Bacterial Assessments on Processed Meat Products sold in Jeli, Kelantan

WONG SHIN SHIAN

F18A0247

A thesis submitted in fulfilment of the requirements for the degree of Bachelor of Applied Science (Product Development Technology) with Honours

Faculty of Agro Based Industry
University Malaysia Kelantan

2022

DECLARATION

I hereby declare that the work embodied in this report is the result of the original research and has not been submitted for a higher degree to any universities or institutions.

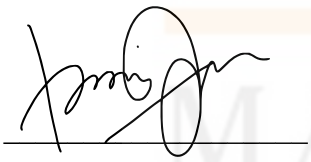
Student

Name: Wong Shin Shian

Date: 27/2/2022

I certify that the report of this final year project entitled "**Bacterial Assessment on Processed Meat Products sold in Jeli, Kelantan**" by Wong Shin Shian, matric number F18A0247 has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Product Development Technology) with Honours, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan.

Approved by:



Supervisor Name: Dr. Khomaizon Abdul Kadir Pahirulzaman
Senior Lecturer
Faculty of AgroBased Industry
Universiti Malaysia Kelantan

Date: 27 February 2022

ACKNOWLEDGMENT

First of all, I would like to express my deepest thankful to my supervisor, Ts. Dr Khomaizon Abdul Kadir Pahirulzaman for her guidance, explanation and encouragement. She patient guiding, teaching lab skill and answer the question that I uncertain. She also sacrifices her time to see our progress. She has helped me with given guidance, encouragement and support in completing final year project. I feel very lucky to have my supervisor who caring much in my work.

Next, I would like to thank to my laboratory assistants, Madam Nor Hidayah Binti Hamzah, Mr. Nik Ahamd Fakruddin Bin Nik Dzulkefli, Mr. Abdul Khaliq Bin Zakar and Mr Abdul Rohimi Bin Mohd Jari for provide assistance and technical support in conducting my experiment. I would also like to thanks Faculty of Agro-Based Industry, Universiti Malaysia Kelantan for providing facilities and supporting my research.

Then I would like to thank my teammate Nur Alia Binti Zakaria for discussing what materials need to be used in the experiments. I would also like to thank my friend Vanessa Carmel Lopez for her encouragement during writing my thesis.

Last but not least, I would thanks to my family member who support me in conducting this research. Especially to my father, Wong Thong Hooi and my mother, Jet Hung Siau who give encouragement though phone calling.

Bacterial Assessments on Processed Meat Products sold in Jeli, Kelantan

ABSTRACT

Salmonella spp., *Staphylococcus aureus* and *Escherichia coli* always detected in beef, poultry, fish and seafood. This study investigate bacterial assessments on processed meat products sold in Jeli, Kelantan. Bacterial from the different ten processed meat products were isolated and identified the possible type of bacteria. The total plate count among the sample, Lekor (unboiled) is highest with 1.2×10^6 cfu/mL followed by Korean Pearl Fish Cake Ball with 9.2×10^5 cfu/mL. Selective media; MacConkey agar, Baird Parker agar and XLD agar were used to isolate *E. coli*, *S. aureus* and *Salmonella* spp. The colonies on selective media isolated from processed meat product were identified by gram staining and biochemical test; catalase test and TSI test. *E. coli* was detected in Korean Pearl Fish Cake Ball, Lekor (unboiled) and Fish cake. Then, *S. aureus* was detected in Surimi scallop, Korean Pearl Fish Cake Ball, Fish ball, Lobster ball, Lekor (unboiled), Fish cake, beef ball and chicken ball. *Salmonella typhi* was detected in Surimi scallop, Fish Ball and Lekor (unboiled). *Salmonella paratyphi* A was detected in Korean Pearl Fish Cake Ball. The presence of *Salmonella* spp., *S. aureus* and *E. coli* found in processed meat product indicate improper hygiene practice in processing and handling.

Keywords: Processed meat product; Bacterial assessment; Total plate count; Pathogen bacterial; Improper hygiene practice

Penilaian Bakteria di Produk Daging Diproses yang dijual di Jeli, Kelantan

ABSTRAK

Salmonella spp., *Staphylococcus aureus* dan *Escherichia coli* sentiasa dikesan di daging lembu, daging ayam, ikan dan makanan laut. Kajian ini dijalankan untuk menyiasat penilaian bakteria di produk daging proses yang dijual di Jeli, Kelantan. Bakteria daripada sepuluh produk daging proses telah diasingkan dan mengenal pasti jenis bakteria yang mungkin. Jumlah kiraan plat di kalangan sampel, Lekor (tanpa rebus) adalah tertinggi dengan 1.2×10^6 cfu/mL diikuti oleh Korean Pearl Fish Cake Ball dengan 9.2×10^5 cfu/mL. Media selective; agar MacConkey, agar Baird Parker dan agar XLD digunakan untuk mengasingkan *E. coli*, *S. aureus* dan *Salmonella* spp. Koloni pada media selective yang diasingkan daripada produk daging proses telah dikenal pasti melalui pewarnaan gram dan ujian biokimia; ujian katalase dan ujian TSI. *E. coli* dikesan di Korean Pearl Fish Cake Ball, Lekor (tanpa rebus) dan Fish cake. Kemudian, *S. aureus* dikesan di kerang Surimi, Bebola Kek Ikan Mutiara Korea, Bebola Ikan, Bebola Lobster, Lekor (tanpa rebus), Kek ikan, Bebola Daging dan Bebola ayam. *Salmonella typhi* dikesan dalam di Surimi, Bebola Ikan dan Lekor (tanpa rebus). *Salmonella paratyphi* A telah dikesan di Korean Pearl Fish Cake Ball. Kehadiran *Salmonella* spp., *S. aureus* dan *E. coli* yang terdapat di produk daging proses menunjukkan amalan kebersihan yang tidak betul dalam pemprosesan dan pengendalian.

Kata kunci: Produk daging proses; Penilaian bakteria; Bakteria patogen; Jumlan kiraan plat; Tidak betul amalan kebersihan

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND SYMBOLS

g	Gram
mL	Milliliter
℃	Degree Celsius
cfu/g	Colony forming unit per Gram
TPC	Total plate count
E. coli	*Escherichia coli*
S. aureus	*Staphylococcus aureus*
XLD agar	Xylose Lysine Deoxycholate agar
TNTC	Too numerous to count
RTC	Ready to cook
RTE	Ready to eat
HACCP	Hazard Analysis Critical Control Point
GMP	Good Manufacturing Practice

CHAPTER 1

INTRODUCTION

1.1 Research Background

Nowadays, various types processed meat products have sold in groceries, such as sausage, nugget, meatball, fish ball, burger patties, and ham. The processed meat product that are sold in groceries usually package in sterile plastic packaging and in can packaging. Processed meat product is a convenience product. Consumers prepare the processed meat product based on the instructions stated on the packaging.

Besides, the processed meat product such as nugget, sausage can also be served as dishes in the buffet because processed meat is ready-made and cook within minutes. Processed meat product also be taken during breakfast, as a snack and also as sandwich filling.

The meat-borne illness also known as foodborne illness. This form of illness is caused by consuming contaminated meat. Meat-borne illnesses have become a public health concern in the developing country include Malaysia (Abdullahi et al., 2016). Meat is easy to cause the growth of microorganism due to abundant nutrition (Subratty, 2003). Foodborne disease symptoms include vomiting, diarrhea, nausea, abdominal pain, cramps and other severe symptoms that may cause permanent damage and threaten human life.

Processed meat product could be contaminated from meat itself, ingredient preparation, processing environment and equipment and the handlers (Melngaile et al., 2014). The lack of personal hygiene food handler is the most common reported factor that cause foodborne illness (Ain Auzureen et al., 2017).

Microbial contamination in process meat product is the main concern in processed meat product quality and safety (Zerabruk et al., 2019). The microorganism in contaminated meat product is identified as hygiene indicator bacteria and pathogen bacteria. The hygiene indicator bacteria are coliforms and *Escherichia coli* while pathogen bacteria are *Salmonella* spp. and *Staphylococcus aureus* (Melngaile et al., 2014).

This study was carried out to investigate bacterial assessments on processed meat products sold in Jeli, Kelantan. The results from this research provide information on the quality and safety of processed meat product whether it is safe for consumer around Jeli, Kelantan.

1.2 Problem Statement

Meat is one of the most common foods for foodborne illness outbreaks. Improper food handling, lack of hygiene in meat product processing, unclean of environment and equipment, and the use of contaminated meat can contribute to the contamination of processed meat products. Processed meat products can contaminated if not concern about the processing method requirements such as improper temperature, improper storage and transportation.

The processed meat products from groceries around Jeli, Kelantan were chosen to conduct the bacterial assessment. The bacterial assessment includes determining the number of bacterial counts and identifying types of bacteria isolated from processed meat products. The number of microbial counts determines whether the amount of bacteria in processed meat products exceeded the maximum limit. The identification of the types of bacterial content in processed meat products can predict the effect of bacterial contamination in processed meat products from groceries in Jeli, Kelantan towards the consumers.

1.3 Hypothesis

H_0: The number of bacterial counts on processed meat products in groceries sold at Jeli, Kelantan is at acceptable amount, $\leq 10^6$ cfu/g.

H_1: The number of bacterial counts on processed meat products in groceries around Kelantan is higher than the recommended maximum limit, $>10^6$ cfu/g.

1.4 Significance of study

There are several significances in this study. Firstly is to ensure the hygiene quality of processed meat products determined by microbiological count. Then, to identify type of bacteria isolated from processed meat product. Though identifying the type of bacteria isolated from processed meat product can predict the risk to consumers of microbial contamination found in processed meat products from groceries in Jeli, Kelantan.

1.5 Objective

This study was carried out in order to:

1) Determine the bacterial count in processed meat products from groceries around Jeli, Kelantan.
2) Identify types of bacterial isolated from the processed meat products.

CHAPTER 2

LITERATURE REVIEW

2.1 Processed Meat Product

Processed meat product is source of protein in human diet with abundant nutrients for physiological stability and biochemical (Badar et al., 2021). The abundant nutrient in processed meat product include essential amino acids, fatty acid, soluble mineral and vitamins. Processed meat product can be directly consumed or cooked in a short time before consumption.

Processed meat products made from beef, chicken, fish, seafood, pork and lamb. The process in making processed meat product in industry include grinding meat, mixing meat with ingredient, molding, partial thermal processing and quick freezing (Krasulya et al., 2020).

Firstly, the meat is grinded. Then, salt and water added in mixing with minced meat. Salt help produce flavour and also help soluble myofibrils protein required to bind water and fat (Lonergan et al., 2019b). A suitable amount of preservative such as sodium nitrate or potassium nitrate added to ensure processed meat product have a longer shelf life. Then, molding into the shape and undergo partial thermal processing. Next, the processed meat product are quick freezing in freezing tunnel. Processed meat products are stored at -18℃ or below to prevent the growth of spoilage microorganisms and maintain quality and freshness (Shamimuzzaman et al., 2022).

2.2 Types of processed meat product

Processed meat products classify into two types, ready to cook (RTC) and ready to eat (RTE) processed meat products (Temgire et al., 2021). Ready to cook meat products are require short time thermal process before consume. Thermal processing such as boiling, frying, grilling and baking. RTC meat products usually are frozen product such as meatball, nugget, fish ball, lobster ball.

While for ready-to-eat processed meat product the meat product can be eaten directly without any thermal processing or require short time thermal process before consumption. RTE meat products are usually packed in canned or vacuum packed, for

canned packaging such as luncheon meat, while vacuum-packed are usually dried type of slides meat such as jerky.

2.3 Foodborne disease caused by processed meat product

Foodborne disease is the most concern in every country, including Malaysia. The most common foods for foodborne disease outbreaks are the food of animal origin, especially beef, poultry and fish (Abdullahi et al., 2016). There is also a strong relation between meat consumption and foodborne disease occur (Abdullahi et al., 2016). Meat is easy growth of microorganisms due to the higher content of nutrient and water activity (Odeyemi et al., 2020).

Poor hygiene food handling process and lack of cleanliness in premises are the main factors that cause foodborne disease in Malaysia, which involves more than 50 % of poisoning events (Soon et al., 2020). Hand washing and using face masks are the least practised habit between food handlers in Malaysia (Ain Auzureen et al., 2017). The moisture and hot climate in Malaysia are factors also for the growth of microorganisms (Abdul-Mutalib et al., 2015).

2.4 Pathogen on processed meat product

The pathogens on processed meat product are foodborne pathogens. The most critical foodborne pathogens related to meat are *Salmonella* spp., *Staphylococcus aureus*, *Escherichia coli*, *Campylobacter jejuni*, *Listeria monocytogenes*, *Clostridium perfringes*, *Yersinia enterocolitica*, and *Aeromonas hydrophila* (Bhandare et al., 2007). The beef, poultry, fish and seafood always detected with the presence of pathogen such as *Salmonella* spp., *S. aureus* and *E. coli* (Pal et al., 2018; Anbudhasan et al., 2012)

The processed meat product is bedding growth of microorganisms including pathogens. The pathogen such as *Salmonella* spp., *S. aureus*, *E. coli* and *Campylobacter* spp. can multiply in human body and cause illness (Pal et al., 2018). The pathogen found in processed meat products causes by unhygienic processing conditions and food handling (Pal et al., 2018). The pathogen on processed meat product should not exist or exceed the microbial limitation since it can cause gastrointestinal disease and will threaten life in a severe situation.

2.4.1 *Salmonella* spp.

