Garlic-Infused Olive Oil as Meat Preservative to Control Bacterial Spoilage

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DECLARATION

I hereby declare that the work embodied in this report is the result of the original research except the excerpt and summaries that I have made clear of the resources.

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ABSTRACT

Garlic and olive are used as food enhancers for a long time and also as a traditional medicine that could help cure certain diseases. Garlic and olive have antimicrobial and antioxidant properties that could help in preserving meat, thus, increasing its shelf life. Meat can spoil in a short time due to microbial growth. Garlic contains allicin that acts as an antimicrobial. The allicin in garlic was extracted by crushing the garlic and mixing it with olive oil to produce garlic-infused olive oil. The meat was cut into 12 pieces of 10 g each and divided into three treatments. Sample A acted as the Control sample. Sample B was meat coated with 25% garlic-infused olive oil of meat weight while Sample C was meat coated with 50% garlic-infused olive oil of meat weight. All samples were refrigerated for 12 days before tested. Then, the meat was homogenised and the solution was used to perform the spread plate method for the microbial count in meat. The analysis was performed using Oneway ANOVA, Duncan test and t-test to obtain significant difference ($p \leq 0.05$). The colour change of meat (ΔE) was determined using the formula proposed by Sharma and Bala. The results showed that Sample B had the best formulation in reducing the microbial growth while Sample C was the most suitable in preserving meat's colour. This study proved that the formulation from Sample B namely meat with garlic-infused olive oil 25% of meat weight warrants further development of research for commercialisation purposes.

Keywords: *Allium sativum*, olive oil, allicin, antimicrobial, meat spoilage.

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ABSTRAK

Bawang putih dan zaitun digunakan sebagai penambah makanan untuk masa yang lama dan juga sebagai ubat tradisional yang boleh membantu menyembuhkan penyakit tertentu. Bawang putih dan zaitun mempunyai ciri antimikrob dan antioksidan yang membantu dalam mengawet daging, meningkatkan jangka hayatnya. Daging mampu rosak dalam masa yang singkat kerana pertumbuhan mikrob. Bawang putih mengandungi allicin yang bertindak sebagai antimikrob. Allicin dalam bawang putih diekstrak dengan menghancurkan bawang putih dan mencampurkannya dengan minyak zaitun untuk menghasilkan minyak zaitun yang diaduk dengan bawang putih. Daging dipotong menjadi 12 keping 10 g setiap satu dan dibahagikan kepada tiga rawatan. Sampel A bertindak sebagai sampel Kawalan. Sampel B ialah daging dengan minyak zaitun yang diselitkan bawang putih 25% daripada berat daging manakala Sampel C adalah daging dengan minyak zaitun yang diselitkan bawang putih 50% daripada berat daging. Semua sampel telah disejukkan selama 12 hari sebelum diuji. Kemudian, daging dihomogenkan dan larutan digunakan untuk melakukan kaedah sebaran ke atas *plate* untuk kiraan mikrob dalam daging. Analisis dilakukan menggunakan ANOVA sehala, ujian Duncan dan ujian-t untuk mendapatkan perbezaan yang signifikan ($p \leq 005$). Perubahan warna daging (ΔE) ditentukan menggunakan formula yang dicadangkan oleh Sharma and Bala. Keputusan menunjukkan bahawa Sampel B mempunyai formulasi terbaik dalam mengurangkan pertumbuhan mikrob manakala Sampel C adalah yang paling sesuai untuk mengekalkan warna daging. Kajian ini membuktikan bahawa formulasi daripada Sampel B iaitu daging dengan minyak zaitun yang diselitkan bawang putih 25% daripada berat daging memerlukan pembangunan penyelidikan selanjutnya untuk tujuan pengkomersialan.

Kata kunci: *Allium sativum*, minyak zaitun, allicin, antimikrob, daging rosak.

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LIST OF ABBREVIATIONS AND SYMBOLS

Abbreviation of Symbol		Pages
ΔE	Delta E (Colour change)	iii, iv, 26, 27, 28
NaCl	Sodium Chloride	15
TBARS	Thiobarbituric acid reactive substances	26

CHAPTER 1

INTRODUCTION

1.1 Research Background

Garlic or *Allium sativum* is known for its usability as a flavouring in cooking to enhance the taste of the cuisine. Some countries even consume raw garlic and some even make it into a pickle to be eaten. Thus, garlic is no stranger to the culinary world. Besides, garlic is also used in the medicinal world for its properties such as antibacterial, antimicrobial, antioxidant, and anti-inflammatory that can help in treating high blood pressure, colon cancer, hypertension, and heart attack (Butler & Newman, 2017). In these circumstances, the antimicrobial and the anti-oxidant properties of garlic can be used as food preservatives peculiarly in preserving meat to prolong its shelf life.

Olive oil also widely used in cooking since it can enhance the taste of the food. Besides, olive oil can give positive effect on health since it is high in monounsaturated fatty acid that give antioxidant and antibacterial properties (Donat-Vargas et al., 2022). These properties could help in treating diseases such as high blood pressure, and heart diseases (Jalarama Reddy et al., 2015). The Mediterranean diet is regarded as the healthiest eating pattern according to Donat-Vargas et al. (2022) as most of Mediterranean diet consist of olive oil. Since olive oil has antimicrobial and antioxidant properties, it also can be used as meat preservative.

Meat preservation has been applied for decades using any kind of preservatives to prolong its shelf life (Shahidi et al., 2014). Garlic as a natural food preservative is not very known among the public, especially in meat preservation (Sarma, 2004). Garlic or *Allium sativum* is commonly used as flavour enhancer and is also used for medicinal purposes. However, its capability in preserving food especially meat is still being analysed. Even though some researchers have already done a study on meat preservation using garlic (Al-Delaimy & Barakat, 1971;Mancini et al., 2020), it is still not commercially introduced to people yet. Researchers did the observation using different states of garlic. Nurwantoro et al. (2015) applied crushed garlic in their research to avoid meat rotten. Meanwhile, Mancini et al. (2020) applied garlic powder incorporated with salt to control microbial growth on rabbit burgers. Briefly, when the meat is marinated with crushed or powdered garlic, it does not absorb the marinade well. This condition might reduce the effectiveness of garlic as a food preservative.

Besides, people opt to use natural preservatives such as salt and sugar to preserve the meat but a high amount of it can lead to health problems. In the modern world, the chemical preservative is introduced to replace the old way. Commonly used chemical preservatives include sodium benzoates, sodium nitrite, and sulphur dioxide.

However, these chemical preservatives are found to be harmful to the health of consumers if taken regularly. Thus, garlic and olive oil can aid in preserving and enhancing the taste of meat as it gives a sweet, mild and nutty taste when cooked, and most importantly it is healthier due to its antioxidant properties.

1.2 Problem Statement

Malaysians are among high meat consumers around the world. Malaysians usually buy the meat when they are about to cook meat-based dishes as the locals wanted to keep the meat fresh. Unfortunately, fresh meat only stands for 5 to 6 days if refrigerated (USDA, 2019). Leftover meat that is not used will be a waste and food waste leads to the emission of greenhouse gases (Oakes, 2020). Therefore, in this study, natural meat preservative which is garlic and olive oil was used to observe its abilities to conserve the meat so it can be kept for a longer time. The meat can be marinated with garlic-infused olive oil and with suitable temperature and packaging, the meat is able to have longer shelf life so that locals can cook the meat whenever they want.

