MICROBIOLOGICAL ANALYSES AND UNDERGRADUATE STUDENT'S ATTITUDES AND PERCEPTIONS TOWARDS MICROBIOLOGICAL RISK OF PLANT-BASED MEAT ITEMS

By

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ABSTRACT

MICROBIOLOGICAL ANALYSES AND UNDERGRADUATE STUDENTS'S ATTITUDES AND PERCEPTIONS TOWARDS MICROBIOLOGICAL RISK OF PLANT-BASED MEAT ITEMS

Wong Siew Ching

Plant-based meat was developed in response to the inefficiencies of meat production when compared to crop harvesting, alongside the increasing public awareness of health-conscious and environmentally friendly dietary choices. Despite its plant-derived nature, the product remained significantly vulnerable to microbial spoilage due to its high moisture and protein levels, as well as its nearly neutral pH. However, the attitudes and perceptions towards the microbiological risks of plant-based meat were not assessed among Malaysian undergraduate students. This study involved the microbiological analyses of plant-based meat items to determine their aerobic colony counts (APC) and yeast and mould counts (YMC) under varying storage temperatures and durations. It was proven that plant-based meat items stored at 4°C exhibited lower microbial load in comparison to those stored at 25°C. As the storage duration increased, a distinct upward trend in the microbial load of plant-based meat items became evident. Therefore, the general public should be aware of the proper storage conditions for plant-based meat items, advocating their storage at 4°C for not longer than 24 hours. The survey regarding attitudes and perceptions obtained a total of 165 responses, revealing a predominant proficiency among Malaysian undergraduate students in comprehending and practising measures about the microbiological risks associated with plant-based meat items. In conclusion, plant-based meat items showed a higher level of safety when stored at 4°C for shorter durations, as opposed to storage at 25°C. Furthermore, the study indicated a positive attitude and perception of Malaysian undergraduate students towards the microbiological risks of plant-based meat items.

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May I continue to strive to live up my dreams in the years to come.

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.

WONG SIEW CHING

APPROVAL SHEET

This project report entitled "**<u>MICROBIOLOGICAL ANALYSES AND UNDERGRADUATE STUDENT'S ATTITUDES AND PERCEPTIONS TOWARDS MICROBIOLOGICAL RISK OF PLANT-BASED MEAT ITEMS</u>**" was prepared by WONG SIEW CHING and submitted as partial fulfilment of the requirements for the degree of Bachelor of Science (Hons) Dietetics at Universiti Tunku Abdul Rahman.

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PERMISSION SHEET

It is hereby certified that **WONG SIEW CHING** (ID No: **19ADB02999**) has completed this final year project entitled "MICROBIOLOGICAL ANALYSES AND UNDERGRADUATE STUDENT'S ATTITUDES AND PERCEPTIONS TOWARDS MICROBIOLOGICAL RISK OF PLANT-BASED MEAT ITEMS" under the supervision of Dr. Chang Sui Kiat from the Department of Allied Health Science, 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 in pdf format into the UTAR Institutional Repository, which may be made accessible to the UTAR community and public.

Yours truly,

(WONG SIEW CHING)

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LIST OF ABBREVIATIONS

ACC	Aerobic Colony Counts
ANOVA	Analyses of Variance
CFU	Colony Forming Units
DNA	Deoxyribonucleic Acid
FAO	Food and Agriculture Organization
FDA	U.S. Food and Drug Administration
ICMSF	International Commission on Microbiological Specifications for Foods
NOs	Nitric Oxide
OECD	The Organization for Economic Cooperation and Development
PCA	Plate Count Agar
PDA	Potato Dextrose Agar
RM	Ringgit Malaysia
SPSS	Statistical Package for Social Science
TFTC	Too Few To Count
TNTC	Too Numerous To Count
UHT	Ultra Heat Treatment
USA	United States of America
USD	US Dollar
USDA-FSIS	United States Department of Agriculture-Food Safety and Inspection Service
YMC	Yeast and Mould Count

CHAPTER 1
INTRODUCTION

1.1 Research Background

Throughout history, there has been a notable consumption of meat due to its great nutritional content and its integral role in human diets. Over the past two decades, there has been a 58% increase in global meat demand, driven by both the global population increase and rapid economic advancements (Whitnall and Pitts, 2019). According to Whitnall and Pitts (2019), global meat consumption reached approximately 320 million tonnes in 2018. These trends are projected to continue, with the OECD/FAO (2018) indicating a potential 15% market expansion by 2025. Therefore, plant-based meat items have been innovated in response to the inefficiencies of meat production compared to crop cultivation. Moreover, the demand for plant-based meat items has gained dramatically due to the growing public consciousness regarding health and environmentally-conscious food choices. To meet the demands of the target consumer, plant-based meat items have been created through a well-defined process that utilises plant proteins in order to closely mimic the sensory qualities of traditional meat. (Joshi and Kumar, 2015; Wild et al., 2014).

Plant-based meat production techniques have been practised for hundreds of years. Ingredients like wheat, gluten, lentils, mushrooms, rice and soy protein were processed together with meat-like flavour enhancers to yield a final product that closely mimics the sensory experience of meat consumption (Joshi and Kumar, 2015). Notable instances of plant-based meat items include tofu and tempeh, which are both derived from soybeans. However, certain items like seitan, which is traditionally made from wheat gluten, pose challenges for those adhering to gluten-free diets. The research conducted by Hu and colleagues (2019) demonstrates the potential of plant-based meat consumption to reduce cholesterol levels, thus mitigating the risks of associated health conditions. Additionally, a shift away from conventional meat consumption could also potentially decrease the susceptibility to colorectal cancers.

In the year 2022, the global market valuation of plant-based meat was approximated to be valued at 10.11 billion USD. This value is expected to experience consistent growth in the upcoming years, with an estimated value of approximately 33.99 billion USD by the year 2025 (Statista Research Department, 2022). Concurrently, investments in plant-based meat substitutes soared to 1.4 billion USD on a global scale in 2020 (Food Frontier, 2021). This surge came about as significant players in the meat industry, along with investors and governmental bodies, sought to establish their presence in a market that is prognosticated to encompass 10% of the expansive 1.4 trillion USD global meat market by the year 2029.

However, the microbial environment within plant-based meat can significantly diverge from that found in animal-derived meat. It is due to the use of distinct methods such as texturization and extrusion during production that contributes to creating a meat-like structure in plant-based meat items (Boukid, et al., 2021). Furthermore, the distinct pH, internal structure and nutritional composition of plant-based meat provide an optimal environment for microorganisms to adhere, infiltrate and proliferate (Hadi and Brightwell, 2021; Luchansky, et al., 2020; Xiang, et al., 2017). Notably, several types of harmful microorganisms, such as *Enterococcus durans*, *Exiguobacterium acetylicum*, *Acinetobacter* spp., *Staphylococcus* spp., *Lactobacillus sakei*, *Carnobacterium divergens* and *E. faecium*, were detected among plant-based meat items (European Commission, 2021; Geeraerts, et al., 2020).

The public must consider the microbiology of plant-based meat items due to the potential health risks posed by harmful microorganisms leading to foodborne illnesses. The impact of consuming spoiled plant-based meat items can result in serious health conditions, including meningitis, bacteremia, and various infections, such as intra-abdominal infections, wound infections, and skin infections (Said, Tirthani and Lesho, 2022; Elbehiry, et al., 2021). Alarming statistics reported by Ceylon (2023) revealed that a plant-based cheese brand was accountable for four cases of *Listeria* infections across the Netherlands, Germany, France and Belgium in 2022. Of particular concern is the fact that out of five cases, four infections occurred among pregnant women who experienced premature birth. Gambino-Shirley and colleagues (2018) demonstrated a widespread outbreak of *Salmonella* Virchow infections. This outbreak was shown to be attributed to a low-moisture powdered meal replacement comprising approximately 40 raw organic ingredients, among which were plant-based proteins.

In light of these concerns, the current study endeavours to comprehensively investigate the microbiological quality of plant-based meat items across various storage temperatures and durations. By doing so, this research intends to contribute to a deeper comprehension of product quality and safety, ultimately ensuring the well-being of consumers and advancing knowledge about plant-based meat items.

1.2 Problem Statements

1.2.1 Microbiological Analyses of Plant-Based Meat Items

Despite their plant-based origin, plant-based meat items remain highly susceptible to spoilage. Ingredients such as vitamins, minerals, flavourings and colourants are frequently incorporated into plant-based meat products to enhance their sensory attributes (Lupo, 2023). However, this incorporation often bypasses the thermal processing, which potentially introduces microorganisms into the final products (Sampson, et al., 2023). Several studies have documented the occurrence of various harmful microorganisms in plant-based meat items. According to the European Commission (2021), the presence of diverse harmful microorganisms, including *Enterococcus durans*, *Exiguobacterium acetylicum*, *Acinetobacter* spp. and *Staphylococcus* spp., have been found among plant-based meat items. Geeraerts and colleagues (2020) also have detected the presence of *Lactobacillus sakei*, *Enterococcus faecium* and *Carnobacterium divergens* among vegetarian as well as vegan meat alternatives. Besides, an investigation of commercial tofu conducted by van Kooij and de Boer (1985) revealed that almost all of the samples (95%) exhibited aerobic colony counts surpassing 10^6 CFU/g, which exceeded the safety standard outlined by the Food Standards Australia/New Zealand (2001). Other than that, *Yersina enterolitica*, *Bacillus cereus* and *Staphylococcus aureus* were all identified in the samples. Notably, a significant prevalence of *Enterococcus faecium* and *Enterobacteriaceae* have been reported, suggesting post-process contamination has occurred. In addition, as demonstrated by Sampson and colleagues (2023), microorganisms can infiltrate plant-based meat items due to the addition of raw ingredients and post-processing contamination resulting from improper handling and storage practices. Storage conditions, including temperature and duration, have demonstrated a significant impact on the microbiological quality of foods, both during preparation and subsequent stages (Zhou, et al., 2022; Suhaili, et al., 2021; Tarek, et al., 2020; Arshad, et al., 2019; Lani, et al., 2015). In light of the aforementioned data, there are only a limited number of studies have been conducted to investigate the impact of storage temperature and duration on the microbial load of plant-based meat items in Malaysia. Given the importance of identifying and characterizing spoilage and potentially hazardous microorganisms in plant-based meat items, the present study endeavours to conduct microbiological analyses of plant-based meat items across different storage temperatures and durations.

1.2.2 Attitudes and Perceptions Towards Microbiological Risks of Plant-Based Meat Items among Undergraduate Students

The evaluation of consumer attitudes and perceptions regarding foods is crucial for the development and promotion of new foods as it will determine whether the product will be accepted or rejected (Guiné et al., 2020). This significance also extends to the domain of plant-based meat, a category gaining substantial attention among the public. Numerous studies have delved into consumer attitudes and perceptions toward plant-based meat items (Vural, et al., 2023; Chen and Zhang, 2022; Zhao, et al., 2022; Bryant and Sanctorum, 2021; Bryant, et al., 2019; Slade, 2018). Overall, these studies predominantly demonstrated positive attitudes and perceptions, indicating a growing public embrace of plant-based meat (Vural, et al., 2023; Chen and Zhang, 2022; Bryant and Sanctorum, 2021; Bryant, et al., 2019). However, a notable portion of individuals maintain a negative attitude and perception of plant-based meat, despite their differences in taste and cost as compared to traditional meat (Zhao, et al., 2022; Slade, 2018). To enhance the positive attitude and perception of plant-based meat items among the public, it is crucial to investigate consumers' perspectives on microbiological risks. Previous research stated that consumers' understanding of food safety and microbiological risks significantly influences their decision-making (Frewer, de Jonge and van Kleef, 2009). Several studies in different regions, such as Slovenia and West Africa, have examined consumer attitudes and perceptions regarding the microbiological risks associated with various food items, including meat and vegetables (Sterniša, et al., 2018; Adjrah, et al., 2011). However, a gap exists in the exploration of consumer attitudes and perceptions of the microbiological risks associated specifically with plant-based meat items. Additionally, limited research has addressed attitudes and perceptions towards plant-based meat items among undergraduate students in Malaysia. Given the prevailing data, it is apparent that there is a dearth of investigations into attitudes and perceptions regarding the microbiological risks of plant-based meat items among Malaysian undergraduate students. Considering the variance in preferences across different countries, it is vital to focus on Malaysian undergraduate students as a distinct group. The present study seeks to bridge this gap and contribute insights into the attitudes and perceptions of microbiological risks associated with plant-based meat items among Malaysian undergraduate students.

1.3 Significance of Study

This study will provide general information on the relationship between storage temperature and the microbiological quality of plant-based meat items which could be used in future work to estimate the shelf-life of plant-based meat items. Consequently, it may further prevent the spread of dangerous bacteria that may lead to serious illness or even death. In addition, this study investigates the attitudes and perceptions towards microbiological risks of plant-based meat items among UTAR undergraduate students. This study may also emphasize the importance of controlling the microbiological pathogens in plant-based meat items. Consequently, the public will be able to regulate the microbial growth of plant-based meat items at all stages of food processing.

1.4 Objectives

1.4.1 General Objective

1. To investigate the effects of storage temperature and storage time on the microbial quality of plant-based meat items in relation to the attitudes and perceptions of UTAR undergraduate students towards the microbiological risks of plant-based meat items.

1.4.2 Specific Objectives

1. To determine the aerobic colony counts as well as yeasts and mould counts of plant-based meat items during storage at refrigerated (4 ± 2 °C) for 24 hours and 48 hours.

2. To investigate the aerobic colony counts as well as yeasts and mould counts of plant-based meat items during storage at room temperature (25 ± 2 °C) for 2 hours, 4 hours, 6 hours, 8 hours and 24 hours.
3. To determine the attitude and perception of UTAR undergraduate students towards the microbiological risks of plant-based meat items.

1.5 Research Questions

1. What are the aerobic colony counts as well as yeasts and mould counts of plant-based meat items during storage at refrigerated (4 ± 2 °C) for 24 hours and 48 hours?
2. What are the aerobic colony counts as well as yeasts and mould counts of plant-based meat items during storage at room temperature (25 ± 2 °C) for 2 hours, 4 hours, 6 hours, 8 hours and 24 hours?
3. Is there any association between storage temperature and microbial load in plant-based meat items during storage at refrigerated (4 ± 2 °C) and room temperature (25 ± 2 °C) for 24 hours?
4. Is there any association between storage duration and microbial load in plant-based meat items during storage at refrigerated (4 ± 2 °C) for 24 hours and 48 hours, and room temperature (25 ± 2 °C) for 2 hours, 4 hours, 6 hours, 8 hours and 24 hours?
5. What are the attitude and perception scores towards microbiological risks of plant-based meat items among UTAR undergraduate students?

1.6 Hypothesis

1.6.1 Null Hypothesis

H_{01}: Storage temperature was not significantly associated with the microbial load in plant-based meat items.

H_{02}: Storage duration was not significantly associated with the microbial load in plant-based meat items.

H_{03}: Attitude and perception towards microbiological risks of plant-based meat items among undergraduate students were negative.

1.6.2 Alternative Hypothesis

H_{A1}: Storage temperature was significantly associated with the microbial load in plant-based meat items.

H_{A2}: Storage duration was significantly associated with the microbial load in plant-based meat items.

H_{A3}: Attitude and perception towards microbiological risks of plant-based meat items among undergraduate students were positive.

CHAPTER 2

LITERATURE REVIEW

2.1 Microbiological Analyses of Plant-Based Meat Items

2.1.1 Factors Affecting Microbial Growth of Plant-Based Meat Items

Although plant-based meat items have entirely different ingredients compared to traditional meat products, they remain highly susceptible to microbial proliferation and reproduction. The emergence of undesirable and sour odours, alterations in colour, texture, appearance, and other spoilage indications can be readily observed in plant-based meat items (Karanth, et al., 2023).

Both intrinsic and extrinsic factors play a role in the deterioration of plant-based meat items. Internal factors include the fact that plant-based meat items are high in moisture and protein content as well as have an almost neutral pH (Toth, et al., 2021). This is further supported by the previous study, which found that plant-based meat items are extremely vulnerable to spoilage due to their nearly neutral pH as well as elevated protein and moisture content (Wild, et al., 2014). According to Alegbeleye and colleagues (2022), the spoilage of plant-based foods is not solely attributed to their high moisture content and almost neutral pH, but also to the presence of oxygen level.