Salmonella spp. is rod-shaped gram-negative microbial which is under the Enterobacteriaceae family. It is facultative anaerobe, able grow with and without oxygen.

The food product can be source of *Salmonella* spp. included seafood, poultry, eggs, beef and pork (Shafini et al., 2017). *Salmonella* spp. is hide in gastrointestinal tract of animals without any clinical sign (USDA, 2020; Meyer et al. , 2010). It causes foodborne disease through infection. The infection occurs through growing in the small intestine and attack intestinal tissue and produce enterotoxin and cause diarrhea (Bell & Kyriakides, 2009).

Salmonella spp. on raw meat is small amount. However the temperature for chilling, storage, or transport more than 7 ℃ causes the multiplication of *Salmonella* spp. (Mendonca et al., 2020). Improper handling, insufficient cooking and cross contamination of raw meat to end product of processed meat product are the factor contaminated by *Salmonella* spp..

2.4.3 *Escherichia coli*

Escherichia coli is a gram-negative, facultatively rod-shaped microbial under the Enterobacteriaceae family member. *E. coli* can grow at temperature 7- 45 ℃ (Ekici & Dümen, 2019).

E. coli presence in food indicates the food is contaminated fecal and not hygiene (Ekici & Dümen, 2019). *E. coli* is also known as faecal coliform because it is related to the colon of warm-blooded animals (Halkman & Halkman, 2014). Improper food handling in processing meat products is the cause of *E. coli* presence in processed meat products. Improper food handling includes not wearing gloves in processing processed meat product and not washing hands after contact with raw meat.

2.4.2 *Staphylococcus aureus*

Staphylococcus aureus is a round-shaped gram-positive microbial under the Firmicutes group. It can grow at a temperature between 7 ℃ to 48.5 ℃, the optimum temperature at 30 ℃ to 37 ℃. It able survive in dry and stressful environment such as skin and non-living surface like surface of equipment (Kadariya et al., 2014).

S. aureus cause human superficial skin infection and food poisoning through production of enterotoxins and other super antigens (Liu, 2015). *S. aureus* can survive on human's skin, nostrils and throat (Mendonca et al., 2020). The food handler does not wear gloves, masks or aprons and touch their nose and face will transmit *S. aureus* into processed meat product. The gloves can be source of contamination if there is no frequently change glove or improper hand washing before wearing glove (Kadariya et al., 2014). Due to glove provide moistly and warn environment suitable *S. aureus* stain on

hand for growth. The symptom of food poisoning included vomiting, nausea and abdominal cramps with diarrhea or not (Kadariya et al., 2014).

2.5 Factors causing processed meat product contamination

In processing processed meat products, the hygiene from raw materials used, environment, equipment used, and food handling could affect the quality and safety of finished products of processed meat products. The processed meat product industry should comply with Hazard Analysis Critical Control Point (HACCP) system in meat industry.

Implement a HACCP system in processed meat product to avoid microbial contamination and ensure food safety with scientific principles (Lonergan et al., 2019). HACCP guidelines for meat safety include refrigerating or freezing meat, separating raw meat from other materials, cleaning equipment at all times and working surfaces after touching raw meat to prevent cross-contamination (Lonergan et al., 2019).

2.5.1 Meat product processing

Meat product processing includes slaughtering animals, cutting meat and processing meat into meat products. However, compliance with food safety requirements in processing meat is insufficient (Abdullahi et al., 2016). The contaminated processed meat product could cause by poor hygiene food handling, equipment and environment processing, poor personal hygiene food handler and lack of education given to food handler.

Proper hand washing is important, and it can decrease transmission of the pathogen from hands to food or other things (Green et al., 2007). Proper hand washing can prevent many microbial contaminations (Kadariya et al., 2014). Before starting in food preparation and handling raw meat, must wash hands properly to avoid contamination. Gloves need to be changed frequently to prevent cross-contamination sources of microbial (Kadariya et al., 2014). Wearing glove without proper handwashing can also lead to microbial contamination inside and outside of gloves (Kadariya et al., 2014).

2.5.2 Temperature

The bacterial pathogen presence in raw meat and meat product includes *Salmonella* spp., *S. aureus,* and *E. coli*. *Salmonella* spp., *S. aureus* and *E. coli* are mesophilic pathogen. The mesophilic pathogen can grow at 10- 45 ℃, moderate temperature (Lonergan et al., 2019). Temperature for cooking the meat product before quick freeze is also important to kill the microbial. The temperature storage of frozen processed meat product at -18 ℃ to prevent microbial pathogen growth. The temperature at which bacterial cannot grow, *E .coli* at below 8 ℃, *Salmonella* spp. at below 5.2 ℃ and *S. aureus* at below 5 ℃ (Kotzekidou, 2016 & Kadariya et al., 2014). While for temperature cooking meat suggest at above 76.7 ℃ to kill microbial pathogen (Lonergan et al., 2019).

2.5.3 Storage and shipping

After finish processing the meat product, the processed meat product store at – 18 ℃ in cold food storage. The cold food storage should keep at -18 ℃ to prevent microbial pathogen growth.

During the transportation of processed meat product to wholesale or retail display, the refrigerated truck must be maintained the temperature at -18 ℃. The refrigerated truck in distribution must have good refrigerated system that able to maintain the required temperature of processed meat product. Maintain the temperature during transportation to avoid microbial spoilage growth and ensure the freshness of processed meat product.

2.6 Microbial Count

The total plate count is also known as aerobic plate count (APC) or standard plate count. Total plate count used to estimate the number of bacteria in food sample (Mendonca et al., 2020). The number of bacteria growth is the bacteria that can grow at mesophilic temperature. The result of the total plate count indicated the hygienic quality of raw materials, processing and storage condition and handling of the finished product. Total plate count is also used to determine the hygiene quality and Good Manufacturing Practices (GMP) compliance. The total plate count exceed 10^6 cfu/mL indicate that the food lacks hygiene quality and microbial growth (Mendonca et al., 2020).

However, the low total plate count no indicates the absence of pathogen bacteria in food product. The unusually high total plate count can be affected by fermented foods such as yoghurt.

2.7 Microbial Isolation

The colonies on selective media are pathogen bacteria isolated from processed meat product. By observing colony morphology on selective media and compared with control plate, it is possible to know which bacteria have been isolated from processed meat product. The selective media used included MacConkey agar, Baird Parker agar and XLD agar.

2.7.1 MacConkey agar

MacConkey agar not only selects for the growth of gram negative bacteria by inhibiting the growth of gram positive bacteria, but also differentiate gram negative by lactose fermentation (Jung & Hoilat, 2021). The lactose fermenter gram negative bacterial form pink colonies. Lactose fermentation produce organic acid lower the pH of agar and change the pH indicator (neutral red) to pink. *E. coli* is lactose fermenter gram negative bacteria. For non-lactose fermenter gram negative bacteria form white opaque colonies due to no change the pH of agar.

2.7.2 Baird Parker agar

Baird Parker agar used to isolate Staphylococci in food product or other material. Lithium and tellurite contain in agar to inhibit contaminate mircoflora, glycine and pyruvate promote the growth of Staphylococci (Jvo Siegrist, n.d.). The colonies of staphylococci form white to grey colour colonies on Baird Parker agar (HiMedia, 2019).

2.7.3 XLD agar

Xylose Lysine Deoxycholate, XLD agar primary used to isolate *Salmonella* spp. and differentiate *Salmonella* spp. with the characteristics included production of hydrogen sulfide and non-lactose fermenter. Sodium deoxycholate content in XLD agar inhibit the gram positive bacteria (Aryal, 2021). The breakdown of xylose, lactose and sucrose produce acid product cause the red colour of medium change to yellow colour. The colour of the majority of *Salmonella* spp. colonies between clear to pink or red (Mikoleit, 2014). *Salmonella typhi* colonies form clear colonies without or with black centre.

2.8 Microbial Identification

The colonies on selective media isolated from processed meat product are identified by gram staining and biochemical test include catalase test and TSI test.

2.8.1 Gram Staining

Gram staining is primary test to classify the characteristic of bacterial. There are four steps of gram staining, primary stain (crystal violet) to heat-fixed bacterial colony smear, add Gram Iodine as mordant, decolorized with 95% Ethyl Alcohol and counterstain with safranin (Thairu et al., 2014).

The gram staining divide bacteria into two groups, gram positive and gram negative. Gram positive bacteria stain purple while gram negative bacteria stain pink. The stain purple or pink depend on cell wall of bacteria. The cell wall of gram positive bacteria consist of thick layers of peptidoglycan while gram negative bacteria consist of thin layers of peptidoglycan and high lipid content.

The crystal violet in an aqueous solution separate into CV^+ and CI^- ions and penetrate the cell wall and membrane. The cell was stained purple due to CV^+ interact with negatively charged components of bacterial cell. Then the iodine added, I^- or I_3^- interacts with CV^+ to create large CVI complexes between cytoplasm and outer layer of cell. The 95% ethyl alcohol added to decolorize though interact with lipid membranes. After decolorization with 95% ethyl alcohol, the gram negative cell's cell wall become leaky and most of the CVI complexes washed from cell. For the gram positive cell, the multilayer of peptidoglycan dehydrate and traps most CVI complexes in cell. Finally is counterstain with safranin, the gram negative cell changed to pink and gram positive cell remain purple.

2.8.2 Biochemical test

a. Catalase test

Catalase test used to detect the enzyme catalase in bacteria. The bacterial colony rapid elaboration of oxygen bubbles that indicates presence of catalase enzyme in the bacterial colony. The catalase accelerate the breakdown of hydrogen peroxide, H_2O_2 into water and oxygen. The chemical reaction breakdown of hydrogen peroxide:

$$2H_2O_2 + \text{Catalase} \rightarrow 2H_2O + O_2.$$

b. TSI test

Embden-Meyerhof-Parnas (EMP) pathway is involved in aerobic reaction in slant and anaerobic reactions in medium to generate to ATP and pyruvate (Lehman, 2000). The EMP pathway is the process of bacteria metabolic utilization of glucose to generate ATP and biosynthetic precursors include pyruvate (Peretó, 2011). The glucose fermentation produce acid, the pH decrease and change the colour indicator phenol red to yellow colour (Wanger et al., 2017). The aerobic reaction in slant, the pyruvate continue metabolized to CO_2, H_2O and energy. After 18 hours of incubation, the glucose was completely consumed, followed by the metabolism of lactose or/and sucrose, and the acidity slant remained unchanged. For the bacteria does not use lactose or sucrose, peptone was used as source energy. The peptone used cause ammonia, NH_3 release, the pH increase and change from yellow colour slant to red colour. In medium, the bacteria convert pyruvate to acetyl CoA, therefore the medium remain acid.

The bubble in medium and displacement of medium indicates gas production due to glucose fermentation. During glucose fermentation, CO_2 gas was produced. The CO_2 gas production splinted the agar and large amount of gas cause the displacement of agar.

The blackening of the medium indicates hydrogen sulfide, H_2S production. The H_2S production requires an acidic environment. The bacteria produce H_2S from sodium thiosulfate and react with ferric ammonium citrate to form ferrous sulfide, $FeSO_4$ as black precipitate.

CHAPTER 3

MATERIAL AND METHOD

3.1 Material

The samples used were Surimi Scallop, Korean Pearl Fish Cake Ball, Lekor (boiled), Lekor (unboiled), fish ball, lobster ball, fish cake, beef ball, chicken ball and chicken nugget.

The chemicals used were peptone powder (Bacto™ Peptone), sodium hydroxide, NaOH pellets (R&M Chemical), potassium dihydrogen phosphate, KH_2PO_4 powder (HmbG®), nutrient agar powder (HIMEDIA®), MacConkey agar powder (HIMEDIA®), Baird Parker agar powder (Merck) and Xylose lysine deoxycholate (XLD) agar powder (Merck).

The materials used were aluminum foil (Diamond), Parafilm M PM999 All-Purpose laboratory film, 15 mL centrifuge tubes (Falcon™ & Biologix®), 70% ethanol, stomacher bag, petri dishes, paper towel roll, A4 paper, black colour manila card.

The apparatus used were 1000 mL media bottle, 500 mL media bottle, 250 mL media bottle, 100 mL beakers, 100 mL & 10 mL measuring cylinders, pipette, Bunsen burner, lighter, Bunsen burner tripod stand and gauze, hockey stick, spatula, 10 mL test tube with caps and test tube racks.

3.2 Equipment

The equipment used were autoclave machine (Hirayama), stomacher (BagMixer®), laminar air flow cabinet (Azteclab), colony counter (Funke Gerber), 37 °C incubator (Lab Companion) and microscope (Leica).

3.3 Methodology

3.3.1 Sample Collection

Ten types of samples were from the grocery store, Jeli, Kelantan. There are three types of samples (beef ball, chicken ball and chicken nugget) with labelled Halal, GMP, HACCP logo on packaging. Seven types of sample (Surimi Scallop, Korean Pearl Fish Cake Ball, Fish Ball, Lobster Ball, Lekor (boiled), Lekor (unboiled) and fish cake) without labelled Halal, GMP, HACCP logo on packaging.