1.3 Research Objectives

i. To determine the antimicrobial and antioxidants properties of garlic-infused olive oil on the meat.

ii. To observe the colour change in meat preserves with garlic-infused olive oil.

iii. To examine the process of slowing down the deterioration of the meat through microbial quantification.

1.4 Research Questions

i. What was the effect of antimicrobial and antioxidants properties of both garlic and olive oil on meat?

ii. Did preserving meat with garlic-infused olive oil change the colour of the meat?

iii. Are the treatment of garlic-infused olive oil slows down the deterioration of meat?

1.5 Hypothesis

H_0 : The preservation of meat with garlic-infused olive oil did not reduce the bacterial spoilage of meat.

H_1 : The preservation of meat with garlic-infused olive oil reduce the bacterial spoilage of meat.

1.6 Scope of Study

The research intends to detect the antimicrobial of the *Allium sativum* and olive oil on raw meat through Plate Count Agar (PCA) using the spread plate method. The meat sample was purchased at a local market in Jeli, Kelantan and was introduced with different amounts of garlic-infused olive oil. The change in meat colour was observed using the CIE Lab Chroma Meter. CIE Lab colour space is one of the colour organisation systems that have been standardised by the Commission Internationale de I'Eclairage (CIE) (Ly et al., 2020). The CIE Lab system shows colour's lightness (L^*), red-green intensity (a^*), and yellow/blue intensity (b^*) of a sample.

1.7 Significance of Study

Through this study, garlic-infused olive oil can be shown as a safe natural food preservative. The garlic-infused olive oil not only preserves the meat due to its antimicrobial and antioxidant properties, thus, it also enhances the taste and flavouring of the meat.

1.8 Limitations

The meat may be treated with a chemical to make it stay bright red colour making the meat colour unnatural and hard to determine its freshness. For the garlic extract, the extraction may be hard to attain a high amount if done manually.

CHAPTER 2

LITERATURE REVIEW

2.1 Meat Spoilage

Meat is categorized as perishable food which has a short shelf life, spoils quickly, rot, or becomes unhealthy to eat (Ndraha et al., 2020). Microbial meat spoilage causes massive losses in the manufacturing, transport, and storage of meat products, responsible for around 21% of all food wastage. Meat spoiling is regarded as a dynamic process that causes physical damage, chemical degradation, and texture and visual losses in meat, making it unattractive or unfit for ingestion (Odeyemi et al., 2020). According to Erkmen and Bozoglu (2016) and Pellissery et al. (2020), the factor that eases the spoilage of meat includes high nutrient content that allows rapid microbial growth, autolytic enzymes that present in the meat, high water activity and pH, and a considerable amount of fat and lipids in the food, which promotes deterioration and spoilage. Though there are a lot of factors, microbial growth always is the one that causes the spoilage of food products.

Spoiled meat usually tastes awful, and declines in colour. For these reasons, the preservation of meat is necessary ever since ancient times to make sure that the meat can be kept for a longer time. Despite significant attempts to minimise or inhibit microbes, such as using hygiene rules, preservatives, and particular procedures, meat deterioration is inevitable owing to microbial cross-contamination. Microbial cells are normally present in the initial refrigerated meat at 2 to 4 log colony-forming units (cfu)/g, but can quickly expand to 6.5 to 7.0 log cfu/g (Shao et al., 2021).

Even though dozens of bacteria species of the genus exist in the meat microbial ecology, only 10% of bacteria are responsible for the spoiling of refrigerated meat, and those bacteria are known as dominant microbial spoilage or particular spoilage bacteria (Shao et al., 2021). In most earlier investigations, the bacteria colonies or gene abundances in a certain meat-related environment, such as a whole poultry carcass, a piece of refrigerated meat, or a packed meat product, were used to determine dominant spoilage bacteria.

The other cause of meat spoilage is lipid oxidation of meat. Natural processes such as lipid oxidation and the generation of free radicals influence fatty acids, causing oxidative degradation of meat and the creation of off-flavours (Simitzis & Deligeorgis, 2010). As the blood flow ceases and metabolic activities are halted after an animal is slaughtered, the fatty acids in the tissues are oxidised. Initiation, propagation, and termination are three stages of free radical processes. The lipids oxidation in meat is influenced by a number of variables, including fatty acid content, vitamin E levels, and prooxidants such as the availability of free iron in tissues (Dave & Ghaly, 2011).

2.2 Meat Colour Change

The redness of meat usually determines its freshness. The quantity of heme pigments particularly myoglobin, the chemical condition of myoglobin, and the physical features of the meat all impact the colour of the meat (Jeong et al., 2009). Other heme components, such as haemoglobin and cytochrome C, may also contribute to meat colour, but myoglobin, the sarcoplasmic heme protein, is the primary factorv (Mancini & Hunt, 2005). The redness of meat occurs when myoglobin, a protein found in meat, contacts oxygen, it transforms into oxymyoglobin, which produces a bright red colour. The meat colour also turns brown if the meat is exposed to air and light for a long time. Muscle fibre parameters such as fibre quantity, fibre composition, cross-sectional surface, and fibre density have an impact on meat quality. One of the most significant meat quality factors is lightness, which is linked to fibre content (Kim et al., 2010).

Meat discoloration also caused by microorganisms, which are also responsible for protein discoloration and pigment build-up (Shao et al., 2021). Myoglobin can become green due to metabolites generated by microbial growth, which are commonly connected with H_2O_2 and H_2S. Bacteria such as *Pseudomonas putida* that present in meat can lead to meat greening (Shao et al., 2021). Certain microbes can exude fat-soluble pigments and generate coloured spots on the surface and other microbes can also induce pigment build-up depending on a variety of circumstances, including temperature, packing conditions, and fat oxidation (Tomasevic et al., 2021).

2.3 Antimicrobial Properties of Garlic

Garlic or *Allium sativum* was mentioned 5000 years ago in Egyptian and Indian civilization, and there is strong archaeological proof for the use of garlic as food and remedy by the Babylonians 4500 years ago and the Chinese 2000 years ago. Garlic may have been cultivated in China as early as 4000 years ago, according to some sources (Simon, 2020). Garlic contains a bioactive ingredient which is Allicin that could act as an antimicrobial. By crushing the clove of garlic, Alliin is transformed to Allicin by the enzyme alliinase (Ankri & Mirelman, 1999 ; Li et al., 2017). The Allicin in garlic was found in 1944 by Cavallito and his colleagues when they tried to determine the cause of antibacterial activities in crushed garlic (Ankri & Mirelman, 1999).