When considering the external factors, storage duration has been shown to change the moisture content, consequently exerting an impact on the microbiological quality of the food (Paul, et al., 2018; Chandravarnan and Mendis, 2016). Besides, recent research conducted by Liu and colleagues (2023) discovered a strong relationship between the microbiological deterioration of plant-based beef products and storage temperature as well as packing conditions. The design, manufacturing method and factory environment all directly affect the microbiological quality of plant-based meat products (Dagnas and Membrè, 2013).

2.1.2 Findings on Microbiological Analyses of Plant-Based Meat Items

The microbiological analyses take the responsibility to analyse the presence and growth of the microorganisms in any that have a high possibility of the sample (Nemati, et al., 2016). The level of microbial contaminants present in plant-based meat items can serve as a gauge for their microbiological quality. Elevated contamination levels signal poor food quality and an increased susceptibility to foodborne illnesses. It also displays the level of cleanliness maintained by food handlers throughout the meal preparation process (Girma, Ketema and Bacha, 2014).

Specifically, yeast and mould are commonly found in plants, fruits and cereals (Alegbeleye, et al., 2022). As outlined by Tóth and colleagues (2021), the *Enterobacteriaceae* as well as yeast and mould were absent in the raw ingredients of plant-based meat items but were detected in the ready-to-eat plant-based meat items. Correspondingly, the European Commission (2021) demonstrated that several microorganisms, including *Enterococcus durans*, *Exiguobacterium acetylicum*, *Acinetobacter* spp. and *Staphylococcus* spp., were identified within plant-based meat items. Additionally, Geeraerts and colleagues (2020) reported the detection of various bacterial contaminants, primarily *Carnobacterium divergens*, *Lactobacillus sakei* and *Enterococcus faecium*, in plant-based meat items. Hence, these findings underscore that plant-based meat items also possess a high risk of contamination, potentially leading to foodborne illnesses.

Unfortunately, information on the aerobic colony counts as well as yeast and mould counts of high-moisture plant-based meat items at different storage temperatures and duration is hardly found in the scholarly literature. It was thus crucial to carry out complete microbiological analyses of the raw materials and the newly created products to identify and characterise any relevant microorganisms that may be dangerous or potentially harmful.

2.1.3 Findings on Storage Temperature on Microbial Growth of Plant-Based Meat Items

Extrusion is a high-temperature, high-pressure processing technique used to produce the majority of plant-based meat products. It reduces the microbial load while producing the required appearance and texture of the products. The findings revealed that each gram of extruded product had, on average, less than 100 colony-forming units. After processing, the plant-based meat products were packaged in a way that reduced the risks of recontamination. For instance, several post-packaging techniques were utilised on the final plant-based meat products, such as sterilising, pasteurisation and freezing (Wild, et al., 2014). However, further post-process contamination might be introduced by the external environment or even through improper food handling processes (Sagoo, et al. 2009). Hence, we may conclude that improper storage temperature has a significant impact on the microbial spoilage of plant-based meat products.

Based on the previous study conducted by Liu and colleagues (2023), storage temperature was shown to be one of the most critical external factors that affected the microbial risks of plant-based meat items. In this study, the microbiological load during the 24 hours of storage temperature at 22 ± 2°C and 32 ± 2°C, while the lower microbial content was found at 4 ± 2°C. The microbial load during storage at 22 ± 2°C was even doubled compared to storage at 4 ± 2°C. Hence, 4 ± 2°C was considered to be the safest temperature for preserving plant-based meat items. This finding was consistent with another study conducted by Cabello-Olmo and colleagues (2020), which demonstrated that a temperature rise harmed the microbial content. In this study, when compared to room temperature, -20 °C and 4 °C were found to be more suitable for preserving the plant-based meat items. This is because these two temperatures can effectively inhibit the proliferation and reproduction of microorganisms in the foods. However, only several types of plant-based meat items have been investigated in the previous study. Also, there are no studies have been done in Malaysia, which probably has different humidity and weather compared to other regions which may affect the microbiological quality of plant-based meat items.

2.1.4 Findings on Storage Duration on Microbial Growth of Plant-Based Meat Items

Storage duration is one of the most extrinsic factors that can affect the microbiological quality of plant-based meat items. The relationship between the storage duration and the microbial growth of other foods has been determined in the previous study. The study conducted on grey oyster mushrooms showed that the total plate count was increased from 3.87 log CFU/g on day 0 to 7.10 log CFU/g on day 6. On the other hand, the yeast and mould counts showed that it increased from 4.73 log CFU/g on day 0 to 6.84 log CFU/g on day 6 (Suhaili, et al., 2021). A similar finding was also found in another study conducted by Chandravarnan and colleagues (2016), which revealed that yeast and mould counts were increased when the storage duration increased. In this study, the yeast and mould counts of black tea were increased from 248 CFU/g at 0 months to 780 CFU/g at 4 months. However, there is limited research that has studied the effect of storage duration on the microbiological quality of plant-based meat items.

2.2 Attitudes and Perceptions Towards Microbiological Risks of Plant-Based Meat Items

2.2.1 Findings on Consumer's Attitudes Towards Plant-based Meat Items

According to Kapoor and Madichie (2012), an attitude is a tendency to judge something positively or negatively. Our behaviour and intentions are influenced by the attitudes and ideas we have. Since the most important barrier in commercializing plant-based meat items is consumer acceptance (Hocquette, 2016), hence, marketers need to understand consumer attitudes in a range of situations and come up with strategies for influencing those attitudes. As a result, marketers can get customers to adopt a more positive attitude regarding the products on sale (Kapoor and Madichie, 2012).

According to Vural and colleagues (2023), both meat and non-meat eaters had a positive attitude towards plant-based meat items. Although meat-eaters expected plant-based meat items to be less satisfying, due to lower expected taste pleasantness and fillingness, they perceived the plant-based meat items to be healthier. According to Bryant and Sanctorum (2021), over 40% of respondents have a positive attitude about plant-based meat alternatives. However, this statement opposed the findings from a study conducted by Slade (2018) as they found that the majority of consumers continue to demonstrate a negative attitude to entirely switching to consuming plant-based meat items. Based on a hypothetical choice experiment, the participants in this study still preferred beef burgers when all the burgers had the same taste. Besides, when all the items priced equally, there were only 21 % of respondents chose a plant-based burger and 11 % chose a cultured meat burger. In short, the research on consumer attitudes towards plant-based meat items remains inconsistent.

2.2.2 Findings on Consumer's Perceptions Towards Plant-Based Meat Items

A consumer's perception is defined as their interpretation of how they feel about a certain product. Marketers need to investigate the perception of consumers as it greatly affects the success of a product or a business. For instance, a restaurant in a town that has a high reputation would do better business, but a business that has a poor reputation will suffer economically (Thiruvenkatraj and Vetrivel, 2017). In light of this, we decided to investigate consumer perceptions because it has an impact on both consumer behaviour and the success and profitability of a business.

A study conducted in China has used social media data to investigate Chinese public interest and the idea of the concept of plant-based meat (Chen and Zhang, 2022). According to this study, personal postings that reflect, positive, neutral and negative perceptions account for around 42.10% of all posts. Out of this 42.10 %, 20.34 % showed a positive perception towards plant-based meat items, 9.65% showed a neutral perception and 12.11% demonstrated a negative perception. Hence, it stands to reason that individuals who have a positive perception towards plant-based meat items are more numerous than those who have a negative perception. This finding was aligned with previous research conducted among the population in the USA, India and China which discovered that the positive perception towards plant-based meat increased from 44 % (2019)

to 51 % (2020) (Bryant, et al., 2019). Yet, this statement conflicted with those made by Zhao and colleagues (2022) in their study. According to the findings, people are more likely to express negative perceptions towards plant-based meat items if they have never eaten any of them or if they love eating meat and are hesitant to give it up completely. However, previous studies did not focus on the Malaysian population.

2.2.3 Findings on Educated People's Attitudes and Perceptions Towards Plant-Based Meat Items

Education level is one of the factors that has been linked to consumer attitudes and perceptions toward plant-based meat items (He, Evans, Liu and Shao, 2020). Thus, the researcher chose to conduct the study among undergraduate students.

A study conducted among Northeast University undergraduate students by Avelino and colleagues (2022) revealed that educated people significantly have a positive attitude towards plant-based meat items. This is supported by the study's findings, which stated that 57% of participants had previously consumed plant-based meat products and that 85.2% were more inclined to give them a try. According to research done in the USA, China and India, more postings are showing a positive attitude towards plant-based meat items (Bryant, et al., 2019). Out of these three countries, China and India had a notably larger proportion of positive attitude postings. It is because people in China and India have better levels of education than Americans do. Another study by Slade (2018) indicated that younger and more educated people showed a higher preference towards plant-based beef rather than beef.

However, only limited academic research works have been undertaken on the attitudes and perceptions of Malaysian undergraduate students towards plant-based meat items. Moreover, the study done by Avelino and colleagues (2022) has several limitations. One of the limitations is that the researcher only focused on undergraduate students at Northeast University. Further research is suggested to be done on various populations since people of different ethnicities would perceive flavours differently. Hence, this is the rationale for the researcher to choose Malaysian undergraduate students for the study.

2.2.4 Findings on Attitudes and Perceptions of Educated People Towards Foodborne Microbial Hazards

Foodborne outbreaks have become a significant global concern, contributing to considerable morbidity and posing risks to human populations. In Malaysia, instances of food poisoning have happened frequently, largely due to the country's hot and humid climate, which provides an ideal environment for the growth of foodborne bacteria (Abdul-Mutalib, et al., 2015). According to the Ministry of Health (2016), Malaysia had an incidence rate of 44.18/100,000 population in 2010, 50.42/100,000 population in 2014 and 47.20/100,000 population in 2016, as well as a mortality rate of 0.041/100,000 population in 2016. Hence, the researcher needs to investigate to conduct the study among Malaysian undergraduate students.

Through multiple linear regressions, Baptista, Rodrigues and ant'Ana (2020) have found that consumers with low educational levels are more likely to present inappropriate food safety practices and negative perception of microbiological risks. According to a study conducted by Sayuti and colleagues (2020) among Management and Science University students at Shah Alam, it showed that undergraduate students exhibited positive attitudes and perceptions towards foodborne microbial hazards. This finding was aligned with another study that investigated the attitude and perception of processors on the microbiological quality of commercially produced traditional fermented cereal beverages (Byakika, et al., 2019). In this study, it was found that the processors had fairly positive perceptions and attitudes with 63.0 ± 2.3% and 52.2% ± 3.0%, respectively. However, this finding contradicted another study conducted among 430 randomly selected college students in Gondar City, northwest Ethiopia (Azanaw, et al., 2021). In that study, it was found that the overall attitude and perception towards food safety among college students were quite low, with only approximately 30% of respondents displaying positive attitudes, as well as good knowledge and practices.

Nevertheless, there are limited research has been done on the attitudes and perceptions towards the microbiological risks of plant-based meat items among Malaysian undergraduate students. In light of this, the researcher decided to conduct the study to assess the attitudes and perceptions of undergraduate students regarding the microbiological risks associated with plant-based meat items. It is due to the fact that microbiology is important to food safety, production, processing, preservation and storage (Lorenzo, et al., 2018).

CHAPTER 3

MATERIALS AND METHODS

3.1 Microbiological Analyses of Plant-Based Meat Items

3.1.1 Experimental Design

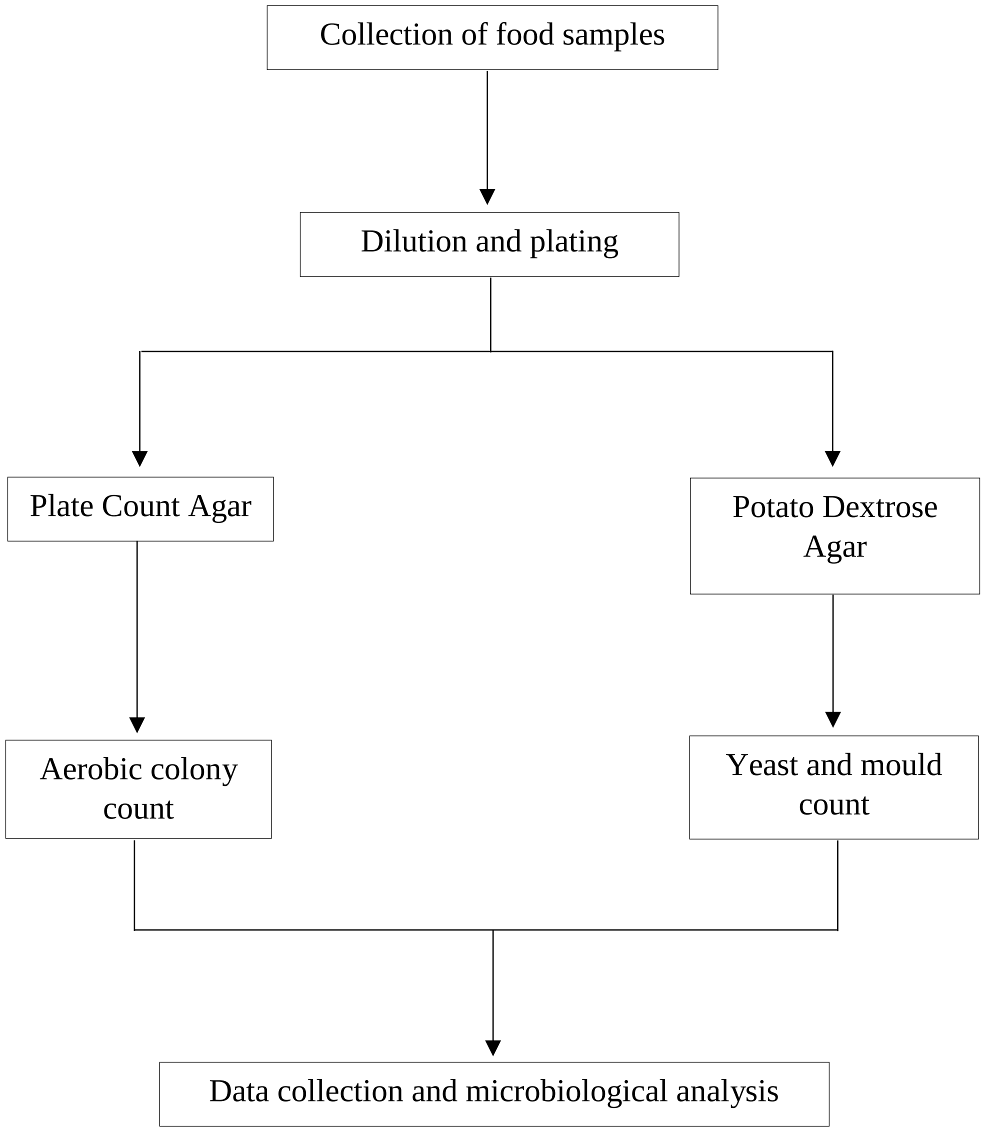

Figure 3.1: Experimental design of this study

3.1.2 Apparatus and Equipment

In Table 3.1, a list of apparatus and equipment utilised in this research is provided along with their respective manufacturers.

Table 3.1: Apparatus and equipment used, along with their respective manufacturers.

Apparatus and Equipment	**Manufacturers**
Incubator	Memmert, Germany
Laminar flow cabinet	Esco
Petri dishes (90 x 15 mm)	Nest
Vortex mixer	Stuart
Weighing balance	Kern, Germany

3.1.3 Chemical and Media

In Table 3.2, a list of chemicals and media utilised in this research is provided along with their respective manufacturers.

Table 3.2: Chemicals and media used, along with their respective manufacturers.

Chemical and media	**Manufacturers**
Plate count agar	Merck KGaA, Germany
Potato dextrose agar	Merck KGaA, Germany

3.1.4 Preparation of Media and Solution

Unless specified otherwise, all media and solutions underwent autoclaving for 15 minutes at 121°C.

3.1.4.1 Plant Count Agar

Plate count agar is a non-selective solid culture medium used for the standardized enumeration of aerobic bacteria in food, dairy products, pharmaceuticals, water as well as other hygienic materials. The ingredients of the medium may differ among each manufacturer, but glucose, peptone, agar and yeast extract are always included (Center for Food Safety and Applied Nutrition, 2021). Amino acids, carbon, vitamins, B-complex vitamins, minerals, nitrogen and energy can all be provided to support the growth of organisms by having these components in the medium (Liofilchem, 2016).

