# MICROBIOLOGICAL ANALYSES AND STUDENTS' ATTITUDES AND PERCEPTIONS TOWARDS MICROBIOLOGICAL RISKS OF PLANT-BASED MEAT ITEMS


By

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# ABSTRACT

## MICROBIOLOGICAL ANALYSES AND STUDENTS' ATTITUDES AND PERCEPTIONS TOWARDS MICROBIOLLOGICAL RISKS OF PLANT-BASED MEAT ITEMS

**Careen Chong Kai Lyn**

Plant-based meat was created to taste, feel, and look like traditional animal meat. It can improve health and reduce the risks of chronic illnesses. In recent years, consumers toward veganism kept increasing and the popularity of plant-based meat products has grown steadily in global market trends. These newly emerging food products were important to be microbiologically investigated, and the consumers' attitudes and perceptions of microbiological risks of plant-based meat were also important for food safety purposes. This study aims to determine the microbiological quality of plant-based meat items in relation to the attitude and perception towards microbiological risks of plant-based meat items among Universiti Tunku Abdul Rahman (UTAR), Kampar students. Aerobic colony count (ACC) and yeast and mold count (YMC) were determined in three plant-based meat meal samples. Collected data were analyzed using SPSS to generate research findings. Questionnaires were distributed to 165 UTAR Kampar students to determine the level of attitude and perception towards microbiological risks on plant-based meat items. Collected data were analyzed by Microsoft Excel to obtain research findings. A significant increase was found in the microbial counts of all food samples as time storage is prolonged at room temperature (25±2°C). In contrast, no significant difference ($p > 0.05$) was found in the microbial loads of all food samples stored for 2 days at refrigeration temperature (4±2°C). A significant difference ($p < 0.05$) was found for the avocado charcoal bun's microbial loads stored for 24 hours at room and refrigeration temperatures. Positive attitudes and perceptions were seen in most respondents toward the microbiological risks of plant-based meat items. These results concluded that microbial counts could differ depending on the specific food item and storage conditions. The survey concluded that students were aware of the microbiological risks of plant-based meat items.

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**DECLARATION**

I hereby declare that the project report is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

CAREEN CHONG KAI LYN

**APPROVAL SHEET**

This final year project report entitled **"MICROBIOLOGICAL ANALYSES AND STUDENTS' ATTITUDES AND PERCEPTIONS TOWARDS MICROBIOLOGICAL RISKS OF PLANT-BASED MEAT ITEMS"** was prepared by CAREEN CHONG KAI LYN and submitted as partial fulfillment of the requirements for the degree of Bachelor of Science (Hons) Dietetics at Universiti Tunku Abdul Rahman.

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It is hereby certified that **CAREEN CHONG KAI LYN** (ID No: **20ADB05306**) has completed this final year project report entitled "MICROBIOLOGICAL ANALYSES AND STUDENTS' ATTITUDES AND PERCEPTIONS TOWARDS MICROBIOLOGICAL RISKS OF PLANT-BASED MEAT ITEMS" under the supervision of Dr. Chang Sui Kiat from the Department of Allied Health Sciences, Faculty of Science, and Dr. Lam Ming Quan from the Department of Biological Science, Faculty of Science.

I hereby give permission to the University to upload the softcopy of my final year project report in pdf format into the UTAR Institutional Repository, which may be made accessible to the UTAR community and public.

Yours truly,

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(CAREEN CHONG KAI LYN)

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| | |
|---|---|
| °C | Degree Celsius |
| g | Gram |
| L | Liter |
| mL | Mililiter |
| % | Percentage |
| ACC | Aerobic Colony Count |
| ANOVA | Analysis of Variance |
| CAGR | Compound Annual Growth Rate |
| CFU | Colony-Forming Units |
| n | Number |
| PCA | Plate Count Agar |
| PDA | Potato Dextrose Agar |
| PDPA | Personal Data Protection Act |
| SD | Standard Deviation |
| SPSS | Statistical Packages for Social Sciences |
| TPC | Total Plate Count |
| US | United States |
| UTAR | Universiti Tunku Abdul Rahman |
| YMC | Yeast and Mold Count |

# CHAPTER 1

# INTRODUCTION

## 1.1 Background of the Study

Plant-based meat items are food products that are designed to imitate the taste, texture, and appearance of traditional animal-based meats like beef, chicken, pork, and seafood. However, instead of being derived from animals, these meat alternatives are made primarily from plant-based ingredients, such as soy, wheat, peas, mushrooms, and other plant proteins (Gayathri, 2022; Sayer, 2022). The goal is to create products that offer a similar sensory experience to animal meat while providing a more sustainable and ethical alternative. Plant-based meat items include burgers, sausages, nuggets, deli slices, ground meat, and even seafood substitutes (Good Food Institute, 2021).

Different diet patterns of people in this modern era have had a considerable effect on the Earth and the natural resources utilization (Tóth et al., 2021). Many people worldwide cannot live without animal origin’s meat products. The consumption of meat products is to gain certain components, especially protein to strengthen muscles and bones, reduce levels of hunger, boost metabolism, maintain weight loss and so on. However, the high consumption of meat, such as red meat and processed meat can affect global greenhouse gas emissions and environmental pollution (Vermeulen, Campbell and Ingram, 2012). Therefore, the number of vegans and vegetarians keep increasing in this current time to reduce environmental and health issues (Wilde, 2022). This phenomenon is also shown in Grand View Research (2022), reported the worldwide plant-based alternative meat had been experiencing substantial growth. The global plant-based meat market was projected to grow at a compound annual growth rate (CAGR) of 24.9% from 2023 to 2030.

Plant-based food items have become important and popular throughout the world as it brings health benefits to humans compared to animal meats. This is because long-term consumption of meat such as sausages, meatballs, burgers, or bacons can cause adverse effects to human health (Toribio-Mateas, Bester and Klimenko, 2021). Many researchers had discovered that there is a strong association between the high consumption of meat and non-communicable diseases' pathogenesis such as cardiovascular disease, cancer, diabetes mellitus, and obesity (Battaglia Richi et al., 2015; Godfray et al., 2018). On the other hand, some researchers have proved that plant-based meat items consumption can help to promote better health and decrease the risk of getting chronic diseases. This is because plant-based meat items are considered a natural food source that contains high fiber and polyphenol content, which helps to promote positive health changes to the gut microbiota (Toribio-Mateas, Bester and Klimenko, 2021).

Generally, plant-based meat items offer a range of health benefits that make them an appealing alternative to traditional animal-derived meats. It can improve the immune system as plants have nutrients that are not found in other foods. Plant-based foods contain vitamins and minerals, phytochemicals, and antioxidants that help keep cells healthy, allowing the immune system to work optimally (Alexander, 2019). The vital nutrients found in plant-based foods help to reduce inflammation in the body. The same phytochemicals and antioxidants that enhance the immune system also circulate throughout the body, neutralizing toxins from pollution, processed foods, bacteria, viruses, and other sources (Alexander, 2019; Haddad-Garcia, 2022; Sayer, 2022).

On the other hand, concerns about the safety and microbiological dangers of these alternative meat options are growing among consumers and food manufacturers as their popularity grows. According to Demetrakakes (2022), there was one plant-based meat processing factory had mold and bacterium infection as a result of the unsanitary conditions, demonstrating the possible microbiological safety and quality concerns connected with plant-based meat alternatives. Plant-based meat alternatives are made of plant proteins, polysaccharides, and several coloring and flavoring agents. Hence, the background microorganisms in plant-based meat items may differ significantly from those in meat of animal origin. Furthermore, plant-based meat products provide a substantially different nutrient environment, pH, and interior structure for microbes to adhere, penetrate, and multiply, potentially influencing the survival and growth of both spoilage and harmful microbes (Luchansky et al., 2020).

Hence, it is vital to ensure that plant-based meat items are safe from microbiological risks. It is essential for public health. Besides, consumers' concerns about microbiological risks demonstrates a commitment for consumer safety. Knowledge about consumers’ attitudes and perceptions can help public health authorities and food manufacturers ensure the consumption of good quality plant-based meat items.

## 1.2 Problem Statements

Food quality could be impacted significantly by the presence of microbial load. It can also be influenced by several factors, especially sanitary and environmental conditions such as storage temperature and duration (Liu et al., 2023). Numerous studies have been carried out to conduct microbiological analyses, aiming to identify spoilage bacteria and pathogens on both animal and plant foods under varying storage temperatures and durations (Sospedra, 2013; Luchansky et al., 2020).

There was one study conducted by Wang et al. (2023), spoilage pathogens in fresh beef were analyzed under different temperature storages, such as chilling (4ºC), superchilling (-2ºC) and frozen (-18ºC). Another study by Pizato et al. (2014) examined the microbial shelf life of industrialized cooked chicken breast meat stored at 7ºC for 15 to 17 days. Caldera and Franzetti (2013) investigated the microbiological quality of ready-to-eat vegetables with different preparations stored at 4ºC and 10ºC for 2 days via microbiological analysis with total plate count (TPC).

However, there is a notable dearth of similar studies focusing on plant-based meat products. Therefore, the problem statement revolves around the limited investigation and understanding of microbial load in plant-based meat items, leaving a gap in knowledge regarding their safety and quality. Addressing this gap is crucial to ensure the overall quality and safety of plant-based meat alternatives and align them with the standards set for conventional meat products.

Furthermore, the awareness on microbiological risk of plant-based meat items can be influenced by consumers' attitudes and perceptions. Numerous studies have been conducted in other countries to understand consumer attitudes and perceptions towards the microbiological risk of both animal and plant foods like meat, vegetables, pastries and so on (Safdar et al., 2022).

According to Odetokun et al. (2021), the researchers conducted a survey study on the consumers’ perception on microbiological risks on hygiene of retail meat such as pork, chicken, beef, mutton, and fish among Nigerian population. A similar research was also carried out by Kumar et al. (2011), showing that public awareness and perception towards the food safety risk of consuming pastries sold in Jalandhar city, India. In addition, there was another survey conducted on consumers’ perception to observe their food safety risk level towards the consumption of vegetables with different preparations in rural and urban areas in Hanoi, Vietnam (Ha, Shakur and Pham Do, 2019).

There is, however, a notable absence of research on this aspect specifically related to plant-based meat items among university students in Malaysia. This research gap highlights the need to investigate the attitudes and perceptions of this specific demographic, as university students represent a significant consumer group that may have unique views and preferences towards plant-based meat products. Addressing this gap can provide valuable insights into factors influencing the acceptance and consumption of plant-based meat alternatives in Malaysia and contribute to the development of targeted strategies for promoting their adoption and hence, ensuring food safety.

## 1.3 Research Objectives

### 1.3.1 General Objective

This study was conducted to determine the microbiological quality of plant-based meat items in relation to the attitude and perception towards microbiological risks on plant-based meat items among UTAR undergraduate students.

### 1.3.2 Specific Objectives

The specific objectives of this study were as follows:

i. To determine the aerobic colony count, and yeast and mold count between plant-based meat items stored at 2, 4, 6, 8 and 24 hours for room temperature (25±2°C), and 24 and 48 hours for refrigeration temperature (4±2°C).
ii. To determine the aerobic colony count, and yeast and mold count between plant-based meat items stored at refrigerator (4±2°C) and room (25±2°C) temperatures for 24 hours.
iii. To determine the attitude and perception level among UTAR Kampar undergraduate students towards microbiological risks of plant-based meat items.

## 1.4 Research Hypotheses

1. $H0_A$: There is no significant difference in the number of microbial loads of food samples at different storage durations at both temperatures.

   $H1_A$: There is a significant difference in the number of microbial loads of food samples at different storage durations at both temperatures.

2. $H0_B$: There is no significant difference in the number of microbial loads of food samples at 2 different storage temperatures for 24 hours.

   $H1_B$: There is a significant difference in the number of microbial loads of food samples at 2 different storage temperatures for 24 hours.

3. $H0_C$: There is a negative attitude and perception toward the microbiological risk of plant-based meat items among UTAR undergraduate students.

   $H1_C$: There is a positive attitude and perception toward the microbiological risk of plant-based meat items among UTAR undergraduate students.

## 1.5 Significance of the Study

This research aims to enhance consumers' understanding of the microbial shelf life of plant-based meat products and assess their safety for consumption through microbiological analyses. By studying this aspect, we can gain valuable insights into how to appropriately store these food items at optimal temperatures to ensure their quality and minimize the risk of foodborne illnesses.

Furthermore, this study will also raise awareness among consumers, particularly students, about the importance of maintaining sanitary and proper storage conditions for plant-based meat items. It will also guide consumers in selecting plant-based meat products of the highest quality available in the current market.

Through this research, the community will gain a deeper understanding of the health benefits associated with plant-based meat consumption and the critical role of temperature control in preventing contamination and foodborne illnesses. Additionally, the study will highlight the advantages of consuming plant-based meat over red and processed meat, offering potential protection against chronic diseases.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 History of Plant-Based Meat Items

Veganism is experiencing a spectacular boom in global popularity, making it a favored lifestyle and nutritional option for many individuals. As a result, the demand for plant-based meat alternatives has skyrocketed, as people actively seek out sustainable and ethical alternatives to traditional animal-based meat products (Lin and Lim, 2023). Plant-based meat has a rich history that spans several decades in Asia, where it has been a vital element of varied diets and was originally consumed in ancient China. This is because during China's Han Dynasty around 206 BC, Mahayana Buddhist monks followed a strict vegetarian diet and cleverly made meat substitutes using "tofu" to avoid eating meat. Tofu, made from soybean curd, has grown in popularity as a flexible and delightful meat substitute, finding its way into a variety of culinary pleasures (Sudofoods, 2023; The Week, n.d.).

Various entrepreneurs and companies have contributed to the invention and creation of plant-based meat products over the years. John Harvey Kellogg, one of the early pioneers in the current plant-based meat industry, produced "Protose," one of the earliest meat replacements in the Western world, in the late 1800s. Protose was created from a combination of peanuts, gluten, and other plant-based substances and was sold as a healthy replacement to meat (Sudofoods, 2023).

Beyond Meat and Impossible Foods are two recent important players that have made significant contributions to the plant-based meat sector. Beyond Meat, created in 2009 by Ethan Brown, has been a key influence in the development of plant-based meat products, notably the well-known Beyond Burger produced from pea protein. Patrick O. Brown started Impossible Foods in 2011 and received global recognition for his Impossible Burger, which employed a heme protein generated from genetically modified yeast to recreate the flavor and texture of beef (Thompson, 2019).

## 2.2 Comparison between Fresh and Processed Plant-Based Meat Products

Fresh plant-based meat alternatives, such as tofu, tempeh, and seitan, are often minimally processed. In terms of texture and flavor, these items are quite similar to their original plant sources (Ishaq et al., 2022; WebstaurantStore, n.d.). Tofu, manufactured from coagulated soy milk, has a delicate texture similar to many animal-based meats (Circulon Uk, n.d.; WebstaurantStore, n.d.). Tempeh, a fermented soy product, has a nutty flavor and a hard yet chewy texture that appeals to a wide range of customer tastes. Furthermore, seitan, which is made from wheat protein, has a meat-like texture, and may be seasoned to taste like various meats (WebstaurantStore, n.d.).

Processed plant-based meat products, on the other hand, use a combination of plant ingredients and various processing techniques to mimic the taste, texture, and appearance of traditional meat products. Plant-based burgers, sausages, and nuggets are just a few examples (Ishaq et al., 2022). These processed alternatives frequently employ technologies such as extrusion and high-pressure processing to attain desirable meat-like characteristics (Ahmad et al., 2022). For instance, the Beyond Burger and the Impossible Burger have become quite famous due to their resemblance to beef patties, both in taste and "bleeding" appearance (Thompson, 2019).

Fresh plant-based meat products retain higher quantities of key nutrients due to minimum processing. Tofu, for example, is a good source of plant-based protein and consist of all essential amino acids that the body is unable to produce (Petre, 2018). Tempeh's fermentation process improves its digestibility and nutrient absorption, making it a good source of protein, fiber, and vitamins (Romulo and Surya, 2021). Seitan, while lower in protein than tofu and tempeh, nonetheless contributes to protein consumption (WebstaurantStore, n.d.).

While processed plant-based meat products successfully replicate the sensory experience of meat eating, nutritional content may differ. These goods frequently contain additional substances like binders, flavor enhancers, colorants, and preservatives, which may have an impact on their overall nutritional profile (Ahmad et al., 2022; Kyriakopoulou, Keppler and van der Goot, 2021). However, formulation advances are made to improve the nutritional value of processed plant-based products, with the goal of matching the protein content and micronutrient profiles of traditional meats (Ahmad et al., 2022; Ishaq et al., 2022).