3.3.2 Growth Media, Buffer and Peptone Water Preparation

a. Nutrient Agar

28 g of nutrient agar powder was suspended in 800 mL distilled water in media bottle and was filled until 1 L with distilled water. The mixture was shake to completely dissolve all nutrient agar powder. The dissolved mixture was sterilized by autoclave at

121 ℃ for 15 minutes. Then, the nutrient agar was poured into plates in laminar air flow, and left to solidify. Media was freshly used or stored at 4 ℃ for future use.

b. MacConkey Agar

24.77 g of MacConkey agar powder was suspended in 300 mL distilled water in media bottle and was filled until 500 mL with distilled water. The mixture was shake to completely dissolve all MacConkey agar powder. The dissolved mixture was sterilized by autoclave at 121 ℃ for 15 minutes. Then, MacConkey agar was poured into plates in laminar air flow, and left to solidify. Media was freshly used or stored at 4 ℃ for future use.

c. Baird Parker Agar

29 g of Baird Parker agar powder was suspended in 275 mL distilled water in media bottle and was filled until 400 mL with distilled water. The mixture was shake to completely dissolve all Baird Parker agar powder. The dissolved mixture was sterilized by autoclave at 121 ℃ for 15 minutes. Then, Baird Parker agar was poured into plates of

petri dish in laminar air flow, and left to solidify. Media was freshly used or stored at 4 ℃ for future use.

d. XLD Agar

27.5 g of XLD agar powder was suspended in 300 mL distilled water in media bottle and was filled until 500 mL with distilled water. The media bottle placed in beaker filled with water and heated. The mixture was heated until there are small bubble. Then, XLD agar was poured into plates in laminar air flow, and left to solidify. Media was freshly used or stored at 4 ℃ for future use.

e. TSI Slant

9.29 g of triple sugar iron powder was mixed with 143 mL distilled water and boiling until in thick texture. Then, poured into test tube with beaker and sterilized by autoclave at 121 ℃ for 15 minutes. After the test tube was placed at an incline, TSI slant was formed in the test tube.

f. Preparation phosphate buffered solution

The 0.2 M KH_2PO_4 solution was prepared by dissolving 27.22 g of KH_2PO_4 powder in 1 L distilled water. Whereas 0.2 M NaOH solution was prepared by dissolving 8 g of NaOH pellet in 1 L distilled water.

225 mL of phosphate buffer solution was prepare in media bottle by adding 56.25 mL of KH_2PO_4 solution, 33.33 mL of NaOH solution, and 135.45 mL of distilled water. Then, the phosphate buffer solution was sterilized by autoclave at 121 ℃ for 15 minutes.

g. Preparation peptone water

The 1 % peptone water was prepared by dissolving 0.4 g of peptone powder in 400 mL distilled water. The peptone water and a 100 mL beaker were sterilized by autoclave at 121 ℃ for 15 minutes. Then, 9 ml of peptone water was poured into falcon tube with using sterile beaker.

3.3.3 Sample Preparation

The frozen processed meat products were thawed overnight at 4 ℃ in chiller. 25 g of each sample were weighted aseptically with sterile spoon and transferred into sterile stomacher bag. Then, 225 ml of phosphate-buffered solution was added to dilute sample to 10^{-1} dilution and homogenized using stomacher (BagMixer®) for 2 minutes (Mohamed et al., 2017). After homogenization, serial dilution was carried out. The ten-fold serial dilutions of each sample were carried out in peptone water up to 10^{-4}.

3.3.4 Total Plate Count by Spread Plate Method

The spread plate method was used to calculate of bacterial isolated from all samples. 0.1 mL of each dilution from section 3.3.3 was spread onto nutrient agar by using a sterile hockey stick. All plates were incubated for 24 hours at 37 ℃.

a. Counting colony

After incubated for 24 hours, colonies on nutrient agar were counted using colony counter (Funke Gerber). Results were reported according to the FDA Guidelines for Aerobic Colony Counting (FDA, 2001):

1. Normal plates (25-250): The spreader-free plate was selected to calculate colony forming units (cfu). cfu including pinpoint size on selected plate. The dilution used was recorded and total number of colonies counted were counted.

2. Plates with more than 250 colonies: The number cfu per plate more than 250 for all dilutions was recorded as too numerous to count (TNTC).

3. Spreaders:

   There are three different types of spreading colonies including: 1) a chain of colonies with not too separated obviously caused by disintegration of a bacterial clump. 2) A water film formed between agar and bottom of plate, and 3) A water film that forms on the edge or surface of agar.

   The plates prepared from sample consist extra spreader growth including (a) Area covered by spreaders include total area inhibiting growth, more than 50 % of plate area or (b) Area of inhibit growth more than 25% of plate area are reported as spreader.

   When required to count the plate with contain spreaders not eliminated by (a) or (b), each of three different spreader types was counted as one source. For Type 1

spreader, if contain only one chain counted as a single colony. One or more chains appear from separate sources, calculate each source as one colony. Each individual growth in the chain does not count as a separate colony. Types 2 and 3 result in different colonies and were counted. The spreader count was combined with the colony count to calculate the total plate count.

4. Plates with no cfu: The plates from all dilutions no colonies was reported as total plate count less than 1 times relevant lowest dilution used. The calculated total plate count was marked with asterisk to represent it was estimated from counts outside the range 25-250 per plate. The plate from a sample was known to be contaminated or dissatisfied, the result was recorded as laboratory accident (LA).

b. Total Plate Count, cfu/mL

The dilution yield the highest colonies in between range 25-250 cfu on nutrient agar was choose and multiplied by dilution factor, then divided by 0.1 mL dilution volume to get the cfu/mL. At the below show the formula cfu/mL:

Formula cfu/mL

$$\text{cfu/mL} = \frac{\text{Number of colonies} \times \text{Dilution factor}}{\text{Volume of culture plated in mL}}$$

$$= \frac{\text{Number of colonies} \times \left(\frac{1}{\text{Dilution}}\right)}{\text{Volume of culture plated in mL}}$$

3.3.5 Bacterial Identification

0.1 mL of sample prepared in Section 3.3.3 was spread onto selective agar media; MacConkey agar, Baird Parker agar and XLD agar. Plates were incubated for 24 hours at 37 ℃. Results were recorded on based on the morphological characteristics of bacteria growth on the selective media. The pink colonies on MacConkey agar indicate the presence of *E. coli*. The white colonies on Baird Parker agar indicate presence of *S. aureus*. The yellow colonies on XLD agar indicate the presence of *Salmonella* spp.

3.3.6 Gram Staining

A smear of bacterial sample was prepared on a clean glass slide. The sample was air-dried and heat fixed. Crystal violet was dropped onto the fixed sample with using dropper and left for 1 minutes before rinsing with distilled water. Iodine solution was dropped on the sample, left for 1 minutes and rinsed with distilled water. Then, it was washed with acetone for 30 seconds followed by rinsing with distilled water. Finally, safranin was added to the sample for 1 minutes, rinsed with distilled water and blotted dry with paper towel. The morphological characteristics of the bacterial sample was observed using microscope (Leica) under 100 x magnification. Results was reported and based on the characteristics; shape and colour.

3.3.7 Biochemical Test

a. Catalase Test

A small bacterial colony was transferred using a sterilized wire loop onto a clean glass slide. A drop of 3 % hydrogen peroxide, H_2O_2 was placed onto bacterial colony. Results were recorded based on the rapid elaboration of oxygen bubbles that indicate presence of catalase enzymes in the bacterial colony.

b. Triple Sugar Iron (TSI) Test

The centre of a well-isolated bacterial colony was touched using a sterile inoculating loop. It was then stabbed through to the bottom of TSI slant. The stab culture was loosely capped and incubated at 37 ℃ for 24 hours. Results were recorded based on the following criteria:

- Yellow slant: indicate acid reaction due to high reduction of acid from glucose.
- Red slant: indicate alkaline reaction due to low production of acid from glucose.
- Blackening of the medium: indicate hydrogen sulfide, H_2S production.

- Bubbles, cracks or displacement of the medium: indicates gas production by the organism due to glucose fermentation.
- No changes on medium: indicates the organism is not capable in fermenting glucose.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Classification sample into group

a. Small Medium industry & Large industry

Table 4.1 shows processed meat product from small medium industry and large industry. Processed meat product from small medium industry without Halal, Hazard Analysis Critical Control Point (HACCP), Good manufacturing practices (GMP) certified logos on package and home based industry, while processed meat product from large industry with Halal, HACCP, GMP certified logo on package.

Table 4.1: Processed meat product from small medium industry & large industry

Small medium industry	Large industry
Surimi scallop	Beef ball
Korean Pearl Fish Cake Ball	Chicken ball
Lekor (boiled)	Chicken nugget
Fish ball	
Lobster ball	
Lekor (unboiled)	
Fish cake	

Halal certification means processing food products from the raw material used, manufacturing, storage, and distribution fulfilling Syariah law and hygienic condition, and food products permissible for Muslim consumption (Ab Talib, 2017). For GMP certification, the manufacturer has implemented guidelines to ensure that food processing is safe and hygienic (Abdullah Sani & Dahlan, 2015). The HACCP certification means the manufacturer follows the guidelines for processing operation and determine parameters for safe and hygiene food processing, maintenance and troubleshooting procedures (Abdullah Sani & Dahlan, 2015).

b. Ready to eat, RTE & Ready to cook, RTC

Table 4.2 shows RTE & RTC processed meat product. Ready to eat (RTE) processed meat product is meat product that has been processed, cooked and frozen, before consumption requires a short time preparation, such as boiling or reheating (Baskaran et al., 2017). Ready to cook processed meat product undergo processing, partial cooking and freezing at -18 ℃ (Shamimuzzaman et al., 2022). Before consumption, RTC processed meat product require thermal process such as boiling or frying.

Table 4.2: RTE & RTC processed meat product

RTE	**RTC**
Lekor (boiled)	Surimi scallop
Fish ball	Korean Pearl Fish Cake Ball
Beef ball	Lobster ball
Chicken ball	Lekor (unboiled)
	Fish cake
	Chicken nugget

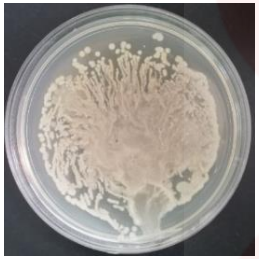
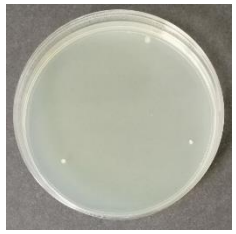
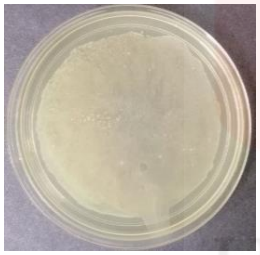
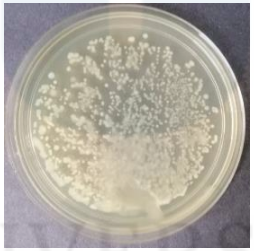
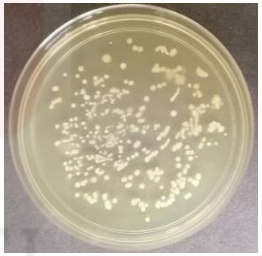
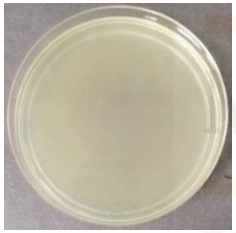
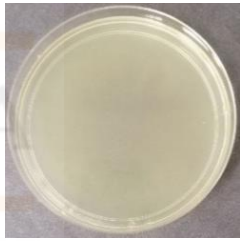
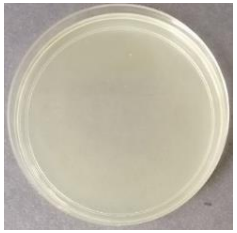
4.2 Determine total plate count of each processed meat product

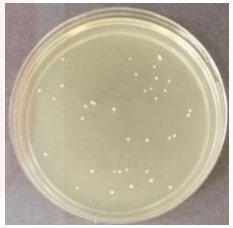
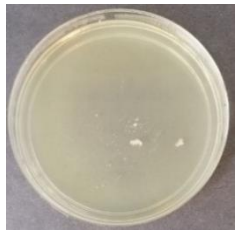
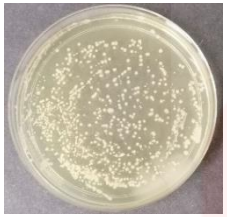
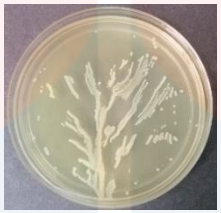
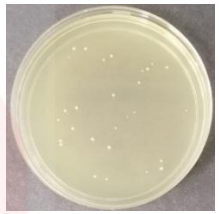
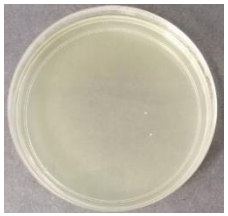
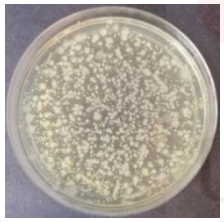
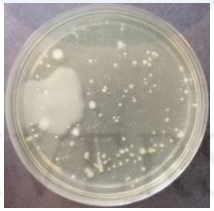
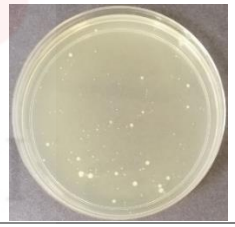
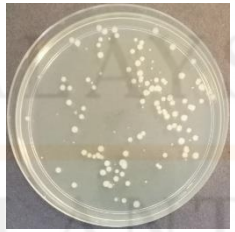
Firstly, the number of colony on nutrient agar isolated from processed meat product is calculated. The colony growth on nutrient agar is aerobic bacteria. The number of aerobic bacteria on nutrient agar plate determine the hygienic quality of processed meat product sample (Mohamed et al., 2017). After getting the colony number, the dilution yield highest colonies in between range 25-250 cfu was selected to calculate total plate count of each processed meat product sample.