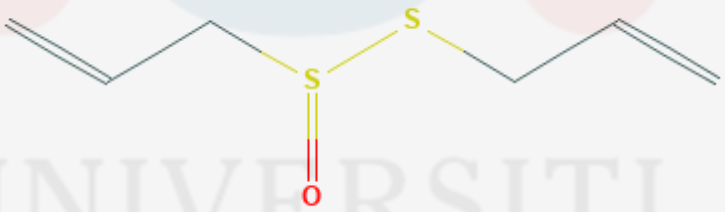

Figure 1: The Structure of Allicin

Source: NCBI (2021)

The antimicrobial activity of garlic is shown to affect a broad range of Gram-negative and Gram-positive bacteria, such as *Escherichia coli*, *Salmonella*, *Staphylococcus, Streptococcus, Klebsiella, Proteus, Bacillus,* and *Clostridium* species. Garlic is toxic to even acid-fast microbes like *Mycobacterium tuberculosis* (Ankri & Mirelman, 1999). The allicin will help to inhibit the growth of these bacteria. This is the main reason garlic is used in medication as viral and fungal infections prevention (Goncagul & Ayaz, 2010). Nonetheless, the antimicrobial properties are also suitable as a meat preservative as meat is usually spoiled by the same species of bacteria.

2.4 Antimicrobial and Antioxidant Properties of Olive Oil.

Although olive oil production is believed to have formed before 4,000 BC, the oldest surviving olive oil proved to date to 3,500 BC. The olive was extensively planted and a highly esteemed crop in Crete by 3,000 BC. Olive tree agriculture became popular in Crete during the post-palatial era, and it played an important role in the island's income, as it did throughout the Mediterranean. The Greeks were the first to use olive oil as food, fuel, skincare, contraceptives, soap, preservative, pesticides, fragrances, and decorations. Mycenaean Greece during 1450 to 1150 BC, also exported a lot of olive oil (Brightland, 2020).

Virgin olive oil has a phenolic component that has been proven to have antibacterial, antioxidant, and anti-inflammatory characteristics that have protective benefits. Substances with antimicrobial characteristics might contribute to the inhibition of microbial development. The dialdehydic form of decarboxymethyl oleuropein aglycon, oleocanthal, hydroxytyrosol, and tyrosol, all contained in virgin olive oil, have been demonstrated to exhibit substantial antibacterial activity against many strains of bacteria (Cicerale et al., 2012).

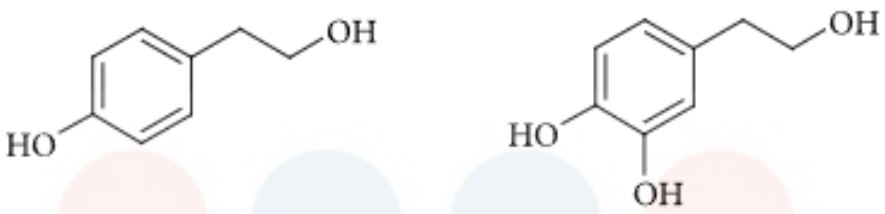

Figure 2: The structure of Tyrosol (left) and Hydroxytyrosol (right).

Source: Abu-Lafi et al. (2017)

Yet, the phenolic components have been reported to have a bactericidal effect on *Lactobacillus acidophilus* and *Bifidobacterium bifidum*, two beneficial microbes (Medina et al., 2006; Cicerale et al., 2012). Additionally, the antioxidant properties of olive oil are able to inhibit lipid oxidation in grounded meat according to DeJong and Lanari (2009).

2.5 The Spread Plate Method of PCA

The spread plate method is a technique for isolating and counting bacteria in a mixed culture and uniformly spreading them on the agar plate. Spread plate method require an aseptic technique to maintain sterility during the procedure. The correct spread plate method can yield recognizable and isolated bacteria colonies that are uniformly distributed and countable throughout the plate.

Spread-plating is commonly employed in enriching, selecting, and screening procedures, in conjunction to viable plate counts, in which the total amount of colony forming units on a single plate is counted and used to quantify the concentration of colonies in the tube from which the sample was plated (Sanders, 2012). The principle of this method is; it includes spreading a small number of bacteria suspended in solvent over an agar plate with a smooth sterilized spreader (Aryal, 2019). To allow the agar to capture the bacteria more efficiently, the plate must be dry and performed at room temperature.

CHAPTER 3

METHODOLOGY

3.1 Material

3.1.1 Chemical and Reagents

Peptone and Sodium chloride (NaCl) was obtained from the laboratory to prepare a 0.1% peptone salt solution.

3.1.2 Apparatus and Instruments

The apparatus that was applied in the laboratory was a knife, and cooking pot. The gas stove and refrigerator were obtained from Panasonic (Osaka, Japan). The food processor was obtained from Panasonic (Osaka, Japan). The Stomacher 400 Circulator Lab Blender was obtained from Seward Limited (West Sussex, United Kingdom). The CIE Lab Chroma Meter CR-400 was supplied by Konica Minolta, INC. (Japan).

3.1.3 Plant Materials

Allium sativum, virgin olive oil and meat were purchased from the local market in Jeli District, Kelantan, Malaysia. A loin section of meat was preferable as it contains less fat. For *Allium sativum*, only firm, crisp, and white colour was chosen as it represents good garlic. Meanwhile, for the type of olive oil used, virgin olive oil was preferable.

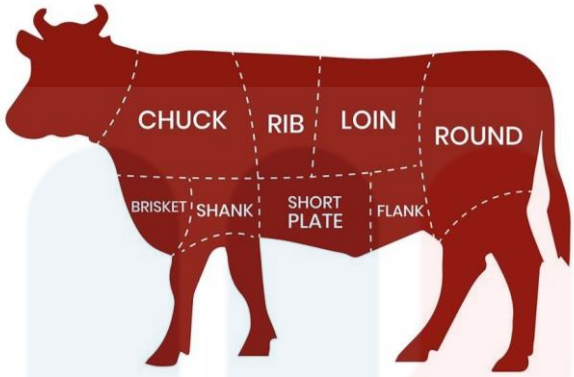

Figure 3: The primal cuts of beef. The loin section is at cow's lower back.

Source: Chang` (2020)

3.2 METHODS

3.2.1 The Extraction of Garlic

To obtain about 500 ml of olive oil infused with concentrated garlic, 95 g of garlic were used. The garlic was peeled using a knife and then was cut into smaller pieces and crushed with a food processor. The crushed garlic was placed in a cooking pot added with 500 ml of virgin olive oil and heated over medium heat (175 ℃) until the garlic turned golden brown. Next, the garlic oil was placed in a closed jar and allowed to cool at room temperature for one hour before being kept in the refrigerator at 4 ℃ (Stewart, 2011).