Sensory characteristics are critical to customer acceptance and market success of plant-based meat products (Fiorentini, Kinchla and Nolden, 2020). According to research, the sensory experience of ingesting both fresh and processed plant-based goods can considerably affect customer preferences. Fresh choices, such as tempeh and tofu, appeal to those looking for a more authentic and lightly processed alternatives (Fiorentini, Kinchla and Nolden, 2020; Ishaq et al., 2022). Processed goods, on the other hand, frequently succeed at emulating the taste, texture, and look of regular meats, thus attracting a wider spectrum of consumers (Fiorentini, Kinchla and Nolden, 2020).

Both fresh and processed plant-based meat products have the potential to be more sustainable than traditional animal-based meats as they were associated with approximately up to 96% to 98% of less greenhouse gas emissions, and 43% to 63% of less ozone depletion (Bryant, 2022). Fresh choices often have lesser processing requirements, resulting in lower energy use and carbon footprints (Bryant, 2022; Shabir et al., 2023). While processed plant-based products require more energy due to the manufacturing procedures involved, they have lower environmental implications than their animal-based counterparts (Bryant, 2022).

Lastly, the comparison of fresh and processed plant-based meat products reveals a broad array of possibilities appealing to various consumer tastes. While fresh plant-based alternatives such as tofu, tempeh, and seitan provide less processed alternatives with different nutritional benefits, processed plant-based goods attempt to recreate the sensory experience of traditional meats. Continuous innovation in the formulation of processed alternatives attempts to create a balance between sensory qualities, nutritional content, and sustainability, leading to the growth of the plant-based meat market.

## 2.3 Microbiological Analyses on Plant-Based Meat Items

Microbiological analysis is the laboratory examination and evaluation of microorganisms present in various samples, including the use of biological, biochemical, or chemical techniques to detect, identify, or enumerate bacteria, fungi, viruses, and other microorganisms in food, water, soil, clinical specimens, and environmental swabs (Elga LabWater, n.d.; Campden BRI, n.d. -b). The major goal of microbiological analysis is to ensure the safety and quality of various goods and surroundings, as well as to detect and monitor the presence of dangerous bacteria that may endanger human health. It is critical in food safety, water quality assessment, medicines, clinical diagnostics, and environmental monitoring. Potential microbial dangers can be discovered through microbiological investigation, and suitable steps can be made to prevent outbreaks of infectious diseases and contamination events. Data from such analyses aid in the establishment of regulatory requirements, the implementation of quality control measures, and the making of informed decisions to protect public health and well-being (Jay, Loessner and Golden, 2005; Nemati et al., 2016).

Several studies have investigated the microbiological quality of plant-based meat products. For instance, Dai et al. (2022) conducted a comprehensive microbial analysis on a variety of plant-based meat samples. Their study showed that the total microbial count exceeded in the control samples stored for 14 days. However, the shelf life of the food samples treated with various combinations of biological preservatives had shown an increase compared to the control samples.

Apart from that, Tóth et al. (2021) found the presence of microbial spoilage in plant-based meat samples such as vegan meatballs and cabbage casserole stored for 36 hours and 24 hours respectively without placing them in the refrigerator. Nevertheless, there were also microbes found in the plant-based spaghetti Bolognese and cabbage casserole samples, but the contamination was low when they were kept in the refrigerator until the $4^{th}$ day.

There were several investigations that have found microbial contamination in plant-based meat products, including common pathogenic bacteria, such as *E. coli, Salmonella spp.*, and *Listeria monocytogenes*. Yeast, mold, and other fungal species have also been found as causes of product spoiling and shorter shelf life (Duthoo et al., 2022; Liu et al., 2022; Luchansky, 2020; Tóth et al., 2021). As a result, this points to the necessity for better production practices and quality control.

Significant research has been conducted on the microbiological quality of plant-based meat products. There is information available on the long-term effects of storage conditions on microbial growth and survival in these food products.

## 2.4 Factors Affecting Microbial Shelf Life of Plant-Based Foods

Shelf life is defined as the amount of time that a product can be consumed after it has been manufactured. The product must stay safe while maintaining appropriate sensory, microbiological, and chemical properties (Campden BRI, n.d. -a). The microbiological shelf life of plant-based food products is an important part of food safety and quality assurance (Guevara, 2022). As the demand for plant-based food items grows, it becomes critical to thoroughly investigate the elements that determine the shelf life of these goods (Alcorta et al., 2021). This literature review aims to identify and synthesize the important parameters influencing the microbiological shelf life of plant-based foods, thereby contributing to a better knowledge of food preservation strategies and consumer health.

Physical, chemical, and environmental factors all have an impact on the microbiological shelf life of plant-based foods. According to numerous studies, the common factors that were discovered include packaging, storage temperature, pH, oxygen, moisture content, preservatives, and processing techniques (Alegbeleye et al., 2022; Dagnas and Membré, 2013; Liu et al., 2023; Wild et al., 2014).

There was one research done by Dagnas and Membré (2013) which stated that molds are frequently encountered in production settings. Airborne spores have been identified as the most common source of contamination during food processing. Air-to-food transfer can happen via dust particles or aerosols, particularly during product packing. Prior to packaging, the likelihood of contamination is determined by various factors that are strictly related to factory design, processing, and environment.

A study by Wild et al. (2014), revealed that endospore-forming microorganisms (such as *Bacillus spp.* and *Clostridium spp.*) may survive the extrusion process of plant-based meat. Relevant microbe concentrations in pre-mixtures and recipes can occur depending on the microbial load of the specified raw materials. This includes the potential presence of pathogenic and toxicogenic microbial species. Plant-based meat items are highly susceptible to spoilage due to several factors, which are their nearly neutral pH, high protein content, and moisture content level.

The study carried out by Alegbeleye et al. (2022) stated that the spoilage of plant commodities occurs on a regular basis all around the world owing to a variety of circumstances, resulting in enormous waste. Several studies have shed light on the elements that contribute to and influence the rotting of plant-based foods, such as environmental factors, pH, temperature, and oxygen, as well as other factors just as manufacturing practices and some consumer attitudes.

According to another research study conducted by Liu et al. (2023), plant-based meat items, similar to ground beef, were also intentionally infected with pathogenic microbes such as *Escherichia coli* O157:H7, *Salmonella spp.*, and *Listeria monocytogenes*, as well as meat spoilage pathogens such as *Pseudomonas fluorescens* and *Brochothrix thermosphacta*. The factors that lead to plant-based meat spoilage were storage temperature and packaging conditions.

Ultimately, the microbiological shelf life of plant-based meals is determined by a complex interplay of many elements such as packaging materials, temperature regulation, and pH levels. While significant work has been made in explaining these factors, there is still a need for additional research to resolve inconsistencies and gaps in the current literature. A thorough understanding of these aspects will allow for the development of successful preservation solutions, as well as the creation of safer and higher-quality plant-based foods.

## 2.5 Effects of Storage Temperatures and Durations on the Microbial Shelf Life of Plant-Based Foods

The development of new functional foods is currently attracting attention due to the rising prevalence of chronic diseases and the essential role of nutrition in the majority of them (Forouzanfar et al., 2016). Plant-based diets are becoming more popular as a result of their health and environmental benefits (Lynch, Johnston and Wharton, 2018). However, because plant-based meals are perishable, preserving their microbiological integrity during storage is difficult. Microbial development can cause spoiling, reducing shelf life and posing health concerns to customers (Luchansky et al., 2020). Storage temperature and duration are crucial for microbial growth management and product quality. They affect the microbiological shelf life of plant-based foods (Liu et al., 2022).

Temperature influences microbial development, with various microbes growing at different rates at different temperatures and durations (Jay, Loessner and Golden, 2005). Psychrotrophic bacteria, which are widespread in refrigerated goods, grow at temperatures ranging from 0°C to 7°C (Erkmen and Bozoglu, 2016). Mesophilic bacteria, which are relevant to foods held at room temperature, grow best at temperatures ranging from 20°C to 45°C (Keenleyside, 2019). Extreme temperatures, such as those experienced when freezing or boiling, can also have an impact on microbial survival and proliferation (Merino et al., 2019).

Plant-based meat alternatives, which are frequently manufactured from plant proteins, are prone to spoiling due to their protein-rich composition.

Temperatures below 4°C have been shown to prevent the growth of spoilage organisms and diseases (Ma et al., 2021). Nonetheless, certain molds can grow in cold temperatures, especially black molds such as *Stachybotrys chartarum* that can produce mycotoxins (Howerton, 2021).

According to study carried out by Cabello-Olmo et al. (2020), the results showed that there was a decline in microbial content as the effect of the storage temperature at 37°C is the most harmful condition for bacterial growth. On the other hand, storage temperatures, -20°C and 4°C were better than room temperature to prevent food spoilage.

Moreover, another study conducted by Liu et al. (2023) showed that the while conducting microbiological analysis, microbial content increased during the 24 hours of storage temperatures at 22°C and 32°C, while the lower microbial content was shown at 4°C, which is considered as the safer temperature for storage of plant-based meat items.

The storage temperature has a significant impact on the microbiological shelf life of plant-based meals. Understanding the growth patterns of various microorganisms and the effect of temperature on their proliferation might aid in the development of successful food preservation systems. Proper storage conditions, adapted to each product's features, can help to reduce food waste while also preserving the safety and quality of plant-based foods.

## 2.6 Yeasts and Molds in Food

Yeast and molds are known as eukaryotic microorganisms that can be unicellular or multicellular. They can also be categorized as fungi. They play a significant role in the food industry and influencing food quality and safety by causing spoilage and sometimes even producing toxins (Lorenzo et al., 2018).

Yeast and mold are found ubiquitously in the environment, including soil, air, and water. Molds are made up of long filamentous structures known as hyphae that grow through apical extension, whereas yeasts are primarily single-celled creatures. Because yeasts and molds are heterotrophic organisms that receive their nutrition from organic matter, food products provide an excellent environment for their growth (McGinnis and Tyring, 1996).

Yeasts reproduce asexually by budding, in which a tiny cell (bud) develops and finally splits from the parent cell. Molds, on the other hand, reproduce by spores, which are discharged into the surrounding environment to stimulate new growth. As a result, understanding their development and reproductive mechanisms is critical for regulating their proliferation in food.

There are various factors that influence the growth of yeasts and molds in food. Temperature, pH, water activity, and the presence of nutrients are critical factors that determine their ability to thrive in different food matrices. Researchers have extensively studied these factors to develop effective preservation strategies and prevent spoilage (Dagnas and Membré, 2013; Microbe Notes, 2021).

Both yeast and mold contribute to the deterioration and decomposition of foods to varying extents. Yeast is mainly involved in fermentation processes, converting sugars into alcohol and carbon dioxide, leading to alterations in taste, texture, and aroma (Maicas, 2020). On the other hand, molds form visible colonies on the food's surface, producing characteristic off-flavors and discoloration. Besides, they can infest and flourish on almost any type of food at any stage (Weishaupt, n.d.). Whether it is invading crops like grains, nuts, beans, and fruits in fields before or during harvest, or growing on processed foods and food mixtures, these microorganisms exhibit their adaptability and widespread impact on the food supply (FDA, 2020; USDA, 2013b).

Apart from causing spoilage, some molds have the potential to produce mycotoxins, which are harmful compounds that can pose serious health risks to consumers. Mycotoxins are particularly dangerous in crops such as grains and nuts, prompting regulatory steps to assure food safety (WHO, 2018). Thus, controlling yeasts and molds in the food sector requires a combination of physical, chemical, and biological approaches. To prevent their proliferation, popular practices include proper hygiene, temperature control, and the use of preservatives. Furthermore, advances in food packaging and storage technology have been investigated in order to improve product shelf life (Conte et al., 2013; Davies et al., 2021).

## 2.7 Attitude towards Plant-Based Meat Items

Consumer attitude can be defined as an individual's favorable or unfavorable feelings towards an object. As we all know, an individual with a positive attitude is more likely to purchase a product, which results in the likelihood of liking or disliking a product (Tutorialspoint, 2019). Consumer attitudes are critical to the success and uptake of these options (Bryant and Barnett, 2020). Understanding the elements that influence attitudes towards plant-based meat products is critical for the food sector to market and promote these products effectively.

Davitt et al. (2021) reported that 55% of Midwest University students had a positive attitude towards plant-based meat items consumption. The top reasons were a desire to try new meals and an interest in the items. Plant-based alternative consumption was strongly linked with out-of-state residency, vegetarian lifestyle, and 10 out of 11 environmental attitude statements ($p < 0.05$). Besides, approximately 30% of students said they wished to eat less meat and believed plant-based options were healthier for the environment. Only 20 to 25% of students gave reasons such as health, animal welfare, or cost towards the consumption of plant-based meat items.

The research conducted by Chung et al. (2022) reported that majority of the China residents had high attitudes towards the consumption of plant-based meat products. Based on the collected data, most of the respondents reported that they chose to consume plant-based meat as it is better for animals and the environment. Some of them also rated plant-based meat products as healthy, safe, convenient and trendy. 41.9% of respondents were willing to try it even though they did not try before, and 31.4% intended to buy it. Among all China residents, younger respondents were more likely to have the highest attitude to buy plant-based meat. Furthermore, those who prepare food for children, those who are married, living with a partner, divorced, or widowed, and those who live in larger cities were considerably more likely to intend to purchase plant-based meat.

According to Michel, Hartmann and Siegrist (2021), results showed that most of the consumers from Germany had high attitude towards plant-based meat if the flavor and feel are very comparable to the animal origin meat. However, female respondents showed more positive attitude towards plant-based meat diet compared to males. This is because they expressed worries about animal welfare and the environment and suggested moderate meat intake; whereas males showed negative attitude as they emphasized the good qualities of real meat.

Consumer views towards plant-based meat products are influenced by health considerations, sensory attributes, environmental concerns, and cultural influences. Views are predicted to move in favor of plant-based alternatives as these elements mature and improve. However, addressing issues like awareness is crucial to moving this adoption process forward.

## 2.8 Perception towards Plant-Based Meat Items

The increased concern for environmental sustainability and health consciousness has resulted in a major movement in consumer preferences towards plant-based diets (Bryant et al., 2019; Michel, Hartmann and Siegrist, 2021). One noticeable component of this trend is the growing popularity of plant-based meat alternatives, which try to mimic the flavor and texture of traditional animal-derived meat products (Ahmad et al., 2022; Gayathri, 2022; Sayer, 2022). As these alternatives become more widely available, studying the aspects impacting customer perception and acceptability of plant-based meat products becomes relevant (Michel, Hartmann and Siegrist, 2021).

The popularity of plant-based meat products worldwide is being driven by environmental sustainability. According to Szenderák, Fróna and Rákos (2022), consumers perceive plant-based patties as a more environmentally responsible option because they have lesser carbon footprint than meat burger patties production. This perception is supported by the idea that plant-based alternatives help to reduce land use and water consumption (Bryant et al., 2019).

Consumer perceptions of plant-based meat are also influenced by health concerns. (Bryant, 2022) stated that health-conscious individuals consider plant-based meats as a healthier alternative due to their lower saturated fat and cholesterol content. Furthermore, according to Szenderák , Fróna and Rákos (2022), consumers connect plant-based goods with superior nutritional content and believe they contribute to general well-being. Besides that, in accordance with Choi (2021) research, around 70% of consumers considered the healthiest protein source is from plants compared to animals, which indicates that their strong perception towards consumption of more plant-based food. Another study conducted among China residents stated that majority of them had higher perception of plant-based meat as it is healthy, having good mouthfeel and food safety benefits (Chung et al., 2022).

Taste and texture are important drivers of consumer acceptability of plant-based meat products. According to studies, the perception of these characteristics is crucial in influencing purchasing decisions (Fiorentini, Kinchla and Nolden, 2020). There was a survey study conducted among US adults, 86% of respondents deemed taste to be the most important determinant of purchase intention (IFIC Foundation, 2019). Based on the study of Hoek et al. (2011), collected data from a consumer survey in the United Kingdom and the Netherlands demonstrate that, while consumers are often aware of the ethical and political consequences of their food choices, purchase intention is ultimately determined by the product's sensory qualities. However, sensory evaluation studies by Waehrens et al. (2023) revealed that some consumers still find some of the plant-based options are inadequate in terms of duplicating the sensory experience of conventional meat, especially for plant-based chicken and beef by adding seasonings to enhance the meaty flavor.

Social and cultural variables also influence how people perceive plant-based meat alternatives. Bryant et al. (2019) discovered that cultural backgrounds and social circles influence customers' willingness to explore plant-based products. Furthermore, social norms and peers also influence individuals' perceptions and attitudes towards plant-based meat items (Paul Fesenfeld et al., 2023).