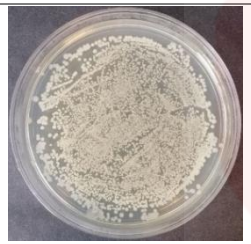
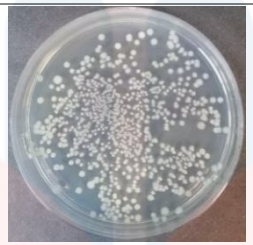
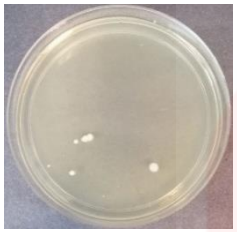
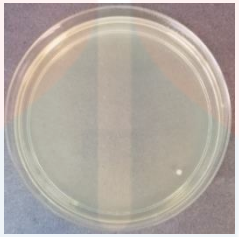
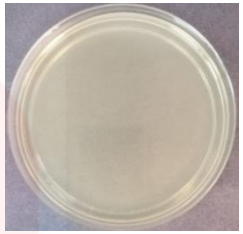
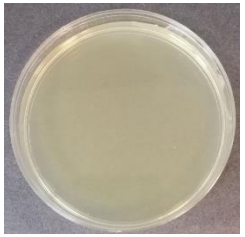
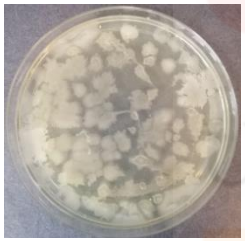
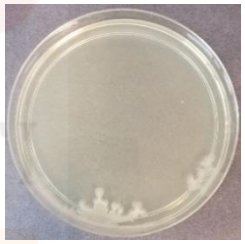
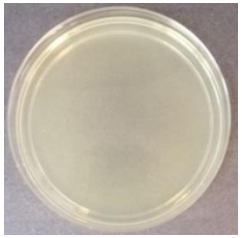
4.2.1 Number of colony on nutrient agar from processed meat product

Table 4.3 shows colonies on nutrient agar isolated from processed meat product. The colonies growth on nutrient agar were greyish-white colour. The number of colony excess 250 was recorded ad TNTC. Lekor (boiled) sample were free of colony at dilutions of 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} on nutrient agar. For the colony on nutrient agar at dilutions of 10^{-1}, the chicken ball sample is lowest colonies with 8, followed by chicken nugget sample with 78. At dilution of 10^{-2}, the chicken ball sample is lowest colonies with 1, followed by chicken nugget with 8 colonies. At dilutions of 10^{-3}, the chicken nugget is lowest colonies with 3, and no colony from chicken ball sample. At dilutions of 10^{-4}, the lobster ball and lekor (unboiled) sample are lowest colonies with 2. The chicken ball and chicken nugget sample are no colony at dilution of 10^{-4}.

Table 4.3: Colonies on Nutrient Agar isolated from processed meat product

Sample	**Dilution**			
	10^{-1}	**10^{-2}**	**10^{-3}**	**10^{-4}**
Surimi Scallop				
No. colony	TNTC/Spreader	110/Spreader	5/Spreader	3
Korean Pearl Fish Cake Ball				
No. colony	TNTC/Spreader	TNTC/Spreader	92	10/Spreader
Lekor (boiled)				
No. colony	-	-	-	-

Fish Ball				
No. colony	TNTC/Spreader	136/Spreader	38	4
Lobster Ball				
No. colony	TNTC/Spreader	120/Spreader	28	2
Lekor (unboiled)				
No. colony	TNTC	TNTC/Spreader	120	2
Fish cake				
No. colony	TNTC	125	22	4/Spreader

Beef ball				
No. colony	TNTC	TNTC	97/Spreader	10/Spreader
Chicken ball				
No. colony	8	1	-	-
Chicken nugget				
No. colony	78/Spreader	8/Spreader	3/Spreader	-

4.2.2 Total Plate Count (cfu/mL)

Table 4.4 shows the bacterial count of the processed meat product sample. Lekor (unboiled) sample is the highest total plate count with 1.2 x 10^6 cfu/mL.

Table 4.4: Bacterial count of the processed meat product

Sample	Colony forming unit, cfu/mL
Surimi scallop	1.1 x 10^5
Korean Pearl Fish Cake Ball	9.2 x 10^5
Lekor (boiled)	0
Fish Ball	1.36 x 10^5
Lobster Ball	1.2 x 10^5
Lekor (unboiled)	1.2 x 10^6
Fish Cake	1.25 x 10^5
Beef Ball	1.0 x 10^{-1}
Chicken Ball	0
Chicken Nugget	7.8 x 10^3

The TPC exceed 10^6 cfu/g mean the microbial growth and microbial produce enzyme content in processed meat product (Mendonca et al., 2020). The TPC exceed 10^6 cfu/g also indicate poor hygienic handling and improper temperature storage. However, the total plate count of processed meat products is not directly related with the presence or absence of the pathogen in processed meat product (Mendonca et al., 2020). Hence,

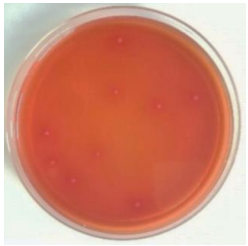
selective media; MacConkey agar, Baird Parker agar and XLD agar are used to isolate pathogen bacterial from processed meat product.

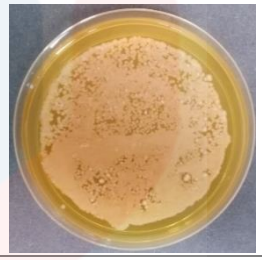
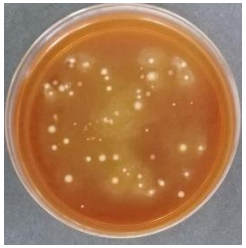
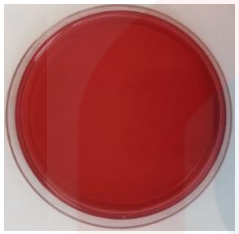
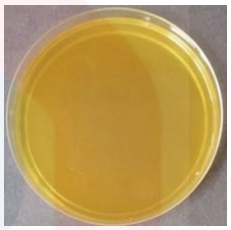
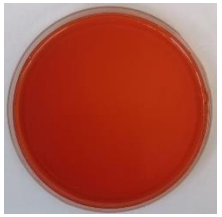
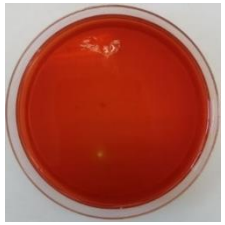
4.3 Isolation bacterial on selective media from processed meat product

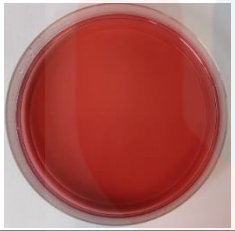
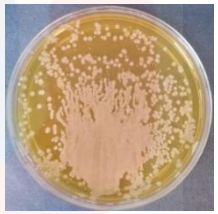
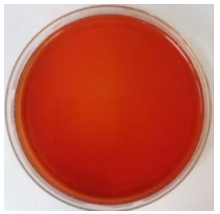
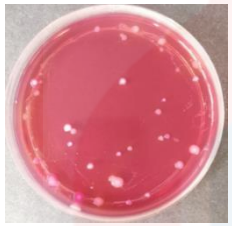
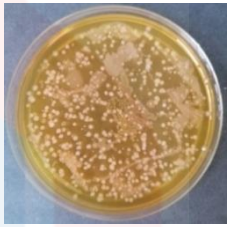
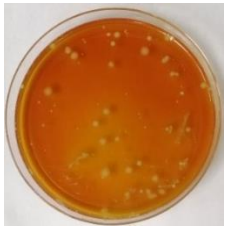
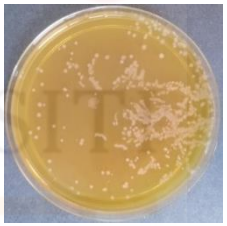
MacConkey agar used for the isolation of *E. coli*, Baird Parker agar for the isolation of *S. aureus* and XLD agar for the isolation of *Salmonella* spp. By observing bacterial morphology on selective media, know the possible type of bacteria is isolated from processed meat product. Table 4.5 shows morphological characteristics of bacterial on selective media. For control plates, *E. coli* growth in pink colonies on MacConkey agar, *S. aureus* growth in white colonies on Baird Parker agar media and *Salmonella typhi* growth in white without or with black centre colonies.

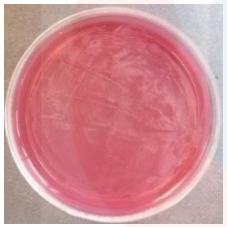
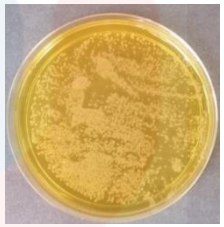
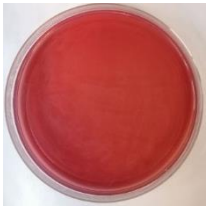
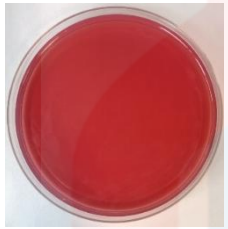
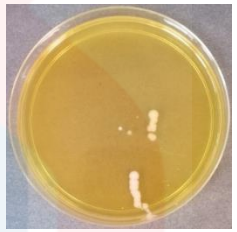
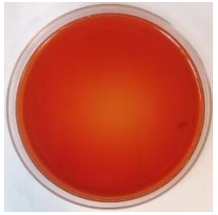
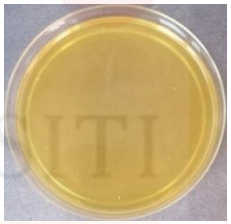
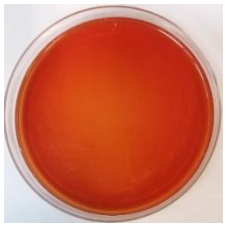
For isolation from processed meat product samples, pink colonies on MacConkey agar, white colonies on Baird Parker agar, and white colonies on XLD agar. The white colonies with or without yellow halo on XLD agar isolated from processed meat product was different colour colonis on control XLD agar plate. The colonies on selective agar identify through gram staining and biochemical tests included catalase test and TSI test.

Table 4.5: Morphological characteristics of bacterial on selective media

Sample	Selective Media		
	MacConkey agar	**Baird Parker agar**	**XLD agar**
Control			
Colony morphology	*E. coli* growth in pink colonies	*S. aureus* growth in white colonies	*Salmonella typhi* growth in white without or with black centre
Surimi scallop			
Colony morphology	No colony	White colonies	White colonies

Korean Pearl Fish Cake Ball			
Colony morphology	Pink colonies	White colonies	White colonies with yellow halo
Lekor (boiled)			
Colony morphology	No colony	No colony	No colony
Fish Ball			
Colony morphology	No colony	White colonies	White colony with yellow halo

Lobster ball			
Colony morphology	No colony	White colonies	No colony
Lekor (unboiled)			
Colony morphology	Pink colonies	White colonies	White colonies with yellow halo
Fish cake			
Colony morphology	Pink colony	White colonies	No colony

Beef Ball			
Colony morphology	No colony	White colonies	No colony
Chicken Ball			
Colony morphology	No colony	White colonies	No colony
Chicken nugget			
Colony morphology	No colony	No colony	No colony

4.4 Label for bacterial isolated from processed meat products

Table 4.6 shows the label for bacterial isolated from processed meat products. The alphabet A to J represent each processed meat product sample. The number 1 represent colonies on MacConkey agar, 2 represent colonies on Baird Parker agar and 3 represent colonies on XLD agar. The '–' indicates no colony on selective media. The label for bacterial isolated from processed meat products are used for gram staining and biochemical test, including catalase test and TSI test.

Table 4.6: Label for bacterial isolated from processed meat products

	Selective media/ Isolation		
Sample	**MacConkey**	**Baird Parker**	**XLD**
Surimi Scallop	-	A2	A3
Korean Pearl Fish Cake Ball	B1	B2	B3
Lekor (boiled)	-	-	-
Fish Ball	-	D2	D3
Lobster Ball	-	E2	-
Lekor (unboiled)	F1	F2	F3
Fish Cake	G1	G2	-
Beef Ball	-	H2	-
Chicken Ball	-	I2	-
Chicken Nugget	-	-	-

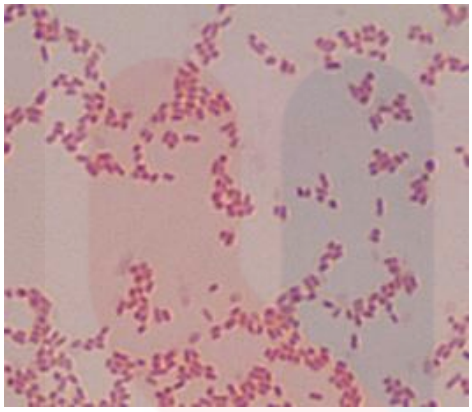
4.5 Identification bacterial type on selective media from processed meat product

Gram staining and biochemical test including catalase test and TSI test are carried out to identify the isolate colony on selective media from processed meat product

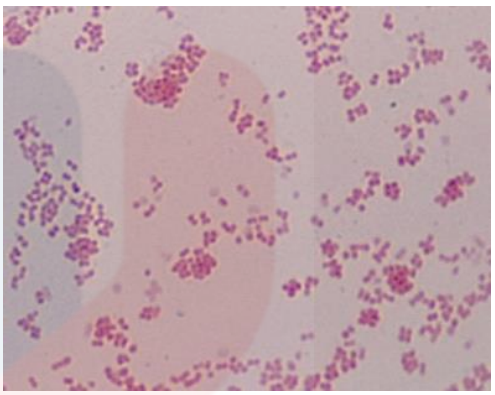
4.5.1 Gram Staining

Figure 4.1 shows bacterial cultures stained pink, rod shaped using Gram Staining method. The morphology of *E. coli* control is rod shaped and pink. Pink colour means gram negative. Based on the observation, the morphology of isolates B1, F1 and G1 are the same as the *E. coli* control. Therefore, isolates B1, F1 and G1 are possible *E. coli*.

