was purchased from a slaughterhouse in

Tamangapa Raya. Coconut water and garlic (*Allium sativum*) were purchased from a local market. Food-grade ammonium sulfate (Lianyungang Zhonghong Chemical Co., Ltd., Lianyungang, China, CAS No: 7783-20-2), yeast extract (Merck, Darmstadt, Germany, CAS No: 8013-01-2), 96% acetic acid (Brenntag Inc, Essen, Germany, CAS No: 64-19-7), *A. xylinum* culture, 5% 1 N NaOH (Brenntag Inc, CAS No: 1310-73-2), sucrose, Bromothymol Blue (BTB) (Merck, Darmstadt, Germany, CAS No: 76-59-5), alcohol (Sd Fine Chem Limited, Chennai, Tamil Nadu, India), aquabides, aquades (Rofa Laboratorium Centre, Bandung, Indonesia) Tashiro's indicator (0.1% Methyl Red [Merck, Darmstadt, Germany, Cas No: 493-52-7] and 0.1% BTB at a ratio of 2:1), 7% trichloroacetic acid (TCA) (Merck, Darmstadt, Germany), Nutrient Agar (NA) (Merck, Darmstadt, Germany), glycerol (Merck, Darmstadt, Germany, CAS No: 56-81-5), food-grade carboxymethyl–cellulose (CMC) (Foodchem, Shanghai, China, E466), and corn starch were used.

2.2. Methods

2.2.1. Production of Bacterial Cellulose from *A. xylinum*

Based on our previous research Dirpan et al. [7], 5% (*w/v*) of food grade Ammonium Sulfate is the best source of Nitrogen in *Acetobacter xylinum* growth media to produce optimal bacterial cellulose membranes. Determination of the composition and type of Nitrogen source. Then, purification of bacterial cellulose was done by removal from the fermentation medium, rinsed in running water, and then soaked for 2 days with periodic water changes. The cellulose was also soaked in 70% alcohol for 1 min, heated to 100 °C in distilled water for 20 min, and reheated in 1 N 5% NaOH solution at 100 °C for 60 min to remove the remaining bacterial cells and substrate attached to the cellulose layer. Afterward, the cellulose was rinsed with running water and soaked in periodically changed water for 24 h until pH reached 7. The purified cellulose appeared transparent [7].

2.2.2. Production of Intelligent Packaging

First, preparation of the indicator solution. BTB indicator solution was chosen for this study because a previous work established this solution as the indicator with the most visually identifiable color change reaction [7]. First, 1% BTB solution (*b/v*) was prepared in 95% ethanol. Then, the pH of the BTB solution was decreased to 2.74 by adding 20% acetic acid. Finally, the BTB solution was stored in a closed container. Second, production of intelligent packaging indicator label. The purified cellulose film was kept in a filter cloth for 24 h to decrease its water content. Half-dried cellulose was cut into 1.5 cm × 4 cm strips and pushed flat against the surface of a Pyrex glass. The cellulose was dried for 30 min at 70 °C until the moisture content reaches 6%. A total of 35 mL of I BTB indicator solution was then absorbed into a dry cellulose via centrifugation at 3000 rpm for 15 min. When the color indicator was successfully absorbed, the BTB indicator solution imparted an orange hue to the cellulose. Afterward, the cellulose was rinsed with distilled water to eliminate any unbound color indicators and then dried [26,28].

2.2.3. Production of Active Packaging Film

First, the production of garlic extract as an active element. The method applied in this research referred to Yolanda et al. [29] with a slight modification. A total of 500 g of garlic was peeled, washed under running water until clean, drained, and then mashed. The minced garlic was extracted via the maceration method by immersing the finely ground garlic in 96% alcohol at a ratio of 1:4 (garlic: alcohol) for 4 days at 3–5 °C and periodically homogenized using a water bath shaker. Afterward, the extract was filtered using a filter paper and then concentrated using a rotary evaporator at 50 rpm at 40 °C to obtain a thick extract. Second, production of active packaging film. The method used referred to Iriani et al. and Indrarti et al. [19,30] with a slight modification. The bacterial cellulose was crushed to form a cellulose pulp. A cellulose suspension was prepared using 30% chitosan (*w/w*), 10% CMC (*w/w*), and 15% corn starch (*w/w*) of cellulose dry weight. The suspension was heated at 50 °C for 60 min with a hot plate stirrer until thoroughly

suspended. At the 50th min, 30% glycerol (w/w) was added. Additionally, the garlic extract was added at quantities of 0% (as the control), 5%, 10%, and 15% (v/v) immediately after the final heating step. Subsequently, 60 g of the suspension was then placed onto a glass plate and dried for 48 h at 37 °C. Finally, the suspension was cooled to room temperature, removed from the glass plate, wrapped in aluminum foil, and placed in a desiccator.

2.2.4. Application of the Intelligent and Active Packaging Indicators to Fresh Beef

Fresh beef tenderloin was collected from a slaughterhouse in Tamangapa Raya Makassar 1 h after the cow was slaughtered. It was immediately placed in a special food box and put into a 38 cm × 29 cm × 30 cm Styrofoam box filled with ice crystals. The samples were promptly transported to the laboratory and processed into 200 g/pack pieces. The meat was packaged in a Styrofoam tray (1.05 g/cm^3) coated with the active packaging film on a Styrofoam base, and an intelligent packaging indicator label was attached to the LDPE plastic wrap film that covered the Styrofoam container (Figure 1). The samples were maintained at room temperature (28 ± 2 °C) with normal light exposure for 24 h.