Consumer perception of plant-based meat products is influenced by a complex interaction of environmental concerns, health considerations, taste and texture experiences, as well as social and cultural variables. Understanding these variables is critical for industry stakeholders and policymakers to properly promote and support the adoption of plant-based diets. As customer preferences fluctuate, more research is required to track changes in perception and develop tactics that respond to the different requirements and preferences of consumers.

## 2.9 Attitude and Perception towards Microbiological Risks of Food

Food safety is a top priority for public health and consumer well-being. The microbiological concerns associated with foodborne pathogens have received much academic and regulatory attention. Understanding consumer attitudes and perceptions of these dangers is critical for improving food safety practices and developing relevant policies (Liguori et al., 2022).

Consumers' attitudes towards food safety have a significant impact on their food purchasing and consumption behavior. According to Feng and Archila (2020), customers who are more aware of microbiological threats exhibit more cautious food handling practices and choose items with greater safety standards. This implies that knowledge and awareness have a substantial impact on consumer attitudes towards food safety. Food manufacturers and retailers, governmental agencies, and health educators are all interested in consumers' attitudes regarding food safety and food-related practices. This interest has manifested itself in debates over how food safety should be defined, as well as how consumers perceive food safety and choose food (Wilcock et al., 2004).

The food processing industry is also critical to guaranteeing food safety. The perception of microbial dangers in this industry is crucial for adopting effective control measures. Van der Vossen-Wijmenga et al. (2022) conducted a survey of food processing professionals and discovered that the majority saw microbial concerns as a key concern. However, the survey indicated differences in attitudes, indicating the necessity for focused training and communication techniques within the industry. Study by Byakika et al. (2019) found that processors in Kampala had fairly good knowledge (63.0 ± 2.3%) and attitudes (52.2 ± 3.0%) towards microbiological quality of commercially produced traditional fermented cereal beverages.

Understanding attitudes and beliefs regarding microbial dangers in food is critical. Consumer knowledge and awareness, opinions within the food processing sector, and trust in regulatory agencies all play important roles in developing these attitudes. Furthermore, differences in demographic groups demand customized methods in food safety education and communication strategies are also factors affecting their attitudes.

# CHAPTER 3

# MATERIALS AND METHODS

## 3.1 Experimental Framework

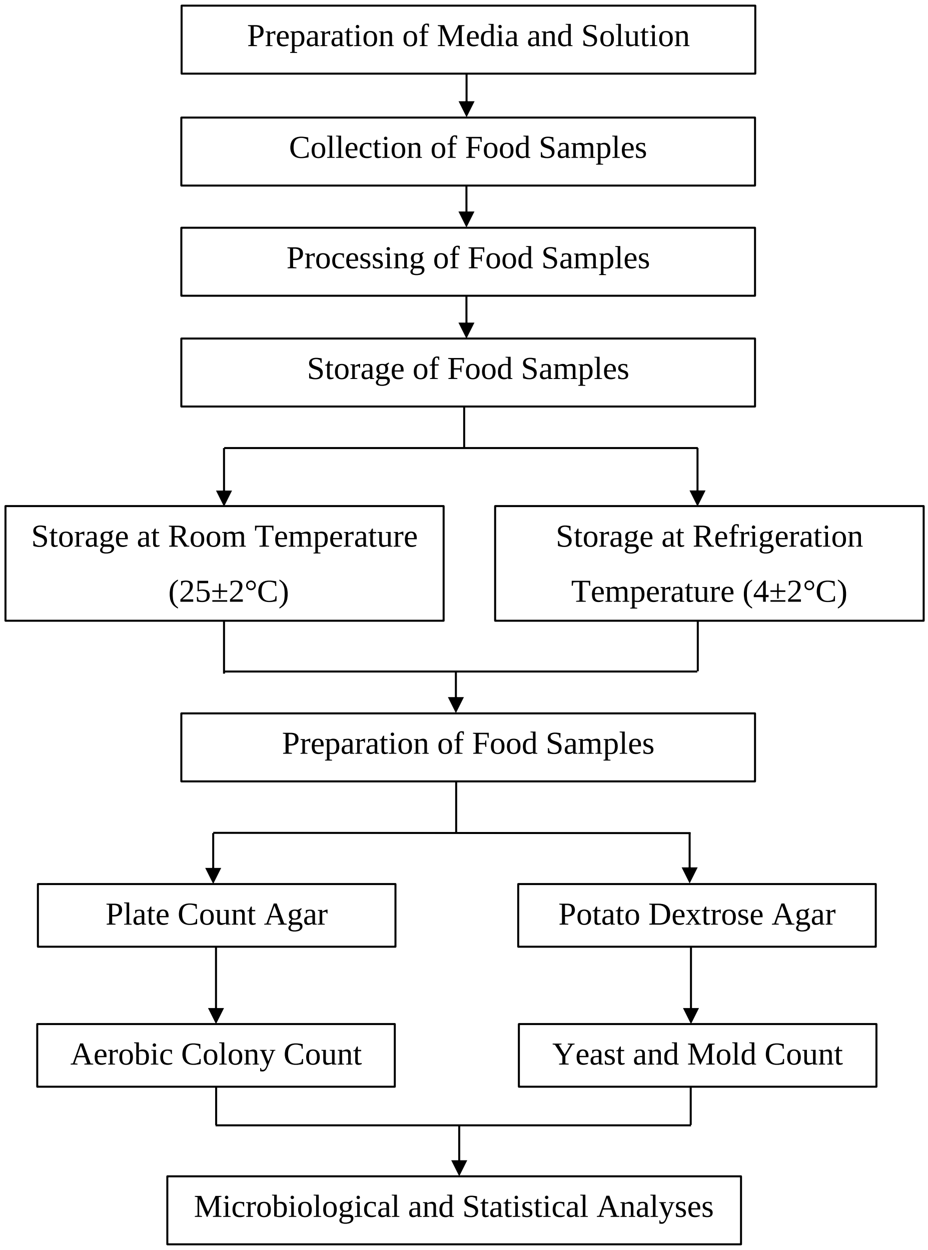


**Figure 3.1:** Experimental framework of this study.

## 3.2 Apparatus and Materials

Methanol ($CH_3OH$), plate count agar (PCA), potato dextrose agar (PDA), sterile distilled water, beakers, falcon tubes, micropipettes, pipette tips, petri dishes, parafilm, glass spreader and Bunsen burners.

## 3.3 Preparation of Media and Solution

All media and solutions used were subjected to autoclaving at 121˚C for 15 minutes, unless otherwise specified.

### 3.3.1 Plate Count Agar (PCA)

Plate count agar (PCA) is a solid growth medium commonly used in microbiology to determine the quantity of viable bacteria within a sample. It creates an optimal environment for bacterial proliferation, facilitating the formation of visible colonies, which aids in precise and accurate counting and quantification of viable microorganisms. PCA is typically composed of tryptone, which provides amino acids and nitrogen to support bacterial growth; yeast extract, which provides essential nutrients and vitamins; glucose, which serves as an energy source for bacterial metabolism; and agar, which acts as a solidifying agent and provides a stable surface for bacterial colony development. To properly use PCA effectively, the sample is appropriately diluted and spread over the agar medium's surface. Subsequent incubation of the agar plates under suitable conditions enables bacterial colonies to grow. The visible colonies are then enumerated after incubation, and the number of viable bacteria in the original food sample is estimated based on the dilution factor (Aryal, 2019a).

PCA was prepared by suspending 22.5 g of the PCA powder in 1 L of distilled water. After the medium cooling from autoclave, it was distributed into petri dishes. All of the agars were cooled and hardened at room temperature.

### 3.3.2 Potato Dextrose Agar (PDA)

Potato dextrose agar (PDA) is a solid growth medium widely utilized in microbiology and mycology for cultivating and isolating fungi and molds. Its popularity stems from its ability to foster the growth of a wide array of fungi, thanks to its simple and nutrient-rich composition. The primary components of PDA consists of potato infusion, which provides essential carbohydrates, vitamins, and minerals for fungal growth; dextrose (glucose), which acts as an energy source for the fungi; and agar, which acts as a solidifying agent and creates a stable surface for fungal development and colony formation. PDA is useful for isolating and identifying fungi from a variety of sources, including air, soil, water, food, and clinical specimens. The fungus develop separate colonies on the surface of the agar, allowing for visual identification and future research. PDA is widely used in laboratory and research settings due to its adaptability in enabling the development of numerous fungal species (Aryal, 2019b).

PDA was prepared by following the same steps as PCA. However, 39 g of the PDA powder was used instead of PCA powder.

## 3.4 Microbiological Analytical Procedures

### 3.4.1 Plant-Based Meat Items' Collection

There were a total of 3 different plant-based meat food items used in this research, which were Hainanese grandpapa's rice, avocado charcoal bun, and popcorn chicken. All cooked plant-based meat items were purchased and collected from a vegan restaurant located in Ipoh, Perak, Malaysia. The plant-based meat items were ordered and delivered to the laboratory within 2 hours in a fresh and good quality condition using disposable paper food containers.

### 3.4.2 Processing of Plant-Based Meat Items

Initially, all cooked plant-based meat items were blended thoroughly using a food blender. Subsequently, each type of blended food sample was placed into separate re-sealable storage bags without vacuum with labeling using sterile spatula for future microbiological analyses.

### 3.4.3 Storage of Plant-Based Meat Items

The blended food samples in re-sealable storage bags were stored at room temperature (25±2°C) and refrigerated temperature (4±2°C), respectively. Subsequently, all samples that were stored at room temperature were analyzed on the $2^{nd}$, $4^{th}$, $6^{th}$, $8^{th}$, and $24^{th}$ hours. Then, samples stored at refrigerated temperature were analyzed on the $24^{th}$ and $48^{th}$ hours for the determination of microbial shelf life. Each experiment was repeated twice to meet the accuracy of the results.

### 3.4.4 Sample Preparation

Blended food samples that were stored at different temperatures were weighed and cut into a standardized portion size of 1 g. A total of seven 10-fold dilution series ($10^{-1}$ to $10^{-7}$) were prepared. One gram of blended food sample was mixed with 9 mL of sterile distilled water and then aseptically transferred into seven falcon tubes ($10^{-1}$ to $10^{-7}$). The mixture was mixed well by shaking. Then, each diluted sample was surface plated on specific agar plates for the detection of different microbes from the food samples.

## 3.5 Microbiological Analyses

The collected food samples underwent comprehensive microbiological analyses, encompassing various tests to assess their safety and quality. The analyses conducted include:

i) Aerobic colony count (ACC) to determine the presence of viable aerobic microorganisms.

ii) Yeast and mold count (YMC) to identify the presence of microorganisms that could impact the safety and shelf life of food.

To ensure accuracy and reliability, each food sample underwent microbiological evaluation twice. Repeated evaluation helps to validate the results and provides a more robust assessment of the microbiological profile of the food samples, enabling the identification of any potential variations or changes in microbial populations over time.

Colony-forming units (CFU) is a unit of measurement commonly used in microbiological analysis to quantify the number of viable microorganisms in a given sample. It is particularly useful when counting bacteria or fungi that form visible colonies on a solid growth medium (Bio-resource, 2011). The CFU value represents the number of viable cells or colony-forming units present in a specific volume or weight of the plant-based meat sample.

The formula for calculating CFU/g is as follows:

CFU/g = (Total number of colony/ volume of inoculum transferred) x total dilution factor

### 3.5.1 Aerobic Colony Count (ACC)

The experiment involved preparing serial dilutions of the food sample, ranging from $10^{-1}$ to $10^{-7}$. These dilutions were thoroughly mixed, and then 0.1 mL of various dilution levels was spread-plated in duplicate on plate count agar (PCA) plates. After spreading, the PCA plates were allowed to dry before being placed in an incubator at 37°C for 24 hours. Following the incubation period, the aerobic colony-forming units (CFUs) were determined using a colony counter pen, and the aerobic colony count was calculated separately from the two duplicated plates. The dilution that resulted in a colony count ranging from 30 to 300 CFUs was selected for further analysis. By using this dilution, colony forming units per gram (CFU/g) of the test sample was able to be determined, providing valuable information about the microbial load and quality of the sample.

### 3.5.2 Yeast and Mold Count (YMC)

After the spread-plating and incubation process at 30°C for 7 days on potato dextrose agar (PDA) plates, the colonies that appeared within the range of 30 to 300 were counted using a colony counter pen. The yeast and mold count (YMC) was obtained by counting the colonies present on two duplicated plates of the same dilution. Subsequently, the calculated YMC was converted into colony-forming units per gram (CFU/g). This conversion allows the quantification of the yeast and mold population present in the test food sample, providing valuable information about its microbial load and quality of the sample.

## 3.6 Statistical Analyses

The one-way ANOVA test followed by Bonferroni as the post hoc test, and paired samples t-test were performed in statistical analysis. This was to determine the effect of storage temperatures and durations on the 3 plant-based food products' microbial load. In this study, the effect of storage temperature was evaluated by making comparisons with the characteristics of the stored food samples at different temperatures. The difference in means was discovered at a significance level of 0.05. All statistical analyses were performed using Statistical Package for Social Sciences (SPSS) version 26. The results were presented as $\log_{10}$CFU/g and followed by mean ± standard deviation.

## 3.7 Students' Attitudes and Perceptions toward Microbiological Risk Survey

### 3.7.1 Research Framework

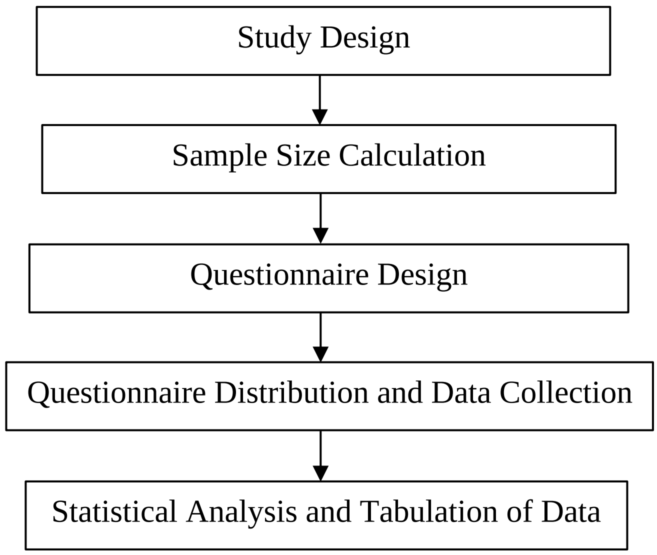


**Figure 3.2:** Research framework of this study.

### 3.7.2 Study Design

This research was carried out at Universiti Tunku Abdul Rahman (UTAR), Kampar Campus using a cross-sectional study design. Cross-sectional design is commonly used in public health research to estimate the prevalence of health outcomes, facilitating better planning, monitoring, and assessment of public health initiatives (Wang and Cheng, 2020). The study was conducted over a period from March 2023 to July 2023.

To select participants for the study, convenience sampling, which is also known as a non-probability sampling method, was employed. This approach was chosen due to time, financial, and workforce constraints. Convenience sampling allows us to reach the target population more easily, considering the practical limitations.

Before data collection commenced, ethical approval from the university was sought (refer to Appendix A). This step was crucial to ensure that the study adhered to ethical guidelines and requirements. Throughout the research process, all gathered information was treated with strict confidentiality and would not be disclosed to the public or unauthorized individuals. Data handling and analysis were carried out exclusively by us and the assigned supervisor, maintaining the confidentiality and integrity of the research findings.

### 3.7.3 Inclusion and Exclusion Criteria

Participants in this study included Malaysian undergraduate students from Universiti Tunku Abdul Rahman (UTAR). On the other hand, postgraduate students, foundation students and undergraduate students that have soy allergies were excluded from this study.

### 3.7.4 Sample Size

The required sample size was estimated using a single population proportion calculation obtained from the website of Pourhoseingholi, Vahedi and Rahimzadeh (2013). The formula used for this calculation is as follows: $n = Z^2 \frac{(p)(1-p)}{d^2}$, where n represents the sample size, Z is the Z-score corresponding to a 95% confidence level (Z = 1.96), d is the precision or accuracy set at 5%, and P denotes the expected prevalence. The expected prevalence of consumption level regarding plant-based meat items use was obtained from a study conducted by Statista Research Department (2022).

$$n = 1.96^2 \frac{(0.11)(1-0.11)}{0.05^2}$$

= 150.44 ≈ 150 subjects

With an assumption of 10% dropout rate:

150 + (150 × 10%) = **165 respondents**

### 3.7.5 Questionnaire Design

The online questionnaire was conducted via Google form. The questions that were used in this study was modified from the questionnaires carried out by Špernjak, Jug Puhmeister and Šorgo (2021) and Sterniša et al. (2018). The questionnaire consists of 3 sections with a total of 16 questions with multiple choices. Section A: Sociodemographic profiles; Section B: Knowledge, self-reported practices, and awareness on controlling the microbiological pathogens in plant-based meat items; Section C: Food microbiology knowledge. The questionnaire was attached in Appendix B.