First, 22.5 g of plate count agar (PCA) powder was dissolved in 1 L of distilled water to prepare PCA agar. The resulting medium was then autoclaved and subsequently cooled to a temperature ranging from 45°C to 50°C. After that, the PCA agar was aseptically dispensed into petri dishes, and these dishes were given time for solidification and cooling at room temperature.

3.1.4.2 Potato Dextrose Agar

Potato dextrose agar is a non-selective, non-differential culture medium that is commonly used to isolate and enumerate yeasts, moulds and other fungi from a variety of samples, including foods and dairy products. Dextrose and potato infusion, which are the components of the Potato dextrose agar, are crucial for providing the energy, carbon and nitrogen essential to promote the luxuriant growth of yeasts and moulds (Westphal et al., 2021). The rich potato infusion and dextrose have also been shown to promote the growth of mould spores as well as pigmentation in particular dermatophytes. Additionally, when tartaric acid is added to potato dextrose agar, the pH of the medium is lowered to a level that prevents bacterial growth but promotes fungus development.

The procedure for preparing potato dextrose agar (PDA) was precisely the same as that for making PCA agar, with the only difference being the use of 39 g of PDA agar powder instead of PCA agar powder.

3.1.5 Collection of Plant-Based Meat Food Samples

A total of three ready-to-eat plant-based meat samples were provided randomly from a plant-based meat restaurant located in Ipoh, Malaysia under the collaboration. At the point of sale, samples were obtained from the restaurants that use their packaging. The foods that going to be analysed were delivered to UTAR, Kampar after being prepared. The duration was approximately 40 minutes. The experiment was conducted at room temperature within 30 minutes upon arrival in the laboratory.

3.1.6 Food Sample Preparation

The food sample was handled and opened aseptically in the laboratory. There is around 10 g of food sample was homogenized using a blender. The homogenized sample was then transferred into two sterile zig bags using a sterile spatula. One of the zig bags was stored in the refrigerator (4 °C) while another zig bag was stored at room temperature (25 °C). For the microbiological examination of the food sample, 1 g of homogenized food sample was weighed and obtained immediately using a sterile spatula after the storage conditions for each sampling time point. Using 9 ml of sterile distilled water, 1 g of the homogenised food sample was serially diluted 10 times (10^{-1} to 10^{-7}). By using a vortex mixer, the mixture was thoroughly mixed.

3.1.7 Microbiological Analyses

The plant-based meat items were subjected to microbiological analyses, which included aerobic colony count as well as yeast and mould count. Each food sample was analysed twice to improve the accuracy of the results.

3.1.7.1 Aerobic Colony Count

After thoroughly mixing the product of serial dilutions (10^{-1} to 10^{-7} dilutions), 0.1 ml of each of the dilution levels was then spread-plated in duplicate on PCA. After allowing the PCA plates to dry, they were incubated at 37°C for 24 hours. After incubation, the number of aerobic colony-forming units has been identified with a colony counter. The average aerobic colony count was then obtained from the two replicated plates. The greatest dilution that produced between 30 and 300 CFUs was used to calculate the colony-units per gram ($\log_{10}$ CFU/g) of the test plant-based meat item.

3.1.7.2 Yeast and Mould Count

Potato dextrose agar plates with colonies ranging below 150 CFUs were determined with a colony counter after being spread-plated and incubated at 30°C for 7 days. The two replicated plates of the same dilution were used to calculate the average yeast and mould count. The colony count is subsequently converted into $\log_{10}$ colony-forming units per gram (CFU/g).

3.1.8 Data Collection and Analyses

The results of the microbiological analyses were presented as $\log_{10}$ CFU/g. A normality test was performed to test the normality of the data. Significant differences between the microbial load and storage time at room temperature were assessed by one-way analyses of variance (ANOVA) test. The significant differences between microbial load and storage time at refrigerated temperature were assessed by paired T-test. Besides, the significant differences between microbial loads and storage temperatures were determined by using a paired T-test. Statistical Package for Social Science (SPSS) version 20 was used to analyse all the data, with a p-value less than 0.05 being regarded as statistically significant. The results were shown as mean ± standard deviation.

3.2 Attitudes and Perceptions Towards Microbiological Risks of Plant-Based Meat Items

3.2.1 Flowchart of Study

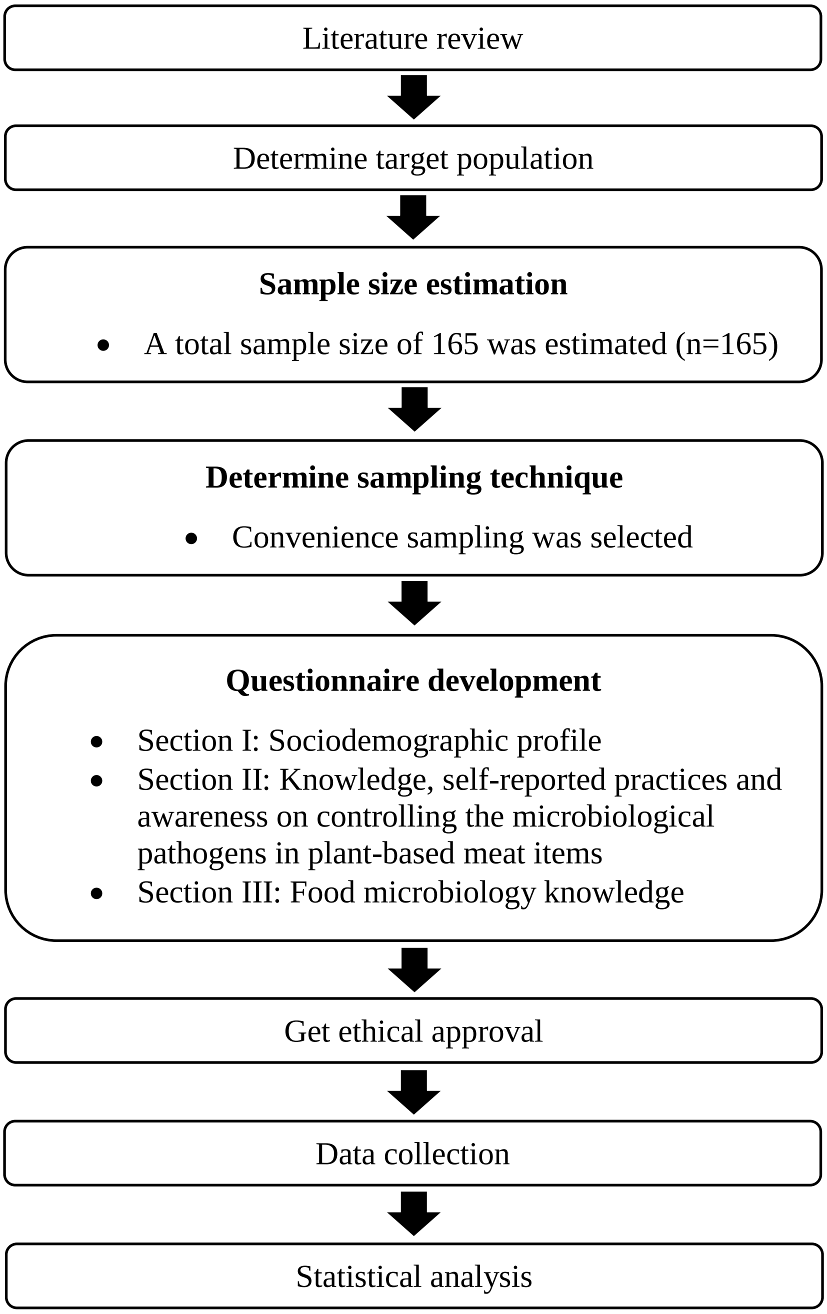

Figure 3.2: Flowchart outlining the research methodology for the attitude and perception of UTAR undergraduate students towards the microbiological risks of plant-based meat items.

3.2.2 Study Design

The researcher decided to implement a descriptive, cross-sectional research approach since it is efficient and cost-effective. Only the data from one population are examined at a time in a cross-sectional study. It can offer fundamental details on prevalence and distribution. Moreover, descriptive research is ideal for use in this study since it intends to classify UTAR Kampar undergraduates' attitudes and perceptions concerning the microbiological risks of plant-based meat products. It can gather information that addresses the what, when, where, and how of an event. However, descriptive research was unable to identify a probable explanation or effect for the phenomena. Descriptive research is also useful for locating characteristics, frequency, trends, and categories (Baker, 2017).

In addition, the researcher chose to employ quantitative data when designing the research as it can be organised, classified, and utilised to identify patterns of behaviour, attitudes, and other characteristics of a population. Using quantitative data, the researcher can describe a population's characteristics in terms of percentage (for instance, the proportion of the population that belongs to certain ethnic groups) or average (for instance, the average household incomes and average scores of the assessments). However, some research biases, such as selection bias, information bias, sample bias or omitted variable bias, might affect the results of quantitative research (McCombes, 2022).

3.2.3 Setting of the Study

The study was carried out by the researcher at the Kampar campus of Universiti Tunku Abdul Rahman (UTAR) in Malaysia. Approximately 1 month of duration has been took to achieve the targeted sample size, which is from 1st June 2023 to 30th June 2023.

3.2.4 Target Population

The intended population of this study was undergraduate students. This particular set of students was chosen as the target population since the researcher may easily contact them when performing the research. Besides, undergraduate students are active users of social media such as WhatsApp, Instagram and Facebook. According to Uma and colleagues (2021), WhatsApp (91.1%) was the application that Malaysian undergraduate students used the most frequently, followed by Instagram (74.3%). Therefore, it will be easier for them to access the questionnaire since the questionnaire is primarily distributed through these two online platforms. Microsoft Teams will also serve as a platform to distribute the questionnaire because UTAR students used Microsoft Teams as their E-learning platform to continue their educational program for their distance learning courses during COVID-19.

3.2.5 Sampling

3.2.5.1 Sampling Method

First of all, non-probability sampling was used in this research. Both the cost and the implementation of this sampling method are minimal. Yet, there is a possibility of sampling error. Convenience sampling was selected for the research. By adopting this sampling method, the researcher might recruit study participants without first needing a list of them. This indicates that the sample, which is UTAR Kampar undergraduate students, is drawn from a population that is accessible to the researcher. Ethical approval was obtained to carry out the cross-sectional study.

3.2.5.2 Sampling Size

According to a study conducted on the consumption of plant-based meat items in Malaysia by Statista Research Department (2021), about 11% of the respondents stated that they have consumed plant-based meat items before. A margin of error of 5% with a 95% confidence level was used in the current study.

Daniel (1999) formula was used to determine the sample size:

$n = Z^2 \frac{p(1-p)}{d^2}$

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

n = 150

After considering a 10% non-response rate:

n = 150 + 10% = 165

Here, n is the estimated sample size;

Z is the confidence interval of 1.96;

p is the expected prevalence of an attribute;

d is an acceptable sample error, d = 0.05.

The actual sample size was 165, up from the expected 150 by an additional 10% to accommodate for turnover.

3.2.6 Sampling Criteria

3.2.6.1 Inclusion Criteria

- UTAR Kampar undergraduate students
- Malaysian citizens

3.2.6.2 Exclusion Criteria

- Students who have soy allergy

3.2.7 Study Instrument

Primary data was collected for this research. A survey questionnaire that was performed in Google Forms was used for data collection. The researcher chose to use Google Forms because it can be distributed easily online to reach more people. Google Forms was then distributed through online platforms, such as WhatsApp, Instagram and Microsoft Teams until the predetermined sample size (n=165) was reached.

The questionnaire was administered in English version and consisted of 16 questions. There were three sections in the questionnaire. Section A: sociodemographic profiles, Section B: knowledge, self-reported practices and awareness on controlling the microbiological pathogens in plant-based meat items and Section C: food microbiology knowledge. The questions inside the questionnaire were referred to Špernjak and colleagues (2021) and Sterniša and colleagues (2018). It is displayed in Appendix B. All information collected from each respondent was kept private and confidential. Besides, the data will be only used for academic purposes.

3.2.7.1 Section I: Sociodemographic Profiles

The sociodemographic profiles consisted of 4 close-ended questions regarding gender, ethnicity, household income and the sources for the respondents to get the safe food preparation.

3.2.7.2 Section II: Knowledge, Self-Reported Practices and Awareness of Controlling the Microbiological Pathogens on Plant-Based Meat Items

A questionnaire from a previous study that assessed knowledge, self-reported practices and awareness of controlling the microbiological pathogens on plant-based meat items was amended and modified (Sterniša, et al., 2017). Six questions that assessed the respondents' knowledge, self-reported practices and awareness of controlling the microbiological pathogens on plant-based meat items mostly required them to select 'Yes' or 'No'. One point was given for a correct response, while 'zero' was given for a false response. The sum of all individual responses to these questions was calculated to get total raw scores, which ranged from 0 to 6 and then converted into percentages.

3.2.7.3 Section III: Food Microbiology Knowledge

A questionnaire on knowledge of food microbiology was amended and modified from a prior study (Špernjak, et al., 2021). Participants were required to select

‘Yes’, ‘No’ or ‘I don’t know’ to six questions related to their knowledge of food microbiology on the questionnaire. One point was given for the right response, while ‘zero’ was given for a false or “I don’t know” response. All individual responses to the questions were added up to get the total raw scores, which ranged from 0 to 6. Those values were then converted into percentages.

3.2.8 Statistical Analyses

3.2.8.1 Descriptive Analyses

Microsoft Excel was used to enter and examine the data. The data in the research may be summarised and measured simply using descriptive analyses. The researcher can better describe, present, and summarise the findings by using tables and figures. Additionally, it aids in the simplification of voluminous data and enables us to provide a summary of the facts under investigation (Trochim, 2020). For each of the attitude and knowledge questions, the researcher estimated the frequency (n) and percentage (%) as part of the analyses. The results will then be categorized into ‘positive’, ‘neutral’ or ‘negative’ by using the bloom’s cut-off points (Feleke, et al., 2017). Participant who had a total score of 10 and above was considered as positive attitude and perception, who scored between 7 and 9 was considered to have a neutral attitude and perception. Last but not least, the participant who scored less than 7 was considered as having a negative attitude and perception towards the microbiological risks of plant-based meat items.

3.2.8.2 Pilot Test

A pilot test is crucial to ensure that the questionnaire and instructions are clear and without ambiguity. A pilot study may also be used to evaluate all other components of the main investigation such as the sequential flow of the questions and the time needed to complete the questionnaire. Consequently, it can greatly reduce the amount of time and money wasted on participants and researchers at the same time (In, 2017). A pilot test is carried out by choosing a small amount of samples from the group and gathering responses. As Julious (2005) suggested that minimum of 12 sample sizes are required for a pilot investigation, the researcher therefore randomly selected 12 UTAR Kampar undergraduate students to participate in the pilot study through an online questionnaire. During the pre-testing, the students took less than 5 minutes to complete the whole set of questionnaires. Since all of the students claimed to have fully understood the questions asked, therefore, no modification was made to the questionnaire.

CHAPTER 4

RESULTS

4.1 Microbiological Analyses of Plant-Based Meat Items

4.1.1 Effect of Storage Temperature on Aerobic Colony Counts of Plant-Based Meat Items

Table 4.1 provides a summary of the effects of different storage temperatures on aerobic colony counts in plant-based meat items. After 24 hours of incubation, all plant-based meat items exhibited aerobic count when stored at both room temperature (25°C) and in the refrigerator (4°C).

The highest aerobic colony count was observed in nasi lemak for both storage conditions, recording counts of $1.33 \pm 0.04 \times 10^{10}$ CFU/g at room temperature (25°C) and $2.88 \pm 0.02 \times 10^{5}$ CFU/g at refrigerated temperature (4°C). Following closely was spaghetti carbonara, displaying counts of $3.12 \pm 0.17 \times 10^{9}$ CFU/g at room temperature (25°C) and $3.10 \pm 0.14 \times 10^{4}$ CFU/g at refrigerated temperature (4°C). Both nasi lemak and spaghetti carbonara demonstrated considerable increases in aerobic counts when comparing storage at room temperature (25°C) and refrigerated temperature (4°C). Among the samples, luncheon meat exhibited the lowest aerobic colony counts, which are $5.10 \pm 0.07 \times 10^{5}$ CFU/g at room temperature (25°C) and $2.92 \pm 0.02 \times 10^{4}$ CFU/g at refrigerated temperature (4°C). Nevertheless, all of the plant-based meat items showed a statistically significant difference ($p<0.05$) in the growth of aerobic colony counts between storage at room temperature (25°C) and refrigerated temperature (4°C).