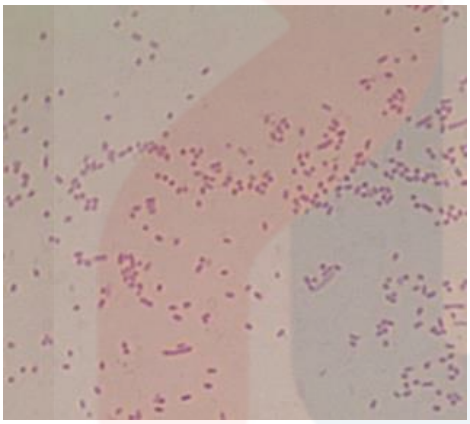
Figure 4.1: Bacterial cultures stained pink, rod shaped using Gram Staining method



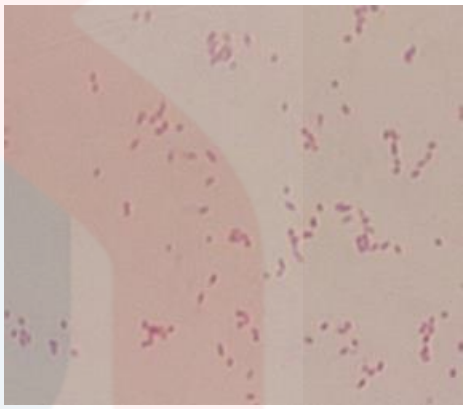
E. coli Control



Isolate B1



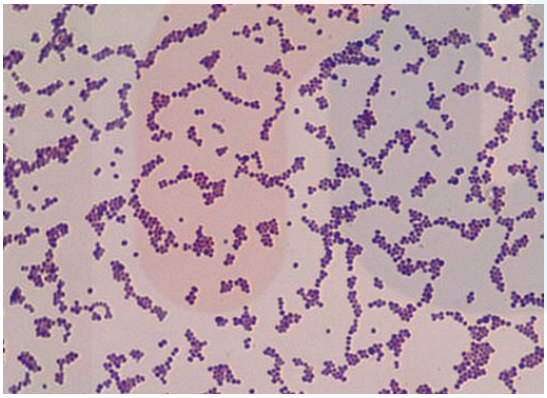
Isolate F1



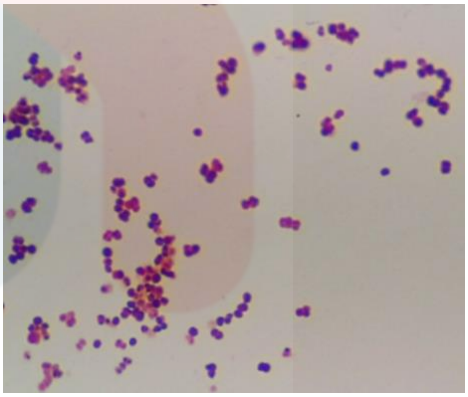
Isolate G1

Figure 4.2 shows bacterial cultures stained purple and pink, cocci shaped using Gram Staining method. The morphology of *S. aureus* control is cocci shape and purple colour. Purple colour mean gram positive. Based on the observation, the morphology of isolates A2, B2, D2, E2, F2, G2, H2 and I2 are the same as the *S. aureus* control. However the isolates A2, B2, D2, E2, F2, G2, H2 and I2 contain some stained pink and cocci shaped due to excessive decolorization (Smith & Hussey, 2005). Therefore, isolates A2, B2, D2, E2, F2, G2, H2 and I2 are possible *S. aureus*.

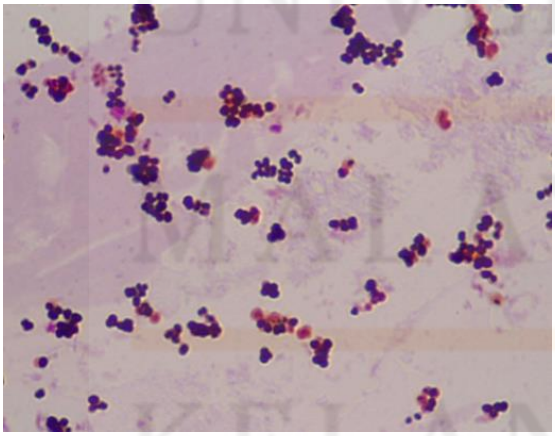
Figure 4.2: Bacterial cultures stained purple, cocci shaped using Gram Staining method



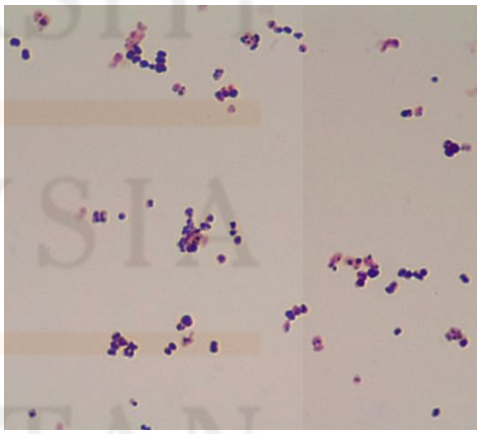
S. aureus Control



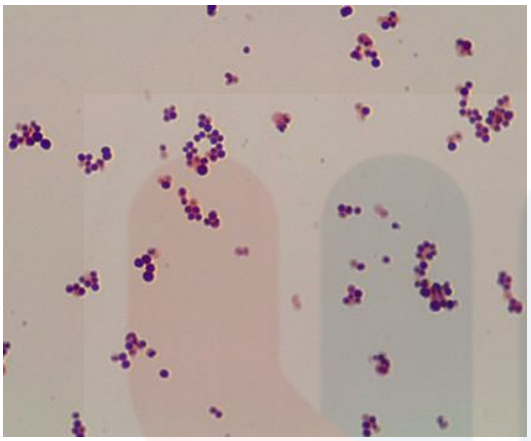
Isolate A2



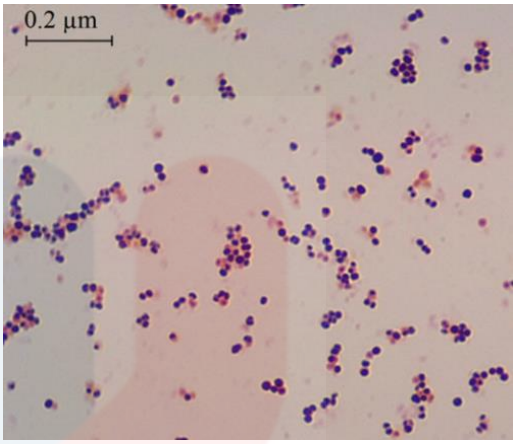
Isolate B2



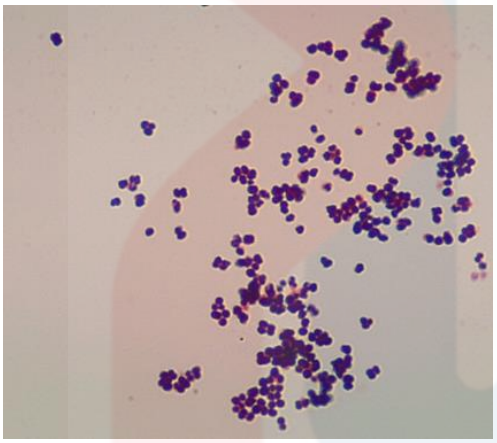
Isolate D2



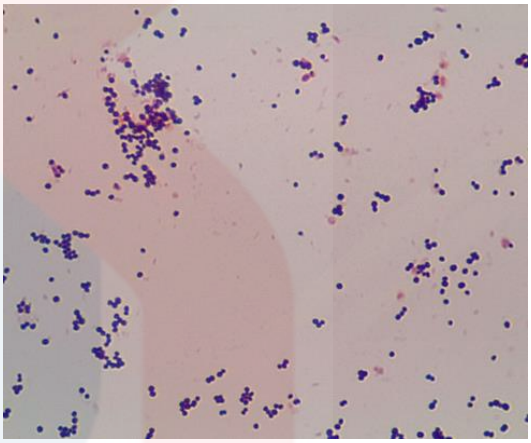
Isolate E2



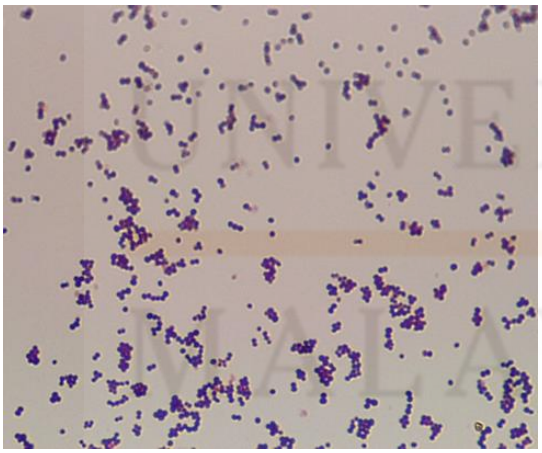
Isolate F2



Isolate G2



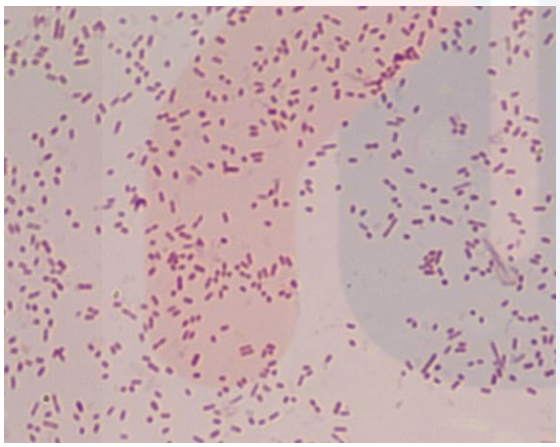
Isolate H2



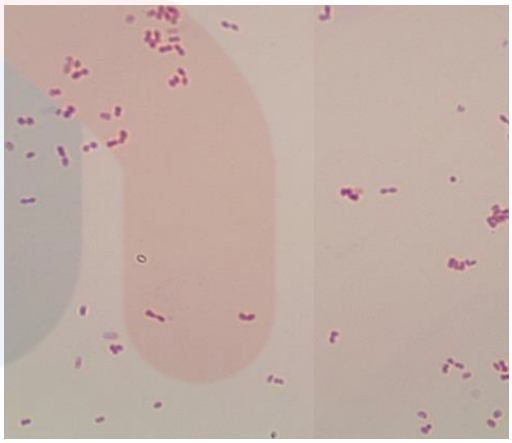
Isolate I2

Figure 4.3 shows bacterial cultures stained pink, rod shaped using Gram Staining method. The morphology of *Salmonella typhi* is rod shape and pink colour. Based on the observation, the morphology isolates A3, D3 and F3 are the same as the *Salmonella typhi* control. Therefore, isolates A3, D3 and F3 are possible *Salmonella typhi*. The morphology isolate B3 rod shape and pink colour. However, the length of the rod is longer than *Salmonella typhi*. The isolate B3 is possible *Salmonella* spp.

