KORESPONDENSI

1. Proofread document received (05-2-2022)
   -Document from proofreading service
2. Submitted to the journal "FOODS" (30-4-2022)
3. Revision: Accepted with minor revision (13-5-2022)
4. Revised version received (17-5-2022)
   -Revisions and Amends
   -Revised version with highlights
   - Manuscript revisions
5. Paper accepted for publication (18-5-2022)
   -Final paper
6. Final proofreading before publication (19-5-2022)
7. Paper published (20-5-2022)
   -Certificate

1. **Proofread document received (05-2-2022)**

-Document from proofreading service

A Simple Combination of Active and Smart Packaging in Extending and Monitoring the Meat Quality Stored at Cold Temperatures (4 ±1ºC)

Abstract

A simple combination of active and smart packaging has been developed using garlic extract as the raw material as well as bromothymol blue (BTB) and phenol red (PR) as the solution. Therefore, this study aims to extend and monitor the beef quality deterioration at temperatures of 4±1ºC by applying a double function in active and smart packaging. The active packaging uses garlic extract with concentrations of 0%, 15%, and 20% (w/w) to release anti-microbial agents. Meanwhile, the smart paper uses a combination of BTB and PR solutions at pH 5.00. On the ninth day, beef packed without adding 15% and 20% garlic extract on active paper is unfit for eating. The smart indication label's color change profile is as follows: dark yellow (fresh), reddish-yellow (to be consumed immediately), and faded red (rotten). The color change of the smart BTB+PR pH 5.00 indicator label in response to all meat deterioration criteria demonstrates a linear correlation for determining the extent of rottenness during storage. Therefore, active paper and smart indicator labels are combined to extend the shelf life and monitor meat quality deterioration.

1. Introduction

Meat is one of the most basic nutrients containing high levels of easily digestible protein, calorie-dense fat, vitamins, and other micronutrients. Its high nutritional value corresponds to the rising demand as the world's most significant food product during the past several years.[1] The high demand should be accompanied by an increase in the fresh meat grade, including the quality and safety to minimize damage due to chemical, physical, and microbiological factors. The decrease in quality is due to the high fat and water content, making it susceptible to microbial contamination and lipid oxidation. Furthermore, quality degradation occurs at several stages of the production chain, including preparation, storage, and distribution.[2,3] The use of active packaging in conjunction with cold storage is one of the efforts that can be made to prolong the shelf life and enhance the quality.

Active packaging applications can be used to inhibit microbial growth in meat.[4–6] These applications incorporate specific active substances into packaging methods to preserve food products from bacteria that cause spoilage during storage.[7] The spoilage inhibition mechanism can be conducted in two ways of direct and indirect contact. The direct contact is between packaging materials and food, while in the indirect contact, the active packaging releases volatile anti-microbial agent.[8,9] Volatile anti-microbial compounds are essential oils derived from herbs and spices, and the anti-microbial activities of different species of Allium have been investigated.[10–12] Furthermore, Zheng Dong et al. (2019) developed an active packaging material based on a PP/LDPE composite film containing Allium sativum essential oil. The addition of essential oils into the packaging extends the shelf life of large yellow croaker *(Pseudosciaena crocea)* fillets for five days stored at 4 ± 1 °C.[13] Seydim and Sarikus (2006) also reported that the anti-microbial properties of whey protein isolate containing essential garlic oil with a ratio of 3.0 – 4.0% (wt/vol) effectively inhibited the growth of *Salmonella Enteritidis, S. aureus, E. coli O157:H7, L. monocytogenes,* and *Lactobacillus Plantarum*.[14] The use of garlic extract in active packaging can inhibit microbial growth due to its volatile *Allicin* constituent.[15] Allicin chemicals suppress microbial development by increasing their permeability in penetrating bacterial cell walls to decrease protease enzyme production and disrupt protein and nucleic acid metabolism.[11]

The application of active packaging on fresh meat can be accompanied by the addition of smart packaging in the form of indicators that monitor changes in quality. This is achieved through chemical reactions between indicators and the results of microbial metabolism or changes in the meat's chemical composition. Smart packaging incorporates indicators that convey information about the product's quality without causing damage to the packaging. This reduces the risk of loss due to product damage and provides a more accurate condition estimate than conventional expired labels. Furthermore, smart packaging also assists distributors in adjusting the prices of the products sold.[16] For example, lowering prices when quality has decreased to avoid losing food products. Previous studies applied this method to avocados (Dirpan et al., 2021) [17], tuna fillets (Agustianti et al., 2021)[18], shrimp (Wu et al., 2021)[19], cherry tomatoes (Kim et al., 2021)[20] and *arummanis* mango (Dirpan et al., 2018)[21]. According to Shukla et al. (2015), color-based pH indicators can be used as smart packaging to monitor the presence of volatile amines from microbial metabolites in meat spoilage. The increase in pH during decay causes a significant change in the indicator's color to be visually observed.[22] Meanwhile, Romero et al. (2019) evaluated the quality of packaged cow's milk using two smart packaging prototypes based on the bromothymol blue pH indicator.[23] Julyaningsih et al. (2020) and Yolanda et al. (2020) combined active and smart packaging based on pH indicators methyl red and bromothymol blue to monitor the tuna fillet's quality. [24,25] Meanwhile, Yong and Liu (2020) combined anthocyanin-rich extracts into biopolymer-based films to extend shelf life and monitor the food product's quality.[26]

The spoilage and contamination of food supplies jeopardize global food security. Therefore, the innovative application of active and smart packaging needs to be developed. As a result of these considerations, the purpose of this research is to evaluate the efficacy of active paper with garlic extract addition when applied to fresh beef during storage at low temperatures (4±1ºC). Furthermore, it investigates the relationship (correlation) between color analysis of smart packaging indicators on various parameters of the beef spoilage test.

2. Material and Methods

2.1. Materials

The beef tenderloin is purchased fresh at the slaughterhouse, Antang. Meanwhile, garlic as an anti-microbial compound in the activated paper was purchased from supermarkets. Bromothymol blue (BTB) and phenol red (PR) color indicators in smart packaging were purchased from Sigma-Aldrich (Merck) with Mueller Hinton Agar (MHA) and Nutrient Agar (NA) media. This research also uses *Escherichia coli, Staphylococcus aureus*, and *Pseudomonas aeruginosa*, and the other chemicals used were of analytical grade.

2.2. Garlic Extract Preparation

Garlic of 50 g was blended until smooth and put into a container containing 250 ml of 96% alcohol with 3 x 24-hour immersion. The stirring process was conducted 14 times in 5 hours during immersion. Subsequently, it is separated from the garlic dregs and the filtrate by filtering. At 45ºC, the filtrate was evaporated with an industrial rotary evaporator (TMAX-2L-1) until it became thick reddish-yellow garlic. [27]

2.3. Active Paper Preparation

Active paper preparation was carried out using Whatman paper no. 1 cut into small pieces of 15 g and soaked in 250 ml of distilled water for 24 hours. The paper soak was added with 250 mL of distilled water and crushed using a blender for 20 minutes until a pulp was formed. The paper pulp obtained was squeezed to remove the water, and 30% (w/w) tapioca starch was dissolved in 50 mL of distilled water, then homogenized with the pulp. Furthermore, 100 mL of acetic acid (1%) was added with 0.45% chitosan powder and homogenized with a blender for 5 minutes. Garlic extract with concentrations of 15% and 20% (w/w) on filter paper was added with 50 mL of distilled water, respectively. Furthermore, 0.205 g of tween 80 was added and mixed at room temperature using a hotplate magnetic stirrer to generate an emulsion. The paper dough is poured over the surface of the printer container following the styrofoam size used as a beef storage container and flattened to make a wet paper sheet. Meanwhile, the wet paper sheet on the filter surface is pressed between the glass surfaces with a load of 2 kg. It was then dried at 40°C for 48 hours, and a paper turning procedure was after 24 hours of drying (Modification of Wiastuti et al., 2016).[28]

2.4. Indicator Solution Preparation

BTB and PR (1:1) indicator solutions were made with 1% (w/v) and dissolved in 95% ethanol. Furthermore, the indicator was adjusted to pH 5 using a solution of glacial acetic acid (Kuswandi et al., 2017).[29]

2.5. Smart Indicator Label Preparation

Whatman paper no. 1 was cut with a size of 2 x 4 cm and immersed in 20 ml of indicator solution for 12 hours at room temperature (28±2°C). The indicator labels were dried using an electric dryer and a closed container with a drying distance of 30 cm.

2.6. Application of Active and Smart Packaging on Fresh Beef

Fresh beef (tenderloin) with normal pH (5.6 – 5.7) was obtained from the Tamangapa Antang Makassar abattoir, which was taken at a relative postmortem time of about 3 hours. The meat was packaged in plastic (PP) containers and put in a cooler, then immediately transported to the laboratory and prepared sterilely into 180-gram pieces/packages. Activated paper with different concentrations was inserted into the styrofoam base. Furthermore, the study used A0: Control (without garlic extract addition); A1: 15% garlic extract; A2: 20% garlic extract. The pieces of meat were packed in styrofoam (1.05 g/cm3) covered with active paper that filled the entire base. The indicator label was placed inside the package by sticking to the LDPE film surface (0.9 g/cm^3) used as a cover. Observation of beef (tenderloin) packaged with smart and active packaging was conducted at a cold temperature (chiller) of 4 ± 1oC every 3 x 24 hours for 21 days of storage. The design for implementing smart and active packaging can be seen in Figure 1.

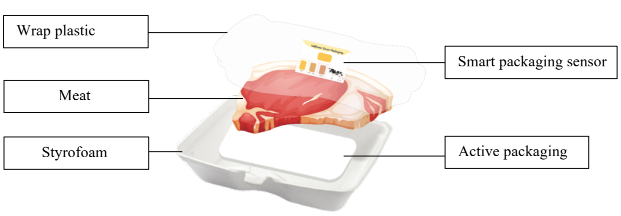


Figure 1. Active and Smart Packaging Design

2.7. Antibacterial Activity Testing of Agar Diffusion Method on Activated Paper

The antibacterial activity of active paper was tested to determine the inhibition toward the growth of gram-positive bacteria *Staphylococcus aureus* (ATCC 29213), gram-negative bacteria *Escherichia coli*, and *Pseudomonas aeruginosa*. Sheets of active paper with a diameter of 5.5 mm were placed on Mueller Hinton Agar (MHA) media, which spread 0.1 ml of microorganism culture on its surface. Furthermore, the Petri dishes were incubated at 37^{o}C for 24 hours, and after incubation, a clear zone (inhibition zone) was formed around the active paper. The resistance diameter was measured in millimeters (mm) using a caliper[30], and each test was conducted in 3 replications to obtain the average result.

2.8. Parameters of Beef Observation packaged with a combination of active and smart packaging

2.8.1. pH Measurement

pH measurements measured the initial and final pH during storage of fresh beef using Horiba Laquatwin Compact pH Meter P-33 with an accuracy of 0.01%. A total of 1 g of the sample was mashed and dissolved in 10 ml of Aquades. Furthermore, it was inserted on the surface of the pH meter sensor until the value was printed on the pH meter screen.

2.8.2. TVBN Value Analysis

The meat sample was weighed at 1 g ± 0.1 g, pulverized in a mortar with 3 ml of 7% TCA solution, and filtered to obtain the filtrate. The boric acid solution of 1 ml was put into the "inner chamber" of the Conway cup, and the lid was placed in a position to cover the cup. Furthermore, the filtrate was fed into the outer chamber on the left. Then 1 ml saturated K2CO3 solution was added to the outer chamber on the right, ensuring that the filtrate and K2CO3 do not mix. The cup was instantly closed and rotated to mix the two liquids in the outer chamber. The blank solution was prepared the same way as the standard solution, except that the filtrate was replaced with a 7% TCA solution. Subsequently, it was stored at 28±2^{o}C for 24 hours, and the boric acid solution in the inner chamber containing the blank was added with two drops of color indicator solution, then titrated with 0.02 N HCl until it turned pink. Conway cup containing the filtrate was added with two drops of color indicator solution and titrated with the same solution until it turned pink such a blank (SNI-2354.8:2009; AOAC, 1995). The formula for determining TVBN is:

$$TVBN\ level\ (mg/100g) = \frac{(V_c - Vb)\ x\ N\ x\ 14.007\ x\ 100}{w}$$

Description:

Vc = volume of HCl solution in sample titration

Vb = volume of HCl solution in blank titration

N = normality of HCl solution

W = sample weight (g)
14.07 = atomic weight of nitrogen

2.8.3. Calculation of Total Bacterial Count

Total bacteria were calculated based on the cup count method (SNI 2897:2008). The sample solution was made from 1 g of meat homogenized with 9 ml of sterile physiological solution (0.85% NaCl). A total of 1 ml of the sample solution was put into the first test tube containing 9 ml of the physiological solution until homogeneous, referred to as the 10^{-1} dilution. Then, the dilution was conducted up to a concentration of $10^{-6,}$ and 1 ml from dilutions 10^{-4}, 10^{-5}, and 10^{-6} were put into separate sterile Petri dishes in duplicate. Similarly, ± 15 ml of sterile NA media was homogenized and incubated at 30oC for 48 hours. The calculation of the Total Plate Count is:

$$N = \frac{\Sigma C}{[(1 \times n_1) + (0.1 \times n_2) + \cdots] \times (D)}$$

Description:
N = number of colonies per ml/ per gram of product
ΣC = total number of colonies counted
n_1 = number of cups in the first dilution
n_2 = number of cups in the second dilution
D = first dilution calculated

2.9. Smart Indicator Color Measurement Quantification

The color measurement of the smart indicator label was analyzed using a Digital color meter (T-135). This quantification is conducted by attaching the colorimeter sensor to the smart indicator label. The tool will then show the values of L (lightness), a (redness), and b (yellowness) on display. These three values are international standards of color measurement published by the Hunterlab Association Laboratory (2008). Furthermore, the smart packaging indicator color is determined by calculating the °Hue value using the formula[31]:

$$°Hue = tan^{-1}\frac{b}{a}$$

Description:
°Hue = parameters for color range
a = is a red-green mixed color
b = is a red-green mixed color yellow-blue

2.10. Level of Relationship (Correlation) between Test Parameters

The smart packaging indicator quantification data will be compared with the data for each beef spoilage parameter presented in a graph using the Sigma Plot software version 14.0.

2.11. *Statistical analysis*

All data from the test parameters were analyzed using analysis of variance (ANOVA) with Duncan's multiple range test using SPSS software version 22.0 (IBM Corp., United States), and the calculated values differed significantly when $p<0.05$. In addition, the relationship (correlation) of intelligent indicator color parameters to all beef spoilage parameters was presented in one graph using Sigma Plot software version 14.0.

3. Results and Discussion

3.1. The antibacterial analysis results of active paper

Antibacterial activity on active paper was tested against several bacteria that generally cause damage to beef, namely *Escherichia coli, Staphylococcus aureus,* and *Pseudomonas aeruginosa*. Figure 2 illustrates the active paper's analytical findings for the bacterial inhibition zone region.

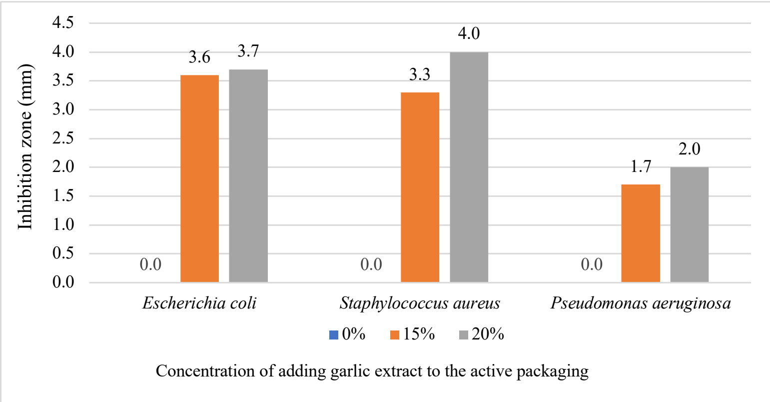


Figure 2. Diameter of Bacterial Growth Barriers on Activated Paper

The garlic extract addition on active paper had a significant effect ($p<0.05$) on the growth inhibition of *Escherichia coli, Staphylococcus aureus,* and *Pseudomonas aeruginosa* bacteria (Figure 2). On the other hand, adding active paper without the garlic extract (0%) did not show any inhibition zones. In comparison, the addition of 15% and 20% extract significantly affected bacterial inhibitory activity, therefore, the garlic extracts have antibacterial activity.

The results obtained for activated paper with the addition of 15% and 20% garlic extract against *E. coli* and *S. aureus* bacteria were classified as a moderate inhibitory response to bacterial growth. Meanwhile, *P. aeruginosa* was classified as a weak inhibitory response to bacterial growth. This is consistent with Pan et al. (2009)'s assessment of the response to bacterial growth inhibition. The antibacterial activity with an inhibitory zone > 6.00 mm, 3-5 mm, and < 3 mm was considered strong, moderate, and weak. [32]

The difference in the diameter of the inhibition zone formed on *Escherichia coli, Staphylococcus aureus*, and *Pseudomonas aeruginosa* was influenced by differences in the structure of bacteria that affect the penetration of cell walls by Allicin compounds. *E. coli* and *P. aeruginosa* are gram-negative bacteria with complex cell walls, therefore, the antibacterial compounds in garlic extract cannot inhibit bacterial growth properly. Gram-negative bacteria have three-layered cell walls consisting of lipoproteins, outer membrane phospholipids, lipopolysaccharides, and the lipid content of the cell walls, which ranges from 11-22%. The phospholipid outer membrane makes it difficult for antibacterial chemical components to penetrate the cell walls of gram-negative bacteria. Meanwhile, *S. aureus* is a gram-positive bacterium with single or simple layered cell walls and a 1-4% lipid content. The low lipid content in the cell membrane causes antibacterial compounds to penetrate the bacterial cell wall. Therefore, their inhibitory power against bacteria is better than gram-negative bacteria. This is consistent with Vijayakumar et al.'s (2019) statement that differences influence the bacteria susceptibility to garlic components in the bacteria structure.[33] *Escherichia coli* bacteria cell membrane consists of 20% lipid, while *Staphylococcus aureus* consists of only 2% lipid. Differences in the lipid content of the bacterial membrane can affect the permeability of allicin and other elements. Salima (2015) confirms that garlic's antibacterial properties are greater against gram-positive bacteria (*Staphylococcus aureus*) than against gram-negative (e*scherichia coli*). [34]

3.2. Meat pH

Analysis of the meat pH value determines the effect of adding various concentrations of garlic extract during cold storage. Therefore, the degree of acidity (pH) is an indicator to examine the meat freshness level. Figure 3 shows the pH changes that occur in beef during storage.