3.2.2 The Preparation of Meat

Meat samples were obtained from Pasaraya Pantai Timur in Bukit Bunga, Jeli, Kelantan. The meat samples were cut into 12 pieces each weighing 10 g. Each piece of meat sample was put in a different zipper bags. The meat was divided into two groups namely 6 pieces for homogenisation and 6 pieces for colour testing. Based on the method performed by Bintoro et al. (2015), all subsequent samples were introduced with different amounts of garlic oil. The first sample (A) was not coated with garlic oil and acted as a control sample. The second sample (B) was coated with garlic-infused olive oil at 25% of the meat weight. The third sample (C) was coated with garlic-infused olive oil at 50% of the meat weight. The sample was then refrigerated at below 5℃ for 12 days. On Day 6 and Day 12 the sample was homogenized for the quantification of microbial. Day 6 and Day 12 were chosen as it passed the stated storage time for fresh meat which was 5 days (USDA, 2019). The meat samples were supposed to have high bacterial development at the studied time. This was because bacteria grow very quickly within 5 and 60℃, where the bacterial growth curve could be as short as 20 minutes (Karabudak et al., 2008). Consequently, the closer the storage temperature of meat gets to the bacterial growth's optimum temperature, the faster the meat degrades (James & James, 2002).

3.2.3 The Quantification of Microbial

The preparation of the serial dilution for the Plate Count Agar (PCA) method was based on the method by Mancini et al. (2020), each 10 g of the meat was homogenized with 90 ml 0.1% peptone salt solution. The dilution was prepared by transferring 1 ml of the homogenised solution into the test tube with a 9 ml 0.1% peptone salt solution. The dilution factor of 10^{-4} and 10^{-5} was plated 0.1 ml on agar medium. The plating technique was done according to Aryal (2019), by using the spread plate method and incubated at 37 °C for 24 hours.

3.2.4 Colour Change Test

The colour change of the meat was determined by using the CIELAB system. The system describes L^* as lightness, a^* as redness, and b^* as yellowness (Mancini et al., 2020). The colour measurement was done using the CIE Lab Chroma Meter CR-400 with aperture 8 mm or less, illuminant D6 and 0^o angle (Mancini et al., 2020). According to Mancini et al. (2020), total colour change (ΔE) was measured using the formula presented by (Sharma & Bala, 2003). The formula proposed was:

$$\Delta E_{(\alpha-\beta)} = \sqrt{(L^*_\alpha - L^*_\beta)^2 + (a^*_\alpha - a^*_\beta)^2 + (b^*_\alpha - b^*_\beta)^2}$$

where α and β = subscripts L^*, a^* and b^* for same or different storage.

3.3 Statistical Analysis

The analysis of the data was done using Oneway ANOVA to determine the significant difference between means where $p \leq 0.05$ to determine if the hypothesis was accepted or rejected. The calculation was performed using SPSS Software. Duncan test and T-test were also performed. The calculation was tested at a 5% level of significance. The colour change test was expressed in ΔE.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Microbial Quantification

The results for microbial quantification with 10^{-4} dilution factor are shown in Table 4.1. It was found that on Day 6, there was no significant difference ($p \geq 0.05$) between Sample A (Control), and C compared to Sample B showing that certain amount of garlic-infused olive oil reduced the bacterial number. On Day 12, there was a significant difference ($p \leq 0.05$) between Sample A and B compared to Sample C (76.00^{ab}). It was also found that there was no significant difference ($p \geq 0.05$) between microbial count on both Day 6 and Day 12 (refer to APPENDIX A, T-Test).

The microbial counts for meat samples with formulation decreased as the storage time increased at 10^{-4} dilution factor. Samples B had the lowest microbial count (56.33 ± 10.50^{a}) at Day 6 compared to Samples A (89.00 ± 12.49^{b}) and C (85.00 ± 11.36^{b}) which had almost similar microbial counts. The number of microbial increased slightly in Sample B (60.33 ± 2.89^{a}) on Day 12. However, Sample C showed a decrease (76.00 ± 31.23^{ab}) in the microbial count at Day 12 despite having a high number at Day 6 showing that Sample C formulation might inhibit the microbe's growth.

Table 4.1 The microbial quantification of meat sample at 10^{-4} dilution factor at different storage times.

Sample	Storage Time	
	Day 6	**Day 12**
A	89.00 ± 12.49^{b}	106.67 ± 11.06^{b}
B	56.33 ± 10.50^{a}	60.33 ± 2.89^{a}
C	85.00 ± 11.36^{b}	76.00 ± 31.23^{ab}

A: Control; B: Control + 25% garlic-infused olive oil of meat weight; C: Control + 50% garlic-infused olive oil of meat weight. *$^{a-b}$ Each value represented as mean ± SD (n=3). Different letters in same row indicates significant differences at $p \leq 0.05$.

As stated by Sarma (2004) in her study, the garlic extract was able to perform antimicrobial properties on meat since the garlic extract inhibit the *E. coli* and *Salmonella* in chicken meat. In addition, the garlic extract's antibacterial effect lasted for almost 15 days while stored at refrigerator temperature, which was the maximum duration investigated in the study. During preservation at 4°C for 5 and 15 days, the

garlic extract substantially reduced the number of live bacteria. Furthermore, this was due to allicin in garlic and also Tyrosol in olive oil that acted as an antioxidant prevented the lipid oxidation of the meat to slow the meat rancidity (Jalarama Reddy et al., 2015).

Table 4.2 shows the result for the 10^{-5} dilution factor. There was no significant difference ($p \geq 0.05$) between Sample A and Sample B compared to C (6.00^{ab}) on Day 6. It was also found that there was no significant difference ($p \geq 0.05$) between Sample A and C compared to Sample B (17.67^{a}) on Day 12. Thus, this indicated that the formulation abled to reduce the bacterial spoilage of meat. Besides, there was a significant difference ($p \leq 0.05$) between microbial count on both Day 6 and Day 12 (refer to APPENDIX A, T-Test).

The number of microbes increased as storage time increased. The microbial counts increased rapidly in Sample A (7.67 ± 2.52^{b} to 28.33 ± 1.53^{b}), B (4.00 ± 0.00^{a} to 17.67 ± 4.16^{a}), and C (6.00 ± 1.00^{ab} to 25.67 ± 5.03^{b}). This was the same as a study by Mancini et al. (2020), where the garlic and salt formulation did not affect the microbial load. The increase in bacterial count from Day 6 to Day 12 may be due to the meat's deterioration where the garlic extract and olive oil concentration were too low to inhibit the microbes grown on meat (Sarma, 2004).

Table 4.2 The microbial quantification of meat sample at 10^{-5} dilution factor at different storage times.

Sample	Storage Time	
	Day 6	**Day 12**
A	7.67 ± 2.52^{b}	28.33 ± 1.53^{b}
B	4.00 ± 0.00^{a}	17.67 ± 4.16^{a}
C	6.00 ± 1.00^{ab}	25.67 ± 5.03^{b}

A: Control; B: Control + 25% garlic-infused olive oil of meat weight; C: Control + 50% garlic-infused olive oil of meat weight. *[a-b] Each value represented as mean ± SD (n=3). Different letters in same row indicates significant differences at $p \leq 0.05$.

After all, Sample B with 25% of garlic-infused olive oil had the lowest microbial counts among other samples. The amount of garlic oil used appeared to affect microbial growth. In this context, too much garlic-infused olive oil led to higher microbial growth. Thus, a moderate amount should be used depending on the meat weight. Additionally, Sample A had an unpleasant odour starting Day 6 to Day 12 but Sample B and C's odours were still pleasant because of the antioxidant properties of olive oil and garlic. Both garlic and olive oil have anti-microbial properties that can prevent microbial growth. Thus, the garlic-infused olive oil may slow down the deterioration process of the meat sample.