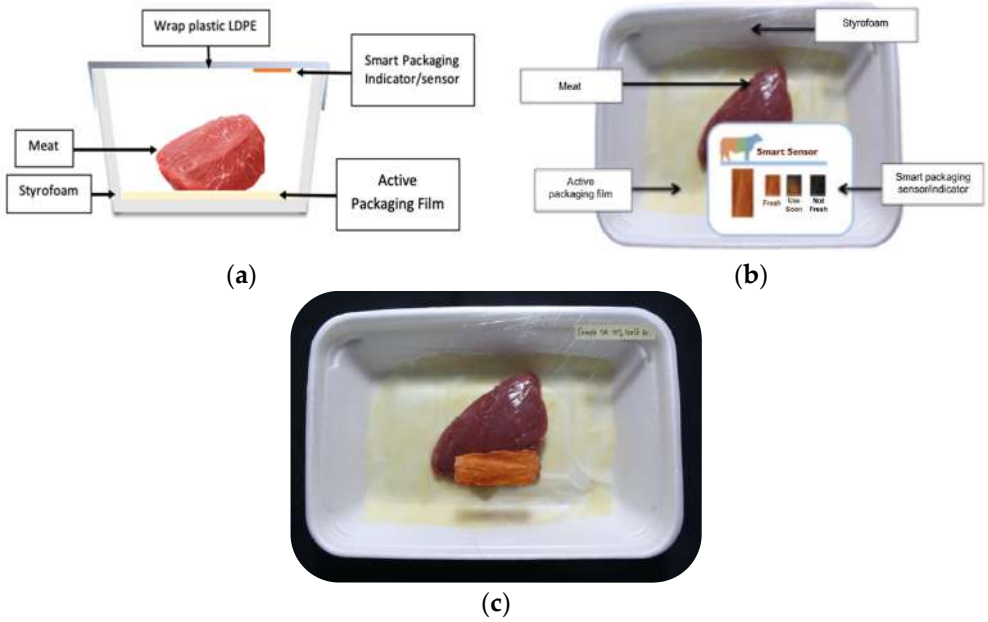

Figure 1. (**a**) Design of the intelligent and active packaging system; (**b**) prototype of the intelligent packaging; (**c**) and its application on fresh beef.

During the entire storage period, the intelligent packaging label changed its color three times that corresponded to three phases of the meat samples' level of quality (Figure 1b). In phase I, its color was orange, indicating that the meat samples were still fresh. In phase II, its color was green with an orange hue, suggesting that the meat samples should be consumed immediately (Use soon). In phase III, its color was dark green, denoting that the meat samples were already spoiled (Not Fresh).

2.2.5. Observation Parameters

Measurement of Intelligent Packaging Indicator Color Response on Meat

The color of the intelligent packaging indicators was quantitatively determined using a chromameter digital color meter (T-135). Intelligent and active packaging system containing meat is placed on a flat black background with homogeneous lighting. The chromameter detector was placed on the surface of the intelligent packaging indicator. The measurement results were expressed according to the notation of the Hunter's Lab Colorimetric System, which is presented in three values, namely L* (lightness), a* (redness),

and b* (yellowness) [31]. The color of the intelligent packaging indicator was determined by calculating the Hue value by using the formula (1) below:

$$\text{Hue (degrees)} = \tan^{-1}\frac{\text{b}*}{\text{a}*} \quad (1)$$

where Hue (360 degrees in a circle) represents the parameters for color range, a* is a red-green mixed color, and b* is a yellow-blue mixed color.

Antimicrobial Activity of the Active Packaging Films

The antimicrobial activity of the active packaging films was determined via the agar diffusion method. Each active packaging film was cut into 5 mm circles in a sterile environment and then placed on NA agar media with 0.1 mL of the test microorganism culture (*Staphylococcus aureus*) containing 10^6 CFU/mL. Petri dishes were incubated for 24 h at 37 °C. After incubation, the inhibitory zone was measured using a caliper, this measurement was carried out with three replicates [32].

Determination of pH of the Beef Samples

The pH of the beef samples was measured using a pH meter (Oakton pH 510). First, 5 g of crushed meat was introduced with 45 mL of distilled water until the mixture became homogenous. The pH meter's electrode was then immersed in the beef suspension until the pH value on the monitor became constant. This measurement was carried out with three replicates.

Measurement of TVBN

The method applied in this research referred to AOAC [33]. A Conway cup with an outer diameter of 10 cm and an inner diameter of 5 cm was utilized in this study. First, 30 mL of 7% TCA solution was added to a meat sample (10 ± 0.1 g) and mixed before filtering. A total of 1 mL boric acid solution was placed in the "inner chamber" of the Conway dish. The lid of the cup was placed in such a way that it almost covered the cup. The 1 mL filtrate was placed into the outer chamber of the Conway dish. Afterward, 1 mL saturated K_2CO_3 solution was put into the outer chamber. The cup was closed and rotated to mix the two liquids in the outer chamber. The blank solution was prepared following the same process but with 7% TCA instead of the filtrate. The solutions were stored at 37 °C for 2 h. Then, 2 drops of methyl red and bromothymol blue (2:1) were added to the inner Conway cup and then titrated with 0.01 N HCl until a pink hue was formed. TVBN was calculated by formula (2) as follows:

$$\text{TVBN content}\left(\frac{\text{mg}}{100\text{ g}}\right) = \frac{(\text{Vc} - \text{Vb}) \times 14.007 \times \text{ df } \times 100}{\text{W}} \quad (2)$$

where Vc is the volume of the HCl solution used in sample titration, Vb is the volume of the HCl solution used in blank titration, N is the normality of the HCl solution, W is the sample's weight (g), 14.007 is the molecular weight of nitrogen, and df is the dilution factor. This measurement was carried out with three replicates.