Table 4.1: Aerobic colony counts of plant-based meat items stored for 24 hours at 4°C and 25°C

Plant-Based Meat Items (n=3)	Mean Aerobic Colony Counts (log CFU/g)	
	4°C	**25°C**
Nasi Lemak	$2.88 \pm 0.02 \times 10^{5\text{ a}}$	$1.33 \pm 0.04 \times 10^{10\text{ b}}$
Spaghetti Carbonara	$3.10 \pm 0.14 \times 10^{4\text{ a}}$	$3.12 \pm 0.17 \times 10^{9\text{ b}}$
Luncheon Meat	$2.92 \pm 0.02 \times 10^{4\text{ a}}$	$5.10 \pm 0.07 \times 10^{5\text{ b}}$

Values are means ± standard deviations, n = 3. Mean values followed by different superscript letters represent significant differences in aerobic colony counts for each type of plant-based meat item among two sampling conditions ($p < 0.05$).

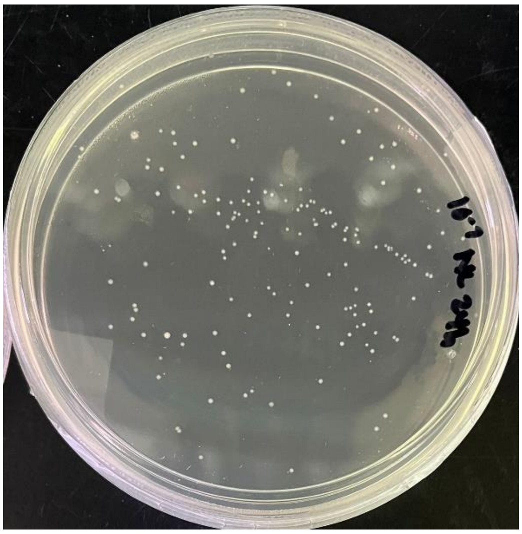

Figure 4.0.1a: Nasi Lemak at 4°C for 24 hours (Dilution factor: 10-^{2})

Figure 4.0.1b: Nasi Lemak at 25°C for 24 hours (Dilution factor: 10^{-7})

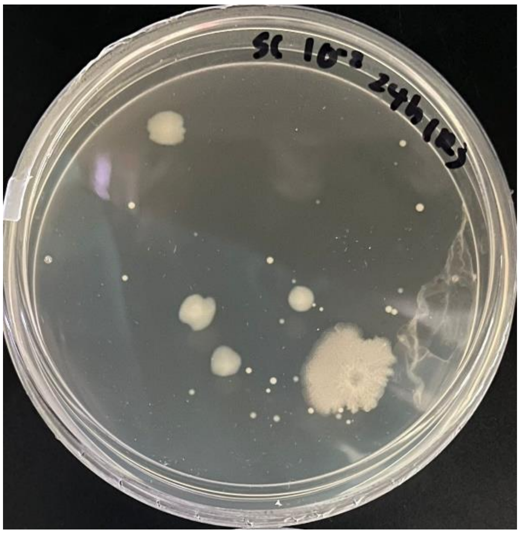

Figure 4.0.1c: Spaghetti Carbonara at 4°C for 24 hours (Dilution factor: 10^{-2})

Figure 4.0.1d: Spaghetti Carbonara at 25°C for 24 hours (Dilution factor: 10^{-7})

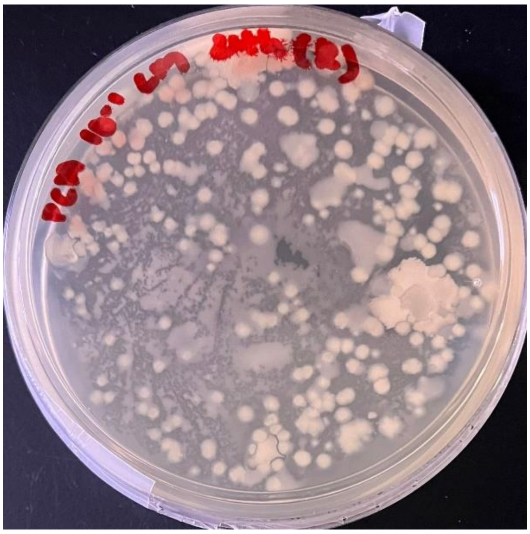

Figure 4.0.1e: Luncheon Meat at 4°C for 24 hours (Dilution factor: 10^{-1})

Figure 4.0.1f: Luncheon Meat at 25°C for 24 hours (Dilution factor: 10^{-1})

4.1.2 Effect of Storage Temperature on Yeasts and Mould Counts of Plant-Based Meat Items

Table 4.2 demonstrates the impact of different storage temperatures on yeast and mould counts in plant-based meat items. After a 7-day incubation period, all tested plant-based meat items, except luncheon meat, displayed noticeable yeast and mould growth under both room temperature (25°C) and refrigerated temperature (4°C). Due to time constraints, the microbiological analyses of yeast and mould counts for refrigerated temperature were not conducted on luncheon meat. Hence, a paired t-test was unable to be performed to assess the significant difference between yeast and mould counts and storage temperature in luncheon meat.

The highest counts of yeast and mould were detected in nasi lemak when stored at room temperature (25°C), registering a substantial $1.29 \pm 0.12 \times 10^{10}$ CFU/g. Apart from that, spaghetti carbonara displayed the highest yeast and mould counts when stored in the refrigerator at 4°C. Overall, both nasi lemak and spaghetti carbonara demonstrated a considerable increase in yeast and mould counts when comparing storage at room temperature (25°C) and refrigerated temperature (4°C). Notably, all plant-based meat items showed statistically significant differences ($p<0.05$) in the growth of yeasts and moulds counts between storage at room temperature (25°C) and refrigerated temperature (4°C).

Table 4.2: Yeast and mould counts of plant-based meat items stored for 24 hours at 4°C and 25°C

Plant-Based Meat Items (n=3)	**Mean Yeast and Mould Counts (log CFU/g)**	
	4°C	**25°C**
Nasi Lemak	$1.10 \pm 0.14 \times 10^{3\ a}$	$1.29 \pm 0.12 \times 10^{10\ b}$
Spaghetti Carbonara	$1.75 \pm 0.07 \times 10^{4\ a}$	$2.41 \pm 0.04 \times 10^{9\ b}$
Luncheon Meat	-	$9.75 \pm 0.35 \times 10^{4}$

Values are means ± standard deviations, n = 3. Mean values followed by different superscript letters represent significant differences in yeast and mould counts for each type of plant-based meat item among two sampling conditions ($p < 0.05$).

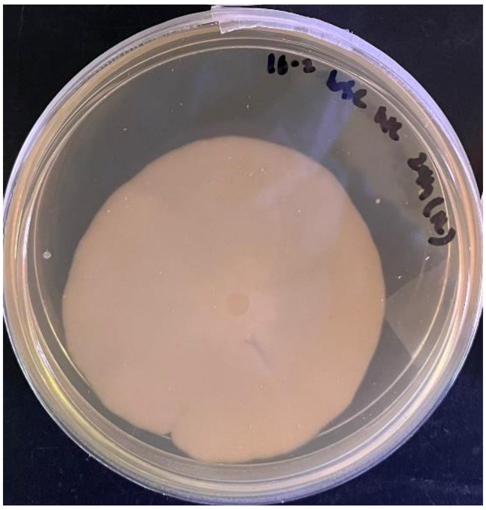

Figure 4.0.2a: Nasi Lemak at 4°C for 24 hours (Dilution factor: 10^{-2})

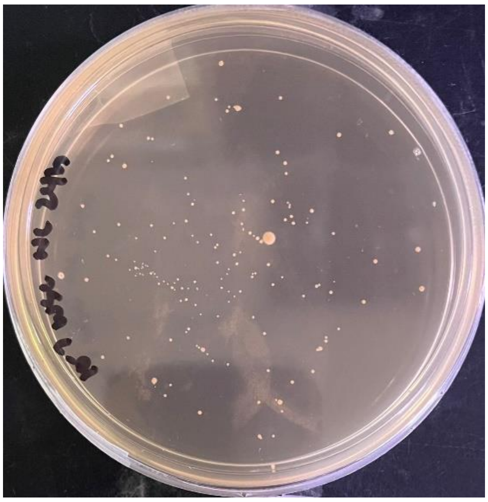

Figure 4.0.2b: Nasi Lemak at 25°C for 24 hours (Dilution factor: 10^{-7})

Figure 4.0.2c: Spaghetti Carbonara at 4°C for 24 hours (Dilution factor: 10^{-2})

Figure 4.0.2d: Spaghetti Carbonara at 25°C for 24 hours (Dilution factor: 10^{-7})

4.1.3 Effect of Storage Duration on Aerobic Colony Counts of Plant-Based Meat Items

Table 4.3 and Table 4.4 have shown the impact of storage duration on aerobic colony counts in plant-based meat items when stored at room temperature (25°C) as well as refrigerated temperature (4°C), respectively. After 24 hours of incubation, all plant-based meat items exhibited aerobic counts when stored at room temperature (25°C) for 2 hours, 4 hours, 6 hours, 8 hours and 24 hours as well as in the refrigerator (4°C) for 24 hours and 48 hours.

Table 4.3 shows the aerobic colony counts of the plant-based meat items stored at room temperature (25°C) for 2 hours, 4 hours, 6 hours, 8 hours and 24 hours. Only luncheon meat exhibited aerobic counts, detectable after just 2 hours of storage at room temperature (25°C). Meanwhile, nasi lemak and spaghetti carbonara were only detected with colonies starting from 6 hours and 4 hours, respectively. As the storage duration increased, nasi lemak demonstrated the most substantial rise in aerobic counts, increasing from $1.23 \pm 0.19 \times 10^4$ CFU/g at 6 hours to $1.18 \pm 0.18 \times 10^{10}$ CFU/g at 24 hours. Notably, nasi lemak also showed the highest aerobic counts after enduring 24 hours of storage at room temperature (25°C). Subsequently, spaghetti carbonara displayed an increment in aerobic counts from $3.40 \pm 0.14 \times 10^4$ CFU/g at 4 hours to $3.17 \pm 1.64 \times 10^9$ CFU/g at 24 hours. However, despite luncheon meat displaying colony growth even after just 2 hours of storage at room temperature (25°C), the aerobic counts did not increase significantly compared to the other two plant-based meat items, even as the storage duration increased. Importantly, a statistically significant difference ($p<0.05$) was observed for all plant-based meat items between aerobic colony counts and storage duration at room temperature (25°C).

The aerobic colony counts of plant-based meat items stored at refrigerated temperatures (4°C) for 24 hours and 48 hours were detailed in Table 4.4. Nasi lemak exhibited the highest aerobic counts for both the 24-hour and 48-hour storage duration in the refrigerator (4°C). It also presented the most substantial increase in aerobic counts among the three plant-based meat items when stored at refrigerated temperature (4°C), ranging from $2.38 \pm 0.69 \times 10^5$ CFU/g at 24 hours to an impressive $1.96 \pm 0.13 \times 10^7$ CFU/g at 48 hours. In contrast, spaghetti carbonara has the lowest aerobic counts after 24 hours of refrigerator storage (4°C), with aerobic counts of $3.00 \pm 0.28 \times 10^4$ CFU/g. However, after 48-hour storage duration, luncheon meat, but not spaghetti carbonara, showed the lowest aerobic counts among the three items, measuring $4.44 \pm 0.06 \times 10^4$ CFU/g. This discrepancy can be attributed to the relatively minor increase in aerobic counts observed in luncheon meat, rising from $3.73 \pm 0.19 \times 10^4$ CFU/g at 24 hours to $4.44 \pm 0.06 \times 10^4$ CFU/g at 48 hours. Although spaghetti carbonara exhibited slightly higher aerobic counts than luncheon meat after 48 hours of refrigerator storage (4°C), the incremental change from 24 hours to 48 hours was not as significant as that observed in nasi lemak. Notably, a significant difference ($p<0.05$) was observed between storage duration and yeast and mould counts when plant-based meat items were stored at refrigerated temperature (4°C), except spaghetti carbonara.

Table 4.3: Aerobic colony counts of plant-based meat items stored for 2 hours, 4 hours, 6 hours, 8 hours and 24 hours at 25°C

Plant-Based Meat Items (n=3)	**Storage Duration (hour)**	**25°C**
		Mean Aerobic Colony Counts (log CFU/g)
Nasi Lemak	2	TFTC
	4	TFTC
	6	1.23 ± 0.19 x $10^{4\,\mathrm{a}}$
	8	1.88 ± 0.06 x $10^{5\,\mathrm{a}}$
	24	1.18 ± 0.18 x $10^{10\,\mathrm{b}}$
Spaghetti Carbonara	2	TFTC
	4	3.40 ± 0.14 x $10^{4\,\mathrm{a}}$
	6	1.08 ± 0.04 x $10^{5\,\mathrm{a}}$
	8	TNTC
	24	3.17 ± 1.64 x $10^{9\,\mathrm{b}}$
Luncheon Meat	2	5.42 ± 0.27 x $10^{4\,\mathrm{a}}$
	4	4.09 ± 0.35 x $10^{4\,\mathrm{b}}$
	6	3.86 ± 0.19 x $10^{4\,\mathrm{bc}}$
	8	3.43 ± 0.03 x $10^{4\,\mathrm{bc}}$
	24	2.92 ± 0.90 x $10^{4\,\mathrm{c}}$

Values are means ± standard deviations, n = 3. Mean values followed by different superscript letters represent significant differences in aerobic colony counts for each type of plant-based meat item among five sampling time points ($p < 0.05$). *TFTC (too few to count) and TNTC (too numerous to count).

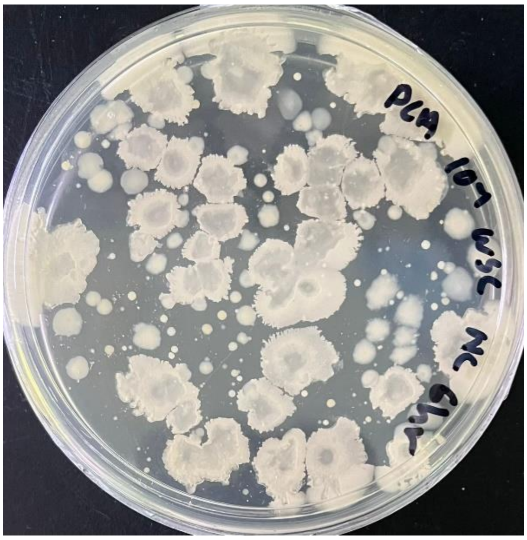

Figure 4.0.3a: Nasi Lemak at 25°C for 6 hours (Dilution factor: 10^{-1})

Figure 4.0.3b: Nasi Lemak at 25°C for 8 hours (Dilution factor: 10^{-2})

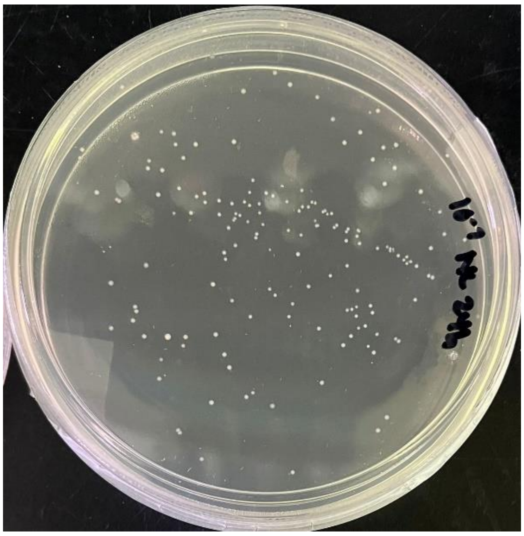

Figure 4.0.3c: Nasi Lemak at 25°C for 24 hours (Dilution factor: 10^{-7})

Figure 4.0.3d: Spaghetti Carbonara at 25°C for 4 hours (Dilution factor: 10^{-2})