### 3.7.6 Ethical Consideration and Approval

This study adhered to ethical guidelines, and ethical approval was obtained from the UTAR Scientific and Ethical Review Committee (U/SERC/62/2023). Before participating, all participants were provided with information about the study through questionnaires. It was explicitly stated that participation was voluntary, and individuals could choose to participate by clicking the "Agree" button on the consent form.

Participants were given the freedom to withdraw from the study any time by simply exiting or closing the browser page (refer to Appendix A). Additionally, all necessary measures were taken to comply with the Personal Data Protection Act 2016 (PDPA) in collecting samples and ensuring data privacy. The participants' identities were kept anonymous, and their responses were treated with strict confidentiality.

### 3.7.7 Pilot Study

A pilot study is the initial phase in the research procedure and is typically a smaller-scale study that aids in the planning and adjustment of the major study (In, 2017). The pre-testing in this study was conducted to assess the reliability of the specific customized questionnaires. A total of 10 UTAR undergraduate Kampar students participated in the pilot study. Consequently, the participants were able to comprehend and respond to the questions in the survey form.

### 3.7.8 Data Collection

During the data collection period, the final questionnaires, along with informed consent, were distributed to eligible UTAR undergraduate students through various platforms, including Microsoft Teams, WhatsApp, Instagram, and WeChat. Ultimately, a total of 165 responses were collected from the participants.

### 3.7.9 Statistical Analysis

The data collected via Google Forms were exported to Microsoft Office Excel 2019 for analysis. In this study, descriptive analyses were employed to present the findings on various aspects, including sociodemographic profiles, knowledge, self-reported practices, awareness of controlling microbiological pathogens in plant-based meat items, and food microbiology knowledge. The results were presented in terms of frequency (n) and percentage (%).

Regarding participants' attitudes and perceptions towards microbiological risk on plant-based meat items, the questions in both section B and C were analyzed. The scores were categorized using Bloom's cut-off point. A total score between 80% and 100% was classified as positive, a score between 60% and 79% as moderate, and a score below 60% as negative (Akalu, Ayelign and Molla, 2020).

# CHAPTER 4

# RESULTS

## 4.1 Microbiological Analyses on Plant-Based Meat Items

### 4.1.1 Effects of Storage Durations on the Microbiological Loads of the Plant-Based Meat Items Stored at Room Temperature (25±2°C)

The results shown in Table 4.1 represent the correlation between the influence of storage durations and the growth of microbiological loads of the food samples stored at room temperature (25±2°C). Each experiment was repeated twice and each plates represented in the form of A and B shown in the figures below (refer to Figures 4.1a and 4.1b). A significant increase in the microbial counts were found in all food samples stored from 2 hours to 24 hours. The microbial loads for Hainanese grandpapa's rice stored at room temperature (25±2°C) for 2, 4, 6, and 8 hours were too few to count in aerobic colony count (ACC) and yeast and mold count (YMC). Thus, the microbial load of the food sample cannot be enumerated. However, the microbial load increased when the storage period increased up to 24 hours, which showed an average of $1.63 \times 10^8$ CFU/g for ACC and $6.07 \times 10^8$ CFU/g for YMC. Subsequently, there were no significant difference ($p > 0.05$) in the microbial loads of avocado charcoal bun stored at room temperature (25±2°C) for 2, 4, 6, and 8 hours in both ACC and YMC. However, ACC and YMC for avocado charcoal bun stored at 24 hours showed a significant difference ($p < 0.05$) compared to the other storage durations. Meanwhile, there was no significant difference ($p > 0.05$) in either ACC or YMC for popcorn chicken stored at room temperature (25±2°C) for 2, 4, 6, and 8 hours.

Nonetheless, there were significant difference ($p < 0.05$) shown in both ACC and YMC for popcorn chicken stored at room temperature (25±2°C) for 24 hours compared to the other storage durations.

**Table 4.1:** Microbial count (log CFU/g) of plant-based meat samples stored at room temperature (25±2°C).

| Microbiological Parameters | Duration (hours) | Log CFU/g | | |
|---|---|---|---|---|
| | | **Hainanese Grandpapa's Rice** | **Avocado Charcoal Bun** | **Popcorn Chicken** |
| Aerobic Colony Count | 2 | TFTC | $^{a}3.05 \pm 0.71 \times 10^{5}$ | $^{a}3.60 \pm 0.71 \times 10^{8}$ |
| | 4 | TFTC | $^{a}3.95 \pm 0.21 \times 10^{5}$ | $^{a}4.45 \pm 0.49 \times 10^{8}$ |
| | 6 | TFTC | $^{a}1.06 \pm 0.47 \times 10^{6}$ | $^{a}4.35 \pm 0.21 \times 10^{8}$ |
| | 8 | TFTC | $^{a}1.67 \pm 0.76 \times 10^{7}$ | $^{a}3.75 \pm 0.49 \times 10^{8}$ |
| | 24 | $^{a}1.63 \pm 0.57 \times 10^{9}$ | $^{b}5.65 \pm 0.21 \times 10^{10}$ | $^{b}1.18 \pm 0.24 \times 10^{10}$ |
| Yeast and Mold Count | 2 | TFTC | TFTC | $^{a}9.15 \pm 0.26 \times 10^{8}$ |
| | 4 | TFTC | $^{a}4.10 \pm 0.71 \times 10^{5}$ | $^{a}9.70 \pm 0.16 \times 10^{8}$ |
| | 6 | TFTC | $^{a}1.73 \pm 0.16 \times 10^{6}$ | $^{a}6.70 \pm 7.07 \times 10^{8}$ |
| | 8 | TFTC | $^{a}1.50 \pm 0.26 \times 10^{7}$ | $^{a}1.07 \pm 0.13 \times 10^{9}$ |
| | 24 | $^{a}6.07 \pm 0.13 \times 10^{9}$ | $^{b}9.30 \pm 0.42 \times 10^{10}$ | $^{b}3.60 \pm 0.71 \times 10^{9}$ |

Data are presented as mean ± standard deviation of samples (n = 3).

TFTC presented as too few to count.

Different letters ($^{a\ \&\ b}$) in the same column indicate significant differences ($p < 0.05$) between durations in a sample.

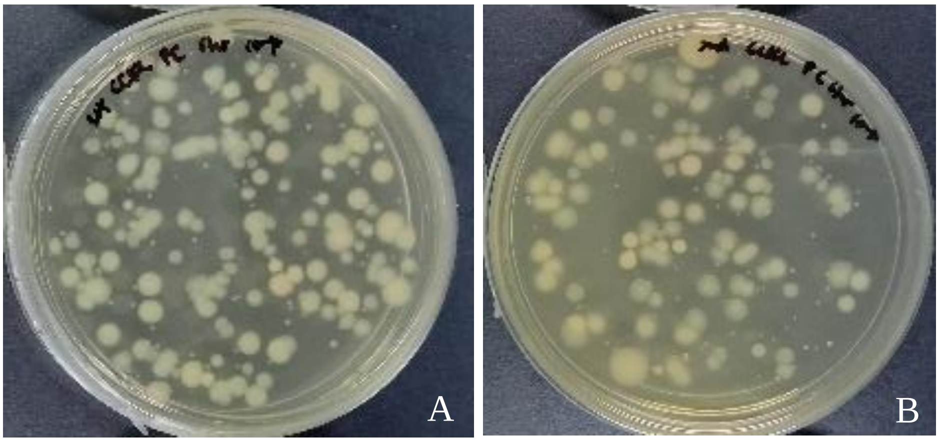


**Figure 4.1a:** Aerobic colonies found in popcorn chicken sample on duplicate $10^{-4}$ PCA plates stored for 6 hours at room temperature (25±2°C).

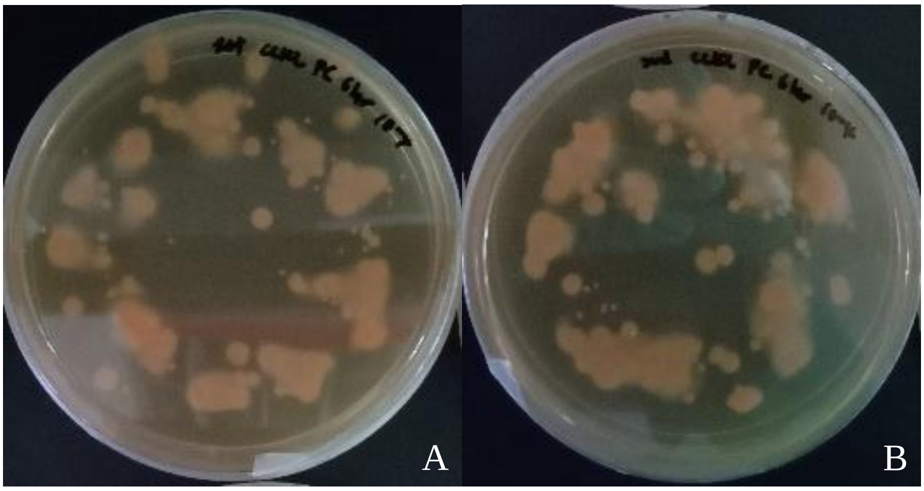


**Figure 4.1b:** Yeasts and molds found in popcorn chicken sample on duplicate $10^{-4}$ PDA plates stored for 6 hours at room temperature (25±2°C).

### 4.1.2 Effects of Storage Durations on the Microbiological Loads of Plant-Based Meat Items Stored at Refrigeration Temperature (4±2°C)

The presented results in Table 4.2 illustrate the correlation between storage durations and the growth of microbiological loads in refrigerated food samples, which was stored at 4°C. Each experiment was repeated twice and each plates represented in the form of A and B shown in the figures below (refer to Figures 4.2a and 4.2b). No statistically significant differences ($p > 0.05$) were observed in microbial loads across all tested food samples, including Hainanese grandpapa's rice, avocado charcoal bun, and popcorn chicken, when stored for 24 and 48 hours in the ACC analysis. However, the avocado charcoal bun sample exhibited detectable microbial growth, while the microbial load in Hainanese grandpapa's rice was too abundant to be enumerated. Notably, YMC analysis was not conducted for the popcorn chicken sample. Conversely, YMC analysis for avocado charcoal bun showed no significant differences in microbial load between the 24-hour and 48-hour storage durations. On the other hand, decline in ACC were found in Hainanese grandpapa's rice, as well as ACC and YMC avocado charcoal bun stored on the second day.

**Table 4.2:** Microbial count (log CFU/g) of plant-based meat samples stored at refrigeration temperature (4±2°C).

| Microbiological Parameters | Duration (hours) | Log CFU/g | | |
|---|---|---|---|---|
| | | Hainanese Grandpapa's Rice | Avocado Charcoal Bun | Popcorn Chicken |
| Aerobic Colony Count | 24 | $^{a}3.80 \pm 0.86 \times 10^5$ | $^{a}5.80 \pm 0.17 \times 10^4$ | $^{a}2.88 \pm 0.22 \times 10^8$ |
| | 48 | $^{a}3.60 \pm 0.57 \times 10^5$ | $^{a}5.75 \pm 0.26 \times 10^4$ | $^{a}5.43 \pm 0.15 \times 10^8$ |
| Yeast and Mold Count | 24 | TNTC | $^{a}1.88 \pm 0.28 \times 10^5$ | N.D. |
| | 48 | TNTC | $^{a}1.73 \pm 0.13 \times 10^5$ | N.D. |

Data are presented as mean ± standard deviation of samples (n = 3).

TNTC presented as too numerous to count; N.D. presented as not done.

Different letters ($^{a\ \&\ b}$) in the same column indicate significant differences ($p < 0.05$) between durations in a sample.

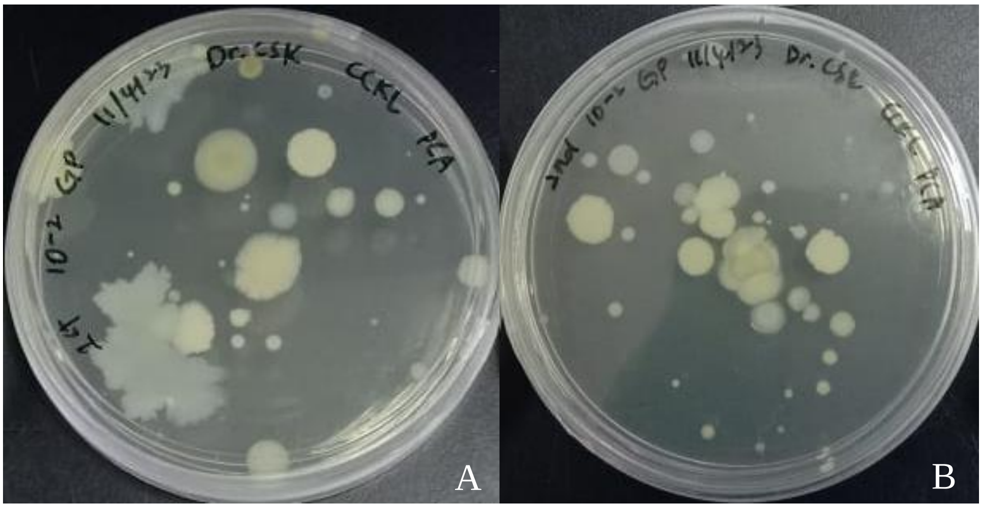


**Figure 4.2a:** Aerobic colonies found in Hainanese grandpapa's rice sample on duplicate $10^{-2}$ PCA plates stored for 24 hours at refrigeration temperature (4±2°C).

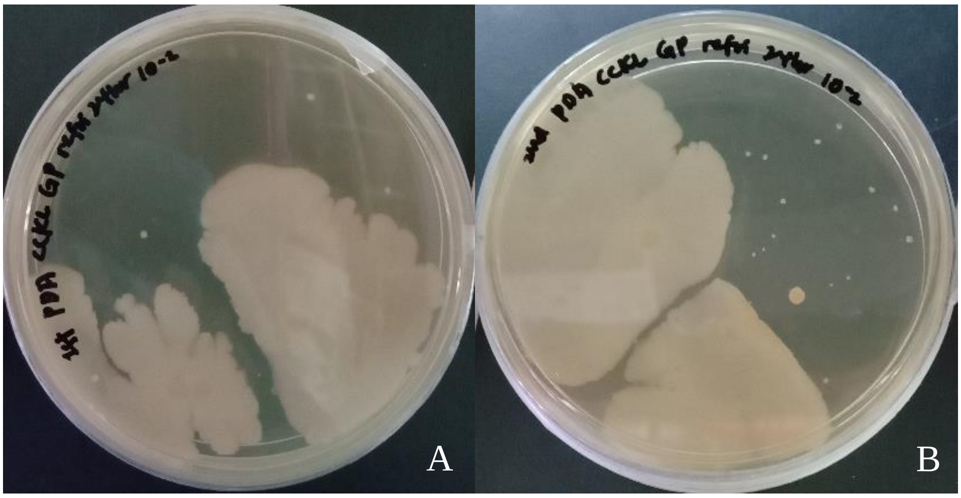


**Figure 4.2b:** Yeasts and molds found in Hainanese grandpapa's rice sample on duplicate $10^{-2}$ PDA plates stored for 24 hours at refrigeration temperature (4±2°C).

### 4.1.3 Effects of Storage Temperatures on the Microbiological Loads of the Plant-Based Meat Items

The results presented in Table 4.3 depict the impact of storage temperatures on microbial growth in food samples after 24 hours. A comparison was made between room (25±2°C) and refrigeration (4±2°C) temperatures for each food sample. Each experiment was repeated twice and each plates represented in the form of A and B shown in the figures below (refer to Figures 4.3a, 4.3b, 4.3c, and 4.3d). Despite the expectation that refrigeration would slow microbial growth, the ACC analysis indicated that the microbial loads in Hainanese grandpapa's rice and popcorn chicken, did not exhibit significant differences ($p > 0.05$) at varying temperatures. However, the avocado charcoal bun displayed significant variances ($p < 0.05$) in microbial loads when subjected to different temperatures.

Moreover, only the avocado charcoal bun exhibited the presence of yeast and mold counts for both temperatures, with a significant variations ($p < 0.05$) between them. Hainanese grandpapa's rice and popcorn chicken lack comparable results between room and refrigeration temperatures due to excessive counts for Hainanese grandpapa's rice and the yeast and mold count plates for popcorn chicken were not done.