Measurement of Total Plate Count

The total amount of microorganisms was determined via the total plate count (TPC) method described in Indonesian National Standard (SNI) 2332.3: 2015. First, 1 g of the sample was added to a test tube containing 9 mL of physiological solution until homogeneous (10^{-1} dilution). The dilution was continued until 10^{-6}, at which point 1 mL of the diluted sample was inoculated on NA media in duplicate via the pour plate technique. After the media solidified, the Petri dishes containing the media and the sample solution

were incubated upside down at 30 °C for 48 h. Afterward, TPC was calculated using the formula (3) below [34]:

$$N = \frac{\sum C}{[(1 \times n_1) + (0.1 \times n_2)] \times (d)} \quad (3)$$

where N is TPC (CFU/mL), ∑C is the number of colonies counted in all Petri dishes, n_1 is the number of colonies counted in all Petri dishes at first dilution, n_2 is the number of colonies counted in all Petri dishes at second dilution, and d is the dilution factor corresponding to the first dilution.

2.2.6. Data Analysis

ANOVA was used to analyze the parameters of the intelligent packaging indicator, antimicrobial activity of the active packaging films, and quality of the beef samples, including pH, TVBN, and TPC. Differences between treatments were determined using Duncan's test. The correlations between the changes in the color of the intelligent packaging indicator and the effects of the active packaging on all parameters of meat spoilage were explored and presented in graphs by using the Sigma Plot 12 software. Data were analyzed using Microsoft Excel 2019, SPSS 19, and Sigma Plot 12.

3. Results and Discussion

3.1. Antimicrobial Activity of the Active Packaging Films against Staphylococcus aureus

The antimicrobial activity of the active packaging films is presented in Figure 2.

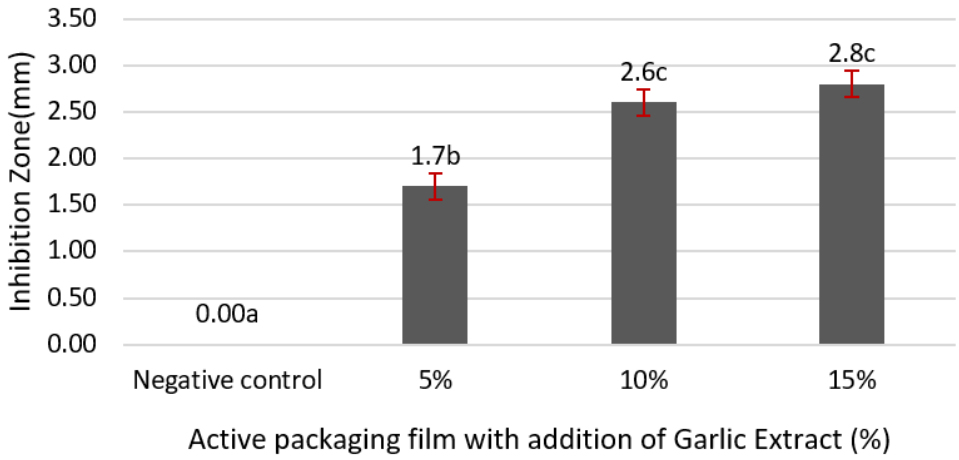

Figure 2. Antimicrobial activity of the active packaging films against *S. aureus*. The mean value followed by different letters showed a significant difference based on the Duncan's test at the 5% level (p-value < 0.05).

The antimicrobial activity of the active packaging films against *S. aureus* was assessed by measuring the diameter of the inhibition zone. As shown in Figure 2, the negative control did not generate an inhibitory zone. However, when high concentrations of the garlic extract were added to the active packaging films, the inhibitory activity against the bacteria increased, although the inhibition zone was not significantly different between 10% and 15% garlic extract. This study demonstrated that 10–15% garlic extract has antibacterial effects. According to Maroles et al. [35], differences in the diameter of inhibitory zones are influenced by the ability and rate of diffusion of antimicrobial compounds in the medium, the growth rate of microorganisms and their sensitivity to antimicrobial chemicals, and the viscosity and thickness of the medium.

The antibacterial effects of garlic extract are due to allicin, which is generated when garlic is damaged. When the flesh of garlic is damaged during the refining process, allicin is rapidly generated because of the release of alliinase, which reacts with nonprotein amino acids, namely, alliin. Allicin is a part of the defense mechanism of garlic that exerts

antimicrobial effects on both Gram-positive and Gram-negative bacteria by inhibiting RNA and lipid syntheses, which in turn inhibit the production of amino acids and proteins and the phospholipid bilayer of bacterial cell wall, thereby preventing bacterial growth and development. Allicin is highly permeable and can easily penetrate bacterial cells across the cell membrane. The thiosulfinate S(=O)S group in allicin then binds to the sulfhydryl groups of bacteria, thus inhibiting the activation mechanism of bacterial proteinases [36,37].