4.2 Colour Change Test

Table 4.3 shows the result of the colour test on the meat sample. There was no significant difference ($p \geq 0.05$) between a^* and b^* compared to L^* (36.2500^b) on Day 6. Meanwhile, there was a significant difference ($p \leq 0.05$) between L^*, a^*, and b^* on Day 12.

From the table, Sample A was lighter (41.98 ± 1.22^b), less red (7.18 ± 0.56^a), and less yellow (5.79 ± 0.17^a) on Day 6 than Day 12. Sample B was lighter (36.84 ± 0.73^c), redder (10.65 ± 0.41^b), and less yellow (6.18 ± 0.15^a) on Day 12 than Day 6. Meanwhile, Sample C was lighter (33.37 ± 0.32^c), less red (5.38 ± 0.23^b), and slightly yellower (5.85 ± 0.12^a) on Day 12.

Table 4.3 L^*, a^*, b^* values of meat sample at different storage times.

	Storage Time					
	D6			**D12**		
Sample	***L****	***a****	***b****	***L****	***a****	***b****
A	41.98 ± 1.22^b	7.18 ± 0.56^a	5.79 ± 0.17^a	35.96 ± 0.20^c	9.28 ± 0.10^b	5.93 ± 0.02^a
B	34.82 ± 1.73^b	5.90 ± 0.25^a	6.91 ± 1.07^a	36.84 ± 0.73^c	10.65 ± 0.41^b	6.18 ± 0.15^a
C	31.95 ± 0.31^b	7.35 ± 0.26^a	5.49 ± 0.049^a	33.37 ± 0.32^c	5.38 ± 0.23^b	5.85 ± 0.12^a

A: Control; B: Control + 25% garlic-infused olive oil of meat weight; C: Control + 50% garlic-infused olive oil of meat weight. L^*: lightness; a^*: redness; b^*: yellowness. $^{a\text{-}c}$ Each value represented as mean ± SD (n=3). Different letters in same row indicates significant differences at $p \leq 0.05$.

Because of protein degradation in the muscle structure of meat, a rise in *L**-values happened since there was not only less myoglobin on the surface of meat samples but also more light dispersion (Warner, 2014). Other than that, redness in meat was the main indicator in purchasing behaviour as it represents fresh meat and was more attractive. The reduction in *a**- values of Sample A and C caused the samples to turn green was because hydrogen peroxide produced from *Leuconostoc* spp. and *Leuconostoc*-like microbes, which oxidised nitrosomyochromogen as a result of exposure to oxygen (Dušková et al., 2013). Thus, the meat samples had low myoglobin concentrations. According to Mitelut et al. (2009), *Pseudomonas aeruginosa* caused the *b**-values in meat to decreased. In this context, decreasing in *b**-values in Sample B indicated *Pseudomonas aeruginosa* might present in the sample. Less oil formulation seemed to be not affective towards those bacteria.

The redness and lightness of both meat samples with garlic-infused olive oil increased as storage time increased indicating that the olive oil was able to conserve the meat colour. The antioxidant properties of olive leaves applied to minced meat was examined by Aouidi et al. (2017). Olive leaves inhibited ($p \leq 0.05$) lipid oxidation and myoglobin oxidation, according to the findings. The olive leaves reduced the thiobarbituric acid reactive substances (TBARS) values resulting in lower myoglobin oxidation (Hayes et al., 2010).

Colour differences (ΔE) quantifies the difference between two colours. In Table 4.4, the colour differences for each sample were observable at a glance. Higher ΔE values imply a large mismatch, while lower ΔE values suggest more precision (View-Sonic, 2021). Sample C with 50% garlic-infused olive oil has the lowest colour difference that was 2.45 signifying that the formulation may prevent higher colour contrast.

Table 4.4 Colour differences (ΔE) within sample and storage time.

Sample	ΔE
A	6.38
B	5.21
C	2.45

A: Control; B: Control + 25% garlic-infused olive oil of meat weight; C: Control + 50% garlic-infused olive oil of meat weight.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

From the result obtained, the null hypothesis (H_0) that indicated the preservation of meat with garlic-infused olive oil did not reduce the bacterial spoilage of meat was accepted for the microbial count at 10^{-4} dilution factor as $p \geq 0.05$ between Day 6 and Day 12 and was rejected for the microbial count at 10^{-5} dilution factor as $p \leq 0.05$ between Day 6 and day 12. The different acceptance in results may due to experimental error where the agar medium or the apparatus may be contaminated before the experiment was done. Aside from that, this study acknowledges that a moderate amount of garlic-infused olive oil in proportion with meat's weight could reduce the bacterial number. Other than that, the colour differences (ΔE) of each sample were detectable at glance but since the values were low enough, the colour contrast might be a bit difficult to distinguish. In this context, the higher the amount of garlic-infused olive oil introduced to meat, the lower the colour contrast. In brief, the formulation that was best

at lowering the microbial number was Sample B which was meat with garlic-infused olive oil 25% of meat weight while Sample C which was meat with garlic-infused oil 50% of meat weight was the best for preserving the meat colour.

5.2 Recommendation

As for the suggestion, further research should be performed in discovering the effectiveness of garlic-infused olive oil in preserving meat. From the research, it was found that the meat with 25% garlic-infused olive oil of its weight seems to be the best formulation in preserving the meat. Since only a moderate amount of garlic-infused olive oil can give the best outcome, more tests can be performed to discover if the amount of the oil and the meat's weight is related to controlling the microbial growth. In addition, the period suggested to perform the test is within 7 days if the meat sample is refrigerated (0-4℃). This is because refrigerated meat tends to deteriorate after a week compared to meat that is kept in the freezer (-18℃).

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APPENDICES

APPENDIX A

Microbial Count: 10^{-4} Dilution Factor

Oneway

Descriptives

Microbial Count Day 6

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
A	3	89.0000	12.49000	7.21110	57.9731	120.0269	75.00	99.00
B	3	56.3333	10.50397	6.06447	30.2400	82.4266	46.00	67.00
C	3	85.0000	11.35782	6.55744	56.7856	113.2144	72.00	93.00
Total	9	76.7778	18.35605	6.11868	62.6681	90.8875	46.00	99.00

ANOVA

ANOVA

Microbial Count Day 6

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1904.889	2	952.444	7.228	.025
Within Groups	790.667	6	131.778		
Total	2695.556	8			

Post Hoc Tests Homogeneous Subsets

Microbial Count Day 6

Duncan[a]

Sample	N	Subset for alpha = 0.05	
		1	2
B	3	56.3333	
C	3		85.0000
A	3		89.0000
Sig.		1.000	.684

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Oneway

Descriptives

Microbial Count Day 12

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
A	3	106.67	11.060	6.386	79.19	134.14	95	117
B	3	60.33	2.887	1.667	53.16	67.50	57	62
C	3	76.00	31.225	18.028	-1.57	153.57	41	101
Total	9	81.00	26.325	8.775	60.76	101.24	41	117