**Table 4.3:** Microbial count (log CFU/g) of plant-based meat samples stored at both room (25±2°C) and refrigeration (4±2°C) temperatures at 24 hours.

| **Microbiological Parameters** | **Log CFU/g** | | |
|---|---|---|---|
| | **Hainanese Grandpapa's Rice** | **Avocado Charcoal Bun** | **Popcorn Chicken** |
| Aerobic Colony Count | $^{a}1.63 \pm 0.57 \times 10^{9}$ | $^{b}5.65 \pm 0.21 \times 10^{10}$ | $^{a}1.15 \pm 0.22 \times 10^{10}$ |
| Yeast and Mold Count | N. A. | $^{b}9.30 \pm 0.42 \times 10^{10}$ | N. A. |

Data are presented as mean ± standard deviation of samples (n = 3).

N.A. presented as not available.

Different letters ($^{a \& b}$) in the same column indicate significant differences ($p < 0.05$) between storage temperatures in a sample.

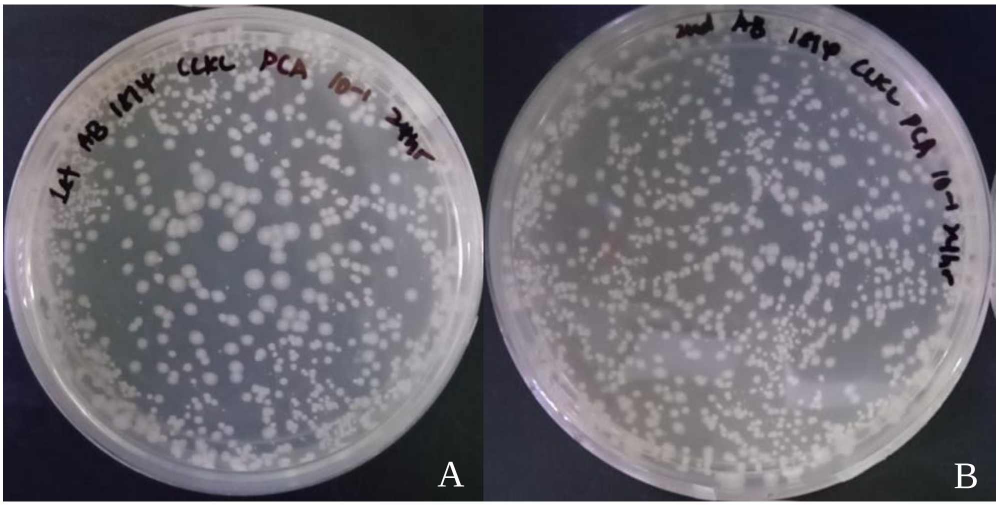


**Figure 4.3a:** Aerobic colonies found in avocado charcoal bun sample on duplicate $10^{-1}$ PCA plates stored for 24 hours at room temperature (25±2°C).

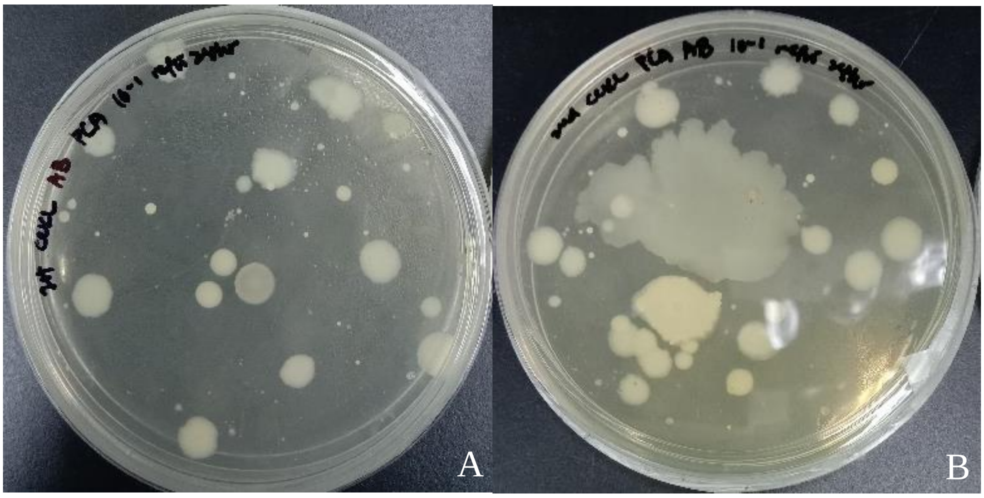


**Figure 4.3b:** Aerobic colonies found in avocado charcoal bun sample on duplicate $10^{-1}$ PCA plates stored for 24 hours at refrigeration temperature (4±2°C).

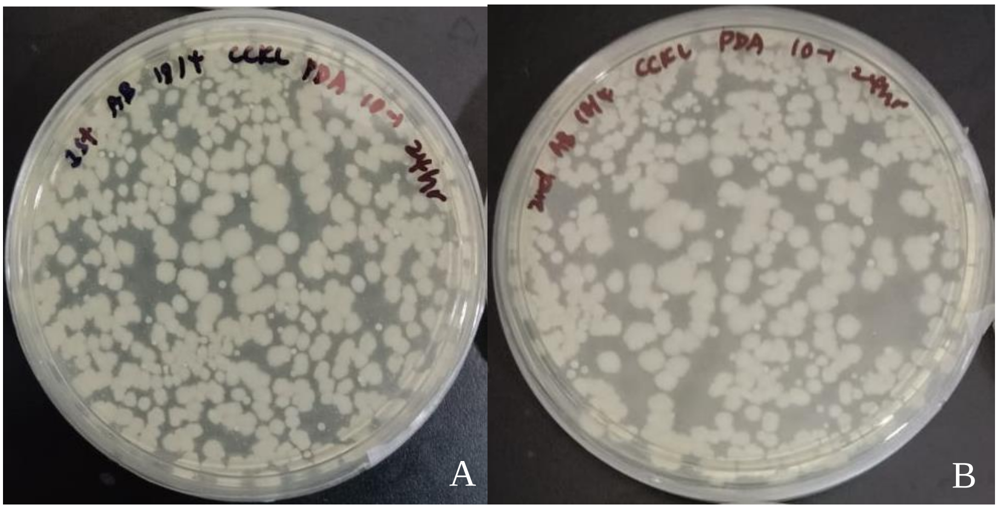


**Figure 4.3c:** Yeasts and molds found in avocado charcoal bun sample on duplicate $10^{-1}$ PDA plates stored for 24 hours at room temperature (25±2°C).

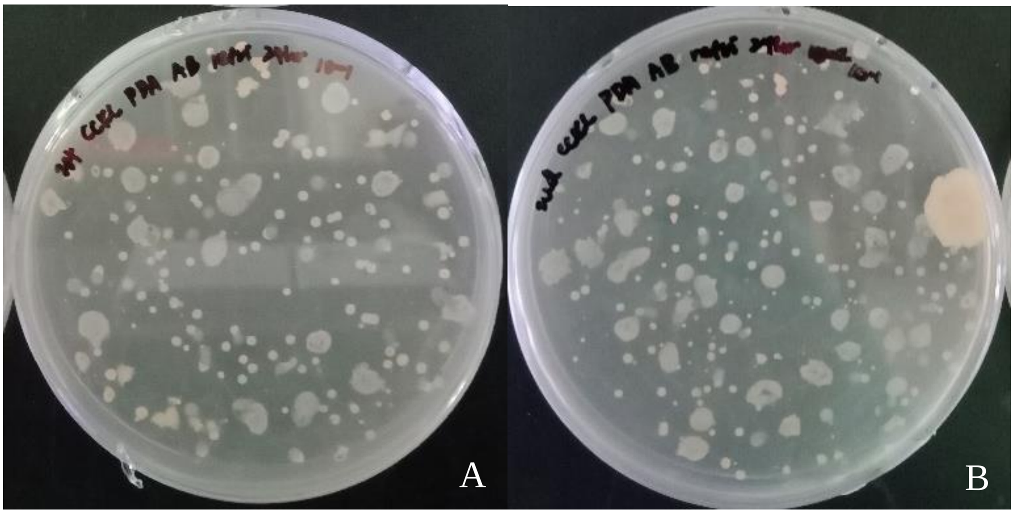


**Figure 4.3d:** Yeasts and molds found in avocado charcoal bun sample on duplicate $10^{-1}$ PDA plates stored for 24 hours at refrigeration temperature (4±2°C).

## 4.2 Analysis on Students' Attitudes and Perceptions toward Microbiological Risk of Plant-Based Meat Items

### 4.2.1 Participants Recruitment

In May 2023, the online questionnaire link was distributed to UTAR undergraduate students via several social media platforms, and the data collection phase ended in the beginning of July 2023. During this period, 165 participants were successfully recruited as all of them met the criteria for this study. Therefore, the final sample for analysis comprised all 165 participants who were included in this research.

### 4.2.2 Socio-demographic Profiles

Table 4.4 presents the socio-demographic characteristics of the study sample. The recruited respondents consisted of more females (63%) than males (37%) for the study. Regarding ethnicity, majority of the respondents were Chinese (92.1%), with smaller representation of Indians (3.0%) and Malays (0.6%). For household monthly income, most of the respondents fell into the M40 category (56.4%), 59 were classified as B40 (35.8%), and only 13 belonged to the T20 category (7.9%). Regarding the source of information on safe food preparation, the data revealed that the majority of respondents (73.3%) acquired this knowledge from school. Additionally, 101 of them learned from the Internet (61.2%), 90 of them learned from their family (54.5%), 48 of them learned from books and magazines (29.1%), and 6 of them learned from their workplace (3.6%).

**Table 4.4:** Socio-demographic information of respondents.

| **Variables** | **Subjects (N=165)** | |
|---|---|---|
| | **Frequency (n)** | **Percentage (%)** |
| **Gender** | | |
| Male | 104 | 63.0 |
| Female | 61 | 37.0 |
| **Ethnicity** | | |
| Chinese | 152 | 92.1 |
| Malay | 1 | 0.6 |
| Indian | 12 | 7.3 |
| **Household Monthly Income** | | |
| B40 (< RM 4,849) | 59 | 35.8 |
| M40 (RM 4,850 – RM 10,959) | 93 | 56.4 |
| T20 (> RM 10,960) | 13 | 7.9 |
| **I learned about safe food preparation……** | | |
| From family | 90 | 54.5 |
| From books and magazines | 48 | 29.1 |
| From the Internet | 101 | 61.2 |
| At school | 121 | 73.3 |
| At work | 6 | 3.6 |

Notes: n = number of respondents that answered the related option.

### 4.2.3 Attitude and Perception towards Microbiological Risks of Plant-Based Meat Items

Table 4.5 presents the knowledge and awareness levels related to controlling microbiological pathogens in plant-based meat items. The findings indicated a high level of consciousness among respondents regarding microbiological risk level and food safety practices. Almost all respondents (97%) indicated that they checked the freshness of plant-based meat items before purchasing them. Additionally, an overwhelming majority of respondents (98.2%) recognized the health risks associated with improper handling or preparation of raw plant-based meat items at home. Regarding heat treatment, a significant number of respondents (90.9%) displayed a positive attitude towards ensuring sufficient cooking temperatures for plant-based meat items. Furthermore, when storing plant-based meat items in their refrigerators, 84.2% of the respondents preferred to keep them separate from other types of food to prevent cross-contamination. As for defrosting methods, 35.2% of the respondents preferred using the refrigerator, 33.9% used kitchen surfaces at room temperature, 26.1% used tap water, and 4.8% used a microwave. Besides, approximately two-thirds of the respondents (67.9%) reported that they froze previously defrosted plant-based meat items, while the remaining 32.1% did not follow this practice.

**Table 4.5:** Knowledge, self-reported practices, and awareness on controlling the microbiological pathogens in plant-based meat items.

| **Variables** | | **Subjects (N=165)** | |
|---|---|---|---|
| | | **Frequency (n)** | **Percentage (%)** |
| I would check for the freshness before purchasing any plant-based meat items. | Yes | 160 | 97.0 |
| | No | 5 | 3.0 |
| Improper home raw plant-based meat items preparation presents a health risk. | Yes | 162 | 98.2 |
| | No | 3 | 1.8 |
| I would pay attention to sufficient heat treatment of plant-based meat items. | Yes | 150 | 90.9 |
| | No | 15 | 9.1 |
| I always store plant-based meat items in the same place in a refrigerator. | Yes | 139 | 84.2 |
| | No | 26 | 15.8 |
| I defrost plant-based meat ...... | On the kitchen surfaces at room temperature | 56 | 33.9 |
| | In a refrigerator | 58 | 35.2 |
| | Under tap water | 43 | 26.1 |
| | In a microwave | 8 | 4.8 |
| Sometimes, I would freeze the previously defrosted plant-based meat items. | Yes | 112 | 67.9 |
| | No | 53 | 32.1 |

Notes: n = number of respondents that answered the related option.

Table 4.6 shows the knowledge level of the respondents towards food microbiology. A substantial proportion of respondents (82.4%) disagreed with the notion that all microorganisms are harmful to humans, while only 9.7% agreed with it, and 7.9% were uncertain. Similarly, majority of the respondents (76.4%) disagreed with the belief that all bacteria are harmful, with 20.0% agreeing and 3.6% being uncertain. Regarding yeast and fungi, around 87.9% of the respondents disagreed with the idea that all yeast and fungi are harmless to humans, while 6.7% agreed with it, and 5.5% did not know the answer. In terms of food safety, 73.9% of the respondents acknowledged the potential for harmful microorganism contamination in retail plant-based meat items, whereas 10.9% disagreed, and 15.2% were unsure. Furthermore, 63.0% of the respondents recognized that microorganisms could multiply in a refrigerator, whereas 28.5% disagreed, and 8.5% were uncertain about this fact. Regarding prepared, heat-treated foods left on a kitchen counter, 84.8% of the respondents agreed that microorganisms can multiply in such conditions, while 7.9% disagreed, and 7.3% were uncertain.

**Table 4.6:** Food microbiology knowledge.

| **Variables** | | **Subjects (N=165)** | |
|---|---|---|---|
| | | **Frequency (n)** | **Percentage (%)** |
| All microorganisms are harmful to humans. | Yes | 16 | 9.7 |
| | No | 136 | 82.4 |
| | I Don't Know | 13 | 7.9 |
| All bacteria are harmful to humans. | Yes | 33 | 20.0 |
| | No | 126 | 76.4 |
| | I Don't Know | 6 | 3.6 |
| All yeast and fungi are harmless to humans. | Yes | 11 | 6.7 |
| | No | 145 | 87.9 |
| | I Don't Know | 9 | 5.5 |
| Plant-based meat items in retail can be contaminated with harmful microorganisms. | Yes | 122 | 73.9 |
| | No | 18 | 10.9 |
| | I Don't Know | 25 | 15.2 |
| Microorganisms can multiply in a fridge. | Yes | 104 | 63.0 |
| | No | 47 | 28.5 |
| | I Don't Know | 14 | 8.5 |
| Microorganisms can multiply in prepared, heat-treated foods that left on a kitchen counter. | Yes | 140 | 84.8 |
| | No | 13 | 7.9 |
| | I Don't Know | 12 | 7.3 |

Notes: n = number of respondents that answered the related option.

### 4.2.4 Level of Attitude and Perception towards Microbiological Risk of Plant-Based Meat Items

The level of attitude and perception towards microbiological risk of plant-based meat items was shown in Figure 4.4 below. The overall attitude and perception were categorized as positive if the score was between 80 and 100%, neutral if the score was between 60 and 79%, and negative if the score was less than 60% (Akalu, Ayelign and Molla, 2020). There were a total of 88 respondents (53.3%) had a positive attitude and perception towards microbiological risk of plant-based meat items, whereas 64 (38.8%) and 13 (7.9%) had a neutral and negative attitude and perception towards microbiological risk of plant-based meat items, respectively.