3.2. pH of the Beef Samples

The pH of the beef samples was measured to investigate the effects of the active packaging films as the meat base in the packaging system. The beef samples were stored at room temperature for 24 h. The results of pH measurements are shown in Table 1.

Table 1. pH values of packaged meat sample stored at room temperature for 24 h.

Storage Time (h)	Addition of Garlic Extract to the Active Packaging Film				Average
	0%	5%	10%	15%	
0	6.56 ± 0.08	6.56 ± 0.08	6.57 ± 0.08	6.57 ± 0.08	6.56 ± 0.00 [a]
4	6.68 ± 0.04	6.70 ± 0.06	6.76 ± 0.10	6.75 ± 0.06	6.72 ± 0.04 [d]
8	6.72 ± 0.05	6.71 ± 0.07	6.72 ± 0.05	6.59 ± 0.20	6.68 ± 0.06 [d]
12	6.57 ± 0.11	6.51 ± 0.03	6.47 ± 0.04	6.39 ± 0.02	6.48 ± 0.08 [b]
16	6.44 ± 0.23	6.58 ± 0.06	6.62 ± 0.08	6.61 ± 0.11	6.56 ± 0.08 [c]
20	6.75 ± 0.05	6.85 ± 0.01	6.85 ± 0.02	6.81 ± 0.02	6.81 ± 0.05 [e]
24	6.88 ± 0.11	6.82 ± 0.11	6.79 ± 0.10	6.8 5 ± 0.03	6.83 ± 0.04 [e]

The mean value followed by different letters showed a significant difference based on the Duncan's test at the 5% level (p-value < 0.05).

A statistical test of the storage time showed a significant difference to the pH value (0.000 < 0.005). However, the statistical test results of the active packaging (0.654 > 0.005) and interaction between active packaging and storage time (0.179 > 0.005), on the other hand, did not show a significant effect on the pH value (Table 1). One of the characteristics that contribute to meat quality reduction is pH. However, pH cannot be used as the single indicator of meat rot. The pH value is used to confirm the results of other meat deterioration parameters such as TPC or TVBN. According to statistics, active packaging had no significant influence on the pH of the meat and the change in pH seemed to fluctuated, but the data still indicated a rise in pH at each increase in time.

The initial pH of the meat samples, which was immediately determined after the cow was slaughtered, was normal (6.57) (Table 1). The pH fluctuated during the storage period, but the trend graph has shown a decrease in pH at 12 h then the pH increased at the 16 h to 24 h storage. After the animal dies, the blood flow that supplies oxygen to the muscles stops causing an anaerobic glycolysis process to occur. During anaerobic glycolysis, glycogen conversion occurs in the muscles to lactic acid which accumulates in the tissues, causing the pH of the meat to decrease (4 h storage), during anaerobically glycolysis, the decrease in pH continues until the glycogen is converted to lactic acid followed by the neutralization of alkaline compounds resulting from the metabolism of microorganisms, so that the pH of the meat rises again (16–24 h storage).

According to Sánchez-Macías et al. [38] and Moreno et al. [39], reported that the lower the content of glycogen in the meat is, the slower the glycolysis process will be and the higher the final pH will be. However, the decrease in pH in muscles can be influenced by internal factors, such as species, muscle type, muscle glycogen content, and livestock variability, as well as external factors, such as environmental temperature, additional treatment prior to slaughter, and pre-slaughter stress.

After 20 h of storage, the meat's pH value ranged from 6.75 and 6.85 and remained steady up to 24 h of storage; at this point, the meat was classified as decayed (Table 1). According to Prache et al. [40], the meat's pH continues to decline until glycogen is depleted into lactic acid and alkaline compounds are neutralized because of microbial metabolism,

resulting in an increase in pH. If the pH reaches 6.8 or higher, protein decomposition will occur, resulting in spoilage.

3.3. TVBN of the Meat Samples

The TVBN values of the meat samples are presented in Table 2.

Table 2. Total volatile basic nitrogen (TVBN) of the packed meat stored at room temperature for 24 h. Average Result of Meat's TVBN value.

Storage Time (h)	Addition of Garlic Extract to the Active Packaging Film				Average
	0%	5%	10%	15%	
	mgN/100 g				
0	8.35 ± 0.96	7.37 ± 0.56	7.51 ± 0.54	7.23 ± 1.24	7.62 ± 0.50 [a]
4	12.27 ± 0.54	9.47 ± 2.80	10.17 ± 2.17	10.73 ± 1.17	10.66 ± 1.19 [b]
8	19.13 ± 2.07	14.65 ± 0.72	14.79 ± 1.40	13.95 ± 0.96	15.63 ± 2.36 [c]
12	20.67 ± 2.68	16.19 ± 0.28	17.31 ± 1.73	16.61 ± 1.21	17.70 ± 2.04 [c]
16	29.91 ± 3.78	29.21 ± 5.57	26.41 ± 3.31	25.43 ± 4.89	27.74 ± 2.16 [d]
20	47.41 ± 3.17	43.21 ± 1.19	42.09 ± 1.19	44.05 ± 0.79	44.19 ± 2.29 [e]
24	80.03 ± 8.65	77.79 ± 3.11	74.99 ± 5.63	76.81 ± 8.26	77.42 ± 2.12 [f]
Average	31.12 ± 25.13 [b]	28.27 ± 25.16 [a]	27.61 ± 23.93 [a]	27.83 ± 24.84 [a]	

The mean value followed by different letters showed a significant difference based on the Duncan's test at the 5% level (p-value < 0.05).