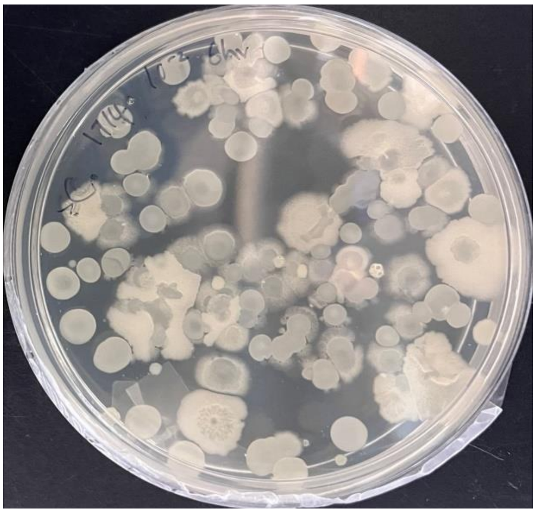

Figure 4.0.3e: Spaghetti Carbonara at 25°C for 6 hours (Dilution factor: 10^{-2})

Figure 4.0.3f: Spaghetti Carbonara at 25°C for 24 hours (Dilution factor: 10^{-7})

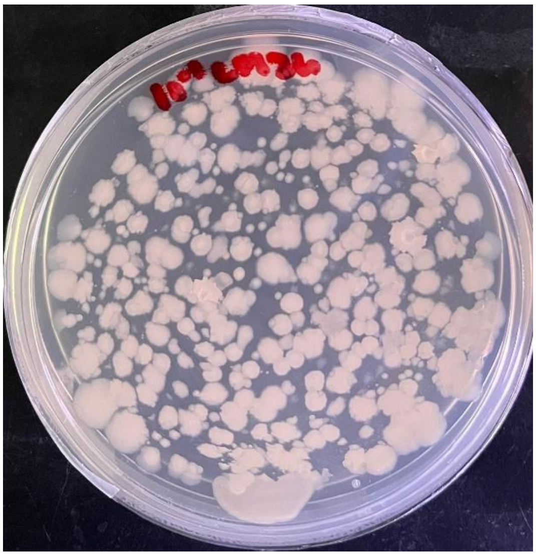

Figure 4.0.3g: Luncheon Meat at 25°C for 2 hours (Dilution factor: 10^{-1})

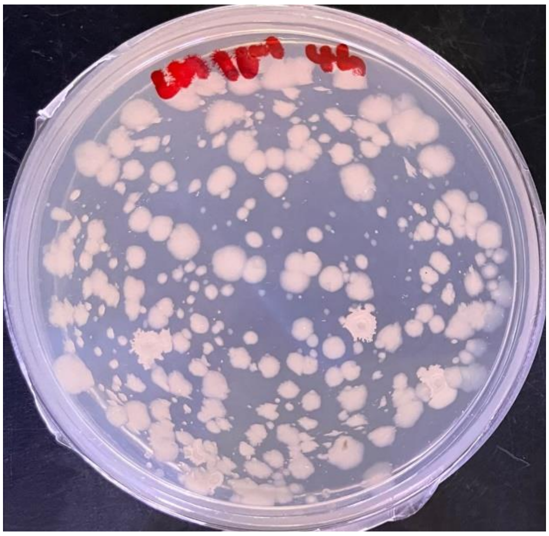

Figure 4.0.3h: Luncheon Meat at 25°C for 4 hours (Dilution factor: 10^{-1})

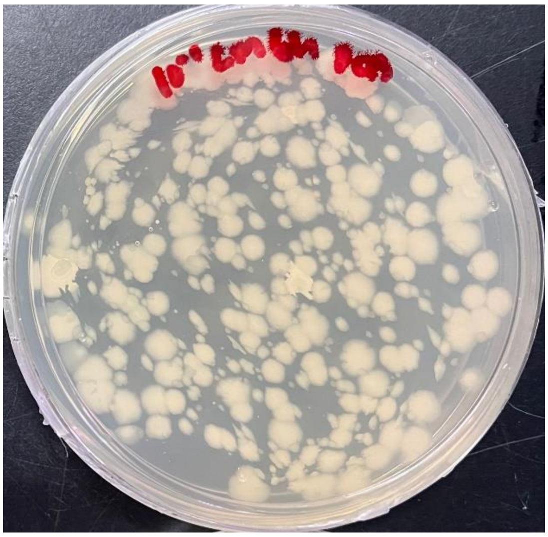

Figure 4.0.3i: Luncheon Meat at 25°C for 6 hours (Dilution factor: 10^{-1})

Figure 4.0.3j: Luncheon Meat at 25°C for 8 hours (Dilution factor: 10^{-1})

Figure 4.0.3k: Luncheon Meat at 25°C for 24 hours (Dilution factor: 10^{-1})

Table 4.4: Aerobic colony counts of plant-based meat items stored for 24 hours and 48 hours at 4°C

Plant-Based Meat Items (n=3)	**Storage Duration (hour)**	**4°C**
		Mean Aerobic Colony Counts (log CFU/g)
Nasi Lemak	24	2.38 ± 0.69 x $10^{5\,a}$
	48	1.96 ± 0.13 x $10^{7\,b}$
Spaghetti Carbonara	24	3.00 ± 0.28 x $10^{4\,a}$
	48	5.00 ± 0.64 x $10^{4\,a}$
Luncheon Meat	24	3.73 ± 0.19 x $10^{4\,a}$
	48	4.44 ± 0.06 x $10^{4\,b}$

Values are means ± standard deviations, n = 3. Mean values followed by different superscript letters represent significant differences in aerobic colony counts for each type of plant-based meat item among two sampling time points ($p < 0.05$).

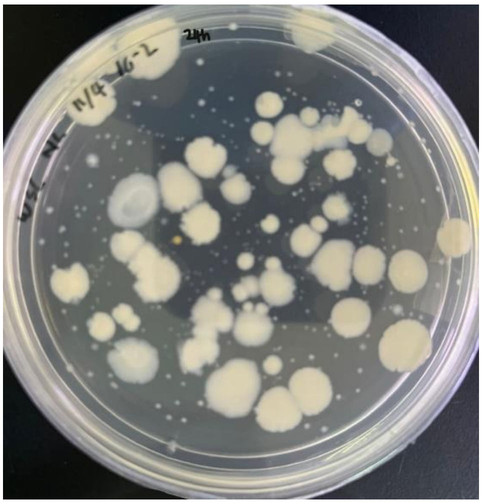

Figure 4.0.3l: Nasi Lemak at 4°C for 24 hours (Dilution factor: 10^{-2})

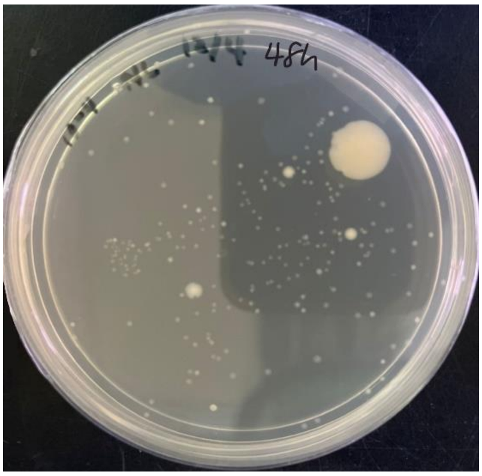

Figure 4.0.3m: Nasi Lemak at 4°C for 48 hours (Dilution factor: 10^{-4})

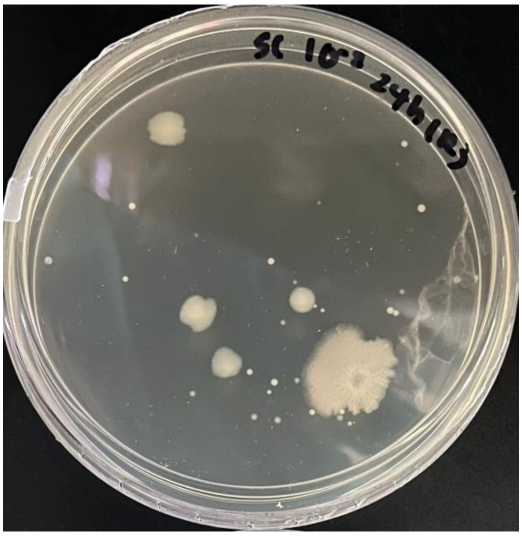

Figure 4.0.3n: Spaghetti Carbonara at 4°C for 24 hours (Dilution factor: 10^{-2})

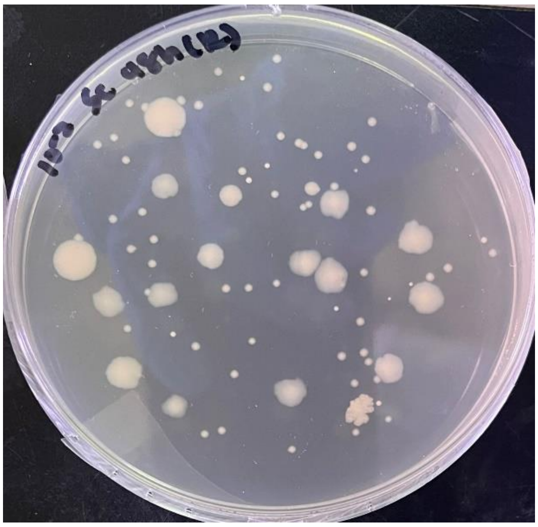

Figure 4.0.3o: Spaghetti Carbonara at 4°C for 48 hours (Dilution factor: 10^{-2})

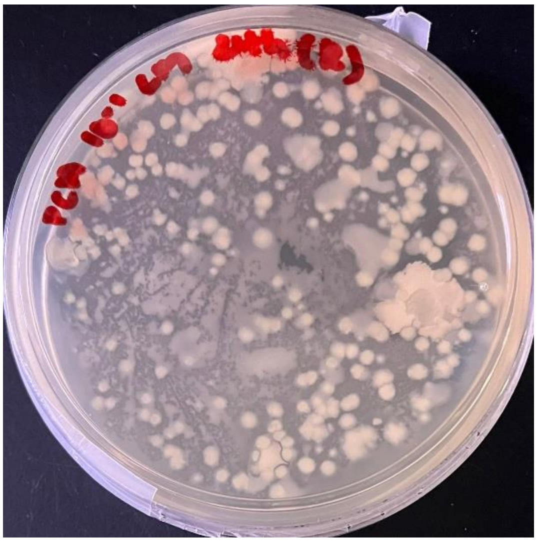

Figure 4.0.3p: Luncheon Meat at 4°C for 24 hours (Dilution factor: 10^{-1})

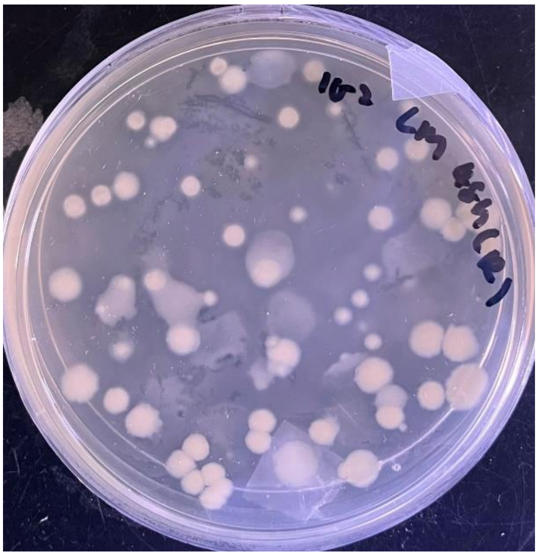

Figure 4.0.3q: Luncheon Meat at 4°C for 48 hours (Dilution factor: 10^{-2})

4.1.4 Effect of Storage Duration on Yeast and Mould Counts of Plant-Based Meat Items

Table 4.5 and Table 4.6 represent the impact of storage duration on yeast and mould development in plant-based meat items. These tables detail the yeast and mould counts under two temperature conditions, which are room (25°C) and refrigerated (4°C). After 7 days of incubation, all plant-based meat items, except for luncheon meat, exhibited yeast and mould counts following storage duration of 2, 4, 6, 8, and 24 hours at room temperature (25°C), as well as 24 and 48 hours at refrigerated temperature (4°C). Due to time constraints, the microbiological analyses of yeast and mould counts for refrigerated temperature (4°C) was not conducted on luncheon meat. As a result, a statistical test was not performed to assess the significant difference between yeast and mould counts and storage temperature in luncheon meat.

Table 4.5 illustrates the yeast and mould counts of plant-based meat items when stored for 2, 4, 6, 8, and 24 hours at room temperature (25°C). All the plant-based meat items showed the presence of yeast and mould counts ranging from 10^3 to 10^{10} CFU/g. Most prominently, nasi lemak showed the highest counts after 24 hours, with an impressive count of $1.34 \pm 0.05 \times 10^{10}$ CFU/g. Another standout was spaghetti carbonara, which observed a notable growth, reaching $2.41 \pm 0.04 \times 10^9$ CFU/g after 24 hours. Conversely, luncheon meat has the smallest growth as the storage duration increased, ranging from $5.98 \pm 0.67 \times 10^3$ CFU/g to $9.75 \pm 0.35 \times 10^4$ CFU/g. Notably, a significant difference ($p<0.05$) between storage duration and yeast and mould counts when stored at room temperature (25°C) was confirmed for all the plant-based meat items.

Table 4.6 details the yeast and mould counts of plant-based meat items after 24 and 48 hours of refrigeration (4°C). Nasi lemak stood out with the highest yeast and mould counts after 48 hours of storage in the refrigerator (4°C). Moreover, it also presented the greatest increase in microbial load as the storage duration increased. Besides, spaghetti carbonara displayed a modest increase from $1.75 \pm 0.07 \times 10^4$ CFU/g at 24 hours to $7.80 \pm 0.14 \times 10^4$ CFU/g at 48 hours. Importantly, a statistically significant difference ($p<0.05$) between storage duration and yeast and mould counts became evident across all samples under refrigerated temperature (4°C).

Table 4.5: Yeast and mould counts of plant-based meat items stored for 2 hours, 4 hours, 6 hours, 8 hours and 24 hours at 25°C

Plant-Based Meat Items (n=3)	**Storage Duration (hour)**	**25°C**
		Mean Yeast and Mould Counts (log CFU/g)
Nasi Lemak	2	$2.38 \pm 0.04 \times 10^{3\ a}$
	4	$7.70 \pm 0.49 \times 10^{3\ a}$
	6	$5.45 \pm 0.07 \times 10^{4\ a}$
	8	$5.60 \pm 1.06 \times 10^{3\ a}$
	24	$1.34 \pm 0.05 \times 10^{10\ b}$
Spaghetti Carbonara	2	$1.75 \pm 0.35 \times 10^{4\ a}$
	4	$3.63 \pm 0.32 \times 10^{4\ a}$
	6	$6.18 \pm 0.46 \times 10^{4\ a}$
	8	$1.68 \pm 0.04 \times 10^{5\ a}$
	24	$2.41 \pm 0.04 \times 10^{9\ b}$
Luncheon Meat	2	$5.98 \pm 0.67 \times 10^{3\ a}$
	4	$1.38 \pm 0.04 \times 10^{3\ ab}$
	6	$8.05 \pm 1.34 \times 10^{4\ c}$
	8	$8.08 \pm 0.11 \times 10^{4\ c}$
	24	$9.75 \pm 0.35 \times 10^{4\ c}$

Values are means ± standard deviations, n = 3. Mean values followed by different superscript letters represent significant differences in yeast and mould counts for each type of plant-based meat item among five sampling time points ($p < 0.05$).