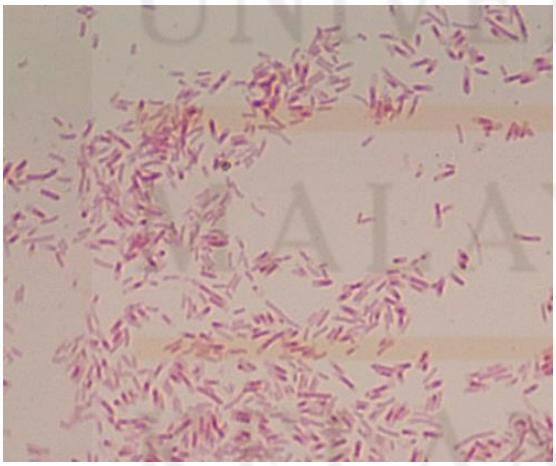
Figure 4.3: Bacterial cultures stained pink, rod shaped using Gram Staining method



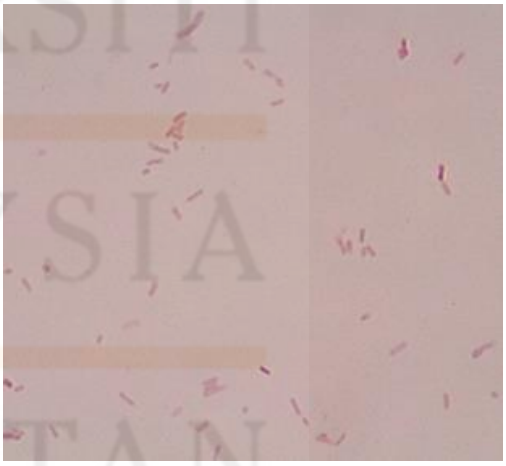
*Salmonella typh*i Control



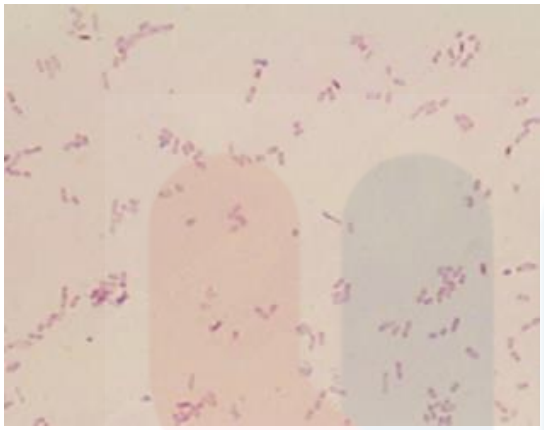
Isolate A3



Isolate B3



Isolate D3



Isolate F3

4.5.2 Biochemical test

a. Catalase test

Table 4.7 shows catalase test with colonies on MacConkey agar. The bacterial colony rapid elaboration of oxygen bubbles that indicates presence of catalase enzyme in the bacterial colony. The result of *E. coli* control shows rapid elaboration of oxygen bubbles. Based on the observation, the result for isolates B1, F1 and G1 are the same as the *E. coli* control. Therefore, isolates B1, F1 and G1 are possible *E. coli*. *E. coli* contain 2 catalase enzymes, hydroperoxide I, HPI and hydroperoxidase II, HPII (Iwase et al., 2013). The role of 2 catalase enzymes is to protect cell from oxidative stress.

Table 4.7: Catalase test with colonies on MacConkey agar

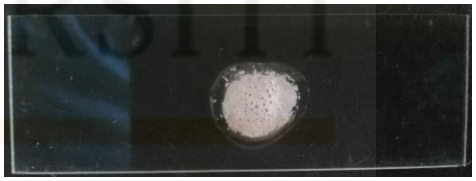
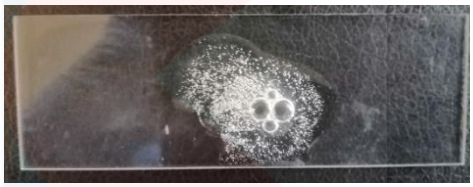
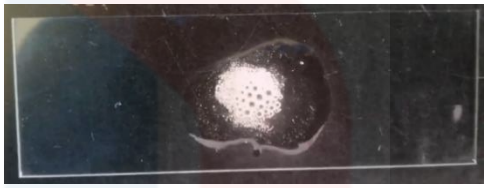
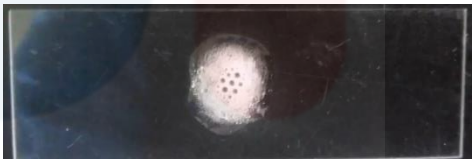
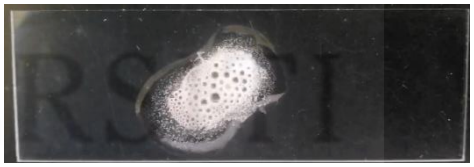
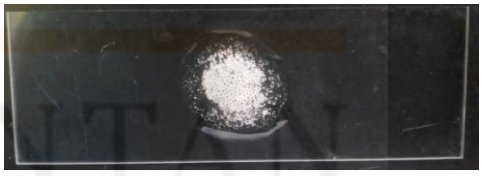
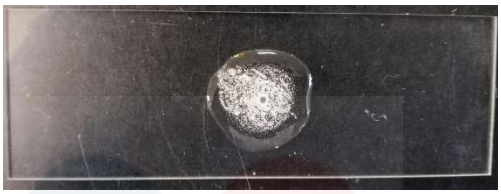
Sample	Observation
E. coli control	
Isolate B1	
Isolate F1	
Isolate G1	

Table 4.8 shows catalase test with colonies on Baird Parker agar. The result of *S.aureus* control shows rapid elaboration of oxygen bubbles. Based on the observation, the result for isolates A2, B2, D2, E2, F2, G2, H2 and I2 are the same as for the *S. aureus* control. The isolates A2, B2, D2, E2, F2, G2, H2 and I2 are possible *S. aureus*. *S. aureus* contain catalase enzyme for cellular detoxification (Mustafa, 2014).

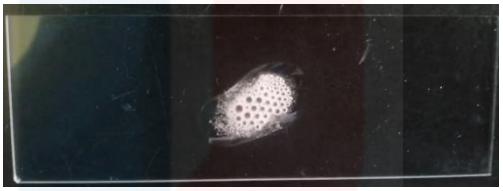
Table 4.8: Catalase test with colonies on Baird Parker agar

Sample	**Observation**
S. aureus control	
Isolate A2	
Isolate B2	
Isolate D2	
Isolate E2	
Isolate F2	

Isolate G2



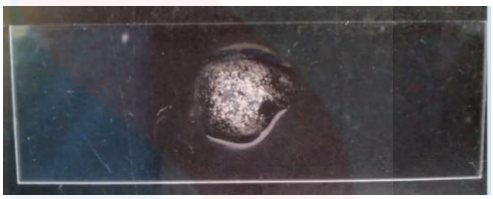
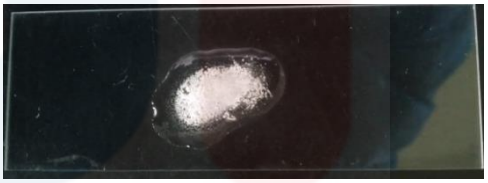
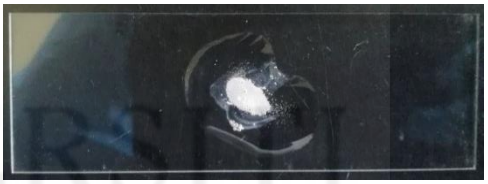
Isolate H2



Isolate I2

Table 4.9 shows catalase test with colonies on XLD agar. The results of *Salmonella typhi* control shows the rapid elaboration of oxygen bubbles. Based on the observation, the result for isolates A3, B3, D3 and F3 are the same as the *Salmonella typhi* control. Therefore, the isolates A3, B3, D3 and F3 are possible *Salmonella typhi.*

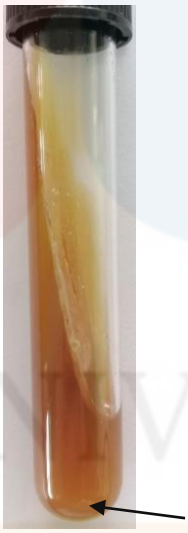
Table 4.9: Catalase test with colonies on XLD agar

Sample	**Observation**
Salmonella typhi control	
Isolate A3	
Isolate B3	
Isolate D3	
Isolate F3	

b. TSI test

Table 4.10 shows TSI agar slant between uninoculated *E. coli* control and isolated colony on MacConkey agar. Uninoculated agar slant is red colour. *E. coli* control agar slant shows yellow slant and have bubble on the medium. B1 agar slant shows yellow slant and have displacement of the medium. F1 and G1 agar slant are show yellow slant and have bubble in medium.

Table 4.10: TSI agar slant between uninoculated, *E. coli* control and isolated colony on MacConkey agar

Uninoculated	***E. coli* control**	**Isolate B1**	**Isolate F1**	**Isolate G1**
				

* ⟶ - bubble, displacement of the medium

The bubble and displacement of medium indicates production of gas by organism during fermentation glucose. The isolates A3, B3, D3 and F3 agar slant are the same as for the *E. coli* control agar slant. Hence the isolates B1, F1 and G1 are possible *E. coli*. *E.coli* fermentation of sugar including glucose, lactose with production of gas (M.Selman et al., 2020).

Table 4.11 shows TSI agar slant between uninoculated, *S. aureus* control and isolated colony on Baird Parker agar. *S. aureus* control, isolates A2, B2, D2, E2, F2, G2, H2 and I2 agar slant show yellow slant.

Yellow slant indicates acid reaction due to the production of acid from glucose. The isolates A2, B2, D2, E2, F2, G2, H2 and I2 agar slant are the same as for the *S. aureus* control agar slant. Hence, the isolates A2, B2, D2, E2, F2, G2, H2 and I2 are possible *S.aureus*. *S .aureus* fermentation of sugar including glucose, lactose, sucrose, maltose, mannitol, and glucose, and formation acids without gas production (Kukhar et al., 2010)

Table 4.11: TSI agar slant between uninoculated, *S .aureus* control and isolated colony on Baird Parker agar

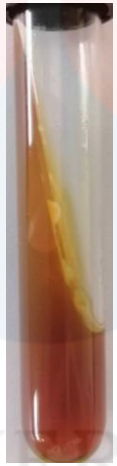
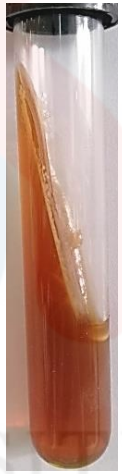
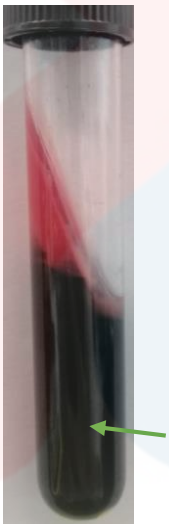
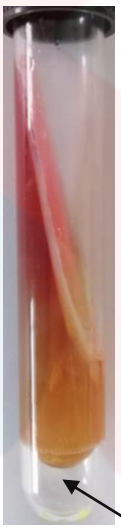
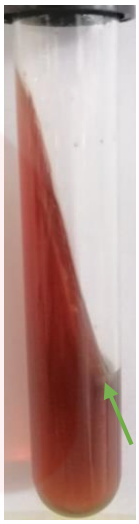
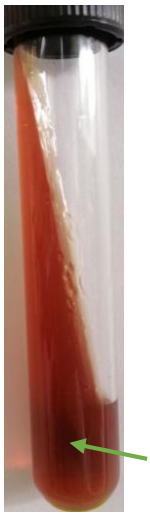
Uninoculated	***S. aureus* control**	**A2**	**B2**	**D2**	**E2**	**F2**	**G2**	**H2**	**I2**
									

Table 4.12 shows the TSI agar slant between uninoculated, *Salmonella typhi* control and isolated colony on XLD agar. *Salmonella typhi* control agar slant shows red slant and blackening of the medium. Isolates A3, D3 and F3 agar slant shows red slant and less blackening of the medium. Isolates B3 agar slant shows red slant and displacement of the medium.

Table 4.12: TSI agar slant between uninoculated, *Salmonella typhi* control and isolated colony on XLD agar

Uninoculated	***Salmonella typhi* control**	**Isolate A3**	**Isolate B3**	**Isolate D3**	**Isolate F3**
					

* → - Blackening of the medium, → - Displacement of medium

Red slant indicate acid reaction due to low production of acid from glucose. The isolates A3, D3 and F3 agar slant are the same as the *Salmonella typhi* control agar slant. The red slant and displacement of medium showed in B3 agar slant indicates it is possible *Salmonella paratyphi* A (M.Selman et al., 2020). Hence, isolates A3, D3 and F3 is possible *Salmonella typhi* whereas isolate B3 is *Salmonella paratyphi* A. *Salmonella* spp. ferment sugar including glucose, sorbitol and manitol, to produce acid without or with gas production (Percival & Williams, 2014). *Salmonella typhi* formation acid without gas whereas *Salmonella paratyphi* A formation acid with gas (M.Selman et al., 2020). The

blackening of medium indicates production of hydrogen sulfide, H_2S. *Salmonella typhi* produce H_2S while *Salmonella paratyphi* A does not produce H_2S (M.Selman et al., 2020).

4.6 Identification of possible bacterial colony on selective media

Gram staining, catalase test and TSI test are carry out to identify possible bacterial colony on selective media isolated from processed meat product. The bacterial type of possible bacterial colony on selective media is identified by comparing gram staining, catalase test and TSI test to bacterial control.

a. Identification of possible *E. coli* colony on MacConkey agar

Table 4.13 shows identification of possible *E. coli* colony on MacConkey agar. Based on the table, isolates B1, F1 and G1 are the same as the *E. coli* control and are *E. coli*. Therefore, Korean Pearl Fish Cake Ball, Lekor (unboiled) and Fish cake presence of *E. coli*.

E. coli is the indicator of faecal contamination. The low amount of *E. coli* in raw meat is normally due to carcasses contaminate with animal skin and faeces when slaughtering and seasoning (Mendonca et al., 2020). Lekor (unboiled) presence of *E. coli* due to uncooked and is raw minced fish paste. Korean Pearl Fish Cake Ball and Fish Cake presence of *E. coli*. Maybe due to cross contamination from raw material, equipment used or food handler (Hamat et al., 2019). To avoid cross contamination of *E. coli*, finished processed meat products should separate place from the raw material. The equipment

after used should wash and sanitize. The food handler should wash hands properly before and after processing processed meat product.