A statistical test revealed a highly significant difference between the active packaging (0.004 < 0.005) and storage time (0.000 < 0.005) on the TVBN value. However, the statistical test results of the interaction between active packaging and storage time (0.986 > 0.005), on the other hand, did not show a significant effect on the TVBN value (Table 2). At 0 h, all meat samples had TVBN values ranging from 7.23 mgN/100 g to 8.35 mgN/100 g (Table 2). Therefore, they were classified as fresh meat. After 12 h of storage, the meat samples that had not been treated with the active packaging films had a TVBN value of 20.67 mg N/100g, indicating that they were rotten. By comparison, the meat samples treated with the active packaging films and added with 5%, 10%, and 15% garlic extract had TVBN values of 16.19, 17.31, and 16.61 mgN/100 g, respectively. Thus, they were categorized as semi-fresh meat (stale meat) or could still be consumed. However, the TVBN values of all meat samples taken between the 16th to 24th h of storage exceeded the threshold for food-grade beef, demonstrating that adding 5%, 10%, and 15% garlic extract to the active packaging films effectively reduced the amount of TVBN. On the other hand, meat samples that were not treated active packaging film had a significant increase in TVBN value at 12 h storage. Beef or livestock is considered fresh if the TVBN value is less than 15 mg/100 g [41] or TVBN is <10 mg N/100 g [42]. Moreover, SNI 2354.8:2009 states that the standard levels of TVBN fit for consumption is 20–30 mg N/100 g [43].

In this study, the values of TVBN increased throughout the storage period (observed every 4 h), indicating that the meat's quality continued to deteriorate owing to the breakdown of proteins into volatile base compounds. According to Bekhit et al. [10], the increase in TVBN value is due to protein degradation by microorganisms that results in the formation of foul-smelling chemicals, such as ammonia (NH_3), basic skatole and indole compounds, mercaptans and H_2S (which are weak acids), and amines and cadaverin (which are strong bases). The results demonstrated that the addition of garlic extract to the active packaging films delayed the spoiling of the meat samples likely because the garlic's active components prevented microbial development, thereby lowering the synthesis of nitrogenous base compounds in the meat caused by bacteria and autolytic enzymes during the rotting process. This conjecture was supported by Al Hakim et al. [44] and Reiter et al. [37], who reported that garlic extract has the ability to block microbe-produced enzymes involved in the breakdown of proteins into volatile base chemicals.

3.4. TPC of the Microbes in the Beef Samples

The TPC of bacteria in the meat samples was determined to assess the utility of the active packaging films (Table 3).

Table 3. Total plate count (TPC) of packed meat stored at room temperature for 24 h.

Storage Time (h)	Addition of Garlic Extract to the Active Packaging Film				Average
	0%	5%	10%	15%	
	log CFU/mL				
0	2.53 ± 0.64	2.53 ± 0.64	2.53 ± 0.64	2.53 ± 0.64	2.53 ± 0.00 [a]
4	5.18 ± 0.20	4.64 ± 0.16	3.83 ± 0.30	3.91 ± 0.10	4.39 ± 0.64 [b]
8	5.43 ± 0.21	5.33 ± 0.07	4.83 ± 0.40	5.04 ± 0.06	5.16 ± 0.27 [c]
12	7.65 ± 0.39	6.20 ± 0.00	5.51 ± 0.10	5.34 ± 0.08	6.18 ± 1.05 [d]
16	8.89 ± 0.67	7.44 ± 0.03	7.47 ± 0.26	6.78 ± 0.67	7.64 ± 0.89 [e]
20	10.04 ± 0.58	8.30 ± 1.35	7.57 ± 0.60	8.28 ± 0.06	8.55 ± 1.08 [f]
24	10.36 ± 0.15	9.53 ± 0.39	9.44 ± 0.68	9.25 ± 0.03	9.65 ± 0.49 [g]
Average	7.15 ± 2.89 [c]	6.28 ± 2.37 [b]	5.88 ± 2.41[a]	5.88 ± 2.39 [a]	

The mean value followed by different letters showed a significant difference based on the Duncan's test at the 5% level (p-value < 0.05).

A statistical test revealed a highly significant difference between the active packaging (0.000 < 0.005) and storage time (0.000 < 0.005) on the TPC value. However, the statistical test results of the interaction between active packaging and storage time (0.09 > 0.005), on the other hand, did not show a significant effect on the TPC value (Table 3). At 0 h of the storage period, the initial TPC value (Log TPC) of all meat samples was 2.53 ± 0.64 CFU/mL (Table 3). Thus, the meat samples were classified as fresh on the basis of microbiological quality. Throughout the storage period, the TPC value increased until it reached the maximum number of meat microbes permitted by SNI 3932:2008 on carcass and beef quality, which is 1 × 10^6 CFU/mL or equivalent to Log TPC 6 CFU/mL. At 12 h of storage, the meat samples packaged with the control film (0%) and those added with 5% garlic extract did not fulfil the microbiological requirements as they had a Log TPC value of 7.65 ± 0.39 and 6.20 ± 0.00 CFU/mL, respectively. By comparison, the meat samples treated with the active packaging films and 10% and 15% garlic extract did not fulfil the microbiological requirements after 16 h of storage as they have a Log TPC value of 7.47 ± 0.26 and 6.78 ± 0.67 CFU/mL, respectively. This result demonstrated that the active packaging films with 10% and 15% garlic extract in the meat packaging system can inhibit microbial growth and extend the shelf life of meat by up to 4 h because allicin can inhibit the growth of both Gram-positive and Gram-negative bacteria by destroying the sulfhydryl group bound to bacterial proteins. This process is important because the sulfhydryl group is required for bacterial cell division or acts as a specific stimulator for cell multiplication. Allicin damaged the RNA and DNA of bacteria and thus inhibits their growth and development in meat. Likewise, Deresse [45] reported that allicin can suppress the growth of both Gram-positive and Gram-negative bacteria by completely inhibiting the syntheses of bacterial RNA, DNA, and proteins.