Figure 4.0.4a: Nasi Lemak at 25°C for 2 hours (Dilution factor: 10^{-1})

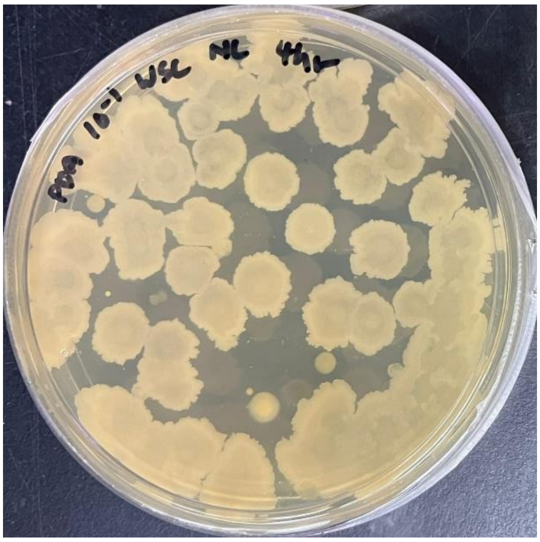

Figure 4.0.4b: Nasi Lemak at 25°C for 4 hours (Dilution factor: 10^{-1})

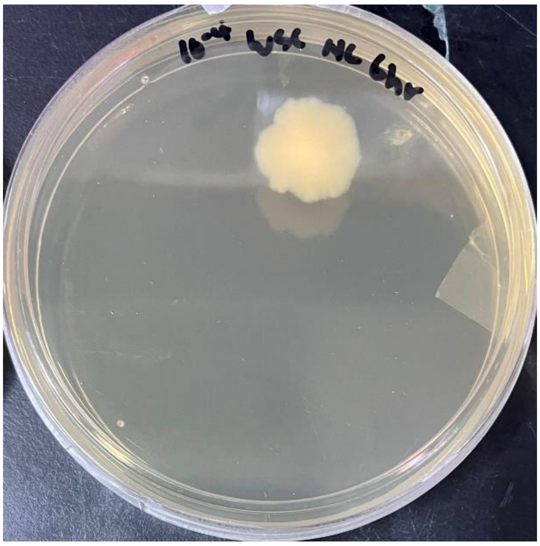

Figure 4.0.4c: Nasi Lemak at 25°C for 6 hours (Dilution factor: 10^{-4})

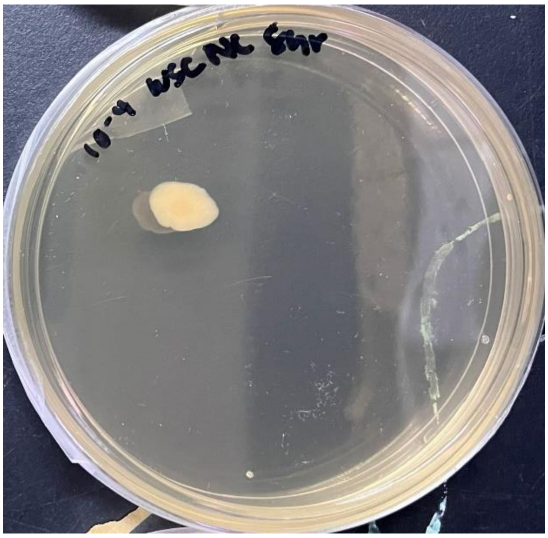

Figure 4.0.4d: Nasi Lemak at 25°C for 8 hours (Dilution factor: 10^{-4})

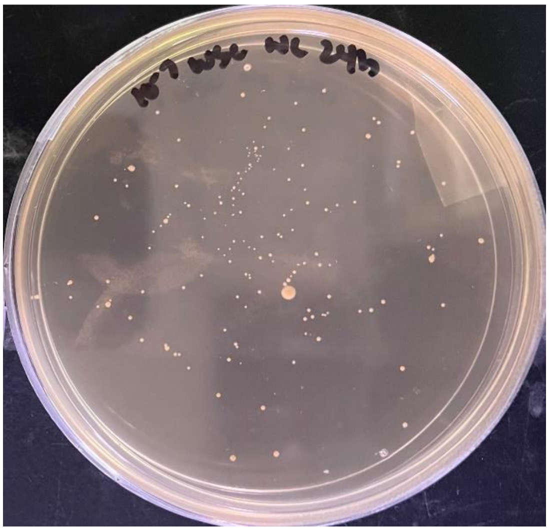

Figure 4.0.4e: Nasi Lemak at 25°C for 24 hours (Dilution factor: 10^{-7})

Figure 4.0.4f: Spaghetti Carbonara at 25°C for 2 hours (Dilution factor: 10^{-2})

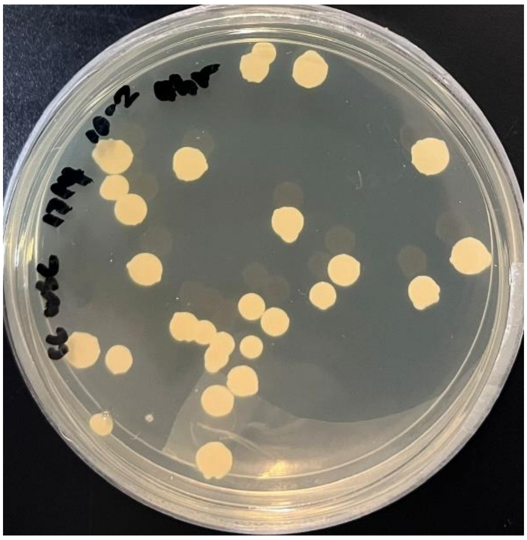

Figure 4.0.4g: Spaghetti Carbonara at 25°C for 4 hours (Dilution factor: 10^{-2})

Figure 4.0.4h: Spaghetti Carbonara at 25°C for 6 hours (Dilution factor: 10^{-3})

Figure 4.0.4i: Spaghetti Carbonara at 25°C for 8 hours (Dilution factor: 10^{-3})

Figure 4.0.4j: Spaghetti Carbonara at 25°C for 24 hours (Dilution factor: 10^{-7})

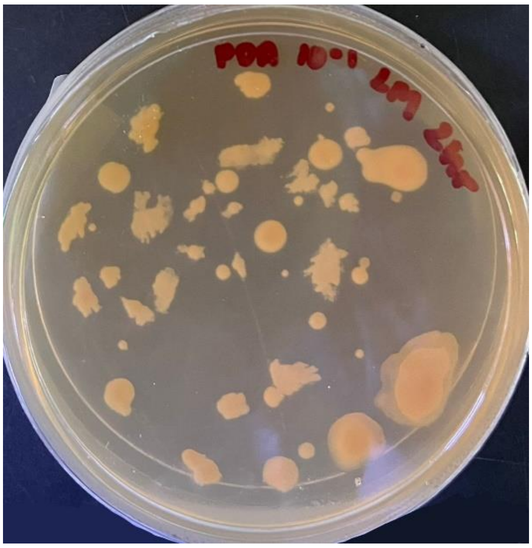

Figure 4.0.4k: Luncheon Meat at 25°C for 2 hours (Dilution factor: 10^{-1})

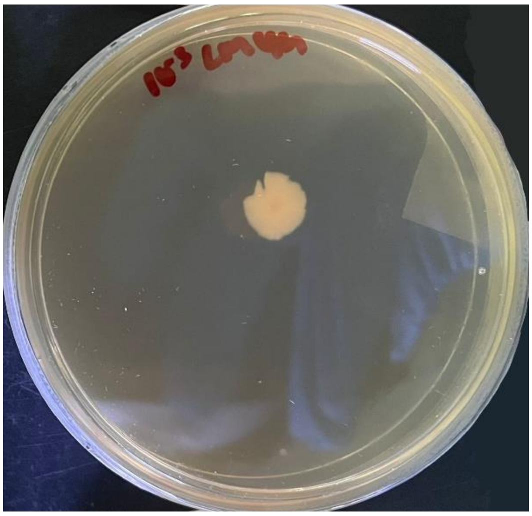

Figure 4.0.4l: Luncheon Meat at 25°C for 4 hours (Dilution factor: 10^{-3})

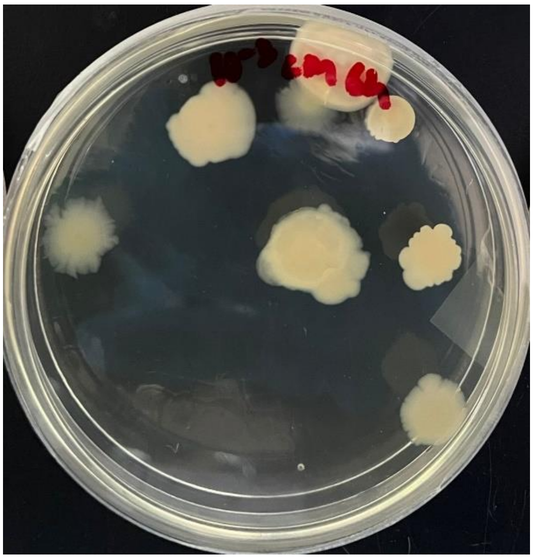

Figure 4.0.4m: Luncheon Meat at 25°C for 6 hours (Dilution factor: 10^{-3})

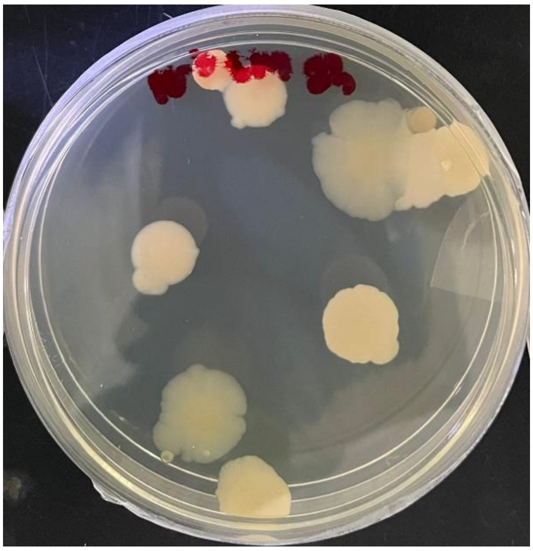

Figure 4.0.4n: Luncheon Meat at 25°C for 8 hours (Dilution factor: 10^{-3})

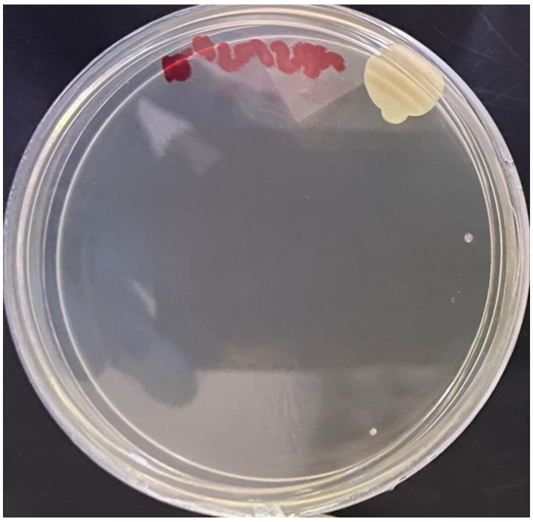

Figure 4.0.4o: Luncheon Meat at 25°C for 24 hours (Dilution factor: 10^{-3})

Table 4.6: Yeasts and moulds counts of plant-based meat items stored for 24 hours and 48 hours at 4°C

Plant-Based Meat Items (n=2)	**Storage Duration (hour)**	**4°C**
		Mean Yeast and Mould Counts (log CFU/g)
Nasi Lemak	24	$1.15 \pm 0.07 \times 10^{3\ a}$
	48	$1.89 \pm 0.12 \times 10^{5\ b}$
Spaghetti Carbonara	24	$1.75 \pm 0.07 \times 10^{4\ a}$
	48	$7.80 \pm 0.14 \times 10^{4\ b}$
Luncheon Meat	24	-
	48	-

*Values are means ± standard deviations, n = 2. Mean values followed by different superscript letters represent significant differences in yeast and mould counts for each type of plant-based meat item among two sampling time points ($p < 0.05$).

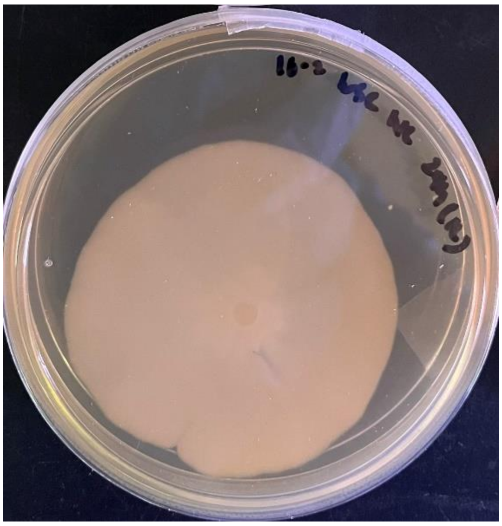

Figure 4.0.4p: Nasi Lemak at 4°C for 24 hours (Dilution factor: 10^{-2})

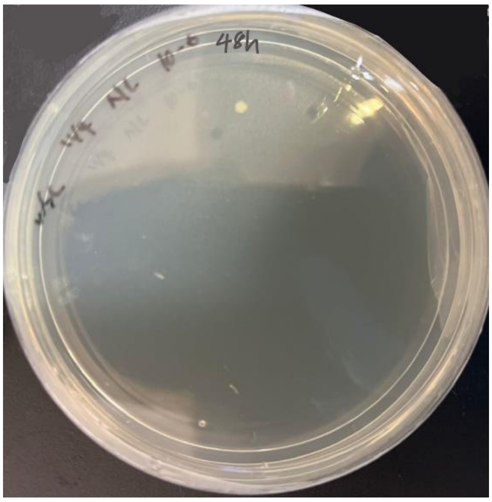

Figure 4.0.4q: Nasi Lemak at 4°C for 48 hours (Dilution factor: 10^{-6})

Figure 4.0.4r: Spaghetti Carbonara at 4°C for 24 hours (Dilution factor: 10^{-2})

Figure 4.0.4s: Spaghetti Carbonara at 4°C for 48 hours (Dilution factor: 10^{-3})

4.2 Attitudes and Perceptions Towards Microbiological Risks of Plant-Based Meat Items among Undergraduate Students

4.2.1 Sociodemographic Characteristics of Respondents

The sociodemographic profile of the 165 respondents is displayed in Table 4.7. In this study, the number of female respondents (n=104) constituted 63% of the total respondents, surpassing the number of male respondents (n=61), who accounted for 37% of the total. With regards to the race of the respondents, the questionnaire was dominated by Chinese respondents with a total of 92.1% (n=152), while a small portion of respondents were Indian (n=12, 7.3%) and Malay (n=1, 0.6%). In terms of the household monthly income classification, more than half of the respondents (n=93, 56.4%) belonged to the M40 group, followed by 35.8% (n=59) of them from the B40 group. Only a minority of them were classified under the T20 group (n=13, 7.9%). Lastly, the primary sources from which the respondents acquired knowledge about safe food preparation were mostly at school (n=121, 73.3%), from the internet (n=101, 61.2%) and family (n=90, 54.45%).

Table 4.7: Sociodemographic profile of respondents (n=165)

Characteristic	**Frequency**	
	n	**%**
Gender		
Male	61	37%
Female	104	63%
Race		
Chinese	152	92.1
Indian	12	7.3
Malay	1	0.6
Household monthly income classification		
B40 (Less than RM 4, 849)	59	35.8
M40 (RM 4, 850 to RM 10, 960)	93	56.4
T20 (More than RM 10, 960)	13	7.9
I learned about safe food preparation ……		
From family	90	54.5
From books and magazine	48	29.1
From the internet	101	61.2
At school	121	73.3
At work	6	3.6

4.2.2 Attitudes and Perceptions of Respondents

The individual score was generated after adding up each of the scores obtained from all twelve questions from Section B and Section C. With a maximum score of 12, the attitudes and perceptions of respondents were categorized into three levels: Negative (< 7), Neutral (7 - 9) and Positive (> 9). Among 165 respondents, a total of 88 respondents (53.3%) had positive attitudes and perceptions whereas 64 respondents (38.6%) had neutral attitudes and perceptions and 13 respondents (7.9%) had negative attitudes and perceptions.