ANOVA

ANOVA

Microbial Count Day 12

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3332.667	2	1666.333	4.521	.063
Within Groups	2211.333	6	368.556		
Total	5544.000	8			

Post Hoc Tests Homogenous Subsets

Microbial Count Day 12

Duncan[a]

Sample	N	Subset for alpha = 0.05	
		1	2
B	3	60.33	
C	3	76.00	76.00
A	3		106.67
Sig.		.356	.098

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Microial Count: 10^{-5} Dilution Factor

Oneway

Descriptives

Microbial Count Day 6

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
A	3	7.67	2.517	1.453	1.42	13.92	5	10
B	3	4.00	.000	.000	4.00	4.00	4	4
C	3	6.00	1.000	.577	3.52	8.48	5	7
Total	9	5.89	2.088	.696	4.28	7.49	4	10

ANOVA

ANOVA

Microbial Count Day 6

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	20.222	2	10.111	4.136	.074
Within Groups	14.667	6	2.444		
Total	34.889	8			

Post Hoc Tests Homogenous Subsets

Microbial Count Day 6

Duncan[a]

Sample	N	Subset for alpha = 0.05	
		1	2
B	3	4.00	
C	3	6.00	6.00
A	3		7.67
Sig.		.168	.240

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Oneway

Descriptives

Microbial Count Day 12

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
A	3	28.33	1.528	.882	24.54	32.13	27	30
B	3	17.67	4.163	2.404	7.32	28.01	13	21
C	3	25.67	5.033	2.906	13.16	38.17	21	31
Total	9	23.89	5.862	1.954	19.38	28.39	13	31

ANOVA

ANOVA

Microbial Count D12

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	184.889	2	92.444	6.163	.035
Within Groups	90.000	6	15.000		
Total	274.889	8			

Post Hoc Tests Homogeneous Subsets

Microbial Count D12

Duncan[a]

Sample	N	Subset for alpha = 0.05	
		1	2
B	3	17.67	
C	3		25.67
A	3		28.33
Sig.		1.000	.431

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Microbial Count Day 6	77.78	9	18.356	6.119
	Microbial Count Day 12	81.00	9	26.325	8.775
Pair 2	Microbial Count Day 6	5.89	9	2.088	.696
	Microbial Count Day 12	23.89	9	5.862	1.954

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Microbial Count Day 6 & Microbial Count Day 12	9	.452	.221
Pair 2	Microbial Count Day 6 & Microbial Count Day 12	9	.489	.182

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Microbial Count Day 6 - Microbial Count Day 12	-4.222	24.345	8.115	-22.936	14.491	-.520	8	.617
Pair 2	Microbial Count Day 6 – Microbial Count Day 12	-18.000	5.172	1.724	-21.976	-14.024	-10.441	8	.000

APPENDIX B

Colour Test

Univariate Analysis of Variance: Day 6

Descriptive Statistics

Descriptive Statistics

Dependent Variable: L*, a*, b* reading on Day 6

Sample	Item	Mean	Std. Deviation	N
A	L	41.9800	1.21655	3
	a	7.1767	.55770	3
	b	5.7933	.17388	3
	Total	18.3167	17.77042	9
B	L	34.8233	1.73451	3
	a	5.9000	.24880	3
	b	6.9133	1.07058	3
	Total	15.8789	14.25214	9
C	L	31.9467	.30860	3
	a	7.3500	.26058	3
	b	5.4867	.04933	3
	Total	14.9278	12.79126	9
Total	L	36.2500	4.60066	9
	a	6.8089	.76191	9
	b	6.0644	.84716	9
	Total	16.3744	14.56447	27

Tests of Between-Subjects Effects

Tests of Between-Subjects Effects

Dependent Variable: L*, a*, b* reading on Day 6

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5502.810[a]	8	687.851	997.947	.000
Intercept	7239.306	1	7239.306	10502.910	.000
Sample	54.996	2	27.498	39.894	.000
Item	5335.503	2	2667.751	3870.420	.000
Sample * Item	112.311	4	28.078	40.736	.000
Error	12.407	18	.689		
Total	12754.523	27			
Corrected Total	5515.217	26			

a. R Squared = .998 (Adjusted R Squared = .997)

Estimated Marginal Means

Sample * Item

Dependent Variable: L*, a*, b* reading on Day 6

Sample	Item	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
A	L	41.980	.479	40.973	42.987
	a	7.177	.479	6.170	8.184
	b	5.793	.479	4.786	6.800
B	L	34.823	.479	33.816	35.830
	a	5.900	.479	4.893	6.907
	b	6.913	.479	5.906	7.920
C	L	31.947	.479	30.940	32.954
	a	7.350	.479	6.343	8.357
	b	5.487	.479	4.480	6.494

Post Hoc Tests Homogeneous Subsets

L*, a*, b* reading on Day 6

Duncan[a,b]

Item	N	Subset 1	Subset 2
b	9	6.0644	
a	9	6.8089	
L	9		36.2500
Sig.		.073	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .689.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Univariate Analysis of Variance: Day 12

Descriptive Statistics

Descriptive Statistics

Dependent Variable: L*, a*, b* reading on Day 12

Sample	Item	Mean	Std. Deviation	N
A	L	35.9600	.19519	3
	a	9.2767	.10116	3
	b	5.9300	.02000	3
	Total	17.0556	14.25263	9
B	L	36.8367	.72597	3
	a	10.6467	.41199	3
	b	6.1767	.15275	3
	Total	17.8867	14.34997	9
C	L	33.3667	.31943	3
	a	5.3800	.23431	3
	b	5.8533	.11719	3
	Total	14.8667	13.87805	9
Total	L	35.3878	1.61513	9
	a	8.4344	2.37877	9
	b	5.9867	.17543	9
	Total	16.6030	13.66788	27

Tests of Between-Subjects Effects

Tests of Between-Subjects Effects

Dependent Variable: L*, a*, b* reading on Day 12

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	4855.202[a]	8	606.900	5813.840	.000
Intercept	7442.776	1	7442.776	71298.548	.000
Sample	43.807	2	21.904	209.827	.000
Item	4790.697	2	2395.349	22946.395	.000
Sample * Item	20.698	4	5.174	49.569	.000
Error	1.879	18	.104		
Total	12299.857	27			
Corrected Total	4857.081	26			

a. R Squared = 1.000 (Adjusted R Squared = .999)

Estimated Marginal Means

Sample * Item

Dependent Variable: L*, a*, b* reading on Day 12

Sample	Item	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
A	L	35.960	.187	35.568	36.352
	a	9.277	.187	8.885	9.669
	b	5.930	.187	5.538	6.322
B	L	36.837	.187	36.445	37.229
	a	10.647	.187	10.255	11.039
	b	6.177	.187	5.785	6.569
C	L	33.367	.187	32.975	33.759
	a	5.380	.187	4.988	5.772
	b	5.853	.187	5.461	6.245

Post Hoc Tests Homogeneous Subsets

L*, a*, b* reading on Day 12

Duncan[a,b]

Item	N	Subset 1	Subset 2	Subset 3
b	9	5.9867		
a	9		8.4344	
L	9			35.3878
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .104.