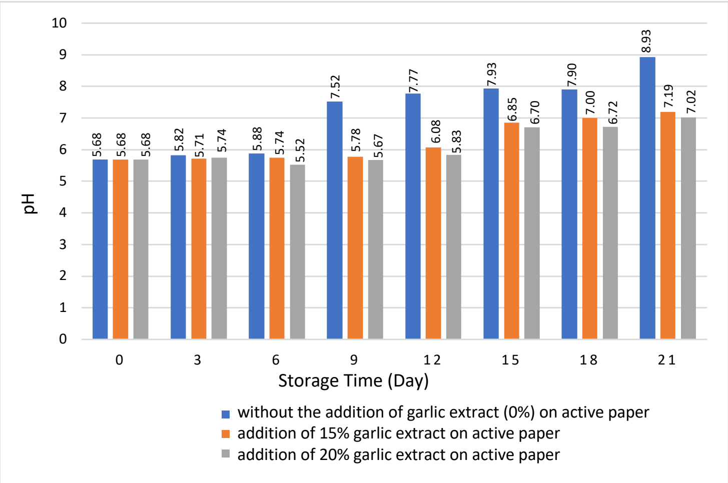


Figure 3. pH value of meat during cold storage

Figure 3 shows that the garlic extract addition with different concentrations affects increasing the pH value of meat during storage. The statistical tests showed that the extract addition on active paper significantly affected the TVBN value ($P < 0.05$). Further test results showed that the treatment without the garlic extract (0%) was significantly different from the other treatments. However, the addition of 15% garlic extract was not significantly different from 20% garlic extract. The initial pH value of fresh meat before storage was 5.68 and classified as rotten after 9 days of storage for treatment without the extract. On the 15th day, 15% and 20% garlic extracts were put to active paper for beef. This is consistent with Ponnampalam et al. (2017) that the pH of normal meat ranges from 5.5 – 5.7, and meat with a pH of > 6 will be easily damaged by microorganisms experiencing some deterioration such as color and texture aroma.[35]

An increase in the pH value of meat during storage indicates a decrease in quality, and anaerobic glycolysis lowers the pH to create lactic acid. The higher the lactic acid produced, the greater the decrease in the meat pH. This is consistent with Kiyimba et al. (2021), which stated that lactic acid production was the cause of the decrease in the pH of the glycolysis phase during postmortem.[36] The pH value of live meat ranges from 6.8-7.2, and after slaughter, it experienced a decrease in pH in the pre rigor condition of pH 7.2 to the ultimate pH between 5.4-5.8. The decrease stopped after the rigor mortise phase was reached in the meat. According to Leygonie et al. (2012), the decrease was also influenced by the ambient temperature (storage).[37] While storing meat at high temperatures accelerates the pace of pH decline, storing at low temperatures slows the rate of pH decrease.

3.4. ***Total Volatile Bases Nitrogen*** **(TVBN)**

Analysis of the TVBN value is one of the parameters used to determine meat freshness level. Figure 4 shows the changes in TVBN values in beef during cold storage.

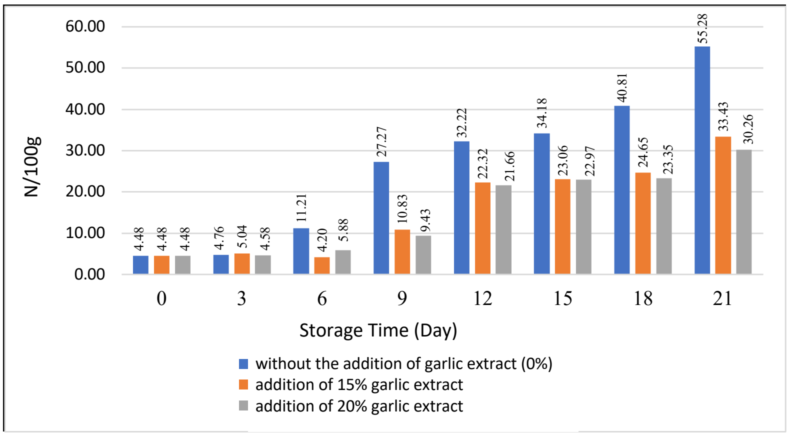


Figure 4. TVBN Value of Beef During Cold Storage

Based on statistical tests, the addition of garlic extract on active paper significantly affected the TVBN value ($P < 0.01$). Further test results showed that the treatment without the extract (0%) addition was significantly different from the other treatments. However, 15% garlic extract was not significantly different from the 20% extract. The TVBN value of meat on the first day of storage was 4.48 mg N/100, indicating a fresh category (Figure 4). On the 9th day of storage, meat treated without the extract had a TVBN value of 27.27 mg N/100g categorized as rotten. Meanwhile, the addition of 15% and 20% garlic extract with TVBN values of 22.32 mg N/100g and 21.66 mg N/100g had crossed the threshold on day 12 and was categorized as rotten meat. TVB-N criteria for identifying rotten beef products are >20 mg/100 g according to the Ministry of Agriculture and Forestry of Korea, 2016[38]. Based on the results obtained, the garlic extract affects the meat TVBN value during storage.

The increase in TVBN value is closely related to the decrease in beef quality. The treatment without onion extract reached the threshold value earlier due to the higher microbial activity in the sample. The high number of microbes in the sample breaks down more meat protein to produce volatile ammonia compounds. This is consistent with Holman et al. (2021), where increased microbial value degrades protein rapidly during storage. Furthermore, it increases the amount of ammonia and other volatile compounds, acting as indicators of decay and increasing the TVB value. [39] The increase in the TVBN value of beef with treatment without and with the garlic extract was also different due to the Allicin constituent and anti-microbial role. The inhibition of microbial growth minimizes the decomposition process of nutritional components in meat to reduce the TVBN value.

3.4. Total Bacteria of Total Plate Count (TPC) Method

The analysis determines the effect of using active paper on the total value of bacteria in meat during cold storage, as shown in Figure 5.

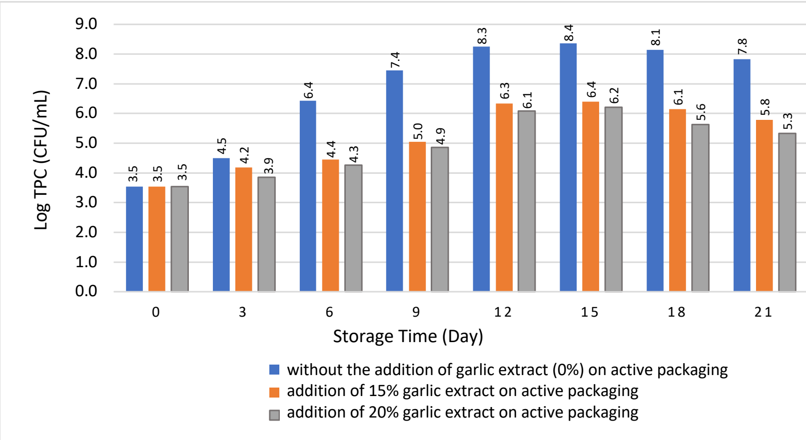


Figure 5. Graph of Beef TPC Value During Cold Storage

The garlic extract added to the active paper had a significant effect on the TPC value of meat ($P < 0.05$). Meanwhile, further test results showed that the treatment without garlic extract (0%) was significantly different from other treatments, but the addition of 15% was not significantly different from the 20% extract. The initial population of TPC meat was 3.5 log CFU/ml, categorized as fresh concerning microbiological quality (Figure 5). However, this value increased to 6.4 log CFU/ml on the 6th day of storage in the control sample, while the recommended maximum bacteriological limit was 6 log CFU/ml (SNI 3932:2008). The meat added with 15% and 20% garlic extract exceeded the maximum bacteriological limit on the 12th day of storage with TPC values of 6.3 log CFU/ml and 6.1 logs CFU/ml, respectively.

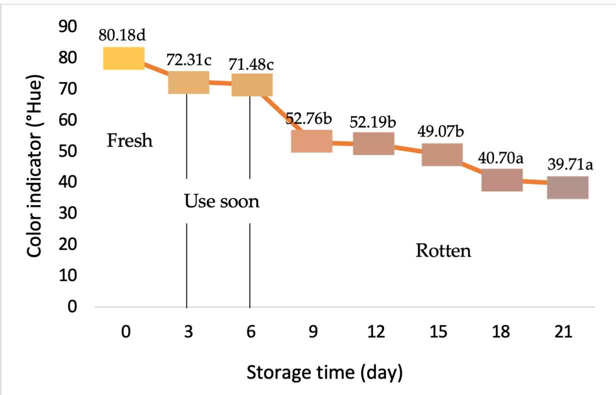
Therefore, the addition of 15% and 20% extract as active packaging inhibits microbial growth and extends the shelf life of meat up to 11 days longer than the samples without the garlic extract in the active packaging. This is because garlic contains an anti-microbial compound in the form of allicin.

Garlic extract applied to the activated paper diffuses to the entire surface of the beef to inhibit the growth of bacteria through total and partial inhibition of RNA, DNA, and protein synthesis. Furthermore, allicin blocks bacterial enzymes with thiol groups to ultimately inhibit bacterial growth. This is consistent with Deresse's (2010) statement that the mechanism of garlic inhibits bacterial growth by completely inhibiting RNA, DNA synthesis, and bacterial protein.[40] Mardiya (2018) also confirmed that the effectiveness of garlic extract with concentrations of 25%, 50%, and 100% was very effective in inhibiting *Staphylococcus aureus* bacteria and bactericidal. Meanwhile, the extract with a concentration of 12.5% was declared as an inhibitor of bacterial growth (bacteriostatic) but less effective at inhibiting bacteria (bacteriocidal).[41]

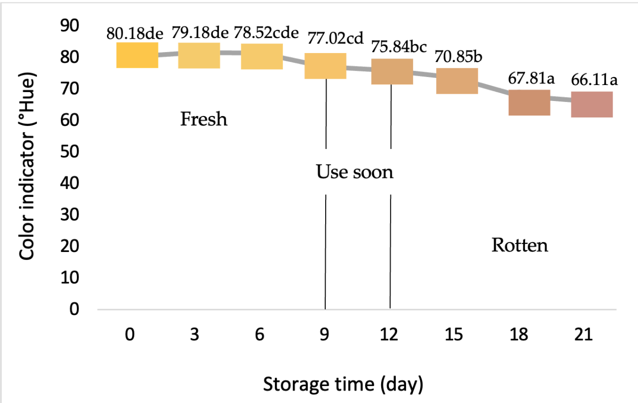
The storage temperature of beef can also affect the meat TPC value. Meat cells in the postmortem phase experience metabolic reactions, which are highly dependent on storage temperature. According to Comi (2017), the storage temperature and the metabolic reactions in meat are inversely proportional.[42] This is supported by Kuswandi and Nurfawaidi (2017), where beef stored at room temperature is categorized as unfit for consumption at 10 hours with a TPC value of 6,711 CFU/mL. Meanwhile, at cold temperature, it is categorized as unfit for consumption on day 7 with a TPC value of 6961 CFU/mL.[29]

3.5. Color Change Response on Smart Indicator Labels for Beef Packaged in Active Packaging at Cold Temperature Storage

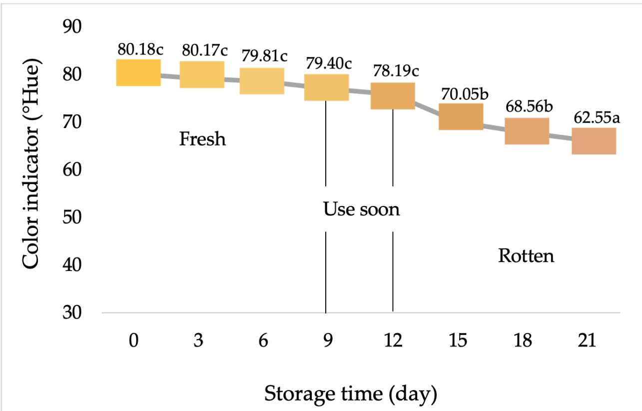
The color change analysis of the smart indicator determines the effect of adding garlic extract to active packaging with different concentrations on beef during storage at cold temperatures (chiller). The color change of the resulting indicator label is information on the freshness condition of the packaged beef. Significant color changes on indicators ease consumers to measure the freshness level based on visual appearance, as seen in Figure 6.



(a)



(b)



(c)

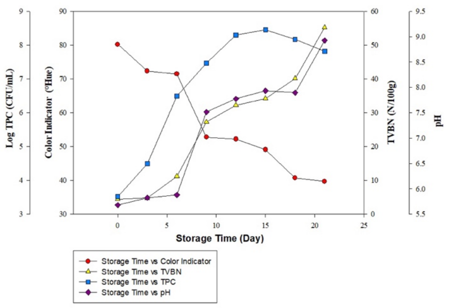
Figure 6. Color Change Profile of Smart Indicator Labels with Garlic Extract Addition on the Concentrations at 0% (a), 15% (b), and 20% (c)

Figure 6 showed that the °Hue value on the smart indicator label decreased during storage, from dark yellow to faded red. The color change is a response to the quality condition. There are three phases of change on smart packaging labels in the description. These include phase I with dark yellow indicating fresh, phase II with yellow and red gradations indicating immediate consumption, and phase III with faded red indicating damaged meat unfit for consumption.

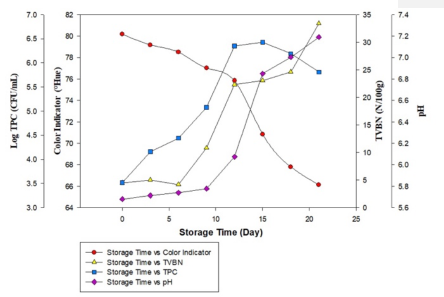
The color change on the smart indicator label is closely related to the increased number of microbes and enzymes in beef during storage. These changes are caused by the decomposition of nutritional components of meat by enzymes and microbes, producing volatile base compounds used as an early sign of damage in packaged beef. This is consistent with Sani et al. (2021) that volatile ammonia compounds in meat can increase the pH.[43] The increase in the pH of meat is caused by ammonia reacting with H+ ions to produce H ions. The OH- ions in the packaging are directly proportional to the pH value, affecting the change in smart indicator labels.

3.6. Correlation of Color Value Changes in Smart Packaging Indicators BTB: PR (1:1) with the Effect of Active Packaging on All Meat Spoilage Parameters

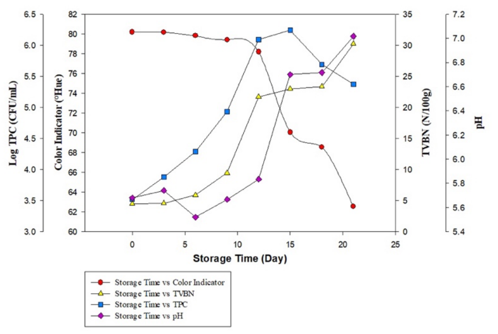
The relationship between the color change response of smart indicator labels with all test parameters on beef, such as TVBN, TPC, and pH, determined the sensitivity of smart indicator labels in detecting quality deterioration. This obtains synchronization between changes in the color of the smart indicator label to the deterioration of beef quality.



(a)



(b)



(c)

Figure 7. Correlation of Color Change on Smart Indicator Label with All Beef Spoilage Parameters with the Garlic Extract Addition (a) Control, (b) 15%, (c) 20%

Based on Figure 7, beef stored without the garlic extract (0%) showed that the increase in the TVBN value and the pH of the meat was in line with the decrease in the color value of the smart packaging indicator. However, it is not consistent with the results of the TPC value since the beef TPC value has crossed the threshold on the 6th day of storage, which is 6.4 CFU/ml. Meanwhile, the TVBN value is suitable for consumption on the 6th day. Mansur et al. (2016) also reported that the TVB-N values were inconsistent with the viable microbial count (TVC). Based on TVB-N, the samples were classified as damaged after seven and eleven days of storage.[44] This is because the microbial calculation method is not selective against spoilage bacteria in meat but counts all the pathogenic microbes. Holman et al. (2021) also stated that the TVB-N value was more precise and accurate in classifying beef in fresh or rotten conditions than the total microbial value (TVC).[39]

Based on Figures 7(b) and (c), beef stored with 15 % and 20% garlic extract treatment showed that the increase in the parameters of the meat quality deterioration, including TPC, TVBN, and pH, was consistent with the decrease in the color of the smart indicator label. The smart indicator changes color from yellow (fresh) to faint red (rotten) during storage. The increase in TVBN, TPC, and pH values is influenced by the high nutritional content, specifically protein broken down into polypeptides and other amino acids through the deamination process to form ammonia. With the increase in microbes, the nutritional components decomposed by microbes are also greater and produce basic volatile compounds increasing the pH value of meat during storage. The resulting volatile compounds accumulate in the packaging and are directly detected by the smart indicator label. The reduction in the color value of the smart indicator label shows the presence of many alkaline volatile chemicals in the package and the rottenness and unfitness for ingestion of the meat. Furthermore, when viewed from the visual appearance on the 9th and 12th day of storage without (0%) and with (15% and 20%) garlic extract, the beef was damaged and unfit for consumption characterized by the formation of mucus, emits a sour/rotten smell, and the texture begins to be sticky to the touch.