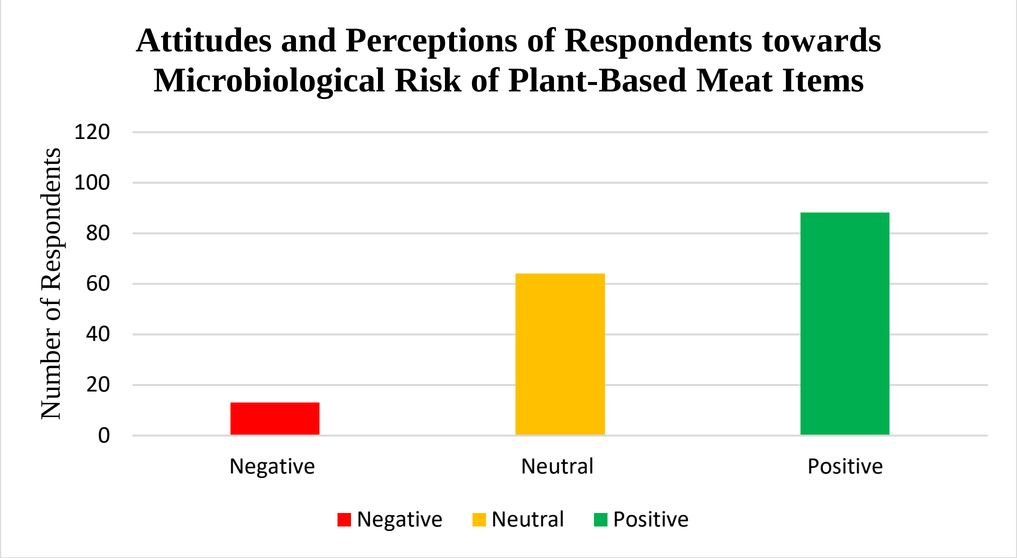

Figure 4.1: Attitudes and perceptions of respondents towards microbiological risks of plant-based meat items (n=165).

According to the data about knowledge, self-reported practices and awareness of controlling the microbiological pathogens in plant-based meat items presented in Table 4.8, a significant majority of the respondents (97%) indicated that they would conduct a freshness check before purchasing any plant-based meat items. Besides, nearly all the respondents (98.2%) acknowledged that improper home raw plant-based meat items preparation poses health risks. In addition, a substantial number of respondents (90.9%) emphasized the importance of ensuring adequate heat treatment for plant-based meat items. When it came to storage practices, most respondents (84.2%) reported that they would store plant-based meat items in the same place in a refrigerator. In terms of defrosting methods, approximately two-thirds (66.1%) of the respondents followed safe defrosting practices, which included defrosting plant-based meat items in a refrigerator (35.2%), under tap water (26.1%) or using a microwave (4.8%). However, a significant portion (33.9%) admitted to defrosting the plant-based meat items in an unsafe manner, which was on the kitchen surfaces at room temperature. Lastly, it was observed that only 32.1% of the respondents would not freeze the previously defrosted plant-based meat items.

Apart from that, the data about food microbiology knowledge among UTAR undergraduate students shown in Table 4.8 demonstrated that majority of the respondents were able to answer the questions correctly. Based on Table 4.8, most of the respondents (82.4%) knew that not all microorganisms are harmful to humans. Besides, 76.4% of the respondents were conscious that not all bacteria are harmful to humans. Other than that, majority of the respondents were well aware that not all yeast and mould are harmless to humans, with 87.9% of total respondents. Moreover, 73.9% of the respondents agreed that plant-based meat items in retail can be contaminated with harmful microorganisms. Next, about two-thirds of the respondents (63%) knew that microorganisms can multiply in the refrigerator and 84.8% of the respondents were aware that cooked, heat-treated plant-based meat items left on the kitchen counter at room temperature can support the growth of microorganisms. The frequency of correct answers for attitudes and perceptions towards microbiological risks of plant-based meat items was outlined in Table 4.8.

Table 4.8: Distribution of correct answers for attitudes and perceptions towards microbiological risks of plant-based meat items.

Questions	Frequency	
	n	**%**
Knowledge, self-reported practices and awareness of controlling the microbiological pathogens in plant-based meat items		
I would check for freshness before purchasing any plant-based meat items.	160	97%
Improper home raw plant-based meat items preparation presents health risks.	162	98.2%
I would pay attention to sufficient heat treatment of plant-based meat items.	150	90.9%
I always store plant-based meat items in the same place in a refrigerator.	139	84.2%
I defrost plant-based meat ……	109	66.1%
Sometimes, I would freeze the previously defrosted plant-based meat items.	53	32.1%
Food microbiology knowledge		
All microorganisms are harmful to humans.	136	82.4%
All bacteria are harmful to humans.	126	76.4%
All yeast and fungi are harmless to humans.	145	87.9%
Plant-based meat items in retail can be contaminated with harmful microorganisms.	122	73.9%
Microorganisms can multiply in a fridge.	104	63%
Microorganisms can multiply in prepared, heat-treated foods that left on a kitchen counter.	140	84.8%

CHAPTER 5

DISCUSSION

5.1 Microbiological Analyses of Plant-Based Meat Items

5.1.1 Effect of Storage Temperature on Microbiological Quality of Plant-Based Meat Items

Based on the data presented in Table 4.1, it is evident that plant-based meat items stored at refrigerated temperature (4°C) exhibit significantly lower aerobic colony counts compared to the storage at room temperature (25°C). This observation aligns with the findings of a study conducted by Liu and colleagues (2023), which found that the aerobic counts of plant-based meat items have increased from 2 or 3 log CFU/g to 6.00 ± 0.30 log CFU/g at storage of room temperature (25°C). Besides, Tóth and colleagues (2021) have revealed that plant-based meat items stored under refrigeration showed contamination levels of $\log_{10}$ CFU/g ranging from 1 to 2. This range was notably lower when contrasted with plant-based meat items stored at room temperature (25°C), where the log10 CFU/g values ranged from 4 to 8. Similar trends were also noted with other ready-to-eat foods in a study conducted by Arshad and colleagues (2019). Besides, Lani and colleagues (2015) also found that after a storage period of 7 days at refrigerated temperature (4°C) and 24 hours at room temperature (25°C), nasi lemak stored in the refrigerator displayed aerobic colony counts similar to those recorded after 24 hours at room temperature (25°C). Notably, when the storage time was standardized at 24 hours, the aerobic counts for nasi lemak stored at room temperature (25°C) were nearly twice as high as those nasi lemak stored under refrigerated conditions.

Based on Table 4.2, after 24 hours of storage, plant-based meat items that were stored at refrigerated temperature (4°C) exhibited lower yeast and mould counts compared to plant-based meat items stored at room temperature (25°C); rather similar to aerobic colony counts. Similar findings were reported by these researchers (Zhou, et al., 2022; Arshad, et al., 2019; Lani, et al., 2015). According to Zhou and colleagues (2022), the growth rate of yeast and mould was reduced when consistently stored at 4°C, resulting in lower yeast and mould counts compared to higher storage temperatures. The lower storage temperature can lead to lower yeast and mould counts as the optimal growth temperature for yeast and mould is typically between 10°C to 35°C (FDA, 2022). Nevertheless, substantial yeast and mould proliferation can occur within the range of 0°C to 30°C or higher, thus contributing to the primary causes of food deterioration in refrigerators (Charpe, et al., 2019).

Several intrinsic and extrinsic factors, such as temperature, water activity and pH, affect the natural defence mechanisms and determine the ability of the microorganisms to proliferate and thrive in a food (ICMSF, 1996). Among these factors, temperature can be considered one of the most critical concerns for controlling the microbiological quality of foods. It is because inappropriate storage of cooked food is a common food safety issue in households (Tóth, et al., 2021). Besides, refrigeration has proven to be one of the most effective practices for controlling microbial growth to preserve food quality (Mercier, et al., 2017). Cooked food stored at room temperature (25°C) for prolonged periods should be considered as a simulation of common household situations (Worsfold, 1997). Hence, it is crucial to avoid storing food within the temperature danger zone, ranging from 5°C and 60°C, which allows the rapid proliferation of bacteria, leading to food spoilage and potential foodborne illnesses (Singh, et al., 2019). Several studies have demonstrated that the optimal growth rate of microorganisms occurs at temperatures ranging from 25 ± 2°C to 30 ± 2°C (Wei, 2020; Wang, et al., 2018; Silva, et al., 2017), which is consistent with the research findings.

Plant-based meat items stored at refrigerated temperature (4°C) did not fall within the optimal temperature range for enzyme activity. Consequently, the items exhibit a lower microbial load due to the reduced enzyme efficiency by decreasing the activity level of the enzyme. As a result, the capability of the enzyme to facilitate cellular biochemical processes was affected (Daniel, et al., 2008). Besides, bacteria encounter difficulties in efficiently absorbing nutrients that are essential for their growth in lower temperature conditions. This challenge arises from the rigidification of cell membranes at lower temperatures, reducing its flexibility and the ability of nutrients to be transported across the cell membrane. Consequently, the uptake of nutrients by bacteria faces impediments (Morita and Moyer, 2001). In addition, the microbial development rates are restricted by sluggish protein synthesis and DNA replication at a lower temperature, extending the lag phase during which microorganisms change their physiology and biochemistry to take advantage of their surroundings (D' Amico et al., 2006). This is supported by Hoel and colleagues (2017), who discovered that aerobic colony counts at refrigerated temperature (4°C) displayed the highest specific rate of growth of 0.06 h^{-1}. In contrast, the maximum specific growth rate showed a notable elevation to 0.37 h^{-1} at room temperature (25°C).

Hence, plant-based meat items stored at refrigerated temperature (4°C) generally possess an extended shelf life compared with those stored at room temperature (25°C), which are often associated with humid environmental conditions (Collin, et al., 1970). This is attributed to the decelerated growth of potentially present bacteria, which necessitates an additional period to attain levels capable of inducing food deterioration or foodborne illness.

5.1.2 Effect of Storage Duration on Microbiological Quality of Plant-Based Meat Items

The aerobic colony counts of plant-based meat items during storage at refrigerated temperature (4°C) and room temperature (25°C) are presented in Table 4.3 and Table 4.4. Most of the plant-based meat items showed an increase in aerobic counts with storage duration from 24 hours to 48 hours for 4°C and 2 hours to 24 hours for 25°C. These findings agreed with those published by Suhaili and colleagues (2021) in grey oyster mushrooms, Tarek and colleagues (2020) in pasteurized coconut milk as well as Lani and colleagues (2015) in nasi lemak. Table 4.5 and Table 4.6 also show the yeast and mould counts at 4°C and 25°C. There was also an increasing trend throughout the storage at both storage temperatures; rather similar to aerobic colony counts. It was found in several studies that increased storage time may have a positive impact on the yeast and mould growth rate (Chandravarnan and Mendis, 2016). However, according to the data presented in Table 4.3, spaghetti carbonara exhibited exceptionally high yeast and mould counts within just 8 hours. This result could be attributed to contamination during the plating process, resulting in a higher microbial load at 8 hours compared to 24 hours of storage time.

The moisture content of plant-based meat items was shown to increase concurrently with their increased time of storage. High moisture content has been proven to enhance the microbial growth of the foods. The study done on several types of traditionally dried marine fish products can provide evidence for this (Paul, et al., 2018). The predominant factor determining the aerobic colony counts in this research was shown to be the relative moisture level of dried products. According to this study, fish with higher moisture content, such as ribbon fish and Bombay duck, had higher aerobic counts than other fish. The relationship between storage duration and moisture content was then discovered by Chandravarnan and Mendis (2016), where it was found that after 4 months of storage, the moisture content of tea samples increased from 4.25% to 4.99%. At the same time, the yeast and mould were also found to be increased from 248 CFU/g to 780 CFU/g. Due to these factors, regulating storage duration is a successful tactic to keep plant-based meat items from spoiling, which greatly raises the risks of contracting foodborne illnesses. Besides, generation duration, also known as doubling duration, is the duration it takes for a colony of bacteria to double through one cycle of binary fission, which is their primary method of reproduction. Bacteria multiply with geometric progression due to binary fission, which doubles their population per generation. In addition, bacteria are noted for their quick reproduction rates. However, the generation duration of bacteria can range from as little as 20 minutes to many hours or even days for slower-growing species. (Kaiser, G., 2023). Therefore, as storage duration increased, more binary fission rounds occurred, which might result in higher microbial load over duration.

However, under room temperature storage conditions (25°C), only luncheon meat demonstrated a declining trend in microbial growth over time. This can be attributed to the abundant presence of antimicrobial preservatives in luncheon meat, such as nitrites and nitrates. These preservatives can impede the proliferation of microorganisms and the production of toxins by interfering with cellular processes, including oxygen and nutrient uptake, formation of nitrous acid and NOs, enzyme functionality as well as oxidative phosphorylation (Weiss, et al., 2010). This interference has the potential to result in the death or suppression of microbial growth (Lin, et al., 2018). Besides, luncheon meat was commonly characterized by a high salt concentration, serving as a preservative to extend its shelf life. The addition of a significant amount of salt generates a hypertonic environment that prompts the removal of water from microorganisms through osmosis, consequently subjecting the microbial cells to osmotic shock (Davidson, Taylor and Schmidt, 2012). The sequence of process subsequently diminishes the water activity within luncheon meat, creating an environment that is less suitable for the thriving of microorganisms (Fennema, 1996). It is plausible that plant-based luncheon meat also contains a significant amount of salt, similar to its counterpart made from meat. This is because the implementation of simple salt reduction in fermented products proves challenging due to the necessity for maintaining lower water activity to manage microbial flora and ensure the dominance of lactic acid bacteria (Ruusunen and Puolanne, 2005). Moreover, fungi and bacteria typically need water activity levels of at least 0.6 and 0.91, respectively (Allen, 2018). Mahmoud, Mona and Bayomey (2016) demonstrated that the blended traditional luncheon meat typically exhibits water activities ranging from 0.47 to 0.5. This indicates that the traditional luncheon meat has been adequately dried to prevent microbial development. To conclude, all of the preservatives present in luncheon meat serve as environmental factors that contribute to the decline in microbial load as storage duration increases.

5.1.3 Overall Microbiological Quality of Plant-Based Meat Items

First and foremost, nasi lemak has been detected with the highest microbial load and the most rapid growth rate among the three plant-based meat items, regardless of the storage temperature. Rice, coconut milk and a variety of spices are commonly involved in the preparation of nasi lemak. This variety of ingredients increases the susceptibility to contamination and foodborne illnesses, particularly if they are handled, prepared or stored improperly. Specifically, rice and coconut milk provide an environment conducive to microbial proliferation because of their moisture content and availability of nutrients. Rice, in particular, plays an important role in the spoilage of nasi lemak. Alongside bacterial contamination, fungal contamination also presents a notable concern for cooked rice (Ayanbimpe, Ogbonna and Abiamugwhe, 2007). The fungal genera such as *Aspergillus* and *Penicillium* can thrive even under refrigeration conditions and produce toxins (Abdel-Nasser, 2022; Ezekiel, et al., 2020). Additionally, coconut milk can exhibit a significant microbial load due to the lack of preservatives that might effectively inhibit microbial growth (Migeemanathan, et al., 2022). In this study, when examining the aerobic counts among coconut milk, pasteurized milk and UHT milk, it was found that coconut milk displayed the highest count at 8.52 ± 0.11 $\log_{10}$ CFU/ml. Subsequently, pasteurised cow's milk demonstrated a lower count at 4.95 ± 0.28 $\log_{10}$ CFU/ml, while UHT milk has not been detected with any microbial growth. In addition to coconut milk, Gilbert and colleagues (1996) also concluded that spices, which are one of the most important ingredients in nasi lemak, may contribute to the spoiling of yeast and mould in nasi lemak.

Furthermore, spaghetti carbonara has been identified as having the second-highest microbial load. The sauce for this classic Italian meal comprised of grated cheese, black pepper and raw egg yolks. Pasta that had just been cooked, drained, and is still boiling hot are all combined with the whisked egg yolks, which are away from the direct heat source. As a result, the sole source of heat for egg yolks is the remaining heat from pasta (Hosking, 2007). The presence of this creamy egg sauce greatly increased the risks of microbial spoilage in plant-based meat items. In contrast to egg whites and whole eggs, which had lower aerobic colony counts, Cwikova and Nedomová (2014) found that egg yolks had the greatest ($p<0.05$) of total aerobic counts, with 2.8 $\log_{10}$ CFU/ml. Besides, the consumption of spaghetti carbonara with the rich egg sauce can enhance the apprehensions regarding its safety due to the potential presence of *Salmonella* in egg yolks (Lopes and Tondo, 2020). The mild cooking necessary to make the silky, creamy sauce for spaghetti carbonara runs the risk of not completely deactivating all of the *Salmonella* that could be present in heavily contaminated yolks. This heat treatment only manages to successfully reduce *Salmonella* spp. by 4.7 $\log_{10}$ CFU/g, leaving approximately 4.0 $\log_{10}$ CFU/g of *Salmonella* that is still alive (Lopes and Tondo, 2020). Therefore, it is particularly crucial to be mindful of the adequate heat treatment and storage temperature of egg yolks when preparing any dishes that include them, extending beyond just spaghetti carbonara. Improper preservation of eggs can result in a marked increase in the presence of naturally occurring yeast and moulds on the surface of eggshell as well as within the egg content (Chousalkar, Khan and McWhorter, 2021; Al-Obaidi, et al., 2011).