The total microbial content of the meat samples continued to increase during the entire storage period (Table 3) because meat contains a high nutrient and water content, which provides an ideal environment for microorganism growth. Moreover, storage at room temperature can accelerate the growth of microorganisms. According to Soeparno [46], meat has the ideal conditions for microorganism growth because it contains a high proportion of water (68–75%), it is rich in nitrogen-containing substances of varying complexity, it contains various fermentable carbohydrates, it is rich in minerals and essential nutrients for microorganism growth, and it has a suitable pH for microorganism growth (pH 5.3–6.5).

3.5. Changes in the Color of the Intelligent Packaging BTB Indicator Solution as a Measure of the Freshness of the Meat Packaged with the Active Packaging Films

Using fresh beef packaged and maintained at room temperature for 24 h, Dirpan et al. [7] determined that BTB solution (pH 2.75) produces the most readily visible color changes to sensitivity tests. In this study, the BTB solution (pH 2.75), as the intelligent packaging indicator, was also utilized to evaluate changes in its color as a reflection of the freshness of the meat samples packed with the active packaging films (Figure 3).

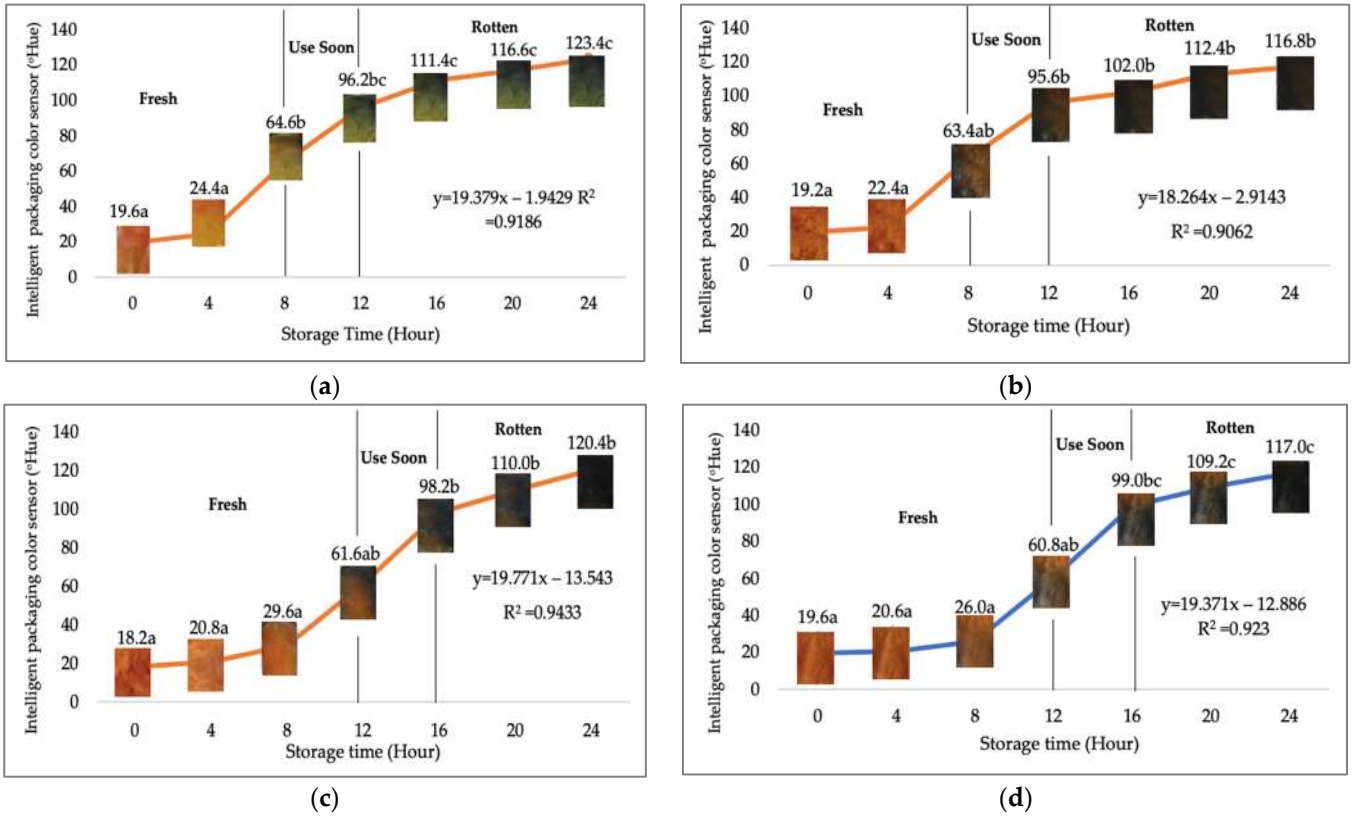

Figure 3. Changes in the color of the BTB solution (pH 2.75) as the intelligent packaging indicator reflecting the freshness of the meat samples packed with the active packaging films with (**a**) 0%; (**b**) 5%; (**c**) 10%; and (**d**) 15% of garlic extract. The mean value followed by different letters showed a significant difference based on the Duncan's test at the 5% level (p-value < 0.05).