The increase in beef quality deterioration parameter value for each treatment was different due to the addition of garlic extract as active packaging. The extract has a bioactive compound in allicin to inhibit microbial growth. This compound in the package diffuses in the meat surface to suppress the microbe growth. According to Deresse (2010), it works by completely inhibiting bacterial RNA synthesis and inhibiting DNA and protein synthesis. [40] The pattern of relationship between the parameters of the decline in beef quality and the color analysis value of indicator labels is also consistent with Kuswandi and Nurfawaidi (2017), where a linear relationship pattern to the parameters of the beef freshness level with changes in the color intensity of the bromocresol purple and methyl red smart labels were observed.[29] In this study, beef treated without the garlic extract experienced a decrease in quality and was not suitable for consumption on the 9th day. Meanwhile, with 15% and 20% garlic extract, the meat was damaged and unfit for consumption on the 12th day of storage. This indicates that garlic extract storing at cold temperatures can extend the shelf life because of the Allicin properties. Visually, the smart indicator label using Whatman paper no. 1 as the base material and the BTB + PR (pH 5.00) used in smart packaging is used for beef freshness with a color change from yellow (beginning) to faint red (late). Therefore, it assists customers in determining freshness without touching the texture or opening the packaging.

4. Conclusion

The garlic extract in active packaging, supported by the microbiological evaluation results of TVBN and pH, extended shelf life during storage. In addition, the results showed that the active paper with 15% and 20% garlic extract extended the shelf life for 12 days of cold storage. The color change profile of the smart indicator label combination of BTB + PR pH 5.00 was easily observed from dark (fresh) to reddish yellow (to be consumed immediately). The red color fades when the beef is rotten and unfit for consumption. Therefore, the combination of active and smart packaging is suggested as a potential packaging application in fresh meat packaging.

References

1. Dave D, Ghaly AE. Meat spoilage mechanisms and preservation techniques: A critical review. Am J Agric Biol Sci. 2011.6(4):486–510. Available from: https://doi.org/10.3844/ajabssp.2011.486.510
2. Ahmed I, Lin H, Zou L, Brody AL, Li Z, Qazi IM, et al. A comprehensive review on the application of active packaging technologies to muscle foods. Food Control. 2017.82:163–78. Available from: https://doi.org/10.1016/j.foodcont.2017.06.009
3. Chen J, Brody AL. Use of active packaging structures to control the microbial quality of a ready-to-eat meat product. Food Control. 2013.30(1):306–10. Available from: https://doi.org/10.1016/j.foodcont.2012.07.002
4. Bahmid NA, Dekker M, Fogliano V, Heising J. Development of a moisture-activated anti-microbial film containing ground mustard seeds and its application on meat in active packaging system. Food Packag Shelf Life. 2021.30:100753. Available from: https://doi.org/10.1016/j.fpsl.2021.100753
5. Umaraw P, Munekata PES, Verma AK, Barba FJ, Singh VP, Kumar P, et al. Edible films/coating with tailored properties for active packaging of meat, fish and derived products. Trends Food Sci Technol. 2020.98:10–24. Available from: https://doi.org/10.1016/j.tifs.2020.01.032
6. Alizadeh-Sani M, Mohammadian E, McClements DJ. Eco-friendly active packaging consisting of nanostructured biopolymer matrix reinforced with TiO2 and essential oil: Application for preservation of refrigerated meat. Food Chem. 2020.322:126782. Available from: https://doi.org/10.1016/j.foodchem.2020.126782
7. Arkoun M, Daigle F, Holley RA, Heuzey MC, Ajji A. Chitosan-based nanofibers as bioactive meat packaging materials. Packag Technol Sci. 2018.31(4):185–95. Available from: https://doi.org/10.1002/pts.2366
8. Quintavalla S, Vicini L. Antimicrobial food packaging in meat industry. Meat Sci. 2002.62(3):373–80. Available from: https://doi.org/10.1016/S0309-1740(02)00121-3
9. Yildirim S, Röcker B, Pettersen MK, Nilsen-Nygaard J, Ayhan Z, Rutkaite R, et al. Active Packaging Applications for Food. Compr Rev Food Sci Food Saf. 2018.17(1):165–99. Available from: https://doi.org/10.1111/1541-4337.12322
10. Santas J, Almajano MP, Carbó R. Antimicrobial and antioxidant activity of crude onion (Allium cepa, L.) extracts. Int J Food Sci Technol. 2010.45(2):403–9. Available from: https://doi.org/10.1111/j.1365-2621.2009.02169.x
11. Kyung KH. Anti-microbial properties of allium species. Curr Opin Biotechnol. 2012.23(2):142–7. Available from: http://dx.doi.org/10.1016/j.copbio.2011.08.004
12. Radusin T, Torres-Giner S, Stupar A, Ristic I, Miletic A, Novakovic A, et al. preparation, characterization and anti-microbial properties of electrospun polylactide films

containing Allium ursinum L. extract. Food Packag Shelf Life. 2019.21(February):100357. Available from: https://doi.org/10.1016/j.fpsl.2019.100357
13. Dong Z, Luo C, Guo Y, Ahmed I, Pavase TR, Lv L, et al. Characterization of new active packaging based on PP/LDPE composite films containing attapulgite loaded with Allium sativum essence oil and its application for large yellow croaker (Pseudosciaena crocea) fillets. Food Packag Shelf Life. 2019.20(November 2018):100320. Available from: https://doi.org/10.1016/j.fpsl.2019.100320
14. Seydim AC, Sarikus G. Antimicrobial activity of whey protein based edible films incorporated with oregano, rosemary and garlic essential oils. Food Res Int. 2006.39(5):639–44. Available from: https://doi.org/10.1016/j.foodres.2006.01.013
15. Yolanda DS, Dirpan A, Rahman ANF, Kamaruddin I, Ainani AF. Determination the best concentration of anti-microbial ingredients with a mixture of paper to create active paper packaging. IOP Conf Ser Earth Environ Sci. 2020.575(1). Available from: https://doi.org/10.1088/1755-1315/575/1/012019
16. Vilas C, Mauricio-Iglesias M, García MR. Model-based design of smart active packaging systems with anti-microbial activity. Food Packag Shelf Life. 2020.24(December 2019):100446. Available from: https://doi.org/10.1016/j.fpsl.2019.100446
17. Dirpan A, Djalal M, Rahman R, Genisa J. The utilization of red cabbage extract (Brassica oleracea) in the production of avocado (Persea Americana Mill) freshness indicator as smart packaging element. Online J Biol Sci. 2021.21(3):261–70. Available from: https://doi.org/10.3844/ojbsci.2021.261.270
18. Agustianti D, Dirpan A, Syarifuddin A. The potential application of red cabbage indicator film as smart packaging on tuna fillet. IOP Conf Ser Earth Environ Sci. 2021.807(2). Available from: https://doi.org/10.1088/1755-1315/807/2/022065
19. Wu Y, Tang P, Quan S, Zhang H, Wang K, Liu J. Preparation, characterization and application of smart packaging films based on locust bean gum/polyvinyl alcohol blend and betacyanins from cockscomb (Celosia cristata L.) flower. Int J Biol Macromol. 2021.191:679–88. Available from: https://doi.org/10.1016/j.ijbiomac.2021.09.113
20. Kim D, Thanakkasaranee S, Lee K, Sadeghi K, Seo J. Smart packaging with temperature-dependent gas permeability maintains the quality of cherry tomatoes. Food Biosci. 2021.41:100997. Available from: https://doi.org/10.1016/j.fbio.2021.100997
21. Dirpan A, Latief R, Syarifuddin A, Rahman ANF, Putra RP, Hidayat SH. The use of colour indicator as a smart packaging system for evaluating mangoes Arummanis (Mangifera indica L. var. Arummanisa) freshness. IOP Conf Ser Earth Environ Sci. 2018.157(1):12031. Available from: https://doi.org/10.1088/1755-1315/157/1/012031
22. Shukla V, Kandeepan G, Vishnuraj MR. Development of On-Package Indicator Sensor for Real-Time Monitoring of Buffalo Meat Quality During Refrigeration Storage. Food Anal Methods. 2015.8(6):1591–7. Available from: https://doi.org/10.1007/s12161-014-0066-6
23. Romero A, Sharp JL, Dawson PL, Darby D, Cooksey K. Evaluation of two intelligent packaging prototypes with a pH indicator to determine spoilage of cow milk. Food Packag Shelf Life. 2021.30(August):100720. Available from: https://doi.org/10.1016/j.fpsl.2021.100720
24. Julyaningsih AH, Latief R, Dirpan A. The making of smart and active packaging on tuna fillet. IOP Conf Ser Earth Environ Sci. 2020.486(1). Available from: https://doi.org/10.1088/1755-1315/486/1/012053

25. Sisilia Yolanda D, Dirpan A, Nur Faidah Rahman A, Djalal M, Hatul Hidayat S. The potential combination of smart and active packaging in one packaging system in improving and maintaining the quality of fish. Canrea J Food Technol Nutr Culin J. 2020.3(2 SE-Articles):74–86. Available from: https://doi.org/10.20956/canrea.v3i2.357
26. Yong H, Liu J. Recent advances in the preparation, physical and functional properties, and applications of anthocyanins-based active and intelligent packaging films. Food Packag Shelf Life. 2020.26(July):100550. Available from: https://doi.org/10.1016/j.fpsl.2020.100550
27. Gosal L, Hutomo S, Sooai CM. Garlic (Allium sativum L.) Ethanolic Extract Capability to Inhibit Pseudomonas aeruginosa Biofilm Formation. J Med Heal. 2021.3(1):1–8.
28. Wiastuti T, Khasanah LU, Kawiji WA, Manuhara GJ, Utami R. Characterization of active paper packaging incorporated with ginger pulp oleoresin. IOP Conf Ser Mater Sci Eng. 2016.107(1). Available from: https://doi.org/10.1088/1757-899X/107/1/012057
29. Kuswandi B, Nurfawaidi A. On-package dual sensors label based on pH indicators for real-time monitoring of beef freshness. Food Control. 2017.82:91–100. Available from: http://dx.doi.org/10.1016/j.foodcont.2017.06.028
30. Mohammed MF, Raman N, Alhoot MA, Alwan MR. Antibacterial activities of allium sativum (Garlic) extracts against staphylococcus aureus and Escherichia coli. Eur J Mol Clin Med. 2020.7(11):526–34.
31. Hunterlab. Colorimeters Versus Spectrophotometers. Virgina: Technical Service Departement Hunter Associates Laboratory, Inc.; 2008.
32. Pan X, Chen F, Wu T, Tang H, Zhao Z. The acid, bile tolerance and anti-microbial property of Lactobacillus acidophilus NIT. Food Control. 2009.20(6):598–602. Available from: https://doi.org/10.1016/j.foodcont.2008.08.019
33. Vijayakumar S, Malaikozhundan B, Saravanakumar K, Durán-Lara EF, Wang M-H, Vaseeharan B. Garlic clove extract assisted silver nanoparticle–Antibacterial, antibiofilm, antihelminthic, anti-inflammatory, anticancer and ecotoxicity assessment. J Photochem Photobiol B Biol. 2019.198:111558. Available from: https://doi.org/10.1016/j.jphotobiol.2019.111558
34. Salima J. Antibacterial Activity of Garlic (Allium sativum L.) on Multi-Drug Resistant. J Major. 2015.4(2):30–9. Available from: http://enterpathog.com/27870.pdf
35. Ponnampalam EN, Hopkins DL, Bruce H, Li D, Baldi G, Bekhit AE din. Causes and Contributing Factors to "Dark Cutting" Meat: Current Trends and Future Directions: A Review. Compr Rev Food Sci Food Saf. 2017.16(3):400–30. Available from: https://doi.org/10.1111/1541-4337.12258
36. Kiyimba F, Hartson SD, Rogers J, VanOverbeke DL, Mafi GG, Ramanathan R. Changes in glycolytic and mitochondrial protein profiles regulates postmortem muscle acidification and oxygen consumption in dark-cutting beef. J Proteomics. 2021.232(September):104016. Available from: https://doi.org/10.1016/j.jprot.2020.104016
37. Leygonie C, Britz TJ, Hoffman LC. Impact of freezing and thawing on the quality of meat: Review. Meat Sci. 2012.91(2):93–8. Available from: http://dx.doi.org/10.1016/j.meatsci.2012.01.013
38. Korean Ministry of Agriculture and Forestry. Processing standards and ingredient specifications for livestock products [Internet]. Ministry of Food and Drug Safety. 2016 [cited 2021 Dec 18]. Available from: www.mfds.go.kr/eng/brd/m_15/v%0Aiew.do?seq=70016&srchFr=&srchTo=&srchWo

rd=&srchTp=&itm_seq_1=0&itm_s%0Aeq_2=0&multi_itm_seq=0&company_cd=&company_nm=&page=2:.
39. Holman BWB, Bekhit AEDA, Waller M, Bailes KL, Kerr MJ, Hopkins DL. The association between total volatile basic nitrogen (TVB-N) concentration and other biomarkers of quality and spoilage for vacuum packaged beef. Meat Sci. 2021.179(May):108551. Available from: https://doi.org/10.1016/j.meatsci.2021.108551
40. Deresse D. Antibacterial effect of garlic (Allium sativum) on Staphylococcu aureus: An in vitro study. Asian J Med Sci. 2010.2(2):62–5.
41. Mardiyah S. Efektivitas Anti Bakteri Perasan Bawang Putih (Allium sativum L.) terhadap Pertumbuhan Staphylococcus aureus. Medicra (Journal Med Lab Sci. 2018.1(2):44–53. Available from: https://doi.org/10.21070/medicra.v1i2.1532
42. Comi G. Spoilage of Meat and Fish [Internet]. The Microbiological Quality of Food: Foodborne Spoilers. Elsevier Ltd; 2017. 179–210 p. Available from: http://dx.doi.org/10.1016/B978-0-08-100502-6.00011-X
43. Alizadeh-Sani M, Tavassoli M, Mohammadian E, Ehsani A, Khaniki GJ, Priyadarshi R, et al. pH-responsive color indicator films based on methylcellulose/chitosan nanofiber and barberry anthocyanins for real-time monitoring of meat freshness. Int J Biol Macromol. 2021.166:741–50. Available from: https://doi.org/10.1016/j.ijbiomac.2020.10.231
44. Mansur AR, Song EJ, Cho YS, Nam Y Do, Choi YS, Kim DO, et al. Comparative evaluation of spoilage-related bacterial diversity and metabolite profiles in chilled beef stored under air and vacuum packaging. Food Microbiol. 2019.77:166–72. Available from: https://doi.org/10.1016/j.fm.2018.09.006

Certificate of Proofreading

This document certifies that the manuscript was edited for proper English language, grammar, punctuation, spelling, and overall style by one or more of the highly qualified native English speaking editors at Good Lingua Center of Education (GLCE)

Manuscript Title

A Simple Combination of Active and Smart Packaging in Extending and Monitoring the Meat Quality Stored at Cold Temperatures (4 ±1oC)

Author(s)

Andi Dirpan

Date Issued

February, 5 2022

GoodLingua
Translation, editing, and proofreading

PT. Internasional Translasi Edukasi, Jakarta

2. Submitted to the journal “FOODS” (30-4-2022)

[Foods] Manuscript ID: foods-1728837 - Submission Received

Sat, Apr 30, 2:09 PM

Dear Dr. Dirpan,

Thank you very much for uploading the following manuscript to the MDPI submission system. One of our editors will be in touch with you soon.

Journal name: Foods
Manuscript ID: foods-1728837
Type of manuscript: Article
Title: A Simple Combination of Active and Intelligent Packaging based on Garlic Extract and Indicator Solution in Extending and Monitoring the Meat Quality Stored at Cold Temperature
Authors: Andi Dirpan *, Muspirah Djalal, Andi Fadiah Ainani
Received: 30 April 2022
E-mails: dirpan@unhas.ac.id, muspirah_djalal@agri.unhas.ac.id, andifadiah97@gmail.com

You can follow progress of your manuscript at the following link (login required):
https://susy.mdpi.com/user/manuscripts/review_info/6a91850571fa17b18e5de65d7113d608

The following points were confirmed during submission:

1. Foods is an open access journal with publishing fees of 2200 CHF for an accepted paper (see https://www.mdpi.com/about/apc/ for details). This manuscript, if accepted, will be published under an open access Creative Commons CC BY license (https://creativecommons.org/licenses/by/4.0/), and I agree to pay the Article Processing Charges as described on the journal webpage (https://www.mdpi.com/journal/foods/apc). See https://www.mdpi.com/about/openaccess for more information about open access publishing.

Please note that you may be entitled to a discount if you have previously received a discount code or if your institute is participating in the MDPI Institutional Open Access Program (IOAP), for more information see https://www.mdpi.com/about/ioap. If you have been granted any other special discounts for your submission, please contact the Foods editorial office.

2. I understand that:

a. If previously published material is reproduced in my manuscript, I will provide proof that I have obtained the necessary copyright permission. (Please refer to the Rights & Permissions website: https://www.mdpi.com/authors/rights).

b. My manuscript is submitted on the understanding that it has not been published in or submitted to another peer-reviewed journal. Exceptions to this rule are papers containing material disclosed at conferences. I confirm that I will inform the journal editorial office if this is the case for my manuscript. I confirm that all authors are familiar with and agree with submission of the contents of the manuscript. The journal editorial office reserves the right to contact all authors to confirm this in case of doubt. I will provide email addresses for all authors and an institutional e-mail address for at least one of the co-authors, and specify the name, address and e-mail for invoicing purposes.