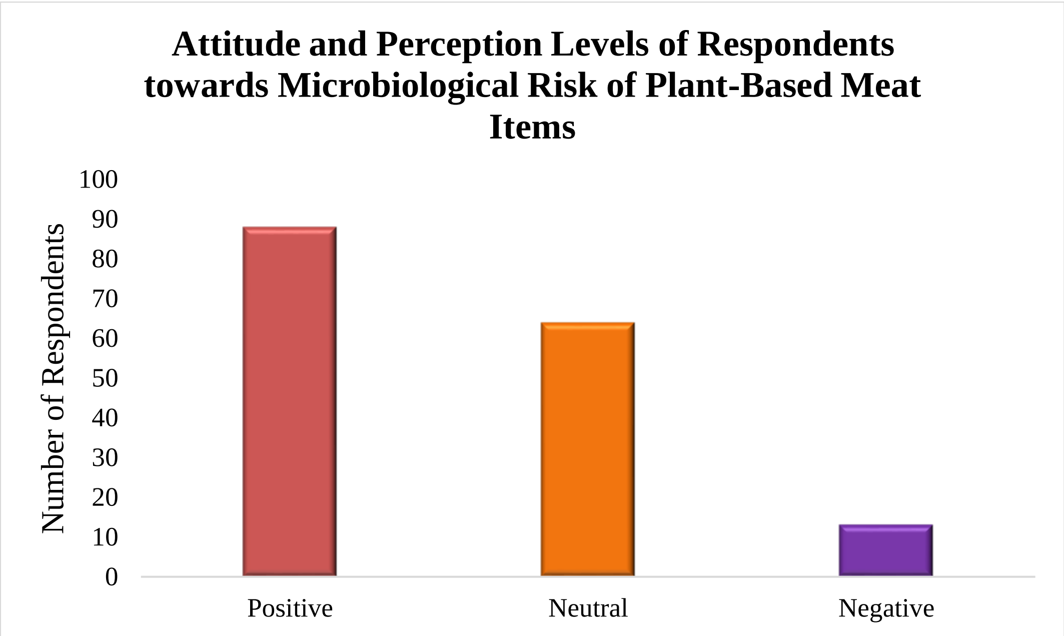


**Figure 4.4:** Level of attitude and perception towards microbiological risk of plant-based meat items.

# CHAPTER 5

# DISCUSSION

## 5.1 Microbiological Analyses of Plant-Based Meat Items

### 5.1.1 Effects of Storage Durations on the Microbiological Qualities of the Plant-Based Meat Products at Room Temperature (25±2°C)

The purpose of this section's study was to see how varied storage periods affect the microbiological characteristics of each tested plant-based meat product stored at room temperature (25±2°C).

The aerobic colony count (ACC) is also known as total viable count or aerobic plate count. It is one of the most commonly used tests to enumerate the aerobic microorganisms, as well as to determine the hygienic condition and microbial shelf life of food items (Microbe Investigations Switzerland, n.d.). Yeast and mold count (YMC) is also a commonly used method to measure the overall cleanliness of the product's life cycle, from cultivation to processing, handling, and storage (Schumacher, Lingle and Silbernagel, 2022).

In this study, all food samples that were kept at room temperature (25±2°C) showed the presence of aerobic colony count (ACC), and yeast and mold count (YMC) after the incubation period. A gradual increase in the microbial counts were shown in all food samples stored at room temperature between 2 to 24 hours of storage. Similar finding was found in a study performed by Yadav et al. (2015) as the microbial growth over prolonged storage on plant-based chicken meat rolls stored at 27°C for 3 days had significantly increased. The ACC and YMC in Hainanese grandpapa's rice sample that were stored for 2 to 8 hours were not significant. This was because the microbes developed were too few to count starting from the first dilution ($10^{-1}$) for the ACC and YMC. This may be due to the ingredients used in plant-based meat items, such as plant proteins, grains, and vegetables. These ingredients may undergo processing methods such as washing, blanching, pasteurization that reduce the initial microbial load (Houška et al., 2022). Besides that, since the food item was cooked at high temperature, heat treatment can effectively kill or inhibit the growth of most microorganisms present in the raw ingredients, as well as inactive the enzymes (Erkmen and Bozoglu, 2016). Hence, initial microbial load reduced. However, the microbial load for Hainanese grandpapa's rice sample stored for 24 hours shown significant differences ($p < 0.05$).

Avocado charcoal bun and popcorn chicken that were kept at the room temperature (25±2°C) had shown no significant differences in the microbial count that were kept for 2, 4, 6, and 8 hours shown in Table 4.1. However, the 2 plant-based meat samples mentioned previously that were kept for 24 hours indicated significant difference ($p < 0.05$). These results are in accordance with the findings of Liu et al. (2023). this study confirmed that the microbial load level of ACC and YMC in pea-based meat samples showed no significant differences for the first 6 hours of storage, but significant changes were shown for 24 hours of storage at 32°C.

The effect of duration on microbial content describes how the amount of time a specific environment is exposed to specific conditions can affect the composition, abundance, and activity of these microorganisms. Microbial populations can undergo dynamic changes throughout time as a result of variables such as growth, competition, adaptability, and environmental alterations (Fink, Held and Manhart, 2023). The microbial growth curve is a basic concept in microbiology that describes how bacteria grow over time (Gonzalez and Aranda, 2023). This curve has 4 different phases, which are lag phase, exponential (log) phase, stationary phase, and death (decline) phase (Jay, Loessner and Golden, 2005). Microbes adapt initially in the lag phase, followed by rapid exponential expansion due to nutrition utilization (Jay, Loessner and Golden, 2005). Over time, the stationary phase occurs with balanced growth and death, and resource shortage leads to steady decline of microbial populations (Bailey, 2018). Due to reduced enzyme activity, lag phase extension can occur in food maintained at room temperature, but exponential growth may be shorter. The existence of the stationary phase at room temperature has an impact on shelf life and safety (Rolfe et al., 2011). Hence, microbes require time to adjust and adapt when entered into a new environment during lag phase for the first few hours. However, as the microbes entered an exponential growth phase to adapt to their new environment, their growth rate accelerates, resulting in an increase in microbial content as the storage duration progresses (D’Amico et al., 2006; Gonzalez and Aranda, 2023). This increases microbial loads in the plant-based food samples stored for 24 hours at room temperature and shorten their shelf life.

According to Jay, Loessner and Golden (2005), extended time storage of perishable foods at danger zone temperatures (4.4°C to 60°C) might promote the fast proliferation of germs, resulting in foodborne diseases. Furthermore, the longer foods are stored, the more likely they will decay, resulting in undesirable changes in taste, odor, and texture. To preserve the safety and quality of food, it is better to adhere to appropriate storage durations and conditions, maintain good hygiene throughout processing, and consume perishable items as soon as possible (Extension, n.d.).

### 5.1.2 Effects of Storage Durations on the Microbiological Qualities of the Plant-Based Meat Products at Refrigeration Temperature (4±2°C)

This purpose of this section is to see how varied storage periods affect the microbiological characteristics of each tested plant-based meat product stored at refrigeration temperature (4±2°C).

The microbial loads for all food samples that were stored for 24 and 48 hours showed no significant differences in Table 4.2. This is comparable to the findings carried out by Liu et al. (2023), the microbial loads in soy- and pea-based meats shown no significant differences between 0 to 5 days at 4°C. In addition, there were no significant changes shown in the microbial load of ready-to-eat salad stored for 2 days at 4°C (Caldera and Franzetti, 2013). These studies suggested that lower temperatures can effectively prevent microbial development and increase product shelf life (Caldera and Franzetti, 2013; Liu et al., 2023).

Temperature, environment, water activity and nutrient content can impact the growth of microorganisms in food. Refrigeration temperature i.e., from 0°C to 5°C falls outside the ideal range of temperature for most bacteria to grow rapidly. The cold environment slows down enzymatic reactions and cellular activities required for rapid growth, resulting in a considerable decrease in growth rate (D'Amico et al., 2006). Refrigerating food effectively slows microbial growth by lowering the temperature. This indicates that the bacteria in the food item are most likely in the lag phase or stationary phase during the storage period. After 2 days, the bacteria are unlikely to have had the time to move from the lag phase to the exponential growth phase in substantial numbers. Furthermore, the low temperature of the refrigerator continues to inhibit their growth. Because the majority of the time is spent in the lag and stationary phases, there is little possibility for significant increase in the microbial population within 2 days at refrigeration temperatures. (D'Amico et al., 2006; Gonzalez and Aranda, 2023). Thus, there is no significant difference in microbe counts over the time length. Cold temperatures prolonged the food items' shelf life.

On the other hand, Hainanese grandpapa's rice and avocado charcoal bun showed a decline in microbial counts stored over 24 hours to 48 hours. The decrease in microbial counts kept at refrigerated temperatures over a 24 to 48-hour period can be attributable to a variety of causes, including microbial growth inhibition, nutrition depletion, and temperature-dependent variables. Most bacteria' metabolic functions are slowed down by refrigeration temperatures. At low temperatures, most bacteria, yeast, and molds have slower growth rates and activity, which can lead to a drop in their numbers over time (Jay, Loessner and

Golden, 2005). Other than that, refrigeration helps to keep moisture levels low, which makes the environment less favorable to microbial growth. Some bacteria and fungus require higher quantities of moisture to grow and multiply (Doyle et al., 2019). Refrigeration can also decrease the availability of nutrients in the food matrix. Enzymatic reactions that break down complex carbohydrates and proteins may slow down at lower temperatures, restricting nutrition availability to bacteria (Jay, Loessner and Golden, 2005). Extended storage in the refrigerator can promote competition between different bacteria species. Some microbes may outcompete others for available nutrients, causing a drop in the population of less competitive species (Tapia, Alzamora and Chirife, 2020).

The YMC for Hainanese grandpapa's rice stored at refrigeration temperature for 2 days were too numerous to count. Contamination is the most prevalent cause of an excessive number of colonies on agar plates. Cross-contamination during plate preparation, handling, or incubation might cause the proliferation of unwanted bacteria. Even a little plate or equipment misused can introduce more bacteria, resulting in a larger colony count (Mkhungo, Oyedeji and Ijabadeniyi, 2018).

This study has practical significance for both manufacturers and consumers of plant-based meat products in the context of food safety and quality. When developing storage recommendations and expiry dates, producers must consider the perishability of these commodities. Based on the findings, consumers can make informed evaluations on the timing of plant-based food consumption.

### 5.1.3 Effects of Storage Temperatures on the Microbiological Qualities of the plant-based meat products stored for 24 hours

This section's study is to see how varied storage temperatures at the same duration (24 hours) affect the microbiological characteristics of plant-based meat products.

Based on the results in Table 4.3, only avocado charcoal bun showed significant differences ($p < 0.05$) in the number of microbial loads stored at room and refrigeration temperatures at 24 hours in both ACC and YMC. This was due to the fact that an increase in temperature increased enzyme activity, thus room temperature is favorable for the development of microbes (LibreTexts, 2019). There was one study conducted by Kandeepan et al. (2012) which found that the total plate count of buffalo meat curry stored at ambient temperature (37°C) for 3 days had significantly increased ($p < 0.01$) compared to refrigeration temperature (4°C). In addition, microbial loads in cream cheese that was stored at 21°C showed significant increased ($p < 0.05$) in comparison with the storage at 4°C (Perveen, 2011). In contrast, another research found that there were no significant changes observed on avocado kept at 5 and 25°C in ACC and YMC (Cabrera-Díaz et al., 2022).

On the other hand, Hainanese grandpapa's rice, and popcorn chicken showed no significant differences in the number of microbial loads stored at room and refrigeration temperatures of 24 hours in ACC. The findings were not consistent with prior study on low-salt fermented fish. The study reported increased microbial growth when stored at room (25°C) and refrigeration (4°C) temperatures. Significant differences ($p < 0.05$) in levels of yeast and aerobic colonies were observed between food samples kept for around 15 days at 35°C. However, there were no significant differences ($p > 0.05$) observed between food samples kept at 4°C and 25°C (Sun et al., 2020). Altunatmaz, Issa and Aydin (2012) confirmed that low-temperature storage inhibits the growth of many bacteria and slows the growth of others. Although certain vegetables, such as green peppers and cucumbers, are sensitive to low temperatures and should be stored at higher temperatures, usually around 7°C, to avoid spoilage. However, psychrotrophic bacteria and fungi are prevalent and have been linked to spoilage under refrigeration conditions.

The storage temperature has a significant impact on the growth of microbial loads in food goods. Different temperature conditions, such as room temperature and refrigeration, can have variable effects on microbial proliferation in plant-based food items (Liang et al., 2021).

Regardless of the reported microbial changes across different storage periods and temperatures, it is critical to consider the unique characteristics of each plant-based food product. Moisture content, pH levels, and nutritional composition can all influence microbial growth and stability (Wild et al., 2014). The considerable microbiological alterations seen in the Hainanese grandpapa's rice sample after 24 hours of storage could be related to its unique composition. The presence of carbohydrates and proteins in the rice-based products may create a favorable environment for long-term microbial development. Due to its nutrient richness, Hainanese sauce in the food sample may create an ideal habitat for microbial growth. Since Hainanese sauce contains sugars, tomato sauce, soy sauce and other ingredients, it could promote the growth of a wide variety of microorganisms (Lorenzo et al., 2018). High moisture foods also provide an ideal habitat for bacteria growth. This is because sauces are liquid or semi-liquid in nature, can offer moisture for bacteria to grow (Alegbeleye et al., 2022). Studies have also found that the microbial load increased in tomato sauce stored at room temperature due to its high moisture and nutrient content (Fatima et al., 2015; Smith and Emmanuel, 2018). Many foods are excellent sources of substrate for many bacteria (The Open University, 2019).

Microorganisms are extremely sensitive to temperature fluctuations, which can have a substantial impact on their growth, activity, and survival (SITNFlash, 2020). Temperature is an important environmental component that influences microbial composition, particularly in terms of food safety and preservation (Lorenzo et al., 2018). The temperature range between 4.4°C and 60°C is generally referred to as the "danger zone" for microbial development. This temperature range is ideal for the rapid growth of dangerous bacteria such as *Salmonella*, *Escherichia coli*, and *Listeria monocytogenes* (USDA, 2017).

Microbial growth is greatly reduced at temperatures below 4.4°C, and some germs may even enter a dormant condition (USDA, 2017). This is why refrigeration is a typical approach for prolonging the shelf life of perishable foods. Cold temperatures limit enzyme activity and metabolic processes, preventing the rapid multiplication of dangerous bacteria that can cause foodborne diseases (Uri.edu, 2015). Temperatures exceeding 60°C are normally considered deadly for most pathogenic bacteria. High temperatures can denature proteins, break cell membranes, and cause irreparable damage to microbiological structures. This is the underlying premise of techniques such as pasteurization and cooking, which aim to eradicate hazardous microbes and assure the safety of food products (USDA, 2017).

## 5.2 Analysis on the Attitude and Perception Levels toward Microbiological Risks of Plant-Based Meat Items among UTAR Undergraduate Students

The present study also focused on the level of UTAR Kampar undergraduate students' attitudes and perceptions towards microbiological risks of plant-based meat items. In this study, various aspects were investigated, including socio-demographic profiles, knowledge, self-reported practices, and awareness on controlling the microbiological pathogens in plant-based meat items, as well as the knowledge of food microbiology.

The results in Figure 4.4 showed that the level of respondents' attitudes and perceptions towards the microbiological risks of plant-based meat items were positive. Around 88 (53.3%) of them indicated a favorable attitude toward it, whereas only 7.9% of respondents indicated a negative attitude. This is due to most respondents have a science background and being more knowledgeable on health and nutrition. Plant-based diets are frequently seen as a healthier option than typical meat-based diets due to decreased saturated fat and cholesterol levels, as well as possible benefits for heart health and weight control (Melina, Craig and Levin, 2016). There was a similar research conducted in Bangladesh, as most health science students were having positive attitude about food safety (Ali et al., 2023).

According to the results shown in Figure 5.1, majority of the respondents (97%) would always double-check plant-based meat items for freshness before purchasing them. This finding shows respondents concerned about food safety, especially if they are new to plant-based diets or have had previous poor experiences with rotten food products. Therefore, they may be more careful while purchasing new products and double-check for signs of freshness to ensure that the food is safe to consume. This result is similar to a survey conducted by Jevnik et al. (2008) had shown that 54.2% of respondents always checked the expired date.

Most of the respondents (98.2%) agreed that the improper handling and preparing home raw plant-based meat items would cause a health risk. This result is similar to another study performed by the same researcher, as around 74.7% had agreed that improper preparation of raw poultry meat could cause the risk of health issues (Sterniša et al., 2018). Raw plant-based meat products, like their animal-based counterparts, may contain the presence of microbes, such as *Salmonella*, *Clostridium perfringens*, *Campylobacter*, *Staphylococcus aureus*, *E. coli*, and *Listeria monocytogenes* can be found in raw, undercooked, or inadequately washed food and poses risk to health (Finn et al., 2013). Raw plant-based meat products can also transfer hazardous bacteria when they come into touch with other foods, surfaces, or kitchen equipment, resulting in cross-contamination (Sterniša et al., 2018).