During the entire storage period, the intelligent packaging indicator changed in three different color that corresponded to three phases of the meat samples' level of quality (Figure 3). In phase I, its color was orange, indicating that the meat samples were still fresh. In phase II, its color was green with an orange hue, suggesting that the meat samples should be consumed immediately. In phase III, its color was dark green, denoting that the meat samples were already spoiled. The change in the indicator's color from orange to green indicated that the quality of the meat samples had deteriorated. The changes in the indicator's color were due to the interactions of alkaline volatile compounds produced by enzyme activity, and the metabolism of the microorganisms present in the meat samples increased with storage time. The early sign of spoilage was indicated by the release of volatile alkaline compounds as the microorganisms and the enzymes degraded the nutritional content of the meat samples. These compounds gradually accumulated in the packaging system, causing an increase in pH, which was detected by the intelligent packaging indicator and displayed as gradual color changes. The change in color of the intelligent packaging indicator (BTB, pH 2.75) from orange to green was induced by deprotonation or the release of a proton from the intelligent packaging indicator dye [47].

The meat samples packaged with the active packaging films without garlic extract (0%) and 5% garlic extract were still fresh from the start of the storage up to 8 h (Figure 3). However, they must be immediately consumed from the 8th h to the 12th h of the storage period. Thereafter (12–24 h of the storage period), they were already spoiled. This result was consistent with that of TPC tests, which showed that the TPC values were above the acceptable threshold for microbial contaminants (1×10^6 or equivalent to 6 CFU/mL) in meat after 12 h. In comparison, the meat samples packaged with the active packaging films containing 10% and 15% garlic extract were still considered fresh from the start of the storage period up to the 12th h. They must be immediately consumed when they had been in storage for 12–16 h. Finally, they were considered rotten when they had been in storage for 16–24 h. This result was also consistent with that of TPC tests (Table 3), which indicated that at the 16th h, the TPC value surpassed the permissible level of microbiological contamination in beef. Statistical analysis revealed that storage duration had a very significant effect on the Hue value, the indicator of color change in the intelligent packaging. The changes in the color of the intelligent packaging indicator (BTB solution, pH 2.75) when used together with the active packaging films to reflect the freshness of meat are presented in Table 4.

Table 4. Changes in the color of the intelligent packaging indicator (BTB solution, pH 2.75) when used together with the active packaging films to reflect the freshness of meat.

Storage Time(h)	Active Packaging Films Added with Garlic Extract			
	0%	5%	10%	15%
0				
4				
8				
12				
16				

Table 4. *Cont.*

Storage Time(h)	Active Packaging Films Added with Garlic Extract			
	0%	5%	10%	15%
20				
24				

3.6. Correlations between Changes in the Color of the Intelligent Packaging Indicator and the Effects of the Active Packaging Films on the Parameters of Meat Freshness

The correlations between changes in the color of the intelligent packaging indicator and parameters of meat quality deterioration (pH, TVBN, and TPC) were explored to ascertain the relationship between the sensitivity of the intelligent packaging indicator to meat freshness and the effectiveness of the active packaging films in slowing the process of meat spoilage.

Based on Figure 4, it is known that the color change of the intelligent packaging indicator which is indicated by an increase in the Hue value is in line with the increase in all values of the meat deterioration parameter. The meat samples packaged with the control film and those treated with 5% garlic extract were rotten and unfit for consumption after 12 h of storage as their Log TPC value was 7.65 ± 0.39 and 6.20 ± 0.00 CFU/mL, respectively, and their TVBN value was 20.67 ± 2.68 and 16.19 ± 0.28 mgN/100g, respectively (Figure 4). In comparison, the meat samples treated with the active packaging films and 10% and 15% garlic extract were rotten and unfit for consumption after 16 h of storage as their Log TPC value was 7.47 ± 0.26 and 6.78 ± 0.67 CFU/mL, respectively, and their TVBN value was 26.41 ± 3.31 and 25.43 ± 4.89 mgN/100 g, respectively.

Meat that was treated with active packaging film without addition (0%) and with the addition of 5% garlic extract experienced a change in indicator color from orange (fresh) with a Hue value of 19.6° and 19.20°, respectively to green (rotten) with Hue values 96.2° and 95.6°, respectively. Meanwhile, the meat that was treated with active packaging film with the addition of 10% and 15% garlic extract experienced a change in indicator color from orange (fresh) with Hue values 18.2° and 19.6°, respectively, to green (rotten) with Hue values 98.2° and 99°, respectively. Wiryawan [48] observed that when garlic extract was added to the active packaging, the values of TPC and TVBN and the pH of the meat increased more slowly, as did the color of the intelligent packaging indicator, compared with those of the meat without the active packaging.

Furthermore, the increase in the values of TPC and TVBN linearly correlates with the increase in Hue value and color changes of the intelligent packaging indicator because the accumulated volatile base compounds raise the pH value of the packaging system, causing the intelligent packaging indicator to experience a color shift. This explanation was in agreement with that of Pacquit et al. [12], who applied active packaging films to cod fish. They stated that the increase in the TPC value of cod fish has a linear correlation with changes in the color of the cellulose-acetate packaging film sensor.