If you have any questions, please do not hesitate to contact the Foods editorial office at foods@mdpi.com

Kind regards,
Foods Editorial Office
St. Alban-Anlage 66, 4052 Basel, Switzerland
E-Mail: foods@mdpi.com
Tel. +41 61 683 77 34
Fax: +41 61 302 89 18

*** This is an automatically generated email ***

3. First revision: Accepted with minor revision (13-5-2022)

[Foods] Manuscript ID: foods-1728837 - Minor Revisions

Fri, May 13, 1:50 PM

Dear Dr. Dirpan,

Thank you again for your manuscript submission:

Manuscript ID: foods-1728837
Type of manuscript: Article
Title: A Simple Combination of Active and Intelligent Packaging based on Garlic Extract and Indicator Solution in Extending and Monitoring the Meat Quality Stored at Cold Temperature
Authors: Andi Dirpan *, Muspirah Djalal, Andi Fadiah Ainani
Received: 30 April 2022
E-mails: dirpan@unhas.ac.id, muspirah_djalal@agri.unhas.ac.id, andifadiah97@gmail.com

Your manuscript has been reviewed by experts in the field. Please find your manuscript with the referee reports at this link:
https://susy.mdpi.com/user/manuscripts/resubmit/6a91850571fa17b18e5de65d7113d608

(I) Please revise your manuscript according to the referees' comments and upload the revised file within 5 days.
(II) Please use the version of your manuscript found at the above link for your revisions.
(III) Please check that all references are relevant to the contents of the manuscript.
(IV) Any revisions made to the manuscript should be marked up using the "Track Changes" function if you are using MS Word/LaTeX, such that changes can be easily viewed by the editors and reviewers.
(V) Please provide a short cover letter detailing your changes for the editors' and referees' approval.

If one of the referees has suggested that your manuscript should undergo extensive English revisions, please address this issue during revision. We propose that you use one of the editing services listed at https://www.mdpi.com/authors/english or have your manuscript checked by a native English-speaking colleague.

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.

Kind regards,
Sasa Novakovic

Dr. Sasa Novakovic
Assistant Editor
MDPI Branch Office, Belgrade
Bulevar Mihajla Pupina 115, 11070 Belgrade, Serbia
Tel. +381 11 7455 363

MDPI
Foods Editorial Office
E-Mail: foods@mdpi.com
http://www.mdpi.com/journal/foods

4. Revised version received (17-5-2022)

-Revisions and Amends

-Revised version with highlights

- Manuscript revisions

[Foods] Manuscript ID: foods-1728837 - Manuscript Resubmitted

Tue, May 17, 6:06 PM

Dear Dr. Dirpan,

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

Manuscript ID: foods-1728837
Type of manuscript: Article
Title: A Simple Combination of Active and Intelligent Packaging based on Garlic Extract and Indicator Solution in Extending and Monitoring the Meat Quality Stored at Cold Temperature
Authors: Andi Dirpan *, Muspirah Djalal, Andi Fadiah Ainani
Received: 30 April 2022
E-mails: dirpan@unhas.ac.id, muspirah_djalal@agri.unhas.ac.id, andifadiah97@gmail.com

https://susy.mdpi.com/user/manuscripts/review_info/6a91850571fa17b18e5de65d7113d608

A member of the editorial office will be in touch with you soon regarding progress of the manuscript.

Kind regards,
Foods Editorial Office
Postfach, CH-4020 Basel, Switzerland
Office: St. Alban-Anlage 66, CH-4052 Basel
Tel. +41 61 683 77 34 (office)
Fax +41 61 302 89 18 (office)
E-mail: foods@mdpi.com
https://www.mdpi.com/journal/foods/

[Foods] Manuscript ID: foods-1728837 - Revised Version Received

Tue, May 17, 6:12 PM

Dear Dr. Dirpan,

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

Manuscript ID: foods-1728837
Type of manuscript: Article
Title: A Simple Combination of Active and Intelligent Packaging based on Garlic Extract and Indicator Solution in Extending and Monitoring the Meat Quality Stored at Cold Temperature
Authors: Andi Dirpan *, Muspirah Djalal, Andi Fadiah Ainani
Received: 30 April 2022
E-mails: dirpan@unhas.ac.id, muspirah_djalal@agri.unhas.ac.id, andifadiah97@gmail.com

https://susy.mdpi.com/user/manuscripts/review_info/6a91850571fa17b18e5de65d7113d608

We will continue processing your paper and will keep you informed about the status of your submission.

Kind regards,
Sasa Novakovic

Dr. Sasa Novakovic
Assistant Editor
MDPI Branch Office, Belgrade
Bulevar Mihajla Pupina 115, 11070 Belgrade, Serbia
Tel. +381 11 7455 363

MDPI
Foods Editorial Office
E-Mail: foods@mdpi.com
http://www.mdpi.com/journal/foods

Reviewer 1

Manuscript ID : **foods-1728837**
Status : Website online
DOI : 10.3390/foods11101495
Publication Certificate : Download Publication Certificate (PDF)
Banner : Download Banner (PDF)
Website Links : Abstract HTML version PDF version Manuscript
Article type : Article
Title : A Simple Combination of Active and Intelligent Packaging Based on Garlic Extract and Indicator Solution in Extending and Monitoring the Meat Quality Stored at Cold Temperature
Journal : *Foods*
Volume : 11
Issue : 10
Topic: : Smart Technologies in Food Packaging and Sensors

Abstract
Safety and quality, as the major concerns of meat, are highly dependent on the ingredients and packaging techniques used. A basic combination of active and intelligent packaging is believed to be capable of preserving product quality, extending shelf life, and monitoring product deterioration. Therefore, this study aimed to extend and monitor the beef quality at cold temperatures (4 ± 1 °C). The active packaging applied garlic extract (0%, 15%, and 20% (*w*/*w*)) to release anti-microbial agents. Meanwhile, the intelligent paper applied a combination of bromothymol blue (BTB) and phenol red (PR) solutions at pH 5.00. The results showed that beef packed without the addition of garlic extract had already deteriorated on the 6th day of storage while, with the addition of garlic extract (15% and 20%) rotted on the 12th day. The intelligent indication label's color profile changed from dark yellow (fresh), to reddish-yellow (to be consumed immediately), to faded red (rotten). The color change of the intelligent indicator label in response to all meat deterioration criteria demonstrates a linear correlation for determining the extent of rottenness during storage. Therefore, this simple combination of active paper and intelligent indicator can be used to extend the shelf life and monitor meat quality.

Keywords
smart packaging; meat; garlic oil; shelf life

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)	( )	( )	( )
Are all the cited references relevant to the research?	( )	(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

Please address the following comments:

- Regarding the packaging structure, shown in Figure 1, Styrofoam is a polystyrene foam whereas the authors indicate that a polypropylene tray is used. The procedure was probalbly not expressed correctly. Moreover, all the pacakging films or products should be included in the Materials section, indicating the commercial reference and main characteristics. Furthermore, the use of PS, LDPE or PP is not sustainable and it is suggested to, at least, indicate that those should be replaced in future developments.
- The organization of the Results section can be improved. For instance, the article can first present results in relation with que quality parameters of meat (pH, color, microbial counts, etc.) and then effect of the additives on the antimicrobial properties and color (active and intelligent properties)
- Results should be discussed and compared with other studies reporting the shelf-life stability of meat packaged. Please see for instance 10.3390/foods11030426
- The potential effect of the garlic extract in the organoleptic properties of meet should be discussed in the Conclusions

Submission Date: 30 April 2022
Date of this review: 12 May 2022 13:51:15

MATRICES OF AMENDMENTS FOR REVIEWER 1

Comments and Suggestions for Authors	Author's responds
Regarding the packaging structure, shown in Figure 1, Styrofoam is a polystyrene foam whereas the authors indicate that a polypropylene tray is used. The procedure was probably not expressed correctly. Moreover, all the packaging films or products should be included in the Materials section, indicating the commercial reference and main characteristics. Furthermore, the use of PS, LDPE or PP is not sustainable and it is suggested to, at least, indicate that those should be replaced in future developments.	Thank you for your comments, For the material we used to transfer the meat from the slaughterhouse to the lab we used polypropylene and for the tray in the packaging system we used Styrofoam (polystyrene), so it is actually for a different purpose. As suggested, all the materials have been included in the materials section and the main characteristics also have been added in line 103-108. For the use of PS and LDPE yes, it is certainly not sustainable and as suggested we already put a recommendation to replace it in future in line 208, thank you.
The organization of the Results section can be improved. For instance, the article can first present results in relation with que quality parameters of meat (pH, color, microbial counts, etc.) and then effect of the additives on the antimicrobial properties and color (active and intelligent properties)	Thank you for your input. We have rearranged the results
Results should be discussed and compared with other studies reporting the shelf-life stability of meat packaged. Please see for instance 10.3390/foods11030426	Thank you for your comment. The result has been compared with other studies and the literature suggested also added in the method.

Reviewer 2

Manuscript ID	: **foods-1728837**
Status	: Website online
DOI	: 10.3390/foods11101495
Publication Certificate	: Download Publication Certificate (PDF)
Banner	: Download Banner (PDF)
Website Links	: Abstract HTML version PDF version Manuscript
Article type	: Article
Title	: A Simple Combination of Active and Intelligent Packaging Based on Garlic Extract and Indicator Solution in Extending and Monitoring the Meat Quality Stored at Cold Temperature
Journal	: *Foods*
Volume	: 11
Issue	: 10
Topic:	: Smart Technologies in Food Packaging and Sensors

Abstract

Safety and quality, as the major concerns of meat, are highly dependent on the ingredients and packaging techniques used. A basic combination of active and intelligent packaging is believed to be capable of preserving product quality, extending shelf life, and monitoring product deterioration. Therefore, this study aimed to extend and monitor the beef quality at cold temperatures (4 ± 1 °C). The active packaging applied garlic extract (0%, 15%, and 20% (*w*/*w*)) to release anti-microbial agents. Meanwhile, the intelligent paper applied a combination of bromothymol blue (BTB) and phenol red (PR) solutions at pH 5.00. The results showed that beef packed without the addition of garlic extract had already deteriorated on the 6th day of storage while, with the addition of garlic extract (15% and 20%) rotted on the 12th day. The intelligent indication label's color profile changed from dark yellow (fresh), to reddish-yellow (to be consumed immediately), to faded red (rotten). The color change of the intelligent indicator label in response to all meat deterioration criteria demonstrates a linear correlation for determining the extent of rottenness during storage. Therefore, this simple combination of active paper and intelligent indicator can be used to extend the shelf life and monitor meat quality.

Keywords

smart packaging; meat; garlic oil; shelf life

Review Report Form

English language and style
( ) Extensive editing of English language and style required
(x) Moderate English changes required
( ) 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)	( )	( )	( )
Are all the cited references relevant to the research?	(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 work concerns an important aspect of food storage and ensuring food safety. In general, the work is written correctly and, after introducing minor corrections.

Line 62 there is"… its volatile Allicin constituent"… should be "…its volatile allicin constituent"…

Line 107-112 This description is not clear. How can blended garlic be immersed in alcohol for several times. The mixing process is not imprecisely described. What was the speed of mixing, was it a mechanical stirrer?

Line 118 Are you sure that tapioca starch was dissolved? Was it soluble starch and real solution or a suspension of starch in water.

Line 122 there is "*tween 80"* should be *"Tween® 80"*

Line 123 "The paper dough is poured" should be "The paper dough was poured"

Line 126 "surface is pressed" should be "surface was pressed"

Line 235 "quantification data will be compared" should be "quantification data was compared"

Please check the description of the methodology in terms of grammar.

Table 1 In my opinion, it does not make sense to give the average pH value, especially in relation to the average pH on the given day (last column). These are different samples, so what would such an average be evidence of? The average in the last line makes mathematical sense, but it might be better to present the difference from the initial pH.

I have the same comments about table 2 and 3.

I have some concerns about the color indicator. Is it certain that the consumer is able to distinguish borderline indicator colors? The colors presented in the charts are quite similar in the borderline ranges. Perhaps the total color difference (delta E) should be determined. For this parameter, the limit values are known which make it possible to conclude whether the consumer will see a color difference or not. Given the results from the CieLab system, it is easy to calculate. Alternatively, a consumer test can be carried out.

Submission Date: 30 April 2022
Date of this review: 11 May 2022 11:46:50

MATRICES OF AMENDMENTS FOR REVIEWER 2

Comments and Suggestions for Authors	Author's responds
Line 62 there is"… its volatile Allicin constituent"… should be "…its volatile allicin constituent"…	Thank you for your response and we greatly appreciate it. We have changed all capital into lowercase except for the one at the beginning of the sentence.
Line 107-112 This description is not clear. How can blended garlic be immersed in alcohol for several times. The mixing process is not imprecisely described. What was the speed of mixing, was it a mechanical stirrer?	Thank you for your detail comment. We are sorry that we create confuse with the way we write the sentence. The 3x24 h actually means 72 hours and not a multiple times immersion. It is already written 72 hours in the paper in line 114. While for the stirring process, we understand that the sentence creates confusion. It is actually a manual gentle stirring process in which during the 72 hours of immersion process the solution was stirred every 5 hours. The changed was made in line 114-115
Line 118 Are you sure that tapioca starch was dissolved? Was it soluble starch and real solution or a suspension of starch in water.	Thank you for the correction. You are right it is suspension so we have changed it into suspended in line 123 instead of dissolved.
Line 122 there is "*tween 80"* should be *"Tween® 80"*	Thank you for the correction. The change has been made in line 128 according to your suggestion.
Line 123 "The paper dough is poured" should be "The paper dough was poured"	Thank you for the correction. The change has been made in line 130 according to your suggestion
Line 126 "surface is pressed" should be "surface was pressed"	Thank you for the correction. The change has been made in line 132 according to your suggestion
Line 235 "quantification data will be compared" should be "quantification data was compared"	Thank you for the correction. The change has been made in line 308 according to your suggestion
Please check the description of the methodology in terms of grammar.	Thank you for your comments. We rechecked the methodology and edit several parts to make it more readable. We hope this meets your expectation.
Table 1 In my opinion, it does not make sense to give the average pH value, especially in relation to the average pH on	Thank you for your suggestion. We appreciate your input and we have deleted the last column.

the given day (last column). These are different samples, so what would such an average be evidence of? The average in the last line makes mathematical sense, but it might be better to present the difference from the initial pH.	
I have the same comments about table 2 and 3.	Thank you for your suggestion. We appreciate your input and we have deleted the last column.
I have some concerns about the color indicator. Is it certain that the consumer is able to distinguish borderline indicator colors? The colors presented in the charts are quite similar in the borderline ranges. Perhaps the total color difference (delta E) should be determined. For this parameter, the limit values are known which make it possible to conclude whether the consumer will see a color difference or not. Given the results from the CieLab system, it is easy to calculate. Alternatively, a consumer test can be carried out.	Thank you for your comments, it very much enlighted us. We added the Delta E value and also an explanation of this value is the result of our study.

foods

MDPI

Article

A Simple Combination of Active and Intelligent Packaging Based on Garlic Extract and Indicator Solution in Extending and Monitoring the Meat Quality Stored at Cold Temperature

Andi Dirpan *, Muspirah Djalal and Andi Fadiah Ainani

Department of Agricultural Technology, Hasanuddin University, Makassar 90245, Indonesia; muspirah_djalal@agri.unhas.ac.id (M.D.); andifadiah97@gmail.com (A.F.A.)
* Correspondence: dirpan@unhas.ac.id

Commented [M3]: Please carefully check the accuracy of names and affiliations.

Commented [MOU4R3]: We confirmed that the names and affiliations are correct.

Abstract: Safety and quality, as the major concerns of meat, are highly dependent on the ingredients and packaging techniques used. A basic combination of active and intelligent packaging is believed to be capable of preserving product quality, extending shelf life, and monitoring product deterioration. Therefore, this study aimed to extend and monitor the beef quality at cold temperatures (4 ± 1 °C). The active packaging applied garlic extract (0%, 15%, and 20% (*w*/*w*)) to release antimicrobial agents. Meanwhile, the intelligent paper applied a combination of bromothymol blue (BTB) and phenol red (PR) solutions at pH 5.00. The results showed that beef packed without the addition of garlic extract had already deteriorated on the 6th day of storage while, and with the addition of garlic extract (15% and 20%) rotted on the 12th day. The intelligent indication label's color profile changed from dark yellow (fresh), to reddish-yellow (to be consumed immediately), to faded red (rotten). The color change of the intelligent indicator label in response to all meat deterioration criteria demonstrates a linear correlation for determining the extent of rottenness during storage. Therefore, this simple combination of active paper and intelligent indicator can be used to extend the shelf life and monitor meat quality.

Keywords: smart packaging; meat; garlic oil; shelf life

Commented [M1]: Please check all author names carefully.

Commented [MOU2R1]: We have checked and all the authors' name and affilition are correct

Citation: Dirpan, A.; Djalal, M.; Ainani, A.F. A Simple Combination of Active and Intelligent Packaging Based on Garlic Extract and Indicator Solution in Extending and Monitoring the Meat Quality Stored at Cold Temperature. *Foods* **2022**, *11*, x. https://doi.org/10.3390/xxxxx

Academic Editor: Michael Kontominas

Received: 30 April 2022
Accepted: 18 May 2022
Published: date

1. Introduction

Meat is one of the most essential nutrients, containing high levels of easily digestible protein, calorie-dense fat, vitamins, and other micronutrients. Its high nutritional value corresponds to its rising demand as the world's most significant food product during the past several years [1]. The high demand should be fulfilled with the availability and an increase in the fresh meat grade, including the quality and safety, since beef is a perishable commodity [2]. The decrease in quality is due to the high fat and water content, making it susceptible to microbial contamination and lipid oxidation.