Table 4.13: Identification of possible *E. coli* colony on MacConkey agar

	***E. coli* control**	**Isolate B1**	**Isolate F1**	**Isolate G1**
Gram staining				
Colour	Pink	Pink	Pink	Pink
Shape	Rod	Rod	Rod	Rod
Catalase test	+	+	+	+
TSI test				
Colour slant	Yellow	Yellow	Yellow	Yellow
Gas production	Present	Present	Present	Present
Bacterial type		*E. coli*	*E. coli*	*E. coli*

Explanation:

- '+' : Rapid elaboration of oxygen bubbles in catalase test

b. Identification of possible *S. aureus* colony on Baird Parker agar

Table 4.14 shows identification of possible *S. aureus* colony on Baird Parker agar. *S. aureus* control is cocci shaped with purple, positive result of catalase test, yellow slant in TSI test. Based on the table, isolates A2, B2, D2, E2, F2, G2, H2 and I2 are the same as the *S. aureus* control and are *S. aureus*. Therefore, Surimi scallop, Korean Pearl Fish Cake Ball, Fish Ball, Lobster ball, Lekor (unboiled), Fish Cake, Beef Ball and Chicken Ball are presence of *S. aureus.*

S. aureus always found on humans' skin, nostrils and throat. The presence of *S.aureus* in processed meat product indicates poor hygiene handling or improper storage temperature. Improper hand washing and improper use of face mask by food handler will transmit *S. aureus* into raw material, the equipment used and finished product through skin, coughing or sneezing (Hamat et al., 2019). The gloves can be a source of *S. aureus* contamination if the glove does not change frequently and there is no proper hand washing before wearing glove (Kadariya et al., 2014).

Table 4.14: Identification of possible *S. aureus* colony on Baird Parker agar

	***S. aureus* control**	**Isolate A2**	**Isolate B2**	**Isolate D2**	**Isolate E2**	**Isolate F2**	**Isolate G2**	**Isolate H2**	**Isolate I2**
Gram staining									
Colour	Purple	Purple	Purple	Purple	Purple	Purple	Purple	Purple	Purple
Shape	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci
Catalase test	+	+	+	+	+	+	+	+	+
TSI test									
Colour slant	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
Bacterial type		*S.aureus*	*S.aureus*	*S.aureus*	*S.aureus*	*S.aureus*	*S.aureus*	*S.aureus*	*S.aureus*

Explanation:

- '+' : Rapid elaboration of oxygen bubbles in catalase test

c. Identification of possible *Salmonella* spp. colony on XLD agar

Table 4.15 shows identification of possible *Salmonella* spp. colony on XLD agar. *Salmonella typhi* control is rod shaped with pink, positive result of catalase test, red slant and production of H_2S in TSI test. The blackening of the medium indicates production of H_2S.

Table 4.15: Identification of possible *Salmonella* spp. colony on XLD agar

	***Salmonella typhi* control**	**Isolate A3**	**Isolate B3**	**Isolate D3**	**Isolate F3**
Gram staining					
Colour	Pink	Pink	Pink	Pink	Pink
Shape	Rod	Rod	Rod	Rod	Rod
Catalase test	+	+	+	+	+
TSI test					
Colour slant	Red	Red	Red	Red	Red
Gas production	Absent	Absent	Present	Absent	Absent
H_2S production	Present	Present	Absent	Present	Present
Bacterial type		*Salmonella typhi*	*Salmonella paratyphi* A	*Salmonella typhi*	*Salmonella typhi*

Explanation:

- '+' : Rapid elaboration of oxygen bubbles in catalase test

Based on the table, the isolates A3, D3 and F3 are the same as the *Salmonella typhi* control. However, isolate B3 is red slant and had a displacement of medium is same as the *Salmonella paratyphi* A (M.Selman et al., 2020). Therefore, isolates A3, D3 and F3 are *Salmonella typhi*. Surimi scallop, Fish ball and Lekor (unboiled) are presence of *Salmonella typhi*. The isolate B3 is *Salmonella paratyphi* A, Korean Pearl Fish Cake Ball presence of *Salmonella paratyphi* A.

Salmonella spp. in processed meat product indicates poor hygienic handling in processing or cross contamination from equipment (Mendonca et al., 2020). The raw meat consist of a low number of *Salmonella* spp. The equipment and utensil in contact with raw meat should wash and sanitize. Food handler should wash hand properly after contact with raw meat.

4.7 Processed meat product from small-medium industry & large industry

The processed meat products from small-medium industries are home-based industries without Halal, GMP, HACCP certified logo on package. Without any food safety certification, the hygienic processing conditions of processed meat products cannot be guaranteed without any food safety certification (Hamat et al., 2019). Surimi scallop, Korean Pearl Fish Cake Ball, Lobster ball, Fish cake and Lekor (unboiled) are RTC

processed meat product. While Fish ball and Lekor (boiled) are RTE processed meat product.

Lekor (boiled) is 0 cfu/g and does not isolate bacteria on selective media. Lekor (boiled) is RTE processed meat product that undergo boiling process at 100 ℃ for 10 min (Lani et al., 2017). The boiling process can eliminate *E. coli*, *S. aureus* and *Salmonella* spp. (Lani et al., 2017 & FDA, 2018). Then, Lekor (unboiled) has the highest total pate count with 1.2 x 10^6 and presence of *E. coli*, *S. aureus* and *Salmonella* spp.. Lekor (unboiled) is uncooked minced fish dough and RTC processed meat product. The presence of pathogen bacteria indicates fish contact with contaminated seawater, which dispose heavy load of sewage into sea, create suitable environment for the growth of pathogen bacteria (Sichewo et al., 2013).

The remaining sample (Surimi scallop, Korean Pearl Fish Cake Ball, Fish ball, Lobster ball and Fish cake), total plate amount at acceptable amount. *E. coli* isolated from Korean Pearl Fish Cake Ball and Fish ball, *S, aureus* isolated from Surimi scallop, Korean Pearl Fish Cake Ball, Fish ball, Lobster ball and Fish cake, and *Salmonella* spp. isolated from Surimi scallop, Korean Pearl Fish Cake Ball and Fish ball. The food safety certification labelled on package play an important role in indicating the hygienic condition of processed meat product processing.

For the processed meat products from large industries, the chicken nugget with the highest total plate count 7.8 x 10^3 cfu/g because it is RTC processed meat product and partially cooked. The beef ball is 1.0 x 10^{-1} cfu/g, and the chicken ball is 0 cfu/g as they

are RTE processed meat products and fully cooked. The beef and chicken ball are boiled at 100 ℃ before freezing.

However, there are no bacteria isolated from chicken nugget and *S. aureus* isolated from beef ball and chicken ball on Baird Parker agar. Due to the chicken nugget package having Halal, HACCP and GMP certified logos. While the beef and chicken ball package only have Halal logo.

Halal logo labelled on processed meat product package not just mean that the food is permissible consumption for Muslim and also indicate the processing of meat product in hygiene condition. However only Halal logo on processed meat product package not sufficient indicate processed meat product processing in perfect hygiene practices. The processing of meat product in perfect hygiene practice is required (Abdullah Sani & Dahlan, 2015). The industry recommends take GMP or/and HACCP certification. The standard scope of GMP includes controlling premises and facilities, creating standard operating procedure (SOP), maintenance and sanitizer, and personal hygiene and training (Abdullah Sani & Dahlan, 2015). While the standard scope of HACCP include analyse biology, chemical and physical hazard, decide critical control points and set up monitoring process for critical control points (Abdullah Sani & Dahlan, 2015).

CHAPTER 5

CONCLUSION & RECOMMENDATION

5.1 Conclusion

Lekor (unboiled) is the highest bacterial count and exceed the recommended maximum limit 10^6 cfu/g. After the bacterial isolation and identification, *E. coli* isolated from Korean Pearl Fish Cake Ball, Lekor (unboiled) and Fish cake. *S. aureus* isolated from Surimi scallop, Korean Pearl Fish Cake Ball, Fish ball, Lobster ball, Lekor (unboiled), Fish cake, Beef ball and chicken ball. *Salmonella* spp. isolated from Surimi scallop, Korean Pearl Fish Cake Ball, Fish Ball and Lekor (unboiled).

The total plate count exceed the recommended maximum limit 10^6 cfu/g indicate bacterial growth in processed meat product. *Salmonella* spp., *S. aureus* and *E. coli* in processed meat product indicate improper storage temperature, poor hygiene processing and handling or cross contamination of raw meat to other materials and finished products.

The processed meat product before freezing, should maintain hygiene condition in processing, storage and handling practice to eliminate or decrease pathogen bacteria content in processed meat product. In this study, RTC and RTE frozen processed meat product were used. Thermal process required before consumption of RTC and RTE processed meat products. Although thermal process can kill pathogen bacteria, long term microbial growth cause microbial toxicity and destroy the nutritional quality of processed meat product.

5.2 Recommendation

In future study**,** molecular analysis can be carry out after isolation and identification bacteria from processed meat product. The molecular analysis will be able to help in confirming the identification of bacteria presence in processed meat product. The confirmation of identification of bacteria is based on DNA. The molecular analysis include lap gene specific Polymerase chain reaction (PCR) amplification and amplification of 16s rRNA gene and sequencing.

Then, in the future, the laboratory test to determine whether the pathogen spores present in processed meat product. Pathogen spore, *Bacillus cereus* and *Clostridium botulinum* are heat resistance and can survive at 100 ℃.

Finally, future studies on a wider range of processed meat products from other countries as well as other states in Malaysia can be carried out. In this research study, only frozen processed meat products that are available at Jeli, Kelantan were used. Therefore, in the future, studies on local and international brands of meat products can be used for bacterial assessment.

REFERENCES

Ab Talib, M. S. (2017). Motivations and benefits of halal food safety certification. In *Journal of Islamic Marketing* (Vol. 8, Issue 4). https://doi.org/10.1108/JIMA-08-2015-0063

Abdul-Mutalib, Syafinaz, A. N., Sakai, & Shirai, Y. (2015). An overview of foodborne illness and food safety in Malaysia. In *International Food Research Journal* (Vol. 22, Issue 3).

Abdullah Sani, N., & Dahlan, H. A. (2015). Current Trend for Food Safety and Halal Measures. *ASEAN Community Conference 2015, November*, 11–12.

Abdullahi, A., Hassan, A., Kadarman, N., Saleh, A., Baraya, Y. S., & Lua, P. L. (2016). Food safety knowledge, attitude, and practice toward compliance with abattoir laws among the abattoir workers in Malaysia. *International Journal of General Medicine*, *9*, 79–87. https://doi.org/10.2147/IJGM.S98436

Ain Auzureen, M. Z., Aklilu, E., Mohd Azam Khan, G. K., Ruhil Hayati, H., Al Sultan, I. I., & Soon, J. M. (2017). Microbiological quality of cooked meat products sold in Kelantan, Malaysia during Ramadhan month. *International Food Research Journal*, *24*(1), 414–421.

Anbudhasan, P., Uma, A., & Rebecca, G. (2012). Assessment of Bacteriological Quality in Selected Commercially Important Processed Sea Foods By Polymerase Chain Reaction (Pcr). *International Journal of Food, Agriculture and Veterinary Sciences*, *June 2014*.

Badar, I. H., Liu, H., Chen, Q., Xia, X., & Kong, B. (2021). Future trends of processed meat products concerning perceived healthiness: A review. *Comprehensive*

Reviews in Food Science and Food Safety, *20*(5), 4739–4778. https://doi.org/10.1111/1541-4337.12813

Baskaran, S., Ayob, S. A., Howe, N. C., & Mahadi, N. (2017). Understanding Purchase Intention of Ready-to-Eat Food among Malaysian Urbanites: A Proposed Framework. *International Journal of Academic Research in Business and Social Sciences*, *7*(11). https://doi.org/10.6007/ijarbss/v7-i11/3496

Bell, C., & Kyriakides, A. (2009). Salmonella. In *Foodborne Pathogens: Hazards, Risk Analysis and Control: Second Edition* (pp. 627–674). Elsevier Ltd. https://doi.org/10.1533/9781845696337.2.627

Bhandare, S. G., Sherikar, A. T., Paturkar, A. M., Waskar, V. S., & Zende, R. J. (2007). A comparison of microbial contamination on sheep/goat carcasses in a modern Indian abattoir and traditional meat shops. *Food Control*, *18*(7), 854–858. https://doi.org/10.1016/j.foodcont.2006.04.012

Ekici, G., & Dümen, E. (2019). Escherichia coli and Food Safety. In *The Universe of Escherichia coli [Working Title]*. IntechOpen. https://doi.org/10.5772/intechopen.82375

Food and Drug Administration (FDA). (2018). Chapter 4: Preventive Controls. In *Hazard Analysis and Risk-Based Preventive Controls for Human Food: Draft Guidance for Industry.* Retrieved from https://www.fda.gov/regulatory-information/search-fda-guidance-documents/draft-guidance-industry-hazard-analysis-and-risk-based-preventive-controls-human-food

Green, L. R., Radke, V., Mason, R., Bushnell, L., Reimann, D. W., Mack, J. C., Motsinger, M. D., Stigger, T., & Selman, C. A. (2007). Factors related to food

worker hand hygiene practices. *Journal of Food Protection*, 70(3), 661–666. https://doi.org/10.4315/0362-028X-70.3.661

Hamat, H. W., Lani, M. N., Hamzah, Y., Alias, R., & Hassan, Z. (2019). Microbiological assessment of keropok lekor production in Kuala Terengganu and Marang, Malaysia. *Asian Journal of Agriculture and Biology*, *7*(1), 74–85.