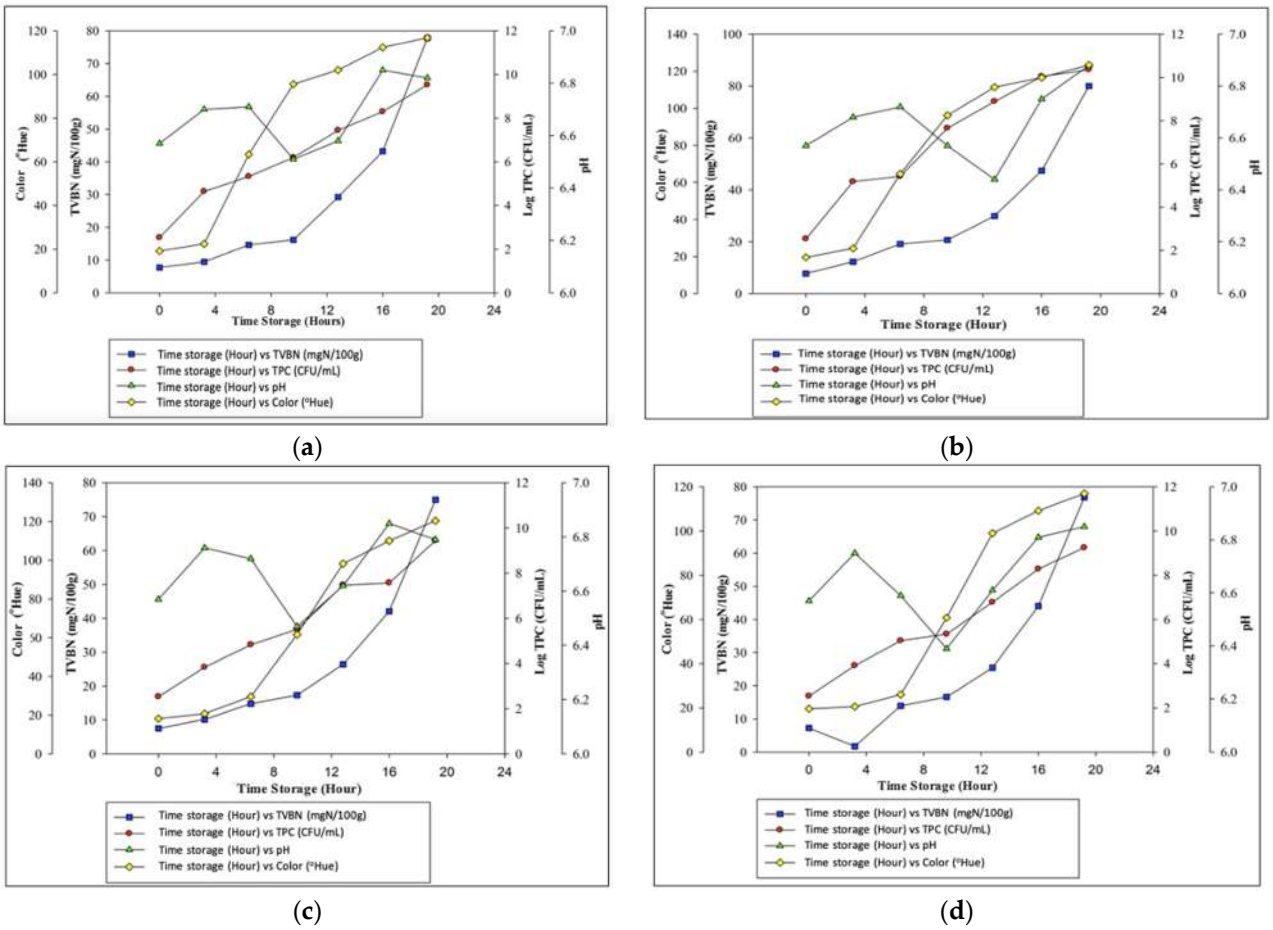

Figure 4. Correlations between changes in the color of the intelligent packaging indicator and the effects of the active packaging films with (**a**) 0%; (**b**) 5%; (**c**) 10%; and (**d**) 15% garlic extract on the parameters of quality deterioration of meat stored for 24 h.

On the other hand, the pH of the sample fluctuated making it difficult to determine the level of quality degradation in meat. However, the interpretation of the TPC and TVBN values, on the other hand, is clear enough to represent a decrease in meat quality which is correlated with an increase in the Hue value of changes in the intelligent packaging indicator. This good correlation demonstrates the accuracy of the film formulation in the monitoring of meat freshness, which is the aim of using intelligent packaging.

4. Conclusions

The paper concludes that intelligent packaging indicators using BTB (Bromothymol blue) pH 2.75 solution can be used as an indicator to identify a decline in the quality of packaged meat. The indicator's color changes are easy to observe visually, namely the orange indicator indicating that the meat is still fresh and the dark green indicator indicating that the meat has rotted and is unfit for consumption. On the other hand, the use of active packaging can extend the shelf life of meat by 4 h longer when using high concentrations of garlic extract. This demonstrates that intelligent and active packaging, which are typically studied separately, have the potential to be combined and researched together using the same basic ingredient, namely bacterial cellulose.

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editing, A.D., M.D. and I.K.; visualization, I.K.; supervision, A.D.; project administration, A.D. All authors have read and agreed to the published version of the manuscript.

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