Proper packaging plays a critical role in preserving the quality and safety of meat. It is critical to select materials that will preserve the packaged product's qualities and assure its safety. The use of active packaging in conjunction with cold storage is one of the efforts that can be made to prolong the shelf life and enhance the quality.

Active packaging applications can be used to inhibit microbial growth in meat [3–8]. These applications incorporate specific active substances into packaging methods to preserve food products by inhibiting bacteria contaminations that contribute to spoilage during storage [9]. The spoilage inhibition mechanism can be conducted in two ways, namely direct and indirect contact. In the direct contact, the packaging materials will preserve food through direct contact, while in the indirect contact, the active packaging releases volatile anti-microbial agents to the packaging system where the food product is placed [10,11]. Camo et al. and Nerin et al. employed an active packaging technique incorporating rosemary and oregano directly touching the meat [12,13], and Campos-Requena et al. placed active packaging containing essential oil to strawberries that was not in direct contact with them [14], and both resulted in a synergistic antibacterial activity.

Commented [MDPI5]: Confirm intended meaning.

Commented [MOU6R5]: Thank you for your attention in this part, we make slight modification in this part. These are example that both direct and indirect contact of active packaging both resulting antibacterial effect.

Deleted: against *Botrytis cinerea* [14]

Volatile anti-microbial compounds are essential oils derived from herbs and spices, such as oregano, thymol, carvacrol, cinnamon, rosemary, ginger, lemongrass, and garlic [15]. Garlic (*allium sativum*) contains the antibacterial component allicin, which is active against both Gram-positive and Gram-negative bacteria [16,17]. The anti-microbial activities of different species of *Allium* have been investigated [18–21]. Several studies have been conducted on the administration of garlic in active packaging, including the following: Zheng Dong et al. developed an active packaging material based on a PP/LDPE composite film containing *Allium sativum* essential oil. The addition of essential oils into the packaging extends the shelf life of large yellow croaker (*Pseudosciaena crocea*) fillets for 5 days stored at 4 ± 1 °C [22]. Seydim and Sarikus also reported that the anti-microbial properties of whey protein isolate containing essential garlic oil with a ratio of 3.0–4.0% (wt/vol) effectively inhibited the growth of *Salmonella enteritidis*, *S. aureus*, *E. coli* O157:H7, *L. monocytogenes,* and *Lactobacillus plantarum* [23]. The use of garlic extract in active packaging can inhibit microbial growth due to its volatile allicin constituent [24]. Allicin chemicals suppress microbial development by increasing their permeability in penetrating bacterial cell walls to decrease protease enzyme production and disrupt protein and nucleic acid metabolism [19].

The application of active packaging on fresh meat can be accompanied by the addition of intelligent packaging in the form of indicators that monitor changes in quality. This is achieved through chemical reactions between indicators and the results of microbial metabolism or changes in the meat's chemical composition. Intelligent packaging incorporates indicators that convey information about the product's quality without causing damage to the packaging and not also in food products. This reduces the risk of loss due to product damage and provides a more accurate condition estimate than expired labels [25,26]. Furthermore, intelligent packaging also assists distributors in adjusting the prices of the products sold [27]. For example, lowering prices when quality has decreased to avoid losing food products. Previous studies applied this method to avocados [28], tuna fillets [29], shrimp [30], cherry tomatoes [31], and *arummanis* mango [32]. Monitoring of color changes can be done due to differences in pH of fresh product and damaged product. According to Shukla et al., color-based pH indicators can be used as intelligent packaging to monitor the presence of volatile amines from microbial metabolites in meat spoilage [33]. The increase in pH during decay causes a significant change in the indicator's color to be visually observed. Meanwhile, Romero et al. evaluated the quality of packaged cow's milk using two intelligent packaging prototypes based on the bromothymol blue pH indicator [34]. Julyaningsih et al. and Yolanda et al. combined active and intelligent packaging based on pH indicators methyl red and bromothymol blue to monitor the tuna fillet's quality [35,36]. Meanwhile, Yong and Liu combined anthocyanin-rich extracts into biopolymer-based films to extend shelf life and monitor the food product's quality [37].

The spoilage and contamination of food supplies jeopardize global food security. Therefore, the innovative application of active and intelligent packaging needs to be developed. As a result of these considerations, the purpose of this research is to evaluate the efficacy of active paper with garlic extract addition when applied to fresh beef during storage at low temperatures (4 ± 1 °C). Furthermore, it investigates the relationship (correlation) between color analysis of intelligent packaging indicators on various parameters of the beef spoilage test.

2. Materials and Methods

2.1. Materials

The beef tenderloin was purchased fresh at the slaughterhouse, Tamangapa Raya Antang. Meanwhile, garlic, as an anti-microbial compound in the activated paper, was purchased from supermarkets. Bromothymol blue (Merck, Darmstadt, Germany, CAS No: 76-59-5) and phenol red (Merck, Darmstadt, Germany, CAS No. 34487-61-1) color indicators in intelligent packaging were purchased from Sigma-Aldrich. Mueller Hinton Agar (Oxoid, CM0405) and Nutrient Agar (Merck, Darmstadt, Germany). This research also uses *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 29213), *Pseudomonas aeruginosa* (ATCC 27853), chitosan food grade (YXchuang, Cina), Tween® 80 (Merck, Darmstadt, Germany), corn starch, 96% glacial acetic acid (Brenntag Inc., Germany), potassium carbonate (K_2CO_3) (Merck, Darmstadt, Germany), 7% trichloroacetic acid (TCA) (Merck, Darmstadt, Germany), hydrochloric acid (HCl) (Merck, Darmstadt, Germany), and 0.85% sodium chloride (NaCl) (Merck KGaA, Germany).

2.2. Garlic Extract Preparation

Fifty grams of garlic was blended until smooth and put into a container containing 250 mL of 96% alcohol for a 72 h immersion. During the immersion time, the solution was gently stirred 14 times or every 5 h. Subsequently, the garlic dregs and the filtrate were separated by filtering. At 45 °C, the filtrate was evaporated with an industrial rotary evaporator (TMAX-2L-1) until it became thick reddish-yellow garlic [38].

2.3. Active Paper Preparation

Active paper preparation was carried out by cutting Whatman paper no. 1 into small pieces, then 15 g of the small pieces paper was soaked in 250 mL of distilled water for 24 h. The soaked paper was added with 250 mL of distilled water and crushed using a blender for 20 min until a pulp was formed. The paper pulp obtained was squeezed to remove the water, meanwhile 30% (*w*/*w*) tapioca starch was suspended in 50 mL of distilled water, then the pulp and tapioca suspension were homogenized. Furthermore, 100 mL of acetic acid (1%) containing 0.45% chitosan was

Commented [M7]: please add city before it.
Commented [MOU8R7]: Thank you, we have made changes as your suggestion.
Formatted: Indonesian
Formatted: Indonesian
Formatted: Indonesian
Formatted: Indonesian
Commented [M9]: Please confirm if the italics should be retained.
Commented [MOU10R9]: The italic is not retained.
Deleted: .
Formatted: Indonesian
Formatted: Font: Not Italic, Indonesian
Formatted: Indonesian
Formatted: Font: Not Italic, Indonesian
Formatted: Indonesian
Commented [M11]: please add city before it.
Commented [MOU12R11]: Thank you, we have made changes as your suggestion.
Formatted: Indonesian
Deleted: ,
Formatted: Indonesian
Formatted: Indonesian
Formatted: Indonesian
Formatted: Indonesian
Formatted: Indonesian
Formatted: Indonesian
Formatted: Indonesian
Formatted: Indonesian
Formatted: Indonesian
Formatted: Indonesian
Formatted: Indonesian

added to the tapioca and pulp mixture and homogenized with a blender for 5 min. Garlic extract with a concentration according to the treatment (0%, 15% and 20%) (*w*/*w*) was added to the mixture; subsequently, 50 mL of distilled water was also added to the mixture. Furthermore, 0.205 g of Tween® 80 was added and mixed at room temperature using a hotplate magnetic stirrer to generate an emulsion. The paper dough was poured over the surface of the printer container following the styrofoam size used as a beef storage container and flattened to make a wet paper sheet. After that, the wet paper sheet was pressed between the glass surfaces with a load of 2 kg. It was then dried at 40 °C for 48 h, and during the drying process the paper was turned over after 24 h of drying (Modification of Wiastuti et al.) [39].

2.4. Indicator Solution Preparation

BTB and PR (1:1) indicator solutions were made by dissolving 1% (*w*/*v*) of the solution in 95% ethanol. Furthermore, the indicator was adjusted to pH 5 using a solution of glacial acetic acid [40].

2.5. Intelligent Indicator Label Preparation

Whatman paper no. 1 was cut with a size of 2 × 4 cm and immersed in 20 mL of indicator solution for 12 h at room temperature (28 ± 2 °C). The indicator labels were then dried using an electric dryer (Philips PH 8102) and in a closed container with a drying distance of 30 cm.

2.6. Application of Active and Intelligent Packaging on Fresh Beef

Fresh beef (tenderloin) with normal pH (5.6–5.7) was obtained from the Tamangapa Antang Makassar abattoir, which was taken at a relative postmortem time of about 3 h. The meat was packaged plastic (PP) containers and put in a cooler box, then immediately transported to the laboratory and prepared sterilely into 180-g pieces/packages. Activated paper with different concentrations was inserted into the styrofoam base. However, since the application of plastic is not sustainable, it is suggested to use more sustainable materials in future. The pieces of meat were packed in styrofoam (1.05 g/cm³) and covered with active paper according to the treatment (0%, 15% and 20% of garlic extract) that filled the entire base. The indicator label was placed inside the package by sticking to the LDPE film surface (0.9 g/cm³) used as a cover. Observation of beef (tenderloin) packaged with active and intelligent packaging was conducted at a cold temperature (chiller) of 4 ± 1 °C every 3 × 24 h for 21 days of storage. The design for implementing active and intelligent packaging is presented in Figure 1.

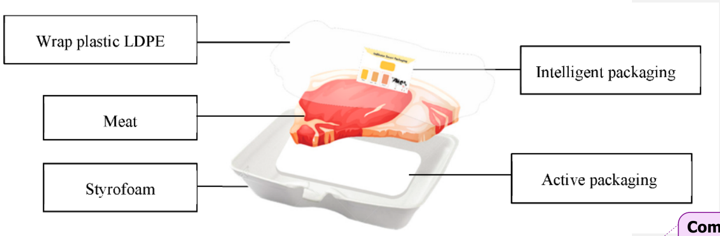


Figure 1. Design of the active packaging and intelligent system.

Formatted: Font: Not Italic

Formatted: Font: Not Italic

Deleted: Meanwhile

Deleted: on the filter surface

Deleted: Furthermore, the study used A0: control (without garlic extract addition); A1: 15% garlic extract; and A2: 20% garlic extract.

Commented [M13]: To avoid any changes to the figure, we have replaced it with a screenshot. Please confirm.

Commented [MOU14R13]: Thank you, we confirm that we accepted the change.

foods

2.7. Antibacterial Activity Testing of Agar Diffusion Method on Activated Paper

The antibacterial activity of active paper was tested to determine the inhibition toward the growth of Gram-positive bacteria *Staphylococcus aureus*, and Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*. Sheets of active paper with a diameter of 5.5 mm were placed on Mueller Hinton Agar (MHA) media, which spread 0.1 mL of the microorganism culture on its surface. Furthermore, the Petri dishes were incubated at 37 °C for 24 h, and after incubation, a clear zone (inhibition zone) was formed around the active paper. The resistance diameter was measured in millimeters (mm) using a caliper [41], and each test was conducted in 3 replications to obtain the average result.

2.8. Parameters of Beef Observation Packaged with a Combination of Active and Intelligent Packaging

2.8.1. pH Measurement

The initial and final pH of fresh beef during storage were measured using Horiba Laquatwin Compact pH Meter P-33 with an accuracy of 0.01%. A total of 1 g of the sample was mashed and dissolved in 10 mL of demineralized water. Furthermore, it was inserted on the surface of the pH meter sensor until the value was displayed on the pH meter screen.

2.8.2. TVBN Value Analysis

The meat sample was weighed at 1 g ± 0.1 g, pulverized in a mortar with 3 mL of 7% TCA solution, and filtered to obtain the filtrate. The boric acid solution of 1 mL was put into the "inner chamber" of the conway cup, and the lid was placed in a position to cover the cup. Furthermore, the filtrate was fed into the outer chamber on the left. Then 1 mL of saturated K_2CO_3 solution was added to the outer chamber on the right, ensuring that the filtrate and K_2CO_3 do not mix. The cup was instantly closed and rotated to mix the two liquids in the outer chamber. The blank solution was prepared the same way as the standard solution, except that the filtrate was replaced with a 7% TCA solution. Subsequently, it was stored at 28 ± 2 °C for 24 h, and the boric acid solution in the inner chamber containing the blank was added with two drops of color indicator solution, then titrated with 0.02 N HCl until it turned pink. The conway cup containing the filtrate was added with two drops of color indicator solution and titrated with the same solution until it turned pink (SNI-2354.8:2009; AOAC, 1995). Equation (1) for determining TVBN:

$$\text{TVBN level (mg/100 g)} = \frac{(V_C - Vb) \times N \times 14.007 \times 100}{W}$$

Description:

Vc = volume of HCl solution in sample titration

Vb = volume of HCl solution in blank titration

N = normality of HCl solution

W = sample weight (g)

14.007 = atomic weight of nitrogen

Deleted: printed

Commented [MDPI15]: Confirm intended meaning.

Commented [MOU16R15]: Thank you for your attention to the this part, we deleted the words or blank.

Deleted: or blank

Commented [M17]: we moved the content into Equation box, please confirm.

Commented [MOU18R17]: We confirm

2.8.3. Calculation of Total Bacterial Count

Total bacteria were calculated based on total plate count method (SNI 2897:2008). The sample solution was made from 1 g of meat homogenized with 9 mL of sterile physiological solution (0.85% NaCl), referred to as the 10^{-1} dilution. Then, the serial dilution was made up to a concentration of 10^{-6}. Microbial cultivation using the pour technique in which 1 mL dilutions of 10^{-4}, 10^{-5}, and 10^{-6} were put into separate sterile petri dishes in duplicate. Subsequently, ±15 mL of sterile NA media was added into the petri dishes, then it was homogenized and incubated at 30 °C for 48 h. Equation (2) for calculation of the total plate count:

Deleted: . A total of 1 mL of the sample solution was put into the first test tube containing 9 mL of the physiological solution until homogeneous,

$$N = \frac{\Sigma C}{[(1 \times n_1) + (0.1 \times n_2) + \cdots] \times (D)} \quad (2)$$

Description:

N = number of colonies per mL/per gram of product

ΣC = total number of colonies counted

n_1 = number of cups in the first dilution

n_2 = number of cups in the second dilution

D = first dilution calculated

2.9. Intelligent Indicator Color Measurement Quantification

The color measurement of the intelligent indicator label was analyzed using a digital color meter (T-135). This quantification is conducted by attaching the colorimeter sensor to the intelligent indicator label. The tool will then show the values of L (lightness), a (redness), and b (yellowness) on display. These three values are international standards of color measurement published by the Hunterlab Association Laboratory (2008). Furthermore, the intelligent packaging indicator color is determined by calculating the °Hue value using the Formula (3), and the difference of total color (ΔE) was measured with this Formula (4) [42,43]:

$$°Hue = \tan^{-1}\frac{b}{a} \quad (3)$$

Description:

°Hue = parameters for color range

a = is a red-green mixed color

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

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (4)$$

2.10. Level of Relationship (Correlation) between Test Parameters

The intelligent packaging indicator quantification data was compared with the data for each beef spoilage parameter presented in a graph using the Sigma Plot software version 14.0.

foods

MDPI

2.11. Statistical Analysis

All data from the test parameters were analyzed using analysis of variance (ANOVA) with Duncan's multiple range test using SPSS software version 22.0 (IBM Corp., United States), and the calculated values differed significantly when $p < 0.05$.

Deleted: In addition, the relationship (correlation) of intelligent indicator color parameters to all beef spoilage parameters was presented in one graph using Sigma Plot software version 14.0.

3. Results and Discussion

3.1. Meat pH

The degree of acidity (pH) in meat is one measure of its freshness, therefore, the analysis of the meat pH value was performed to determine the effect of adding various concentrations of garlic extract during cold storage. Table 1 shows the pH changes that occur in beef during storage.

Table 1. The pH value of packaged meat samples stored in cold storage for 21 days.

Storage Time (Day)	Addition of Garlic Extract on Active Paper		
	0%	15%	20%
0	5.68 ± 0.06 ^{g}	5.68 ± 0.06 ^{g}	5.68 ± 0.06 ^{g}
3	5.82 ± 0.16 ^{g}	5.71 ± 0.01 ^{g}	5.74 ± 0.16 ^{g}
6	5.88 ± 0.0 ^{g}	5.74 ± 0.01 ^{g}	5.52 ± 0.16 ^{g}
9	7.52 ± 0.72 ^{b,c,d}	5.78 ± 0.07 ^{g}	5.67 ± 0.16 ^{g}
12	7.77 ± 0.52 ^{b,c}	6.08 ± 0.01 ^{f,g}	5.83 ± 0.13 ^{g}
15	7.93 ± 0.20 ^{b}	6.85 ± 1.27 ^{d,e,f}	6.70 ± 0.62 ^{e,f}
18	7.90 ± 0.13 ^{b}	7.00 ± 0.66 ^{b,c,d,e}	6.72 ± 0.03 ^{e,f}
21	8.93 ± 0.14 ^{a}	7.19 ± 0.58 ^{b,c,d,e}	7.02 ± 0.60 ^{c,d,e}
Average	7.18 ± 1.22 ^{A}	6.25 ± 0.65 ^{B}	6.11 ± 1.35 ^{B}

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).