HiMedia. (2019). *Baird Parker Agar Base*. HiMedia Laboratories. https://www.britanialab.com/back/public/upload/productos/upl_607060f71db9b.pdf

Iwase, T., Tajima, A., Sugimoto, S., Okuda, K. I., Hironaka, I., Kamata, Y., Takada, K., & Mizunoe, Y. (2013). A Simple Assay for Measuring Catalase Activity: A Visual Approach. *Scientific Reports*, *3*. https://doi.org/10.1038/SREP03081

Jung, B., & Hoilat, G. J. (2021, October 5). *MacConkey Medium*. StatPearls; StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK557394/

Jvo Siegrist. (n.d.). *Detection and Differentiation of Staphylococcus Aureus on Baird Parker Agar*. MERCK. Retrieved January 20, 2022, from https://www.sigmaaldrich.com/MY/en/technical-documents/technical-article/food-and-beverage-testing-and-manufacturing/microbiological-analysis-for-food-and-beverage/detection-and-differentiation

Kadariya, J., Smith, T. C., & Thapaliya, D. (2014). Staphylococcus aureus and Staphylococcal Food-Borne Disease: An Ongoing Challenge in Public Health. *BioMed Research International*, *2014*. https://doi.org/10.1155/2014/827965

Kotzekidou, P. (2016). Factors influencing microbial safety of ready-to-eat foods. In *Food Hygiene and Toxicology in Ready-to-Eat Foods*. Elsevier Inc.

https://doi.org/10.1016/B978-0-12-801916-0.00003-0

Krasulya, O., Bogush, V., Potoroko, I., Tsirulnichenko, L., & Vostrikova, N. (2020). The Use of Ultrasound for Novel Meat Processing. *Innovative Food Processing Technologies: A Comprehensive Review*, *Krasulya*, 465–493. https://doi.org/10.1016/B978-0-08-100596-5.22936-3

Kukhar, V. P., Ajayi, A. A., Adejuwon, A. O., Akintunde, O. O., & Olutiola, P. O. (2010). Antibiotics resistance and susceptibility pattern of a strain of Staphylococus aureus associated with acne. *International Journal of Medicine and Medical Sciences*, *2*(9), 277–280. http://www.academicjournals.org/ijmms

Lani, M. N., Peng, T. A., Suhaili, Z., & Hassan, Z. (2017). Microbiological evaluation on raw materials and food contact surfaces of 'keropok lekor' premises in Kuala Nerus, Terengganu and their prevalence of antibiotic resistant bacteria. *Malaysian Applied Biology*, *46*(3), 71–80.

Larry Maturin & James T. Peeler. (2001, January). BAM Chapter 3: Aerobic Plate Count. *Food and Drug Administration (FDA)*. Retrieved from https://www.fda.gov/food/laboratory-methods-food/bam-chapter-3-aerobic-plate-count

Lehman, D. (2000). *Triple Sugar Iron Agar*. American Society Microbiology. https://www.asmscience.org/content/education/protocol/protocol.2842

Liu, D. (2015). Enterotoxin-Producing Staphylococcus aureus. *Molecular Medical Microbiology: Second Edition*, *2–3*, 979–995. https://doi.org/10.1016/B978-0-12-397169-2.00055-X

Lonergan, S. M., Topel, D. G., & Marple, D. N. (2019a). Meat microbiology and safety.

In *The Science of Animal Growth and Meat Technology* (pp. 183–204). Elsevier. https://doi.org/10.1016/b978-0-12-815277-5.00012-3

Lonergan, S. M., Topel, D. G., & Marple, D. N. (2019b). Sausage processing and production. *The Science of Animal Growth and Meat Technology*, 229–253. https://doi.org/10.1016/b978-0-12-815277-5.00014-7

M.Selman, H., A.Mahdi, A., A.Abugroun, H., & M.Mutwali, E. (2020). Isolation and Identification of Microorganisms in Processed Meats in Khartoum State. *International Journal of Agriculture & Environmental Science*, *7*(5), 7–13. https://doi.org/10.14445/23942568/IJAES-V7I5P102

Mendonca, A., Thomas-Popo, E., & Gordon, A. (2020). Microbiological considerations in food safety and quality systems implementation. In *Food Safety and Quality Systems in Developing Countries*. https://doi.org/10.1016/b978-0-12-814272-1.00005-x

Mikoleit, M. L. (2014). Biochemical Identification of Salmonella and Shigella Using an Abbreviated Panel of Tests. In *WHO GFN Laboratory Protocol*. https://doi.org/10.13140/RG.2.1.1183.3443

Mohamed, A. D., Mohamed, R. D., & Ahmed, S. (2017). Microbiological quality of fresh and frozen ground meat , Alexandria city , Egypt. *Saudi Journal of Pathology and Microbiology*, *3362*, 286–290. https://doi.org/10.21276/sjpm.2017.2.9.1

Mustafa, H. S. I. (2014). Staphylococcus aureus Can Produce Catalase Enzyme When Adding to Human WBCs as a Source of H2O2 Productions in Human Plasma or Serum in the Laboratory. *Open Journal of Medical Microbiology*, *04*(04), 249–251. https://doi.org/10.4236/ojmm.2014.44028

Nastasijević, I., Lakićević, B., & Petrović, Z. (2017). Cold chain management in meat storage, distribution and retail: A review. *IOP Conference Series: Earth and Environmental Science*, *85*(1). https://doi.org/10.1088/1755-1315/85/1/012022

Odeyemi, O. A., Alegbeleye, O. O., Strateva, M., & Stratev, D. (2020). Understanding spoilage microbial community and spoilage mechanisms in foods of animal origin. *Comprehensive Reviews in Food Science and Food Safety*, *19*(2), 311–331. https://doi.org/10.1111/1541-4337.12526

Pal, M., Ayele, Y., Patel, A. S., & Dulo, F. (2018). (PDF) Microbiological and hygienic quality of Meat and Meat Products. *Beverage & Food World*, *45*(5), 21–27. https://www.researchgate.net/publication/325216644_Microbiological_and_hygienic_quality_of_Meat_and_Meat_Products

Percival, S. L., & Williams, D. W. (2014). Salmonella. *Microbiology of Waterborne Diseases: Microbiological Aspects and Risks: Second Edition*, 209–222. https://doi.org/10.1016/B978-0-12-415846-7.00010-X

Peretó, J. (2011). Embden-Meyerhof-Parnas Pathway. *Encyclopedia of Astrobiology*, 485–485. https://doi.org/10.1007/978-3-642-11274-4_503

Shafini, A. B., Son, R., Mahyudin, N. A., Rukayadi, Y., & Tuan Zainazor, T. C. (2017). Prevalence of Salmonella spp. in chicken and beef from retail outlets in Malaysia. *International Food Research Journal*, *24*(1), 437–449.

Shamimuzzaman, M., Roy, R. K., Majumder, T. R., Barman, N. C., Lina, N. N., Hasan, M. T., & Dash, B. K. (2022). Microbial profile of some ready-to-cook frozen food items sold in Dhaka city, Bangladesh. *Food Science and Human Wellness*, *11*(2), 289–296. https://doi.org/10.1016/J.FSHW.2021.11.021

Sichewo, P. R., Gono, R. K., Muzvondiwa, J. V, & Sizanobuhle, N. (2013). Isolation and Identification of Pathogenic Bacteria in Edible Fish : A Case Study of Fletcher Dam in. *International Journal of Science and Research*, *2*(9), 269–273.

Soon, J. M., Wahab, I. R. A., Hamdan, R. H., & Jamaludin, M. H. (2020). Structural equation modelling of food safety knowledge, attitude and practices among consumers in Malaysia. *PLoS ONE*, *15*(7 July). https://doi.org/10.1371/journal.pone.0235870

Temgire, S., Borah, A., & Kumthekar, S. (2021). Recent trends in ready to eat/cook food products: A review Sushant Temgire, Anjan Borah, Sandip Kumthekar and Aniket Idate. *The Pharma Innovation Journal*, *10*(5), 211–217. https://doi.org/10.22271/tpi.2021.v10.i5c.6207

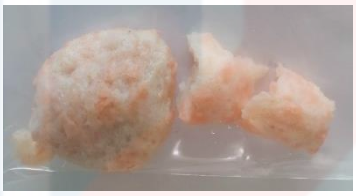
Thairu, Y., Usman, Y., & Nasir, I. (2014). Laboratory perspective of gram staining and its significance in investigations of infectious diseases. *Sub-Saharan African Journal of Medicine*, *1*(4), 168. https://doi.org/10.4103/2384-5147.144725

USDA. (2020). *Beef From Farm To Table | Food Safety and Inspection Service*. Food Safety and Inspection Service U.S. DEPARTMENT OF AGRICULTURAL. https://www.fsis.usda.gov/food-safety/safe-food-handling-and-preparation/meat/beef-farm-table

Wanger, A., Chavez, V., Huang, R. S. P., Wahed, A., Actor, J. K., & Dasgupta, A. (2017). Media for the Clinical Microbiology Laboratory. *Microbiology and Molecular Diagnosis in Pathology*, 51–60. https://doi.org/10.1016/b978-0-12-805351-5.00004-1

APPENDIX A

Table A.1: Processed meat product samples

Sample	Diagram
Surimi Scallop	
Korean Pearl Fish Cake Ball	
Lekor (boiled)	
Fish Ball	
Lobster Ball	

Lekor
(Unboiled)

Fish Cake

Beef Ball

Chicken Ball

Chicken
Nugget

APPENDIX B

Table B.1: Number of colony on nutrient agar for each sample

Sample		10^{-1}	10^{-2}	10^{-3}	10^{-4}
Surimi Scallop	1	TNTC/Spreader	110/Spreader	5/Spreader	3
	2	TNTC/Spreader	96/Spreader	4/Spreader	1
Korean Pearl Fish Cake Ball	1	TNTC/Spreader	TNTC/Spreader	92	10/Spreader
	2	TNTC/Spreader	TNTC/Spreader	30/Spreader	7/Spreader
Lekor (Boiled)	1	-	-	-	-
	2	-	-	-	-
Fish Ball	1	TNTC/Spreader	135/Spreader	36	3
	2	TNTC/Spreader	136/Spreader	38	4
Lobster Ball	1	TNTC/Spreader	118	25	1
	2	TNTC/Spreader	120	28	2
Lekor (Unboiled)	1	TNTC	TNTC/Spreader	120	5
	2	TNTC/Spreader	TNTC/Spreader	80/Spreader	6
Fish Cake	1	TNTC	114/Spreader	22	4
	2	TNTC	125	23/Spreader	2/Spreader
Beef Ball	1	TNTC	TNTC/Spreader	97/Spreader	10/Spreader
	2	TNTC	TNTC	96/Spreader	10/Spreader
Chicken Ball	1	8	1	-	-
	2	12	1	-	-
Nugget Chicken	1	78/Spreader	8/Spreader	3/Spreader	-
	2	72/Spreader	14/Spreader	2/Spreader	-

APPENDIX C

Table C.1: Calculation total plate count for each sample

Sample	Calculation
Surimi scallop	$\text{CFU/mL} = \frac{110 \times (\frac{1}{1/100})}{0.1\ \text{mL}}$ $= 1.1 \times 10^5\ \text{CFU/mL}$
Korean Pearl Fish Cake Ball	$\text{CFU/mL} = \frac{92 \times (\frac{1}{1/1000})}{0.1\ \text{mL}}$ $= 9.2 \times 10^5\ \text{CFU/mL}$
Fish Ball	$\text{CFU/mL} = \frac{136 \times (\frac{1}{1/100})}{0.1\ \text{mL}}$ $= 2.27 \times 10^5\ \text{CFU/mL}$
Lobster Ball	$\text{CFU/mL} = \frac{120 \times (\frac{1}{1/100})}{0.1\ \text{mL}}$ $= 1.2 \times 10^5\ \text{CFU/mL}$
Lekor (Unboiled)	$\text{CFU/mL} = \frac{120 \times (\frac{1}{1/1000})}{0.1\ \text{mL}}$ $= 1.2 \times 10^6\ \text{CFU/mL}$
Lekor (boiled)	-
Fish Cake	$\text{CFU/mL} = \frac{125 \times (\frac{1}{1/100})}{0.1\ \text{mL}}$ $= 1.25 \times 10^5\ \text{CFU/mL}$
Beef ball	$\text{CFU/mL} = \frac{97 \times (\frac{1}{1/1000})}{0.1\ \text{mL}}$ $= 1 \times 10^{-1}\ \text{CFU/mL}$
Chicken ball	-
Chicken nugget	$\text{CFU/mL} = \frac{78 \times (\frac{1}{1/10})}{0.1\ \text{mL}}$ $= 7.8 \times 10^3\ \text{CFU/mL}$

APPENDIX D

Figure D.1: Halal logo on packaged beef ball

Figure D.2: Halal logo on packaged chicken ball

Figure D.3: Halal, HACCP & GMP certified logos on packaged chicken nugget

Bacterial Assessments on Processed Meat Products sold in Jeli, Kelantan

ORIGINALITY REPORT

7%	6%	4%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Runchao Yu, Xiaowei Tu, Jianming Yang, Qinghua Yang. "Optical Image Encryption Vulnerability Detection by Using Generative Adversarial Networks", 2020 Chinese Automation Congress (CAC), 2020 Publication	1%
2	www.ncbi.nlm.nih.gov Internet Source	<1%
3	s.northlincs.gov.uk Internet Source	<1%
4	www-pub.iaea.org Internet Source	<1%
5	www.coursehero.com Internet Source	<1%
6	hl-128-171-57-22.library.manoa.hawaii.edu Internet Source	<1%
7	Noor Hafizoh Saidan, Mohd Amir Afiq Kartayasa, Nur Ilham Atiqah Mohd Lokman, Nurul Fadzlin Ab Llah et al. "Effect of Chengal	<1%