Furthermore, 90.9% of the respondents showed a positive attitude in paying attention to heat treatment that is sufficient for the plant-based meat items. Respondents may be interested in the safety of plant-based meat products, particularly in terms of potential pathogens, as well as ensuring that plant-based meat products preserve their nutritional content after heat treatment. Besides, the respondents may be aware of the significance of heat treatment in plant-based meat preparation. This could be because they have been taught about the correct cooking temperatures for the specific food. This finding is consistent with a prior survey performed by Szymkowiak et al. (2020), in which most of the respondents showed positive attitudes towards conventional thermal preservation while preparing food (Szymkowiak et al., 2020).

In regards with storing plant-based meat items in their refrigerators, around 84.2% of the respondents had a positive attitude and perception towards the practice of food storage in the refrigerator. They would keep plant-based meat items separated from other food in a refrigerator. This is because keeping different types of food separated from each other can prevent cross-contamination (WA Health, n.d.).

In addition, about 67.9% reported that they froze previously defrosted plant-based meat items, while the other 32.1% did not follow this practice. This indicated that there were more respondents who had negative attitude towards the practice of thawing food. This may cause contamination of the food if left at room temperature for more than 2 hours (USDA, 2013a). One possibility is related to time management and lifestyle of a student. University students sometimes lead hectic lives filled with academic coursework, part-time employment, and extracurricular activities. This might lead to inconsistent scheduling, making it difficult to prepare meals ahead of time. As a result, they may end up defrosting more food than they can consume at once, increasing the likelihood of freezing leftovers (Fernandez, Webster and Cornett, 2019). In comparison with the research performed by Osaili, Al-Nabulsi and Al-Jaberi (2022), 91.7% of respondents claimed that they did not refreeze the defrosted food. Thus, this shows that the respondents in this study have negative attitudes and perceptions toward the practice of freezing previously defrosted food.

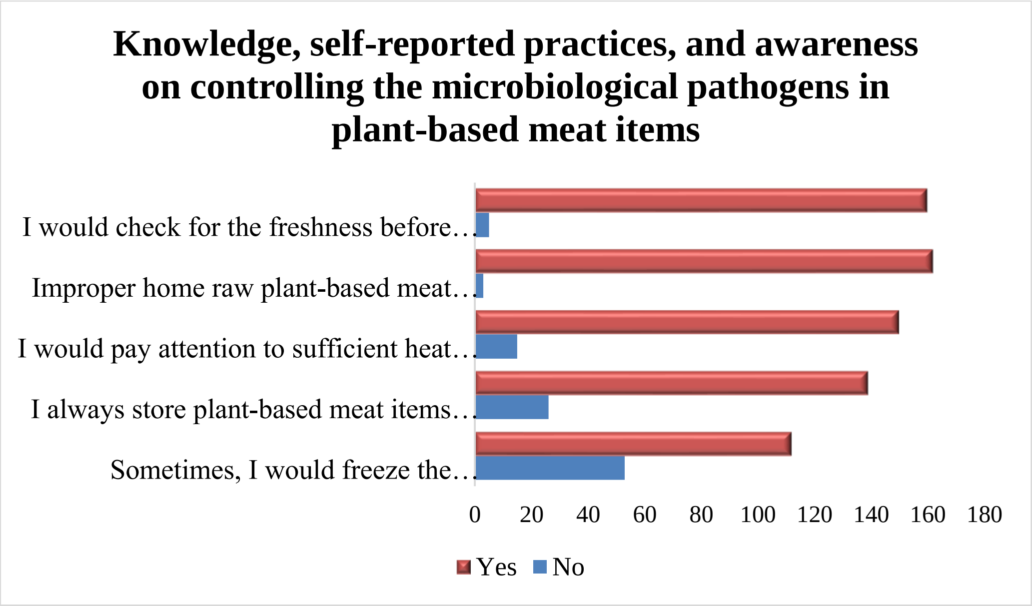


**Figure 5.1:** Knowledge, self-reported practices, and awareness of respondents on controlling the microbiological pathogens in plant-based meat items (n=165).

As for the methods of defrosting food items shown in Figure 5.2, 35.2% of the respondents preferred to use the refrigerator, 33.9% used kitchen surfaces at room temperature, 26.1% used tap water, and 4.8% used microwave. According to USDA (n.d.), refrigerator, microwave and tap water are the safe and proper methods to defrost food items as could reduce the risk of bacterial contamination.

Basic food handling practices are often considered common knowledge. Respondents may have learned these skills from family members, friends, or through general societal knowledge. Hence, respondents were aware about the importance of proper food handling to prevent foodborne illnesses, which includes defrosting food safely to avoid bacterial growth. Therefore, 66.1% of respondents that chose either of these 3 defrost methods showed positive attitudes and perceptions as they were cautious towards microbiological risk.

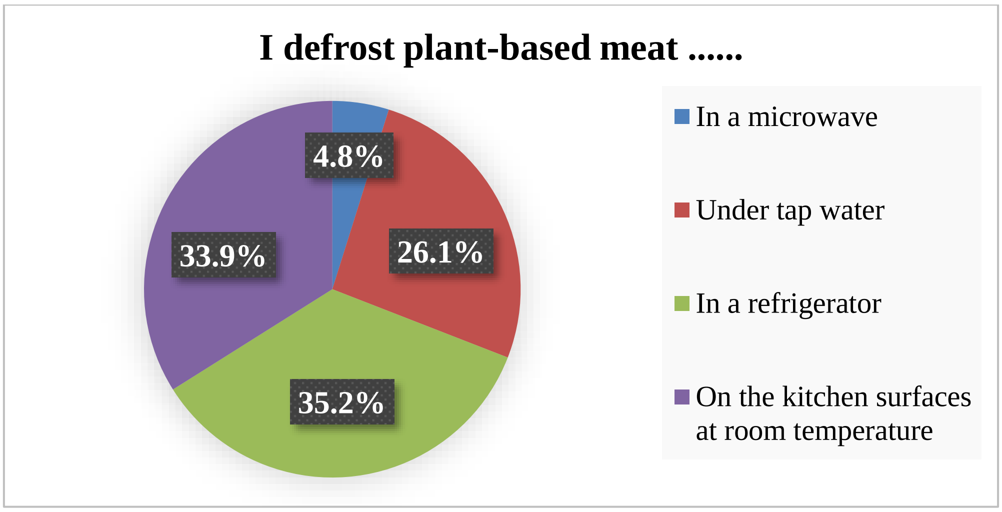


**Figure 5.2:** Different practice of respondents on defrosting plant-based meat items (n=165).

Figure 5.3 showed the findings of the respondents toward the knowledge of food microbiology. Positive attitudes and perceptions of the respondents were seen from the findings. 82.4% of respondents disagreed with the statement that all microorganisms are harmful to humans. A study had discovered that majority of the students (68.7%) believe that microorganisms can be both harmful and harmless (Aydin, 2015). Furthermore, 76.4% disagreed with the belief that all bacteria are harmful. This is because bacteria were not particularly hazardous, and that they only caused health problems when they were plentiful (Simonneaux, 2000). In addition, 87.9% disagreed that all yeast and fungi are harmless to humans. There was one study that stated that only a few of the millions of fungal species can naturally infect humans. However, medical advancements have rendered fungi that were formerly deemed dangerous to be harmless to many people (Kohler et al., 2014).

Furthermore, the respondents showed a positive attitude and perception about plant-based meat items in retail outlets can be contaminated with microorganisms that are harmful to us (73.9%). Meanwhile, 63.0% of the respondents were aware that microorganisms could multiply in a refrigerator. This is because microbes can grow if food is left out at room temperature, which is one of the favorable conditions for them to grow (USDA, 2017). Respondents may recognize the significance of understanding how microbes might affect food safety. This is similar to Sterniša et al. (2018) study which reported that 57.4% of consumers knew that harmful microorganisms could contaminate food, and 75.1% of consumers were also aware that microorganisms can proliferate in a refrigerator. Lastly, 84.8% of the respondents agreed that microorganisms can multiply in conditions like prepared, heat-treated foods left on a kitchen counter. They have a positive attitude and perception towards leaving food at room temperature provides an environment where some microorganisms can multiply rapidly. The "danger zone" for bacterial growth is typically between 4.4°C and 60°C (D’Amico et al., 2006). There was a similar finding about more than 90% of Turkish customers were cautious of bacterial growth on food (Sanlier, 2009).

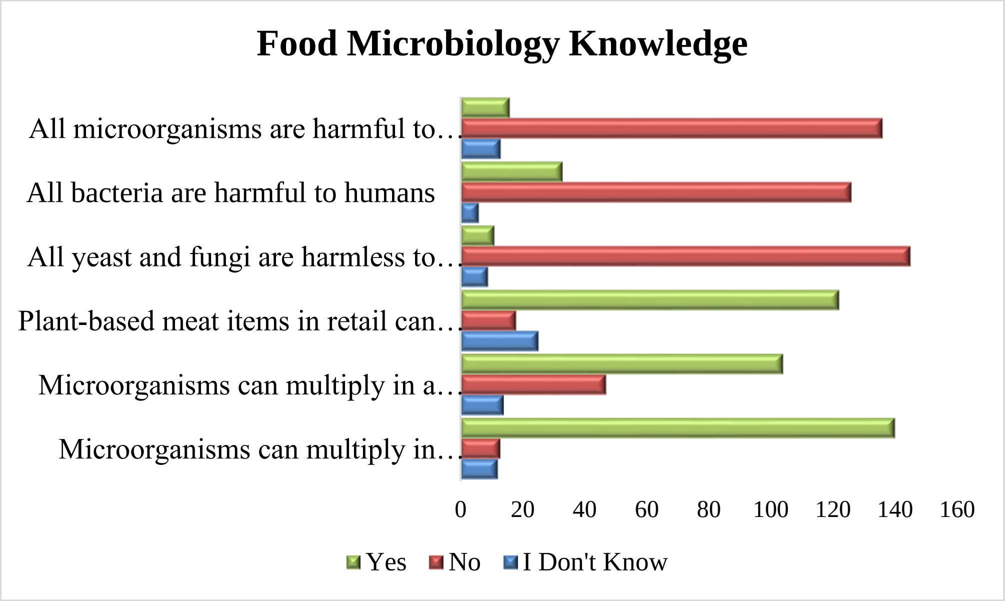


**Figure 5.3:** Food microbiology knowledge of respondents (n=165).

### 5.3 Significance of Results

This study addresses a significant research gap by integrating microbiological analyses and UTAR undergraduate student perceptions in the context of plant-based meat items. Existing research often focuses solely on either the scientific aspect or the consumer aspect, and this study provides a comprehensive view which is essential for shaping food safety strategies.

The results of this study provide an understanding on how storage conditions affect the microbiological quality of plant-based meat helps in ensuring that the product remains safe for consumption throughout its shelf life. This is particularly important for plant-based meats as they can still harbor microorganisms that may cause foodborne illnesses.

Results can also provide valuable information on the shelf life of plant-based meat products. For instance, food items that were stored at refrigeration temperature had lower microbial counts compared to room temperature. This knowledge is crucial for setting appropriate expiration dates and for advising consumers on storage and consumption practices. Researchers can use these findings to innovate and develop improved plant-based meat products with better stability and extended shelf life.

In a consumer-driven industry, the findings about respondents' attitudes and views are critical. The results had shown the understanding on how respondents perceive microbiological risks in plant-based meat items can help in identifying gaps in consumer knowledge. This information can be used to develop targeted educational programs to enhance awareness about safe food handling practices.

Respondents' attitudes and perceptions towards microbiological risks can significantly impact their consumption behavior. Positive attitudes and perceptions may lead to increased consumption, while negative attitudes and perceptions may stop individuals from incorporating plant-based meat into their diets. Research findings on students' attitudes towards microbiological risks can guide future research endeavors. It can help prioritize areas of concern and drive innovation in food safety technologies.

As a results, the combination of microbiology and consumer behavior in this study supports collaboration between scientific and social fields. The importance of the study stems not only from its individual findings, but also from its potential to develop a more holistic approach to addressing difficult challenges at the interface of science and society.

## 5.4 Limitations of Study

Microbiological analyses are critical for understanding the presence and behavior of microorganisms in various food samples. However, as with any scientific approach, they come with inherent limitations. Sampling errors may introduce biases and fail to accurately represent the true microbial population in the entire sample.  There was no result taken for 0 hours as the food samples were delivered from Ipoh to Kampar. Additionally, external microbial contamination during sampling, handling, or processing can distort results and lead to inaccurate interpretations. Contamination may have occurred when delivering the food to the laboratory. The sensitivity and specificity of methods vary, which may result in some bacteria going undetected or producing false results. Therefore, guaranteeing microbial viability is crucial, as some microorganisms may perish during transportation or preparation, resulting in inaccurate numbers. Additionally, I was also facing time constraints that make it challenging to complete all the experiments within the allocated benchwork period. This is particularly so due to the time-intensive nature of spreading plates and incubation period. Spread plating could take longer durations to complete, especially when analyzing food samples stored at room temperature.

Apart from that, the relatively small and homogeneous sample of students from a single undergraduate program and university is one of the limitations of this study, which may limit the generalizability of the findings to a larger student population. This study is only limited to UTAR undergraduate Kampar students.

It cannot reflect the attitude and perception towards microbiological risk of plant-based meat items among all university students.

## 5.5 Recommendations for Future Studies

Based on the outcomes of this research project, it is clear that additional research is needed to support and improve the findings of future studies. Firstly, expanded microbiological investigations are recommended to include a wider range of plant-based meat products. This expanded investigation could broaden the knowledge of elucidating various forms of microorganisms in a particular food product. Subsequently, the yeast and mold count using PDA is suggested to be performed by the next batch of students for popcorn chicken as I did not have sufficient time to carry out that microbial analysis. Thirdly, future students can conduct microbiological analyses on more than two types of microbes. For instance, *Staphylococcus spp.*, *Escherichia coli*, Lactic acid bacteria and so on.

As for students' attitude and perception survey, additional cross-sectional studies can be conducted in other local and private institutions. This is to improve the accuracy and dependability of research focusing on knowledge, attitude, and perception towards microbiological risk of plant-based meat items among undergraduate students. A longitudinal study is recommended as it provides a comprehensive and dynamic view for analyzing changes in student perceptions and attitudes. This study design could provide useful insights into how attitudes and perceptions of students evolve over time and what variables may impact those changes. Instead of using convenient sampling, random sampling is recommended as it ensures that every member of the population has an equal chance of being chosen. This aids in the creation of a sample that accurately represents the greater population. Face-to-face interviews with paper surveys are also recommended as an alternative to self-reported online surveys. This can help minimize response bias and provide assurance that the data collected truly represents the participants' responses.

# CHAPTER 6

# CONCLUSION

In this research study, extensive microbiological investigations were conducted on a variety of plant-based meat products while also investigating students' attitudes and perceptions of the associated microbiological risks. The findings provided insight into the importance of microbiological safety and consumer perceptions.

Based on the findings, all tested food samples were exposed to storage temperatures at 4±2°C and 25±2°C, which affected their nutritional value, quality, and shelf life. No matter what food was stored at varied temperatures, they were all having microbial growth. A significant increase was found in microbial counts of plant-based meat items as storage time was prolonged at room temperature (25±2°C). Nevertheless, no significant difference was found in microbial loads of all food samples stored for 2 days at refrigeration temperature (4±2°C). This was due to a reduction in enzymatic reactions and cellular processes, which prevented bacteria from proliferating quickly. Significant difference was only found for avocado charcoal bun’s microbial loads level stored for 24 hours at room and refrigeration temperatures. This was due to the fact that refrigeration created a regulated environment that prevented the growth and reproduction of most bacteria. Room temperature is frequently more favorable for microbial growth and activity. These results indicated that microbial counts could differ depending on the specific food product and storage conditions.

Most of the respondents showed positive attitudes and perceptions towards microbiological risk of plant-based meat items. They had good knowledge, self-reported practices, and awareness on controlling the microbiological pathogens of plant-based meat items. The majority of respondents were aware of food safety, yet more than half were unaware of the practice of freezing previously defrosted food products. Food contamination may occur when food is kept at temperature danger zone (4.4°C to 60°C).

In conclusion, this study highlights the dynamic nature of microbial populations in various food items during storage. It underscores the significance of considering both time and temperature as critical factors influencing microbial development and food safety. Future research may concentrate on understanding the specific mechanisms underlying these microbial changes and developing focused interventions to ensure the microbiological quality and safety of plant-based meat food products.