Table 1 shows that the garlic extract addition with different concentrations affects the increase in pH value of meat during storage. The statistical tests showed that the extract addition on active paper significantly affected the pH value ($p < 0.05$). Further test results showed that the treatment without the garlic extract (0%) was significantly different from the other treatments on average. However, the addition of 15% garlic extract was not significantly different from 20% garlic extract. Ponnampalam et al. stated that the pH of normal meat ranges from 5.5–5.7, and meat with a pH of >6 will be easily damaged by microorganisms, experiencing some deterioration such as color and texture aroma [44]. In Table 1, it can be seen that the initial pH value of fresh meat before storage was 5.68 and categorized as normal, and after storage it was classified as rotten on the 9th days of storage for treatment without garlic extract and on the 15th day for treatment with 15% and 20% garlic extracts.

Commented [M19]: References should be cited in numerical order. Thus, "Ref 44–47" should be cited after "43", not 48. Please revise.

Commented [MOU20R19]: Thank you, we have already rearranged the reference list.

Deleted: 48

Deleted: and categorized

3.2. Total Volatile Bases Nitrogen (TVBN)

The TVBN value is one of the factors used to measure the level of freshness in meat. Table 2 shows the changes in TVBN values in beef during cold storage.

foods

MDPI

Table 2. The TVBN value of packaged meat samples stored in cold storage for 21 days.

Storage Time (Day)	Addition of Garlic Extract on Active Paper		
	0%	15%	20%
0	4.48 ± 0.00 [i]	4.48 ± 0.00 [i]	4.48 ± 0.00 [i]
3	4.76 ± 0.00 [i]	5.04 ± 1.40 [i]	4.58 ± 1.38 [i]
6	11.21 ± 1.46 [g]	4.20 ± 0.00 [i]	5.88 ± 0.56 [h,i]
9	27.27 ± 2.72 [d,e]	10.83 ± 0.65 [g]	9.43 ± 0.32 [g,h]
12	32.22 ± 9.54 [c]	22.32 ± 1.38 [f]	21.66 ± 2.83 [f]
15	34.18 ± 1.40 [c]	23.06 ± 0.71 [f]	22.97 ± 1.22 [f]
18	40.81 ± 1.17 [b]	24.65 ± 1.84 [e,f]	23.35 ± 1.71 [e,f]
21	55.28 ± 4.66 [a]	33.43 ± 1.13 [c]	30.26 ± 1.28 [c,d]
Average	26.27 ± 18.19 [A]	16.00 ± 11.26 [B]	15.33 ± 10.30 [B]

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).

Based on statistical tests, the addition of garlic extract on active paper significantly affected the TVBN value ($p < 0.05$). Further test results showed that the treatment without the extract (0%) addition was significantly different from the other treatments. However, 15% garlic extract was not significantly different from the 20% extract. TVBN criteria for identifying rotten beef products are >20 mg/100 g according to the Ministry of Agriculture and Forestry of Korea, 2016 [45]. The TVBN value of meat on the first day of storage was 4.48 mg N/100, indicating a fresh category (Table 2). On the 9th day of storage, meat treated without the extract had a TVBN value of 27.27 mg N/100 g, categorized as rotten. Meanwhile, the addition of 15% and 20% garlic extract with TVBN values of 22.32 mg N/100 g and 21.66 mg N/100 g had crossed the threshold on day 12. Based on the results obtained, the garlic extract affects the meat TVBN value during storage.

Deleted: 49

The increase in TVBN value is closely related to the decrease in beef quality. The treatment without garlic extract reached the threshold value earlier due to the higher microbial activity in the sample. The high number of microbes in the sample breaks down more meat protein to produce volatile ammonia compounds. This is consistent with Holman et al., where increased microbial value degrades protein rapidly during storage. Furthermore, it increases the amount of ammonia and other volatile compounds, as well as increases the TVBN value, where these compounds indicate the decay in meat [46]. The increase in the TVBN value of beef with treatment without and with the garlic extract was also different due to the allicin constituent and anti-microbial role. The inhibition of microbial growth minimizes the decomposition process of nutritional components in meat to reduce the TVBN value.

Deleted: 50

3.3. Total Bacteria of Total Plate Count (TPC) Method

The analysis determines the effect of using active paper on the total value of bacteria in meat during cold storage, as shown in Table 3.

Table 3. The TPC value of packaged meat samples stored in cold storage for 21 days.

Storage Time (Day)	Addition of Garlic Extract on Active Paper		
	0%	**15%**	**20%**
0	3.52 ± 0.05 ^{i}	3.52 ± 0.05 ^{i}	3.52 ± 0.05 ^{i}
3	4.49 ± 0.04 ^{f,g,h}	4.16 ± 0.08 ^{g,h,i}	3.88 ± 0.06 ^{h,i}
6	6.49 ± 0.06 ^{c}	4.44 ± 0.03 ^{f,g,h}	4.29 ± 0.18 ^{g,h,i}
9	7.47 ± 0.02 ^{b}	5.08 ± 0.07 ^{e,f}	4.93 ± 0.48 ^{f,g}
12	8.30 ± 0.04 ^{a}	6.35 ± 0.71 ^{c,d}	6.09 ± 0.20 ^{c,d}
15	8.45 ± 1.22 ^{a,b}	6.43 ± 1.27 ^{c,d,e}	6.24 ± 0.69 ^{c,d}
18	8.17 ± 0.61 ^{a,b}	6.19 ± 0.15 ^{c,d}	5.69 ± 0.41 ^{d,e}
21	7.82 ± 0.16 ^{a,b}	5.81 ± 0.24 ^{c,d,e}	5.37 ± 0.54 ^{e,f}
Average	6.84 ± 1.87 ^{A}	5.25 ± 1.11 ^{B}	5.00 ± 1.02 ^{B}

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 garlic extract added to the active paper had a significant effect on the TPC value of meat ($p < 0.05$). Meanwhile, further test results showed that the treatment without garlic extract (0%) was significantly different from other treatments, but the addition of 15% was not significantly different from the 20% extract. The initial population of TPC meat was 3.52 log CFU/mL, which is categorized as fresh concerning microbiological quality (Table 3). However, this value increased to 6.49 log CFU/mL on the 6th day of storage in the control sample; this value is above the recommended maximum bacteriological limit, which was 6 log CFU/mL (SNI 3932:2008). The meat added with 15% and 20% garlic extract exceeded the maximum bacteriological limit on the 12th day of storage with TPC values of 6.35 log CFU/mL and 6.09 logs CFU/mL, respectively. Therefore, the addition of 15% and 20% extract as active packaging inhibits microbial growth and extends the shelf life of meat up to 6 days longer than the samples without the garlic extract in the active packaging. This is because garlic contains an anti-microbial compound in the form of allicin.

Garlic extract applied to the activated paper diffuses to the entire surface of the beef to inhibit the growth of bacteria through total and partial inhibition of RNA, DNA, and protein synthesis. Furthermore, allicin blocks bacterial enzymes with thiol groups to ultimately inhibit bacterial growth [47]. Mardiya also confirmed that the effectiveness of garlic extract with concentrations of 25%, 50%, and 100% was very effective in inhibiting *Staphylococcus aureus* bacteria and bactericidal [48]. Meanwhile, the extract with a concentration of 12.5% was declared as an inhibitor of bacterial growth (bacteriostatic), but less effective at inhibiting bacteria (bacteriocidal) [48].

The storage temperature of beef can also affect the meat TPC value. Meat cells in the postmortem phase experience metabolic reactions, which are highly dependent on storage temperature. According to Comi, the storage temperature and the metabolic reactions in meat are inversely proportional [49]. This is supported by Kuswandi and Nurfawaidi, where beef stored at room temperature is categorized as unfit for consumption at 10 h with a TPC value of 6.711 CFU/mL. Meanwhile, at cold temperature, it is categorized as unfit for consumption on day 7 with a TPC value of 6.961 CFU/mL [40].

Deleted: 51
Field Code Changed
Deleted: 52
Field Code Changed
Field Code Changed
Deleted: 52
Deleted: 53

3.4. The Antibacterial Analysis Results of Active Paper

Antibacterial activity on active paper was tested against several bacteria that generally cause damage to beef, namely *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Figure 2 illustrates the active paper's analytical findings for the bacterial inhibition zone region.

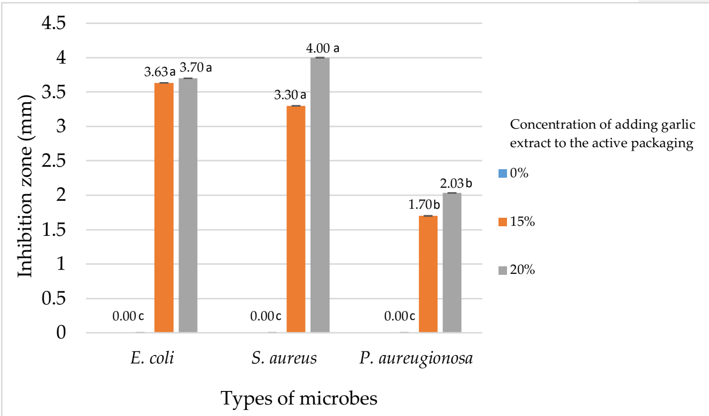


Figure 2. Diameter of bacterial growth barriers on activated paper. The mean value followed by different letters showed a significant difference based on the Tuckey's test at the 5% level (*p*-value < 0.05).

It can be seen from Figure 2 that with no addition of garlic extract to active paper (0%), the paper shows no inhibition zones to all three strains of bacteria tested. In comparison to active paper with the addition of both 15% and 20% garlic extract, it displayed bacterial inhibitory activity, implying that garlic extract possessed antibacterial activity.

According to Pan et al., the response to bacterial growth inhibition was divided into three categories: antibacterial activity with zones of inhibition greater than 6.00 mm (strong), 3–5 mm (moderate), and less than 3 mm (weak) [50]. Figure 2 provides information that shows active packaging with no addition of garlic extract had no inhibitory activity to all bacteria tested. While the addition of 15% and 20% of garlic extract to the active packaging showed a moderate response against *E. coli* (3.6 mm and 3.3 mm, respectively) and also moderate against *S. aureus* (3.7 mm and 4 mm, respectively). While for *P. aeruginosa* the active packaging categorized has a weak inhibitory response both in the administration of 15% and 20% garlic extract with 1.7 mm and 2 mm, respectively.

The diameter of the inhibitory zone created against *Escherichia coli*, *Stapylococcus aureus*, and *Pseudomonas aeruginosa* bacteria varies and could be due to the differences in bacterial structure that affect the penetration of allicin compounds through bacterial cell walls. The inhibitory zone formed by active packaging with the addition of both 15% and 20% garlic extract was classified as weak against *P. aeruginosa* due to the fact that gram-negative bacteria such as *P. aeruginosa* have complex cell walls, which prevent antibacterial compounds from penetrating the bacteria cell sufficiently to inhibit bacterial growth [51]. However, the findings of this study indicated that the application of garlic extract to the active package had the same inhibitory zone category against *E.coli* and *S.aureus*, even

Commented [M21]: References should be cited in numerical order. Thus, "Ref 44" should be cited after "43", not 53. Please revise.

Commented [MOU22R21]: Thank you, we have already rearranged the reference list.

Deleted: 44

Field Code Changed

Field Code Changed

Deleted: 4

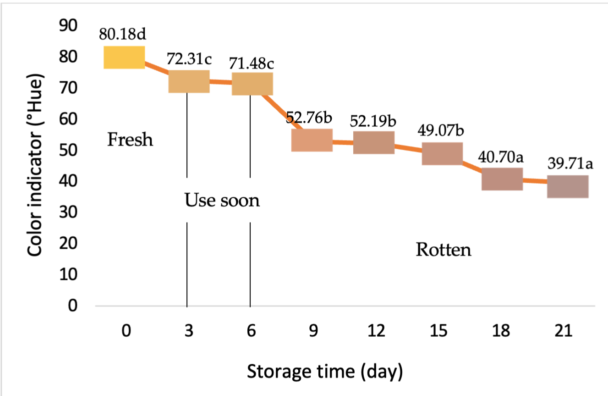
Deleted: 5

though *E. coli* also includes Gram-negative bacteria. Even though the previous explanation for *P. aeruginosa* does not support the result obtained in this research for *E. coli*, these findings are consistent with the findings of Garba et al. and Safithri et al., who discovered that the inhibitory action to *E.coli* bacteria was typically more or equal to that of *S. aureus* [52-53].

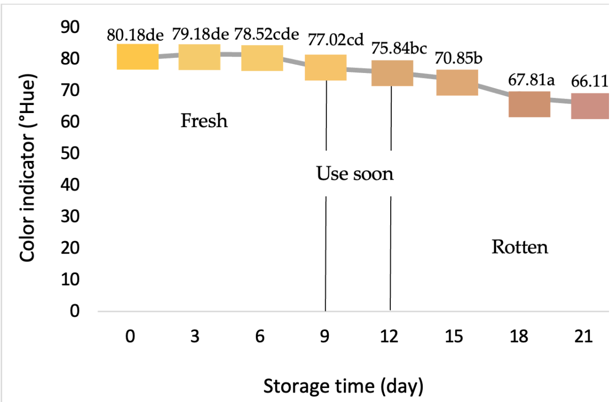
Deleted: 46,47

3.5. Color Change Response on Intelligent Indicator Labels for Beef Packaged in Active Packaging at Cold Temperature Storage

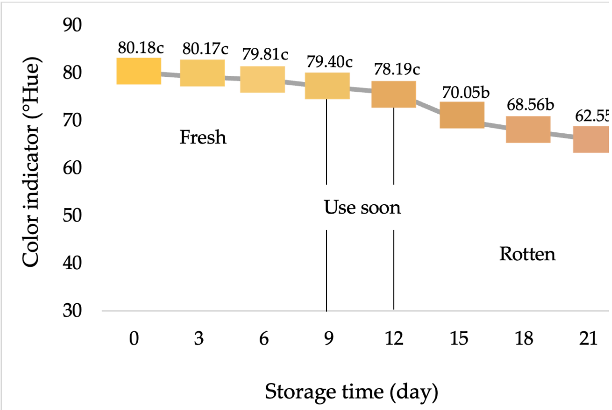
The color change analysis of the intelligent indicator determines the effect of adding garlic extract to active packaging with different concentrations on beef during storage at cold temperatures (chiller). The color change of the resulting indicator label is information of the freshness condition of the packaged beef. Significant color changes on indicators ease consumers to measure the freshness level based on visual appearance, as seen in Figure 3.



(**a**)



(**b**)



(**c**)

Figure 3. Color change profile of intelligent indicator labels with garlic extract addition on the concentrations at 0% (**a**), 15% (**b**), and 20% (**c**). 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). Phase I with dark yellow indicating fresh, phase II with yellow and red gradations indicating have to be consumed soon, and phase III with faded red indicating rotten meat.

Figure 3 showed that the °Hue value on the intelligent indicator label decreased during storage, from dark yellow to faded red. The color change is a response to the quality condition. There are three phases of change on intelligent packaging labels in the description.

The color change on the intelligent indicator label is closely related to the increased number of microbes and enzymes in meat during storage. These changes are caused by the decomposition of nutritional components of meat by enzymes and microbes, producing volatile base compounds used as an early sign of damage in packaged beef. This is consistent with Sani et al. who showed that volatile ammonia compounds in meat can increase the pH [54]. The increase in the pH of meat is caused by ammonia reacting with H+ ions to produce H ions. The OH- ions in the packaging are directly proportional to the pH value, affecting the change in intelligent indicator labels.

Delta E is a standard measurement for quantifying the difference between two colors. The standard delta E perception consists of five scales: 1.0 is invisible to the human eye; 1–2 visible through observation; 2–10 at a glance; 11–49 colors are more similar than opposite; and 100 colors are opposite [55]. Based on Figure 4, the smart indicator shows the value of delta E with a range of 1–18 for each treatment, except on storage days 3–6, the value of delta E is 0.77. This shows that smart indicators are generally easy to visualize based on color changes in smart packaging systems. Based on this, consumers will easily make visual assessments and also monitor the freshness of the meat through intelligent packaging sensors.

Commented [M23]: 1. please add explanations for different letters in Figure body.
2. please add explanations for different color block in Figure body

Commented [MOU24R23]: Thank you, we have already added the explanation for different letters and color block.

Formatted: MDPI_3.1_text

Deleted: These include phase I with dark yellow indicating fresh, phase II with yellow and red gradations indicating immediate consumption, and phase III with faded red indicating damaged meat unfit for consumption.

Commented [M25]: References should be cited in numerical order. Thus, "Ref 51–52" should be cited after "50", not 54. Please revise and check numerical order throughout the entire manuscript.

Commented [MOU26R25]: Thank you, we have already rearranged the reference list.

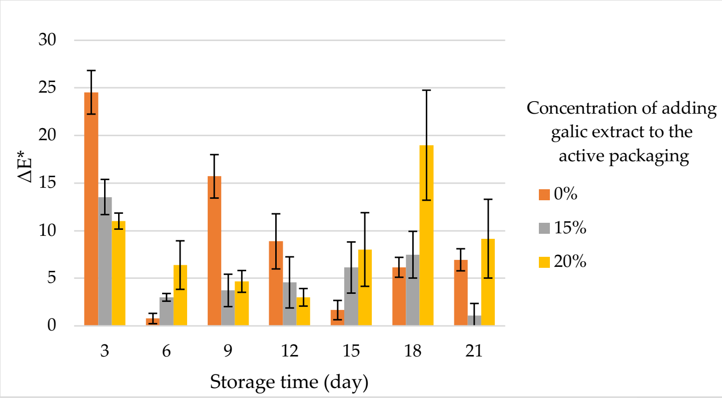


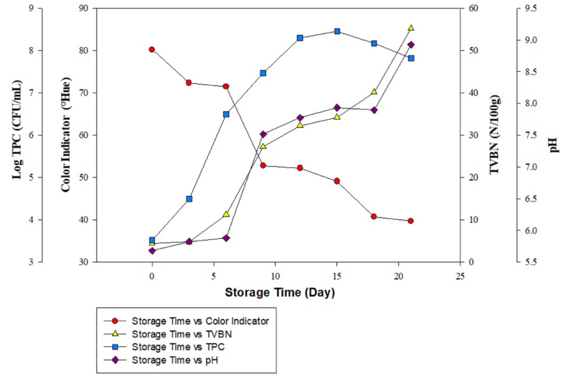
Figure 4. Total color difference (ΔE*).