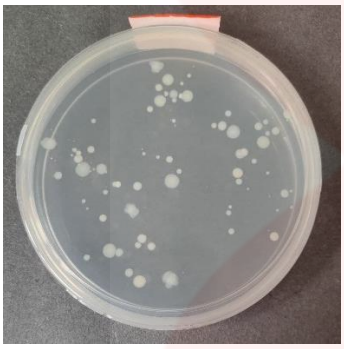
a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

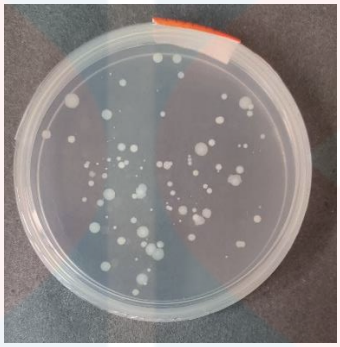
APPENDIX C

Microbial Growth: Day 6

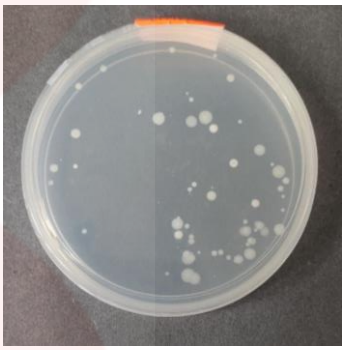
10^{-4} Dilution Factor.