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# APPENDICES

## Appendix A

Ethical Approval for Research from UTAR

**UNIVERSITI TUNKU ABDUL RAHMAN** DU012(A)
Wholly owned by UTAR Education Foundation Co. No. 578227-M

Re: U/SERC/62/2023

20 March 2023

Dr Teh Lai Kuan
Head, Department of Allied Health Sciences
Faculty of Science
Universiti Tunku Abdul Rahman
Jalan Universiti, Bandar Baru Barat
31900 Kampar, Perak.

Dear Dr Teh,

**Ethical Approval For Research Project/Protocol**

We refer to the application for ethical approval for your students' research projects from Bachelor of Science (Honours) Dietetics programme enrolled in course UDDN3108. We are pleased to inform you that the application has been approved under Expedited Review.

The details of the research projects are as follows:

| No | Research Title | Student's Name | Supervisor's Name | Approval Validity |
|---|---|---|---|---|
| 1. | Nutritional Compositions, Total Phenolics, and Antioxidant Capacities of Plant-Based Meat Items | 1. Chen Yu Wei<br>2. Elisa Bong Tsyr Yin<br>3. Oo Xing Joe | Dr Chang Sui Kiat<br>Dr Ee Kah Yaw | 20 March 2023 – 19 March 2024 |
| 2. | Microbiological Analyses and Undergraduate Student's Attitude and Perceptions About Microbiological Risk of Plant-based Meat Items | 1. Careen Chong Kai Lyn<br>2. Siew Fei Kie<br>3. Wong Siew Ching | Dr Chang Sui Kiat<br>Dr Lam Ming Quan | |

The conduct of this research is subject to the following:

(1) The participants' informed consent be obtained prior to the commencement of the research;

(2) Confidentiality of participants' personal data must be maintained; and

(3) Compliance with procedures set out in related policies of UTAR such as the UTAR Research Ethics and Code of Conduct, Code of Practice for Research Involving Humans and other related policies/guidelines.

(4) Written consent be obtained from the institution(s)/company(ies) in which the physical or/and online survey will be carried out, prior to the commencement of the research.

**Kampar Campus** : Jalan Universiti, Bandar Barat, 31900 Kampar, Perak Darul Ridzuan, Malaysia
Tel: (605) 468 8888 Fax: (605) 466 1313
**Sungai Long Campus** : Jalan Sungai Long, Bandar Sungai Long, Cheras, 43000 Kajang, Selangor Darul Ehsan, Malaysia
Tel: (603) 9086 0288 Fax: (603) 9019 8868
**Website**: www.utar.edu.my

Should the students collect personal data of participants in their studies, please have the participants sign the attached Personal Data Protection Statement for records.

Thank you.

Yours sincerely,

**Professor Ts Dr Faidz bin Abd Rahman**
Chairman
UTAR Scientific and Ethical Review Committee

c.c Dean, Faculty of Science
Director, Institute of Postgraduate Studies and Research

**Kampar Campus** : Jalan Universiti, Bandar Barat, 31900 Kampar, Perak Darul Ridzuan, Malaysia
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Tel: (603) 9086 0288 Fax: (603) 9019 8868
**Website**: www.utar.edu.my

# Appendix B

Online Questionnaire of Study



# Attitude and Perception Towards Microbiological Risk of Plant-Based Meat Items Among Undergraduate Students in UTAR Kampar Campus

Dear Utarians,

Good Day! You are cordially invited to participate in this study survey conducted by Year 3 students taking Bachelor of Science (Hons) Dietetics program from Universiti Tunku Abdul Rahman (UTAR), Kampar Campus in Perak, Malaysia.

This study is conducted by Careen Chong Kai Lyn, Siew Fei Kie and Wong Siew Ching led by Dr. Chang Sui Kiat from the Department of Allied Health Sciences, Faculty of Science from UTAR Kampar Campus in Perak, Malaysia.

This study is aimed to evaluate the level of attitude, awareness and perception among undergraduate students from UTAR Kampar Campus towards microbiological risk of plant-based meat items.

Target Population (Inclusion Criteria):

1. Undergraduate students from UTAR Kampar Campus
2. Malaysian Citizen
3. Students that do not have soy-allergy

This questionnaire consists a total of 3 sections:

1. Section A: Sociodemographic Profiles
2. Section B: Knowledge, self-reported practices and awareness on controlling the microbiological pathogens in plant-based meat Items
3. Section C: Food Microbiology Knowledge

This questionnaire would take about less than 5 minutes to complete, and it would be highly appreciated if you could spent a little of your precious time to fill in this survey.

Please be consent that your participation is considered voluntary in this study. You may withdraw your participation in this survey at anytime. All information collected will be kept in private and confidential. All collected information will only be used for our Final Year Project purpose.

Thank you very much for your participation, it is really appreciated. If you have any inquiries about this study, please do not hesitate to contact us at MS Teams (CAREEN CHONG KAI LYN/ SIEW FEI KIE/ WONG SIEW CHING) or Email (careen.lynn@1utar.my/ siewfeikie@1utar.my/ si3wching26@1utar.my).

* Indicates required question

1. Agreement in participating in this study *

*Mark only one oval.*

- ( ) I was informed and have hereby understood, consented and agreed to participate in this study.
- ( ) I disagreed to participate in this study.

**Personal Data Protection Notice**

Please be informed that in accordance with Personal Data Protection Act 2016 ("PDPA") which came into force on 15 November 2013, Universiti Tunku Abdul Rahman ("UTAR") is hereby bound to make notice and require consent in relation to collection, recording, storage, usage and retention of personal information.

**NOTICE**

1. Personal data refers to any information which may directly or indirectly identify a person which could include sensitive personal data and expression of opinion. Among others it includes:

a) Name

b) Identity card

c) Place of Birth

d) Address

e) Education History

f) Employment History

g) Medical History

h) Blood Type

i) Race

j) Religion

k) Photo

l) Personal information and Associated Research Data

2. The purposes for which your personal data may be used are inclusive but not limited to:

a) For assessment of any application to UTAR For processing any benefits and services

b) For communication purposes

c) For advertorial and news

d) For general administration and record purposes

e) For enhancing the value of education

f) For educational and related purposes consequential to UTAR

g) For the purpose of our corporate governance

h) For consideration as a guarantor for UTAR staff/ student applying for his/ her scholarship/ study loan

3. Your personal data may be transferred and/ or disclosed to third party and/ or UTAR collaborative partners including but not limited to the respective and appointed outsourcing agents for purpose of fulfilling our obligations to you in respect of the purposes and all such other purposes that are related to the purposes and also in providing integrated services, maintaining and storing records. Your data may be shred when required by laws and when disclosure is necessary to comply with applicable laws.

4. Any personal information retained by UTAR shall be destroyed and/or deleted in accordance with our retention policy applicable for us in the event such information is no longer required.

5. UTAR is committed in ensuring the confidentiality, protection, security and accuracy of your personal information made available to us and it has been our ongoing strict policy to ensure that your personal information is accurate, complete, not misleading and updated. UTAR would also ensure that your personal data shall not be used for political and commercial purposes.

**CONSENT**

6. By submitting this form, you hereby authorize and consent to us processing (including disclosing) your personal data and any updates of your information for the purpose and / or for any other purposes related to the purpose.

7. If you do not consent or subsequently withdraw your consent to the processing and disclosure of your personal data, UTAR will not be able to fulfill our obligations or to contact you or to assist you in respect of the purposes and/ or for any other purposes related to the purpose.

8. You may access and update your personal data by writing to us at E-mail.
(careen.lynn@1utar.my/ siewfeikie@1utar.my/ si3wching26@1utar.my)

2. Acknowledgement of Notice *

*Mark only one oval.*

- I have been notices and that I hereby understood, consentent and agreed per UTAR above notice.
- I disagree, my personal data will not be processed.

**Section A: Sociodemographic Profiles**

This section consists of 4 questions.
Please fill in your answer to each question accordingly.

3. Gender *

*Mark only one oval.*

- Male
- Female

4. Race *

*Mark only one oval.*

- Malay
- Chinese
- Indian
- Others

5. Household Monthly Income Classification *

*Mark only one oval.*

- B40 (Less than RM 4, 849)
- M40 (RM 4,850 to RM 10, 959)
- T20 (More than RM 10, 960)

6. I learned about safe food preparation ...... *

*Check all that apply.*

- From family
- From books and magazine
- From the Internet
- At school
- At work

**Section B: Knowledge, self-reported practices and awareness on controlling the microbiological pathogens in plant-based meat items**

This section consists of 6 questions.
Kindly select "Yes" if you can answer YES to the question asked.
Kindly select "No" if you have to answer NO to the question asked.

7. 1. I would check for the freshness before purchasing any plant-based meat items. *

*Mark only one oval.*

- Yes
- No

8. 2. Improper home raw plant-based meat items preparation presents a health risk. *

*Mark only one oval.*

- Yes
- No

9. 3. I would pay attention to sufficient heat treatment of plant-based meat items. *

*Mark only one oval.*

- Yes
- No

10. 4. I always store plant-based meat items in the same place in a refrigerator. *

*Mark only one oval.*

- Yes
- No

11. 5. I defrost plant-based meat ...... *

*Mark only one oval.*

- on the kitchen surfaces at room temperature
- in a refrigerator
- under tap water
- in a microwave

12. 6. Sometimes, I would freeze the previously defrosted plant-based meat items. *

*Mark only one oval.*

- Yes
- No

**Section C: Food Microbiology Knowledge**

This section consists of 6 questions that evaluate food microbiology knowledge of the respondent.
Kindly select "I Don't Know" if you are unsure/don't know about the answer.

13. 1. All microorganisms are harmful to humans. *

*Mark only one oval.*

- Yes
- No
- I Don't Know

14. 2. All bacteria are harmful to humans. *

*Mark only one oval.*

- Yes
- No
- I Don't Know

15. 3. All yeast and fungi are harmless to humans. *

*Mark only one oval.*

- Yes
- No
- I Don't Know

16. 4. Plant-based meat items in retail can be contaminated with harmful microorganisms. *

*Mark only one oval.*

- Yes
- No
- I Don't Know

17. 5. Microorganisms can multiply in a fridge. *

*Mark only one oval.*

- Yes
- No
- I Don't Know

18. 6. Microorganisms can multiply in prepared, heat-treated foods that left on a kitchen counter. *

*Mark only one oval.*

- Yes
- No
- I Don't Know

**Attitude and Perception Towards Microbiological Risk of Plant-Based Meat Items Among Undergraduate Students in UTAR Kampar Campus**

Thank you very much for your participation in this survey!
Wishing you a sweet day full of joy and happiness. Have a very nice day and stay safe!

# Appendix C

FM-IAF-005 Form

| Universiti Tunku Abdul Rahman | | | |
|---|---|---|---|
| **Form Title : Supervisor's Comments on Originality Report Generated by Turnitin for Submission of Final Year Project Report (for Undergraduate Programmes)** | | | |
| Form Number: FM-IAD-005 | Rev No.: 1 | Effective Date: 3/10/2019 | Page No.: 1of 1 |

UTAR UNIVERSITI TUNKU ABDUL RAHMAN
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**FACULTY OF SCIENCE**

| | |
|---|---|
| **Full Name(s) of Candidate(s)** | Careen Chong Kai Lyn |
| **ID Number(s)** | 20ADB05306 |
| **Programme / Course** | Bachelor of Science (Hons) Dietetics |
| **Title of Final Year Project** | Microbiological Analyses and Students' Attitudes and Perceptions Towards Microbiological Risk of Plant-Based Meat Items |

| **Similarity** | **Supervisor's Comments** **(Compulsory if parameters of originality exceeds the limits approved by UTAR)** |
|---|---|
| **Overall similarity index:** 15 **%**<br><br>**Similarity by source**<br>Internet Sources: 7 %<br>Publications: 7 %<br>Student Papers: 8 % | |
| **Number of individual sources listed** of more than 3% similarity: 0 | |
| **Parameters of originality required and limits approved by UTAR are as follows:**<br>**(i) Overall similarity index is 20% and below, and**<br>**(ii) Matching of individual sources listed must be less than 3% each, and**<br>**(iii) Matching texts in continuous block must not exceed 8 words**<br>***Note: Parameters (i) – (ii) shall exclude quotes, bibliography and text matches which are less than 8 words.*** | |

Note Supervisor/Candidate(s) is/are required to provide softcopy of full set of the originality report to Faculty/Institute

***Based on the above results, I hereby declare that I am satisfied with the originality of the Final Year Project Report submitted by my student(s) as named above.***

Signature of Supervisor
Name: Dr. Chang Sui Kiat

Date: 14/9/2023

Signature of Co-Supervisor
Name: Dr. Lam Ming Quan

Date: 14/9/2023

# Appendix D

Summary page of the Turnitin Originality Report

Document Viewer

## Turnitin Originality Report

Processed on: 11-Sep-2023 15:03 +08
ID: 2162941544
Word Count: 15926
Submitted: 1

Microbiological analyses student perceptions ... By Careen Chong

| Similarity Index | Similarity by Source | |
|---|---|---|
| 15% | Internet Sources: | 7% |
| | Publications: | 7% |
| | Student Papers: | 8% |

exclude quoted | include bibliography | excluding matches < 8 words | mode: quickview (classic) report | print | refresh | download

5% match (student papers from 07-Sep-2023)
Class: DT FYP thesis submission
Assignment: DT FYP Thesis
Paper ID: 2159703652

1% match (student papers from 21-Sep-2016)
Submitted to Universiti Tunku Abdul Rahman on 2016-09-21

<1% match (student papers from 29-Apr-2023)
Class: Dietetics FYP
Assignment: FYP Thesis
Paper ID: 2079155495

<1% match ()
Zenghui Dai, Linna Han, Zhe Li, Mengqing Gu, Zhigang Xiao, Fei Lu. "Combination of Chitosan, Tea Polyphenols, and Nisin on the Bacterial Inhibition and Quality Maintenance of Plant-Based Meat", Foods

<1% match ()
PLoS One. 2015 Aug 12; 10(8):e0135304

<1% match (Oluwadara Alegbeleye, Olumide Adedokun Odeyemi, Mariyana Strateva, Deyan Stratev. "Microbial spoilage of vegetables, fruits and cereals", Applied Food Research, 2022)
Oluwadara Alegbeleye, Olumide Adedokun Odeyemi, Mariyana Strateva, Deyan Stratev. "Microbial spoilage of vegetables, fruits and cereals", Applied Food Research, 2022

<1% match (Andreja Špernjak, Anja Jug Puhmeister, Andrej Šorgo. "Public opinions and knowledge about microorganisms", Research in Science & Technological Education, 2021)
Andreja Špernjak, Anja Jug Puhmeister, Andrej Šorgo. "Public opinions and knowledge about microorganisms", Research in Science & Technological Education, 2021

<1% match (student papers from 01-Jun-2022)
Submitted to BATANGAS STATE UNIVERSITY on 2022-06-01

<1% match (Internet from 04-Jan-2023)
https://www.researchgate.net/publication/351116434_Evaluation_of_Street_Food_Safety_and_Hygiene_Practices_of_Food_Vendors_in_Can_Th

<1% match (Internet from 21-Feb-2023)
https://www.researchgate.net/publication/282570159_Kenaf_Hibiscus_cannabinus_L_seed_oil-in-water_Pickering_nanoemulsions_stabilised_by_mixture_of_sodium_caseinate_Tween_20_and_b-cyclodextrin

<1% match (Internet from 28-Jan-2023)
https://www.researchgate.net/publication/313630216_A_tutorial_on_pilot_studies_The_what_why_and_how

<1% match (Jah Ying Chung, Christopher J. Bryant, Kathryn E. Asher. "Plant-based meats in China: a cross-sectional study of attitudes and behaviours", Journal of Human Nutrition and Dietetics, 2022)
Jah Ying Chung, Christopher J. Bryant, Kathryn E. Asher. "Plant-based meats in China: a cross-sectional study of attitudes and behaviours", Journal of Human Nutrition and Dietetics, 2022

<1% match (Internet from 17-Mar-2023)
https://fsct.modares.ac.ir/article-7-8099-en.pdf

<1% match (Internet from 03-Jun-2023)
https://www.coursehero.com/file/p72gbho/local-cultural-practices-have-contributed-to-the-high-incidence-of-poverty-among/

<1% match (Internet from 22-Dec-2021)
https://www.coursehero.com/file/115070074/RMSS-Reviewerpdf/

<1% match (Internet from 07-Jun-2023)
https://www.coursehero.com/file/p3acsta4/9-Determine-the-total-colony-forming-unit-cfu-per-ml-or-g-for-the-samples-tested/

<1% match (student papers from 29-Apr-2023)
Submitted to Kaplan Professional on 2023-04-29

<1% match (Internet from 22-May-2023)
https://www.science.gov/topicpages/w/whole-grain+ready-to-eat+oat