3.6. Correlation of Color Value Changes in Intelligent Packaging Indicators BTB: PR (1:1) with the Effect of Active Packaging on All Meat Spoilage Parameters

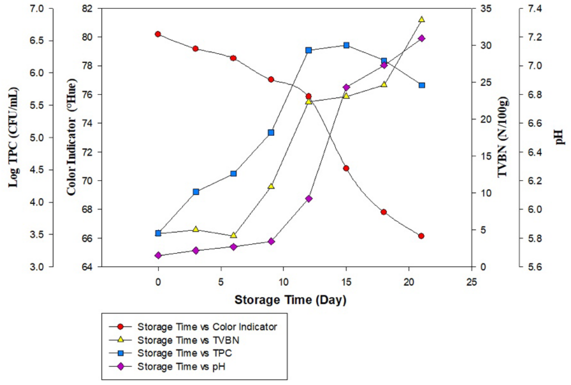
The relationship between the color change response of intelligent indicator labels with all test parameters on meat, such as TVBN, TPC, and pH, determined the sensitivity of intelligent indicator labels in detecting quality deterioration. This obtains synchronization between changes in the color of the intelligent indicator label to the deterioration of meat quality.

Based on Figure 5, beef stored without the garlic extract (0%) showed that the increase in the TVBN value and the pH of the meat was in line with the decrease in the color value of the intelligent packaging indicator. However, it is not consistent with the results of the TPC value since the beef TPC value crossed the threshold on the 6th day of storage, which is 6.4 CFU/mL. Meanwhile, the TVBN value is suitable for consumption on the 6th day. Mansur et al. also reported that the TVB-N values were inconsistent with the viable microbial count (TVC) [56]. Based on TVB-N, the samples were classified as damaged after 7 and 11 days of storage [56]. This is because the microbial calculation method is not selective against spoilage bacteria in meat but counts all the pathogenic microbes. Holman et al. also stated that the TVB-N value was more precise and accurate in classifying beef in fresh or rotten conditions than the total microbial value (TVC) [46].

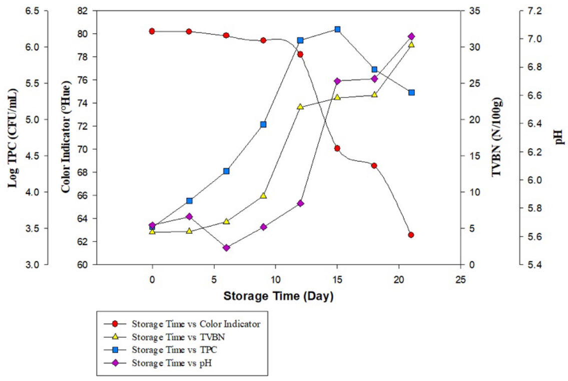
Deleted: 50



(**a**)



(**b**)



(**c**)

Figure 5. Correlation of color change on intelligent indicator label with all beef spoilage parameters with the garlic extract addition: (**a**) control, (**b**) 15%, and (**c**) 20%.

Based on Figure 5b,c, beef stored with 15% and 20% garlic extract treatment showed that the increase in the parameters of the meat quality deterioration, including TPC, TVBN, and pH, was consistent with the decrease in the color of the intelligent indicator label. The intelligent indicator changes color from yellow (fresh) to faint red (rotten) during storage. The increase in TVBN, TPC, and pH values is influenced by the high nutritional content, specifically protein broken down into polypeptides and other amino acids through the deamination process to form ammonia. With the increase in microbes, the nutritional components decomposed by microbes are also greater and produce basic volatile compounds, increasing the pH value of meat during storage. The resulting volatile compounds accumulate in the packaging and are directly detected by the intelligent indicator label. The reduction in the color value of the intelligent indicator label shows the presence of many alkaline volatile chemicals in the package and the rottenness and unfitness for the ingestion of the meat. Furthermore, when viewed for the visual appearance on the 6th and 12th day of storage without (0%) and with (15% and 20%) garlic extract, the beef was damaged and unfit for consumption, which is characterized by the formation of mucus, emitting a sour/rotten smell, and the texture beginning to be sticky to the touch.

The increase in the beef quality deterioration parameter value for each treatment was different due to the addition of garlic extract as active packaging. The extract has a bioactive compound in allicin to inhibit microbial growth. This compound in the package diffuses in the meat surface to suppress the microbe growth. According to Deresse, it works by completely inhibiting bacterial RNA synthesis and inhibiting DNA and protein synthesis [47]. The pattern of relationship between the parameters of the decline in beef quality and the color analysis value of indicator labels is also consistent with Kuswandi and Nurfawaidi, where a linear relationship pattern to the parameters of the beef freshness level with changes in the color intensity of the bromocresol purple and methyl red intelligent labels was observed [40]. In this study, beef treated without the garlic extract experienced a decrease in quality and was not suitable for consumption on the 6th day. Meanwhile, with 15% and 20% garlic extract, the meat was damaged and unfit for consumption on the 12th day of storage. Previous research applying garlic extract as active packaging in meat was performed and resulted as the 15% garlic extract enhancing the shelf life of meat to 16 h from 12 h without garlic extract at room temperature [57]. This indicates that garlic extract storing at cold temperatures can extend the shelf life of packaged meat. Visually, the intelligent indicator label used Whatman paper no. 1 as the base material, and the BTB + PR (pH 5.00) used in intelligent packaging for beef freshness, with a color change from yellow (beginning) to faint red (late). Therefore, it assists customers in determining freshness without touching the texture or opening the packaging.

Deleted: 51

Commented [M27]: no ref 57 in ref part but citation, please make a revision.

Commented [MOU28R27]: Thank you, we have already rearranged the reference list.

4. Conclusions

The garlic extract in active packaging, supported by the microbiological evaluation results of TVBN and pH, extended shelf life during storage. In addition, the results showed that the active paper with 15% and 20% garlic extract extended the shelf life to 12 days of cold storage. The color change profile of the intelligent indicator label combination of BTB + PR pH 5.00 was easily observed from dark (fresh) to reddish yellow (to be consumed immediately). The red color fades when the beef is rotten and unfit for consumption. Therefore, the combination of active and intelligent packaging is suggested as a potential packaging application in fresh meat packaging.

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

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

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

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

References

1. Dave, D.; Ghaly, A.E. Meat spoilage mechanisms and preservation techniques: A critical review. *Am. J. Agric. Biol. Sci.* **2011**, *6*, 486–510. https://doi.org/10.3844/ajabssp.2011.486.510.
2. Yu, H.H.; Chin, Y.-W.; Paik, H.-D. Application of Natural Preservatives for Meat and Meat Products against Food-Borne Pathogens and Spoilage Bacteria: A Review. *Foods* **2021**, *10*, 2418. https://doi.org/10.3390/foods10102418.
3. Djenane, D.; Gómez, D.; Yangüela, J.; Roncalés, P.; Ariño, A. Olive Leaves Extract from Algerian Oleaster (*Olea europaea* var. *sylvestris*) on Microbiological Safety and Shelf-life Stability of Raw Halal Minced Beef during Display. *Foods* **2019**, *8*, 10. https://doi.org/10.3390/foods8010010.
4. Bahmid, N.A.; Dekker, M.; Fogliano, V.; Heising, J. Development of a moisture-activated antimicrobial film containing ground mustard seeds and its application on meat in active packaging system. *Food Packag. Shelf Life* **2021**, *30*, 100753. https://doi.org/10.1016/j.fpsl.2021.100753.
5. Umaraw, P.; Munekata, P.E.S.; Verma, A.K.; Barba, F.J.; Singh, V.P.; Kumar, P.; Lorenzo, J.M. Edible films/coating with tailored properties for active packaging of meat, fish and derived products. *Trends Food Sci. Technol.* **2020**, *98*, 10–24. https://doi.org/10.1016/j.tifs.2020.01.032.
6. Alizadeh-Sani, M.; Mohammadian, E.; McClements, D.J. Eco-friendly active packaging consisting of nanostructured biopolymer matrix reinforced with TiO_2 and essential oil: Application for preservation of refrigerated meat. *Food Chem.* **2020**, *322*, 126782. https://doi.org/10.1016/j.foodchem.2020.126782.
7. Lin, L.; Luo, C.; Li, C.; Chen, X.; Cui, H. Application in Beef Preservation. *Foods* **2022**, *11*, 438. https://doi.org/10.3390/foods11030438.
8. Kapetanakou, A.E.; Pateraki, G.-L.; Skandamis, P.N. Developing a Commercial Antimicrobial Active Packaging System of Ground Beef Based on "Tsipouro" Alcoholic Distillate. *Foods* **2020**, *9*, 1171. https://doi.org/10.3390/foods9091171.
9. Arkoun, M.; Daigle, F.; Holley, R.A.; Heuzey, M.C.; Ajji, A. Chitosan-based nanofibers as bioactive meat packaging materials. *Packag. Technol. Sci.* **2018**, *31*, 185–195.
10. Quintavalla, S.; Vicini, L. Antimicrobial food packaging in meat industry. *Meat Sci.* **2002**, *62*, 373–380. https://doi.org/10.1016/S0309-1740(02)00121-3.
11. Yildirim, S.; Röcker, B.; Pettersen, M.K.; Nilsen-Nygaard, J.; Ayhan, Z.; Rutkaite, R.; Radusin, T.; Suminska, P.; Marcos, B.; Coma, V. Active Packaging Applications for Food. *Compr. Rev. Food Sci. Food Saf.* **2018**, *17*, 165–199. https://doi.org/10.1111/1541-4337.12322.
12. Camo, J.; Beltrán, J.A.; Roncalés, P. Extension of the display life of lamb with an antioxidant active packaging. *Meat Sci.* **2008**, *80*, 1086–1091.
13. Nerín, C.; Tovar, L.; Djenane, D.; Camo, J.; Salafranca, J.; Beltrán, J.A.; Roncalés, P. Stabilization of beef meat by a new active packaging containing natural antioxidants. *J. Agric. Food Chem.* **2006**, *54*, 7840–7846.
14. Campos-Requena, V.H.; Rivas, B.L.; Pérez, M.A.; Figueroa, C.R.; Sanfuentes, E.A. The synergistic antimicrobial effect of carvacrol and thymol in clay/polymer nanocomposite films over strawberry gray mold. *LWT-Food Sci. Technol.* **2015**, *64*, 390–396. https://doi.org/10.1016/j.lwt.2015.06.006.
15. Drago, E.; Campardelli, R.; Pettinato, M.; Perego, P. Innovations in Smart Packaging Concepts for Food: An Extensive Review. *Foods* **2020**, *9*, 1628. https://doi.org/10.3390/foods9111628.
16. Putnik, P.; Gabrić, D.; Roohinejad, S.; Barba, F.J.; Granato, D.; Mallikarjunan, K.; Lorenzo, J.M.; Bursać Kovačević, D. An overview of organosulfur compounds from *Allium* spp.: From processing and preservation

Commented [M29]: In this section, you should add the Institutional Review Board Statement and approval number, if relevant to your study. You might choose to exclude this statement if the study did not require ethical approval. Please note that the Editorial Office might ask you for further information. Please add "The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of NAME OF INSTITUTE (protocol code XXX and date of approval)." for studies involving humans. OR "The animal study protocol was approved by the Institutional Review Board (or Ethics Committee) of NAME OF INSTITUTE (protocol code XXX and date of approval)." for studies involving animals. OR "Ethical review and approval were waived for this study due to REASON (please provide a detailed justification)." OR "Not applicable" for studies not involving humans or animals.

Commented [MOU30R29]: Thank you, we have put not applicable.

Commented [SN31]: Please check all citations and references and make changes accordingly. Please NEVER use EndNote or other tools to re-arrange the reference order.

Commented [MOU32R31]: Thank you, we have already checked and rearranged the reference order manually.

to evaluation of their bioavailability, antimicrobial, and anti-inflammatory properties. *Food Chem.* **2019**, *276*, 680–691. https://doi.org/10.1016/j.foodchem.2018.10.068.

17. Shang, A.; Cao, S.-Y.; Xu, X.-Y.; Gan, R.-Y.; Tang, G.-Y.; Corke, H.; Mavumengwana, V.; Li, H.-B. Bioactive Compounds and Biological Functions of Garlic (*Allium sativum* L.). *Foods* **2019**, *8*, 246. https://doi.org/10.3390/foods8070246.
18. Santas, J.; Almajano, M.P.; Carbó, R. Antimicrobial and antioxidant activity of crude onion (*Allium cepa*, L.) extracts. *Int. J. Food Sci. Technol.* **2010**, *45*, 403–409. https://doi.org/10.1111/j.1365-2621.2009.02169.x.
19. Kyung, K.H. Antimicrobial properties of allium species. *Curr. Opin. Biotechnol.* **2012**, *23*, 142–147. https://doi.org/10.1016/j.copbio.2011.08.004.
20. Radusin, T.; Torres-Giner, S.; Stupar, A.; Ristic, I.; Miletic, A.; Novakovic, A.; Lagaron, J.M. Preparation, characterization and antimicrobial properties of electrospun polylactide films containing *Allium ursinum* L. extract. *Food Packag. Shelf Life* **2019**, *21*, 100357. https://doi.org/10.1016/j.fpsl.2019.100357.
21. Angane, M.; Swift, S.; Huang, K.; Butts, C.A. Essential Oils and Their Major Components: An Updated Review on Antimicrobial Activities, Mechanism of Action and Their Potential Application in the Food Industry. *Foods* **2022**, *11*, 464. https://doi.org/10.3390/foods11030464.
22. Dong, Z.; Luo, C.; Guo, Y.; Ahmed, I.; Pavase, T.R.; Lv, L.; Li, Z.; Lin, H. Characterization of new active packaging based on PP/LDPE composite films containing attapulgite loaded with *Allium sativum* essence oil and its application for large yellow croaker (*Pseudosciaena crocea*) fillets. *Food Packag. Shelf Life* **2019**, *20*, 100320. https://doi.org/10.1016/j.fpsl.2019.100320.
23. Seydim, A.C.; Sarikus, G. Antimicrobial activity of whey protein based edible films incorporated with oregano, rosemary and garlic essential oils. *Food Res. Int.* **2006**, *39*, 639–644. https://doi.org/10.1016/j.foodres.2006.01.013.
24. Yolanda, D.S.; Dirpan, A.; Rahman, A.N.F.; Kamaruddin, I.; Ainani, A.F. Determination the best concentration of antimicrobial ingredients with a mixture of paper to create active paper packaging. *IOP Conf. Ser. Earth Environ. Sci.* **2020**, *575*, 012019. https://doi.org/10.1088/1755-1315/575/1/012019.
25. Lee, S.J.; Rahman, A.T.M.M. *Intelligent Packaging for Food Products*; Elsevier Ltd.: Amsterdam, The Netherlands, 2013; ISBN 9780123946010.
26. Pacquit, A.; Crowley, K.; Diamond, D. Smart Packaging Technologies for Fish and Seafood Products. In *Smart Packaging Technologies for Fast Moving Consumer Goods*; Kerry, J., Ed.; John Wiley & Sons, Ltd.: Hoboken, NJ, USA, 2008; pp. 75–98, ISBN 9780470028025.
27. Vilas, C.; Mauricio-Iglesias, M.; García, M.R. Model-based design of smart active packaging systems with antimicrobial activity. *Food Packag. Shelf Life* **2020**, *24*, 100446. https://doi.org/10.1016/j.fpsl.2019.100446.
28. Dirpan, A.; Djalal, M.; Rahman, R.; Genisa, J. The utilization of red cabbage extract (*Brassica oleracea*) in the production of avocado (*Persea Americana Mill*) freshness indicator as smart packaging element. *Online J. Biol. Sci.* **2021**, *21*, 261–270. https://doi.org/10.3844/ojbsci.2021.261.270.
29. Agustianti, D.; Dirpan, A.; Syarifuddin, A. The potential application of red cabbage indicator film as smart packaging on tuna fillet. *IOP Conf. Ser. Earth Environ. Sci.* **2021**, *807*, 022065. https://doi.org/10.1088/1755-1315/807/2/022065.
30. Wu, Y.; Tang, P.; Quan, S.; Zhang, H.; Wang, K.; Liu, J. Preparation, characterization and application of smart packaging films based on locust bean gum/polyvinyl alcohol blend and betacyanins from cockscomb (*Celosia cristata* L.) flower. *Int. J. Biol. Macromol.* **2021**, *191*, 679–688. https://doi.org/10.1016/j.ijbiomac.2021.09.113.
31. Kim, D.; Thanakkasaranee, S.; Lee, K.; Sadeghi, K.; Seo, J. Smart packaging with temperature-dependent gas permeability maintains the quality of cherry tomatoes. *Food Biosci.* **2021**, *41*, 100997. https://doi.org/10.1016/j.fbio.2021.100997.
32. Dirpan, A.; Latief, R.; Syarifuddin, A.; Rahman, A.N.F.; Putra, R.P.; Hidayat, S.H. The use of colour indicator as a smart packaging system for evaluating mangoes Arummanis (*Mangifera indica* L. var. Arummanisa) freshness. *IOP Conf. Ser. Earth Environ. Sci.* **2018**, *157*, 12031. https://doi.org/10.1088/1755-1315/157/1/012031.
33. Shukla, V.; Kandeepan, G.; Vishnuraj, M.R. Development of On-Package Indicator Sensor for Real-Time Monitoring of Buffalo Meat Quality During Refrigeration Storage. *Food Anal. Methods* **2015**, *8*, 1591–1597. https://doi.org/10.1007/s12161-014-0066-6.
34. Romero, A.; Sharp, J.L.; Dawson, P.L.; Darby, D.; Cooksey, K. Evaluation of two intelligent packaging prototypes with a pH indicator to determine spoilage of cow milk. *Food Packag. Shelf Life* **2021**, *30*, 100720. https://doi.org/10.1016/j.fpsl.2021.100720.
35. Julyaningsih, A.H.; Latief, R.; Dirpan, A. The making of smart and active packaging on tuna fillet. *IOP Conf. Ser. Earth Environ. Sci.* **2020**, *486*, 012053. https://doi.org/10.1088/1755-1315/486/1/012053.
36. Sisilia Yolanda, D.; Dirpan, A.; Nur Faidah Rahman, A.; Djalal, M.; Hatul Hidayat, S. The potential combination of smart and active packaging in one packaging system in improving and maintaining the quality of fish. *Canrea J. Food Technol. Nutr. Culin. J.* **2020**, *3*, 74–86. https://doi.org/10.20956/canrea.v3i2.357.