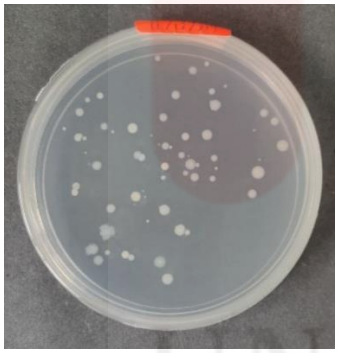
A_1



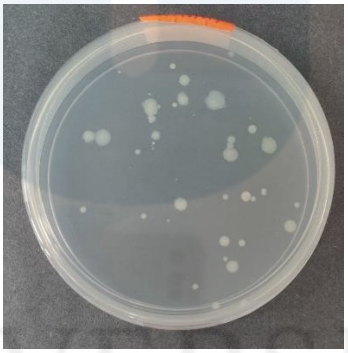
A_2



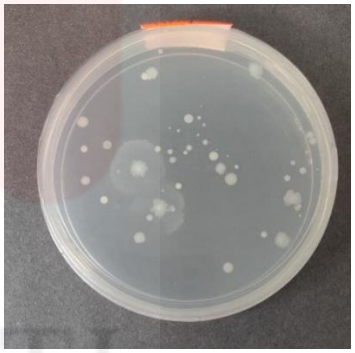
A_3



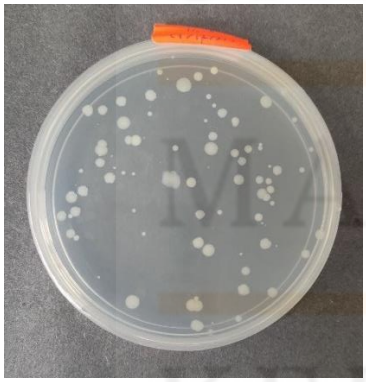
B_1



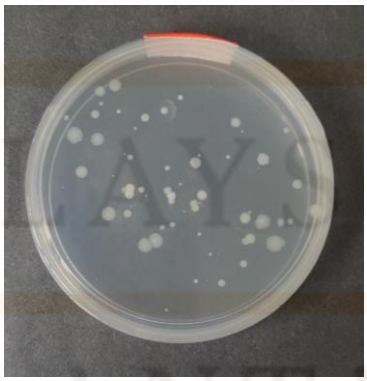
B_2



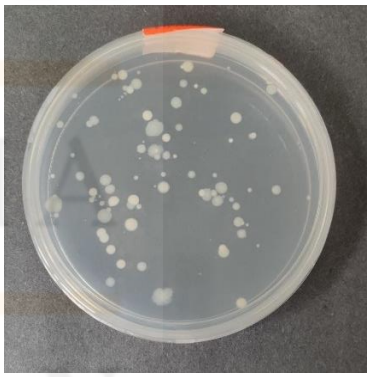
B_3



C_1

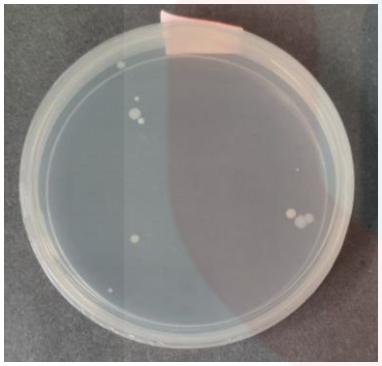


C_2

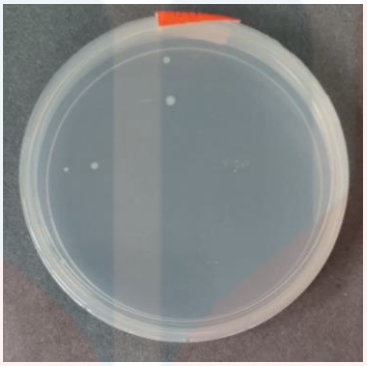


C_3

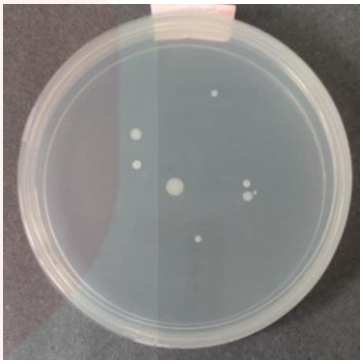
10^{-5} Dilution Factor



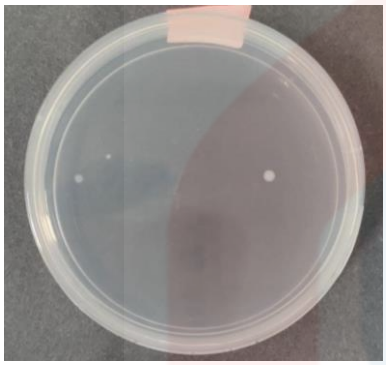
A_1



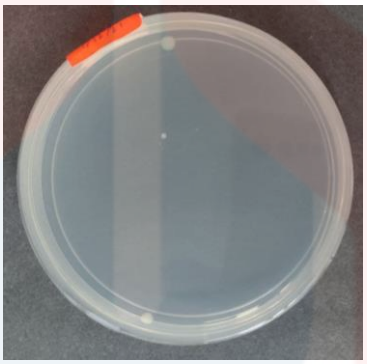
A_2



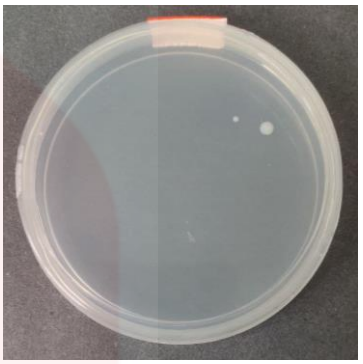
A_3



B_1



B_2



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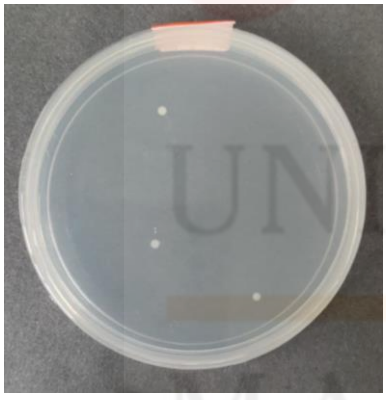
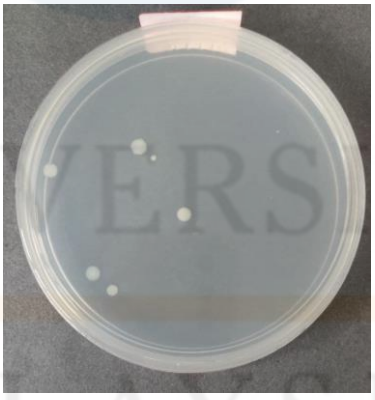


C_1



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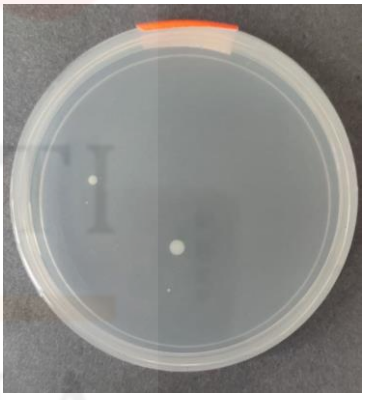


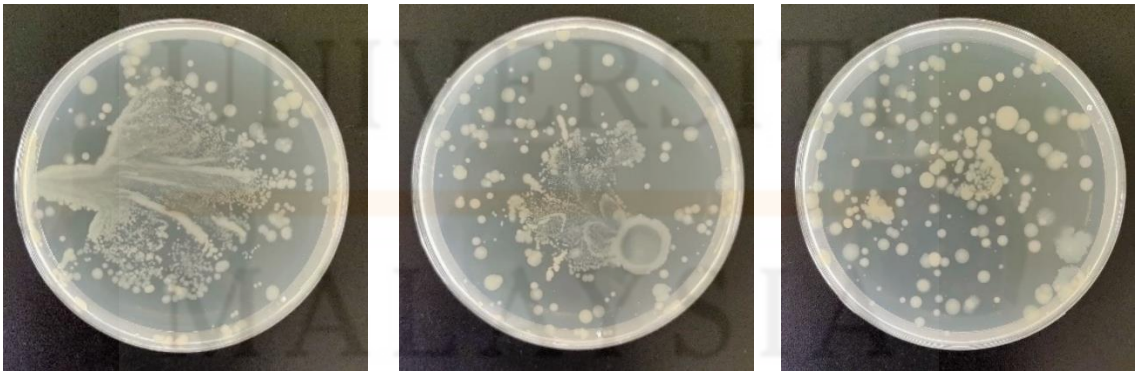
C_3

Microbial Growth: Day 12

10^{-4} Dilution Factor.

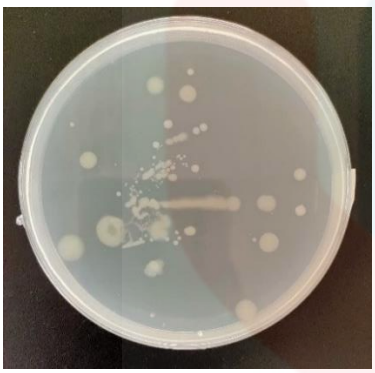
A_1 A_2 A_3

B_1 B_2 B_3



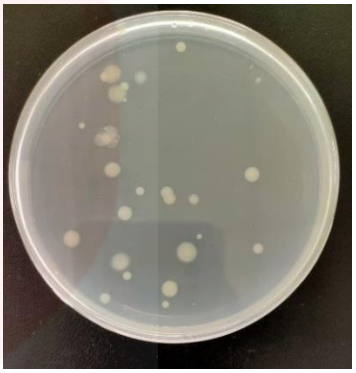
C_1 C_2 C_3

10^{-5} Dilution Factor.



A_1

A_2



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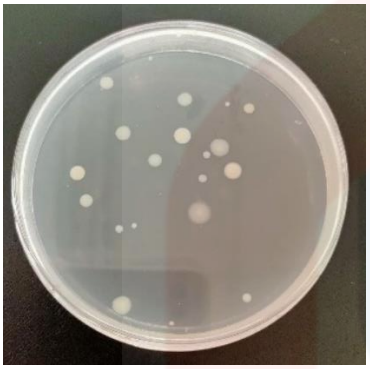

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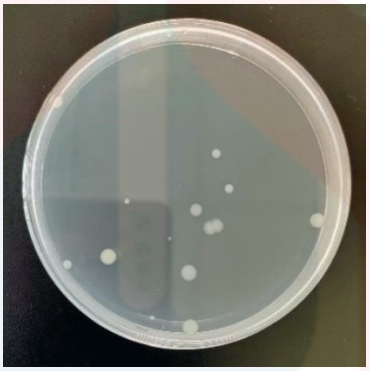
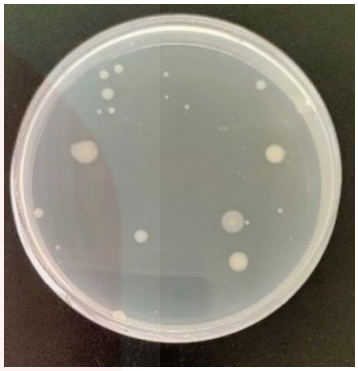


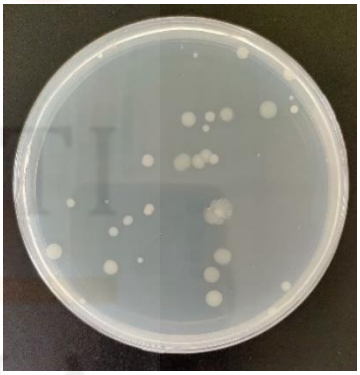
B_2



B_3

C_1

C_2

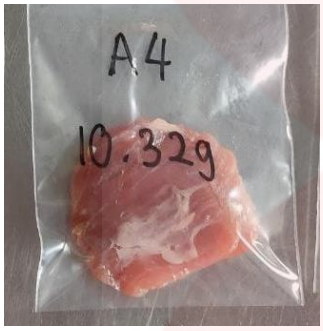


C_3

APPENDIX D

Meat Colour Change (Day 1 and Day 12).

Day 1

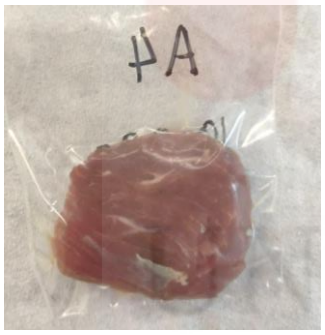


A

B

C

Day 12



A

B

C