Commented [M33]: Newly added information. Same as below, Please check all highlights and confirm.

Commented [MOU34R33]: Yes we confirmed.

37. Yong, H.; Liu, J. Recent advances in the preparation, physical and functional properties, and applications of anthocyanins-based active and intelligent packaging films. *Food Packag. Shelf Life* **2020**, *26*, 100550. https://doi.org/10.1016/j.fpsl.2020.100550.
38. Gosal, L.; Hutomo, S.; Sooai, C.M. Garlic (*Allium sativum* L.) Ethanolic Extract Capability to Inhibit Pseudomonas aeruginosa Biofilm Formation. *J. Med. Health* **2021**, *3*, 1–8. https://doi.org/10.28932/jmh.v3i1.3143.
39. Wiastuti, T.; Khasanah, L.U.; Kawiji, W.A.; Manuhara, G.J.; Utami, R. Characterization of active paper packaging incorporated with ginger pulp oleoresin. *IOP Conf. Ser. Mater. Sci. Eng.* **2016**, *107*, 012057. https://doi.org/10.1088/1757-899X/107/1/012057.
40. Kuswandi, B.; Nurfawaidi, A. On-package dual sensors label based on pH indicators for real-time monitoring of beef freshness. *Food Control* **2017**, *82*, 91–100. https://doi.org/10.1016/j.foodcont.2017.06.028.
41. Mohammed, M.F.; Raman, N.; Alhoot, M.A.; Alwan, M.R. Antibacterial activities of allium sativum (Garlic) extracts against staphylococcus aureus and *Escherichia coli*. *Eur. J. Mol. Clin. Med.* **2020**, *7*, 526–534.
42. Hunterlab. *Colorimeters Versus Spectrophotometers*; Technical Service Departement Hunter Associates Laboratory, Inc.: Reston, VA, USA, 2008.
43. Hernández-García, E.; Vargas, M.; Torres-Giner, S. Quality and shelf-life stability of pork meat fillets packaged in multilayer polylactide films. *Foods* **2022**, *11*, 426. https://doi.org/10.3390/foods11030426.
44. Ponnampalam, E.N.; Hopkins, D.L.; Bruce, H.; Li, D.; Baldi, G.; El-din Bekhit, A. Causes and Contributing Factors to "Dark Cutting" Meat: Current Trends and Future Directions: A Review. *Compr. Rev. Food Sci. Food Saf.* **2017**, *16*, 400–430. https://doi.org/10.1111/1541-4337.12258.
45. Korean Ministry of Agriculture and Forestry. Processing Standards and Ingredient Specifications for Livestock Products Available online: https://www.mfds.go.kr/eng/brd/m_15/view.do?seq=70016 (accessed on 18 December 2021).
46. Holman, B.W.B.; Bekhit, A.E.D.A.; Waller, M.; Bailes, K.L.; Kerr, M.J.; Hopkins, D.L. The association between total volatile basic nitrogen (TVB-N) concentration and other biomarkers of quality and spoilage for vacuum packaged beef. *Meat Sci.* **2021**, *179*, 108551. https://doi.org/10.1016/j.meatsci.2021.108551.
47. Deresse, D. Antibacterial effect of garlic (*Allium sativum*) on Staphylococcu aureus: An in vitro study. *Asian J. Med. Sci.* **2010**, *2*, 62–65.
48. Mardiyah, S. Efektivitas Anti Bakteri Perasan Bawang Putih (*Allium sativum* L.) terhadap Pertumbuhan Staphylococcus aureus. *Med. (J. Med. Lab. Sci.)* **2018**, *1*, 44–53. https://doi.org/10.21070/medicra.v1i2.1532.
49. Comi, G. *Spoilage of Meat and Fish*; Elsevier Ltd.: Amsterdam, The Netherlands, 2017; ISBN 9780081005033.
50. Pan, X.; Chen, F.; Wu, T.; Tang, H.; Zhao, Z. The acid, bile tolerance and antimicrobial property of Lactobacillus acidophilus NIT. *Food Control* **2009**, *20*, 598–602. https://doi.org/10.1016/j.foodcont.2008.08.019.
51. Costa, A.L.R.; de Oliveira, A.C.S.; Azevedo, V.M.; Medeiros, E.A.A.; Soares, N.d.F.F.; Borges, S.V. Essential oils of garlic and oregano incorporated in cellulose acetate films: Antimicrobial activity and physical properties. *Res. Soc. Dev.* **2020**, *9*, e329108304.
52. Garba, I.; Umar, A.; Abdulrahman, A.; Tijjani, M.; Aliyu, M.; Zango, U.; Muhammad, A. Phytochemical and antibacterial properties of garlic extracts. *Bayero J. Pure Appl. Sci.* **2014**, *6*, 45. https://doi.org/10.4314/bajopas.v6i2.10.
53. Safithri, M.; Bintang, M.; Poeloengan, M. Antibacterial activity of garlic extract against some pathogenic animal bacteria. *Media Peternak.* **2011**, *34*, 155–158. https://doi.org/10.5398/medpet.2011.34.3.155.
54. Alizadeh-Sani, M.; Tavassoli, M.; Mohammadian, E.; Ehsani, A.; Khaniki, G.J.; Priyadarshi, R.; Rhim, J.W. PH-responsive color indicator films based on methylcellulose/chitosan nanofiber and barberry anthocyanins for real-time monitoring of meat freshness. *Int. J. Biol. Macromol.* **2021**, *166*, 741–750. https://doi.org/10.1016/j.ijbiomac.2020.10.231.
55. Schuessler, Z. What Is Delta E? and Why Is It Important for Color Accuracy? Available online: http://zschuessler.github.io/DeltaE/learn/ (accessed on 15 May 2022).
56. Mansur, A.R.; Song, E.J.; Cho, Y.S.; Nam, Y.D.; Choi, Y.S.; Kim, D.O.; Seo, D.H.; Nam, T.G. Comparative evaluation of spoilage-related bacterial diversity and metabolite profiles in chilled beef stored under air and vacuum packaging. *Food Microbiol.* **2019**, *77*, 166–172. https://doi.org/10.1016/j.fm.2018.09.006.
57. Dirpan, A.; Djalal, M.; Kamaruddin, I. Application of an intelligent sensor and active packaging system based on the bacterial cellulose of Acetobacter xylinum to meat products. *Sensors* **2022**, *22*, doi:10.3390/s22020544.

Moved (insertion) [1]

Deleted: www.mfds.go.kr/eng/brd/m_15/v%0Aiew.do?seq=70016&srchFr=&srchTo=&srchWord=&srchTp=&itm_seq_1=0&itm_s%0Aeq_2=0&multi_itm_seq=0&company_cd=&company_nm=&page=2:.

Formatted: Not Highlight

Formatted: Font: 9 pt

Moved up [1]: <#>Ponnampalam, E.N.; Hopkins, D.L.; Bruce, H.; Li, D.; Baldi, G.; El-din Bekhit, A. Causes and Contributing Factors to "Dark Cutting" Meat: Current Trends and Future Directions: A Review. *Compr. Rev. Food Sci. Food Saf.* **2017**, *16*, 400–430. https://doi.org/10.1111/1541-4337.12258.
Korean Ministry of Agriculture and Forestry Processing Standards and Ingredient Specifications for Livestock Products Available online: www.mfds.go.kr/eng/brd/m_15/v%0Aiew.do?seq=70016&srchFr=&srchTo=&srchWord=&srchTp=&itm_seq_1=0&itm_s%0Aeq_2=0&multi_itm_seq=0&company_cd=&company_nm=&page=2:. (accessed on 18 December 2021).
Holman, B.W.B.; Bekhit, A.E.D.A.; Waller, M.; Bailes, K.L.; Kerr, M.J.; Hopkins, D.L. The association between total volatile basic nitrogen (TVB-N) concentration and other biomarkers of quality and spoilage for vacuum packaged beef. *Meat Sci.* **2021**, *179*, 108551. https://doi.org/10.1016/j.meatsci.2021.108551.
Deresse, D. Antibacterial effect of garlic (*Allium sativum*) on Staphylococcu aureus: An in vitro study. *Asian J. Med. Sci.* **2010**, *2*, 62–65.
Mardiyah, S. Efektivitas Anti Bakteri Perasan Bawang Putih (*Allium sativum* L.) terhadap Pertumbuhan Staphylococcus aureus. *Med. (J. Med. Lab. Sci.)* **2018**, *1*, 44–53. https://doi.org/10.21070/medicra.v1i2.1532.
Comi, G. *Spoilage of Meat and Fish*; Elsevier Ltd.: Amsterdam, The Netherlands, 2017; ISBN 9780081005033.

Formatted: English (US)

Formatted: Font: Palatino Linotype

Formatted: Font: Palatino Linotype

Formatted: Indent: Left: 0.75 cm, No bullets or numbering

5. Paper accepted for publication (18-5-2022)

- **Final Paper**

[Foods] Manuscript ID: foods-1728837 - Accepted for Publication

Wed, May 18, 3:20 PM

Dear Dr. Dirpan,

Congratulations on the acceptance of your manuscript, and thank you for your interest in submitting your work to Foods:

Manuscript ID: foods-1728837
Type of manuscript: Article
Title: A Simple Combination of Active and Intelligent Packaging based on Garlic Extract and Indicator Solution in Extending and Monitoring the Meat Quality Stored at Cold Temperature
Authors: Andi Dirpan *, Muspirah Djalal, Andi Fadiah Ainani
Received: 30 April 2022
E-mails: dirpan@unhas.ac.id, muspirah_djalal@agri.unhas.ac.id, andifadiah97@gmail.com

https://susy.mdpi.com/user/manuscripts/review_info/6a91850571fa17b18e5de65d7113d608

We will now edit and finalize your paper, which will then be returned to you for your approval. Within the next couple of days, an invoice concerning the article processing charge (APC) for publication in this open access journal will be sent by email from the Editorial Office in Basel, Switzerland.

If, however, extensive English edits are required to your manuscript, we will need to return the paper requesting improvements throughout.

We encourage you to set up your profile at SciProfiles.com, MDPI's researcher network platform. Articles you publish with MDPI will be linked to your SciProfiles page, where colleagues and peers will be able to see all of your publications, citations, as well as other academic contributions.

We also invite you to contribute to Encyclopedia (https://encyclopedia.pub), a scholarly platform providing accurate information about the latest research results. You can adapt parts of your paper to provide valuable reference information, via Encyclopedia, for others both within the field and beyond.

Kind regards,
Arun K. Bhunia
Editor-in-Chief

6. Final proofreading before publication (19-5-2022)

[Foods] Manuscript ID: foods-1728837 - Final Proofreading Before Publication

Thu, May 19, 4:40 PM

Dear Dr. Dirpan,

We invite you to proofread your manuscript to ensure that this is the final version that can be published and confirm that you will require no further changes from hereon:

Manuscript ID: foods-1728837
Type of manuscript: Article
Title: A Simple Combination of Active and Intelligent Packaging based on Garlic Extract and Indicator Solution in Extending and Monitoring the Meat Quality Stored at Cold Temperature
Authors: Andi Dirpan *, Muspirah Djalal, Andi Fadiah Ainani
Received: 30 April 2022
E-mails: dirpan@unhas.ac.id, muspirah_djalal@agri.unhas.ac.id, andifadiah97@gmail.com

Please read the following instructions carefully before proofreading:

1) Download the manuscript from the link provided at the end of this message and upload the final proofed version at the same link within 24 hours (1 working day). If you experience any difficulties, please contact the Foods Editorial Office.

2) Please use Microsoft Word's built-in track changes function to highlight any changes you make, or send a comprehensive list of changes in a separate document. Note that this is the *last chance* to make textual changes to the manuscript. Some style and formatting changes may have been made by the production team, please do not revert these changes.

3) All authors must agree to the final version. Check carefully that authors' names and affiliations are correct, and that funding sources are correctly acknowledged. Incorrect author names or affiliations are picked up by indexing databases, such as the Web of Science or PubMed, and can be difficult to correct.

After proofreading, final production will be carried out. Note that changes to the position of figures and tables may occur during the final steps. Changes can be made to a paper published online only at the discretion of the Editorial Office.

Please download the final version of your paper for proofreading here:

https://susy.mdpi.com/user/manuscripts/proof/file/6a91850571fa17b18e5de65d7113d608

and upload here:

https://susy.mdpi.com/user/manuscripts/resubmit/6a91850571fa17b18e5de65d7113d608

Supplementary and other additional files can be found at the second link. We look forward to hearing from you soon.

Kind regards,
Sasa Novakovic

Dr. Sasa Novakovic
Assistant Editor
MDPI Branch Office, Belgrade
Bulevar Mihajla Pupina 115, 11070 Belgrade, Serbia
Tel. +381 11 7455 363

MDPI
Foods Editorial Office
E-Mail: foods@mdpi.com
http://www.mdpi.com/journal/foods

8. Paper published (20-5-2022)

-Certificate

[Foods] Manuscript ID: foods-1728837; doi: 10.3390/foods11101495. Paper has been published.

Fri, May 20, 8:11 PM

Dear Authors,

We are pleased to inform you that your article "A Simple Combination of Active and Intelligent Packaging Based on Garlic Extract and Indicator Solution in Extending and Monitoring the Meat Quality Stored at Cold Temperature" has been published in Foods and is available online:

Abstract: https://www.mdpi.com/2304-8158/11/10/1495
PDF Version: https://www.mdpi.com/2304-8158/11/10/1495/pdf
The meta data of your article, the manuscript files and a publication certificate are available here (only available to corresponding authors after login):
https://susy.mdpi.com/user/manuscripts/review_info/6a91850571fa17b18e5de65d7113d608

Please note that this is an early access version. The complete PDF, HTML, and XML versions will be available soon. Please take a moment to check that everything is correct. You can reply to this email if there is a problem. If any errors are noticed, please note that all authors must follow MDPI's policy on updating published papers, found here:
https://www.mdpi.com/ethics#16.

To encourage open scientific discussions and increase the visibility of published articles, MDPI recently implemented interactive commenting and recommendation functionalities on all article webpages (side bar on the right). We encourage you to forward the article link to your colleagues and peers.

We encourage you to set up your profile at www.SciProfiles.com, MDPI's researcher network platform. Articles you publish with MDPI will be linked to your SciProfiles page, where colleagues and peers will be able to see all of your publications, citations, as well as your other academic contributions. Please also feel free to send us feedback on the platform that we can improve it quickly and make it useful for scientific communities.

You can also share the paper on various social networks by clicking the links on the article webpage. Alternatively, our Editorial Office can post an announcement of your article on our Twitter channel, please send us a text of up to 200 characters with spaces. Please note that our service Scitations.net will automatically notify authors cited in your article. For further paper promotion guidelines, please refer to the following link:

https://www.mdpi.com/authors/promoting.

We would be happy to keep you updated about new issue releases of foods. Please enter your e-mail address in the box at https://www.mdpi.com/journal/foods/toc-alert/ to receive notifications. After issue release, a version of your paper including the issue cover will be available to download from the article abstract page.

To order high quality reprints of your article in quantities of 25-1000, visit: https://www.mdpi.com/2304-8158/11/10/1495/reprints

We support the multidisciplinary preprint platform /Preprints/, which permanently archives full text documents and datasets of working papers in all subject areas. Posting on the platform is entirely free of charge, and full details can be viewed at http://www.preprints.org.

We are dedicated to providing an outstanding publishing service, and we invite you to complete our author satisfaction survey https://www.surveymonkey.com/r/authorfeedbackmdpi. The survey contains 20 short questions and will only take a couple of minutes to complete.

To help us improve our Production and English editing service, provided as part of MDPI's editorial process, please take a few minutes to participate in the following survey: https://www.surveymonkey.com/r/38TKGWF (for Production and English editing service).

Thank you for choosing Foods to publish your work, we look forward to receiving further contributions from your research group in the future.

Kind regards,

--
MDPI
Postfach, CH - 4020 Basel, Switzerland
Office: St. Alban-Anlage 66, 4052 Basel, Switzerland
Tel. +41 61 683 77 34
Fax: +41 61 302 89 18
E-mail: website@mdpi.com
https://www.mdpi.com/

foods
an Open Access Journal by MDPI

Indexed in: PubMed
CITESCORE 4.1 SCOPUS
IMPACT FACTOR 5.561

CERTIFICATE OF PUBLICATION

Certificate of publication for the article titled:

A Simple Combination of Active and Intelligent Packaging Based on Garlic Extract and Indicator Solution in Extending and Monitoring the Meat Quality Stored at Cold Temperature

Authored by:

Andi Dirpan; Muspirah Djalal; Andi Fadiah Ainani

Published in:

Foods **2022**, Volume 11, Issue 10, 1495

MDPI Academic Open Access Publishing since 1996

Basel, September 2022