Last but not least, luncheon meat exhibits the lowest microbial load and a slower rate of microbial growth. Several factors contribute to luncheon meat's initiation with a lower microbial count compared to other plant-based meat items. One of the factors is the presence of various preservatives that serve as antimicrobial agents within the luncheon meat (Lin, et al., 2018). Additionally, the high concentration of salt in luncheon meat performs a crucial role in microbial growth inhibition by reducing water activity (Davidson, 2001). Moreover, the preparation of luncheon meat commonly involves the use of the high-heat frying method. During this process, the combination of heat and oil penetrates the luncheon meat, releasing moisture from the food into the surrounding oil in the form of steam (Chen et al., 2021; Varela, Bender, and Morton, 1988). As the cooking temperature rises, the water content within the food diminishes (Math et al., 2004). This leads to a decrease in the moisture content of luncheon meat to a certain extent, which subsequently reduces microbial growth (FDA, 2014). This process also concurrently results in a notable increase in the fat content of luncheon meat. Consequently, luncheon meat, which undergoes frying at higher temperatures compared to nasi lemak and spaghetti carbonara, both of which predominantly involve boiling and steaming at lower temperatures, displays the potential for a lower microbial load. Furthermore, the high fat and low water content resulting from frying have been demonstrated to be unfavourable for the proliferation and reproduction of microorganisms (Wang et al., 2021). Consequently, this successfully limits the initial microbial load and ensures a more consistent microbial load during storage. A similar finding was shown when comparing the microbial load of eggs and oiled eggs when stored at room temperature (Eke, Olaitan and Ochefu, 2013). In conclusion, any subsequent increase in luncheon meat might not reach the levels observed in nasi lemak and spaghetti carbonara due to its lower initial microbial count.

5.1.4 Food Safety Level of Plant-Based Meat Items

The determination of safety levels for aerobic colony counts is commonly based on regulations that apply to cooked, ready-to-eat foods. For instance, 10^6 CFU/g was the safety standard established by the Food Standards Australia/New Zealand (2001). The Microbiological Standard for Malaysia Food Law 1983 and Food Regulation 1985 both specify a comparable value. According to Table 4.1, all plant-based meat items stored at a refrigerated temperature of 4ºC for 24 hours remained within the safety limits. However, when stored at ambient temperature (25ºC), except for luncheon meat, all other plant-based meat items exceeded their safety limits within 24 hours. Apart from that, Table 4.3 indicates that spaghetti carbonara exhibited a shorter life, reaching the end of its safe duration within just 8 hours, while nasi lemak ended its shelf life after 24 hours at room temperature. Among the three plant-based meat items analysed, luncheon meat emerged as the safest sample since it was still within the safe shelf life even after 24 hours of storage at room temperature (25°C). Examining Table 4.4, it is evident that all plant-based meat items are within the safety limits after 24 hours of storage at refrigerated temperature (4°C). However, when extending the refrigerated storage to 48 hours, only nasi lemak has reached the unsatisfactory level. In short, refrigerated temperature (4°C) emerged as the safest approach to extend the shelf life of most plant-based meat items, effectively minimizing the risks of food spoilage and foodborne illnesses.

Regarding yeast and mould counts, the threshold for ready-to-eat food is set at 1.0 x 10^5 CFU/g, as established by Curiale (1998). As outlined in Table 4.2, yeast and mould counts for all samples stored for 24 hours at refrigerated temperature (4°C) were within the satisfactory level. However, when stored at room temperature (25°C), all plant-based meat items, except for luncheon meat, have reached the end of satisfactory level within 24 hours. Further insights into yeast and mould growth over time are presented in Table 4.5 and Table 4.6. Based on Table 4.5, it becomes evident that spaghetti carbonara reached an unsatisfactory level of 10^5 CFU/g after 8 hours, while nasi lemak exceeded the satisfactory level after 24 hours of storage. The safest sample, which is luncheon meat, maintained satisfactory levels even after 24 hours at room temperature (25°C). On the other hand, when stored at refrigerated temperature (4°C), all plant-based meat items were found to remain within the safety levels after 24 hours of refrigeration storage. Notably, nasi lemak exceeded the safe level after 48 hours of refrigeration storage (4°C), which distinguishes it from other plant-based meat items. In summary, except for luncheon meat, storage duration of 24 hours appears to be the critical threshold leading to food spoilage, regardless of whether the storage is conducted at refrigerated (4°C) or room temperatures (25°C).

5.2 Attitudes and Perceptions Towards Microbiological Risks of Plant-Based Meat Items among Undergraduate Students

5.2.1 Sociodemographic Characteristics

This study involved 104 respondents, with approximately two-thirds of them being female. The research findings indicated that women exhibited a higher response rate compared to men, which is consistent with earlier studies highlighting that women have having greater inclination to participate in mail- or online-based surveys (Becker and Glauser, 2018). Additionally, multiple studies have demonstrated that women tend to exhibit a greater likelihood to respond frequently to the invitations of online surveys compared to men (Becker, 2022; Becker and Mayer, 2019). Moreover, a significant proportion of Chinese participants were included in this study. In terms of the household income classification, more than 50% of the respondents belonged to the M40 group, followed by the B40 group (35.8%) and T20 group (7.8%). As per the Department of Statistics Malaysia (2020), household income encompasses the combined earnings received by all members of a household. This includes cash income as well as non-cash income that is received regularly during the designated reference period. The findings of this study align closely with the data provided by the Department of Statistics Malaysia (2020), where B40, M40 and T20 represent the bottom 40%, middle 40%, and top 20% of the Malaysian household income, respectively. Also, the study further revealed that the most important sources of knowledge for the respondents were school, the internet and family. The finding was similar to previous research conducted among the students of Ondo State University of Science and Technology, Okitipupa, Nigeria (Oyadeyi, 2014).

5.2.2 Attitudes and Perceptions Towards Microbiological Risks of Plant-Based Meat Items

According to a review of the existing research, there is a lack of studies focusing on the attitudes and perceptions of undergraduate students regarding the microbiological risks associated with plant-based meat products. Consequently, the researcher acknowledges that greater effort needs to be carried out in this area. Therefore, the study offers basic information about the attitudes and perceptions of Malaysian undergraduate students towards the microbiological risks of plant-based meat items.

The researcher conducted an online survey to investigate the attitudes and perceptions of undergraduate students at the Universiti Tunku Abdul Rahman, a not-for-profit private university in Malaysia. Overall, the current study discovered generally positive attitudes and perceptions towards the microbiological risks of plant-based meat items with an average score of 9.4 among 165 respondents. Majority of the respondents (53.3%) were able to provide accurate and correct answers, indicating a reassuring majority awareness. This result was consistent with the prior research that looked at various food products, such as commercially produced traditional fermented cereal beverages and vegetables. Byakika and colleagues (2019) revealed that processors who have relevant knowledge showed a fairly positive perception and attitude toward the microbiology quality of commercially produced traditional fermented cereal beverages, with 63.0 ± 2.3% and 52.2% ± 3.0%, respectively. Similarly, Ivey and colleagues (2012) demonstrated that vegetable producers who have related knowledge have positive attitudes and perceptions.

This can be shown in the producers' level of agreement regarding the importance of management practices ($p \leq 0.0001$) for the prevention of on-farm food contamination varied significantly.

It is an encouraging finding because plant-based meat items can also contribute to the growth of potentially dangerous microorganisms if the respondents do not adhere to proper and safe practices. This is because the harmful microorganisms found in plant-based meat items might come from the basic ingredients. Certain endospore-forming bacteria, including *Clostridium* spp. or *Bacillus* spp., can tolerate the elevated temperature regime even if the majority of these bacteria may be rendered inactive by the heat produced during the extrusion process (Wild, et al., 2014). Despite *Carnobacterium* spp. being identified to be capable of growing in meat products at temperatures as low as 1.5 °C (Jones, 2004), a different study discovered that various bacterial contaminants, primarily *Carnobacterium divergens, Lactobacillus sakei* as well as *Enterococcus faecium*, were detected in plant-based meat items (Geeraerts, et al., 2020). Besides, post-process contamination, such as food processing, cooking and storage, takes an important place in supporting microbial growth in plant-based meat items.

Nevertheless, it is important to highlight that a significant percentage of the respondents expressed either neutral (38.6%) as well as negative (7.9%) attitudes and perceptions concerning the microbiological risks associated with plant-based meat items. This inconsistency becomes evident when reviewing their responses to specific questions, as the correct answer percentage of some questions was below 70%, with some questions dropping even below 40%.

Specifically, Table 4.8 showed a particularly challenging question regarding whether respondents would refreeze previously defrosted plant-based meat items, with only 32.1% answering correctly. This finding starkly contrasts with a previous study by Sterniša, et al. (2018), where 91.9% of participants correctly followed good storage practices for food safety by not refreezing previously defrosted meat items. One significant reason for this contradiction may be attributed to the distinct demographics of participants in the current study, as the previous study focused on Slovenian consumers. Variances in cultural backgrounds, educational levels and food safety experiences likely influenced their attitudes and perceptions of microbiological risks associated with food. It is important to emphasize that a study conducted by Rahman, et al. (2014) discovered that repeated freeze-thaw cycles resulted in a substantial growth ($p<0.05$) in the total number of viable bacteria, coliforms as well as yeast and moulds counts as compared to the initial count. This underscores the microbiological risks associated with refreezing previously defrosted plant-based meat items, despite the general perception of freezing as a safe practice (Sterniša, et al., 2018).

Additionally, the methods that are preferred by the respondents for defrosting plant-based meat items raise concerns. According to Table 4.8, the majority of the respondents are defrosting plant-based meat items in a refrigerator (35.2%), followed by on kitchen surfaces at room temperature (33.9%), under tap water (26.1%) and in a microwave (4.8%). This finding was consistent with earlier research findings, where the refrigerator was the most commonly used method for defrosting (Mazengia, et al., 2015; Ergönül, 2013; Sampers, et al., 2012).

However, it is essential to highlight that the USDA-FSIS (2013) approved only three safe methods for defrosting perishable food, including in the refrigerator, cold water and the microwave, while kitchen counter as well as in hot water thawing is not recommended. Despite these guidelines, a considerable number of respondents (33.9%) still opt for the risky method of defrosting plant-based meat items on the kitchen surface. This is very dangerous as it can allow the temperature of the plant-based meat items to enter into the temperature danger zone, resulting in enhanced microbial growth.

Furthermore, a lack of knowledge about microorganisms can give rise to various challenges in the life of an individual. As indicated in Table 4.8, a substantial 63% of the participants agreed that microorganisms can multiply in fridge. This finding aligns with previous research, which has suggested that individuals with higher levels of education tend to possess a better understanding of microorganisms and their behaviours (Špernjak, Puhmeister and Šorgo, 2021). However, it is crucial to acknowledge that a notable portion of participants in the current study provided incorrect answer or lacked the necessary knowledge, with 28.5% and 8.5%, respectively. This highlights the existence of significant gaps in public awareness and understanding of microorganisms, particularly their growth and behaviour in common settings like refrigerator. This is particularly concerning given the confirmed presence of microorganisms in refrigerators, as demonstrated by the study conducted by Otu-Bassey, et al. (2017). In their research, all samples stored in the refrigerator exhibited bacterial contamination, along with 32% of samples also showing fungal contamination, and 8% of samples consisted of parasitic microorganisms.

Therefore, it is necessary to emphasize the need for enhanced education and awareness campaigns to prevent significant numbers of the population from continuing to engage in risky behaviours. These efforts aim to dispel misconceptions and promote safer practices among the general population. Even a small percentage of negative attitudes and perceptions towards the microbiological risks of plant-based meat items can have a major impact as it may inadvertently encourage people to adopt improper handling and preparation practices. Consequently, it can elevate the risks of microbiological contamination and related illnesses.

5.3 Significance of Results

The present study holds primary significance in identifying the optimal storage temperatures and durations that effectively minimize the proliferation and reproduction of harmful microorganisms, thereby reducing the susceptibility to foodborne illnesses. This study makes a substantial contribution to the understanding of how storage temperature and time impact microbial growth by comparing the changes in microbial load across 24 hours of room temperature storage and 48 hours of refrigerated storage. This, in turn, enhances scholarly discussions and paves the way for future investigations in the area of food microbiology. Furthermore, the study's findings possess the potential for public dissemination through educational campaigns, enhancing awareness regarding safe food storage practices. Consequently, this collective awareness stands to elevate overall food safety standards within households. The comprehensive analyses of microbial load changes across varied storage temperatures and durations form a pivotal cornerstone of this research, thereby substantiating its reliability and validity. These resultant insights can be used in determining the optimal storage parameters for plant-based meat items, ensuring the preservation of their quality and safety.

Apart from that, this study delves into the attitudes and perceptions of undergraduate students regarding the microbiological risks associated with plant-based meat items. The present study boasts a strong level of reliability and is devoid of measurement errors, attributed to the utilization of a validated questionnaire for evaluating attitudes and perceptions towards microbiological risks of plant-based meat items among UTAR students in the Kampar campus. Consequently, the acquired data bears the potential to enrich the currently limited literature concerning university students' attitudes and perceptions towards the microbiological risks of plant-based meat items. Additionally, this contribution extends to public health, as the present study identifies conceivable gaps in knowledge that could culminate in inappropriate food handling practices. Overall, the present study transcends disciplines, merging microbiology, consumer behaviour and public health perspectives to shape the future of plant-based meat consumption and fortify its safety measures.

5.4 Limitations of Study

There are several limitations in this research. First, the utilization of restaurant's own packaging for freshly cooked plant-based meat items brings about the potential for cross-contamination if the packaging is not properly sanitized. Moreover, the use of a convenience sampling method raises the likelihood of bias in selection. Also, due to its non-random character, the final sample could not correctly reflect the complete target population. It is important to note that the sample displayed a lack of diversity, being predominantly comprised of Chinese female respondents. The findings therefore restrict the application of the study's findings to a larger Malaysian population that includes people from various ethnic backgrounds.

5.5 Recommendations for Future Studies

Future research recommendations were made in several ways. First, it is advised that additional plant-based meat items be the subject of future research that investigates the impact of storage temperature and duration. It is also advised to identify the microorganisms that are present in plant-based meat items. Moreover, the use of sterile packagings, such as sterile plastic containers, during collection is crucial to prevent contamination. Last but not least, it is advisable to choose a random sampling method rather than a convenience sampling method to avoid selection bias. By using the random sampling method, the participants from the selected group are chosen at random, facilitating more accurate generalizations of the target population.

CHAPTER 6

CONCLUSION

In conclusion, the present study has analysed the effect of different storage temperatures (refrigeration temperature, 4°C; room temperature, 25°C) and varying storage durations on the microbiological quality and safety of plant-based meat items. The findings clearly demonstrate that the differences in storage temperature and time have a major impact on the microbial growth observed in plant-based meat items. Specifically, maintaining lower temperatures and shorter storage time contributed to better control over microbial growth compared to instances with higher temperatures and longer storage durations. The findings of this study underscore the critical importance of using appropriate storage conditions to ensure the safety and quality of plant-based meat items. In order to properly maintain the integrity of plant-based meat items and avoid the emergence of foodborne illnesses, this safety alert might be useful for the general public and food operators to follow proper handling and storage practices to effectively preserve the microbiological quality of plant-based meat items as well as prevent the occurrence of foodborne illnesses.

The present study also shows that UTAR undergraduate students at the Kampar campus generally hold a positive attitude and perception towards the microbiological risks of plant-based meat items. However, a notable portion of participants exhibit neutral or even negative attitudes and perceptions on this matter. As a result, targeted interventions are warranted to address and rectify these unfavourable attitudes and perceptions regarding the microbiological risks of plant-based meat items, both among college students and the public. Consequently, it can improve the awareness and management of microbiological pathogens in plant-based meat items. In light of these findings, it becomes imperative to delve deeper into the factors influencing these attitudes and perceptions, in order to attain a more nuanced understanding in this area.

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APPENDICES

Appendix A

Ethical Approval

UNIVERSITI TUNKU ABDUL RAHMAN DU012(A)
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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 2. Elisa Bong Tsyr Yin 3. Oo Xing Joe	Dr Chang Sui Kiat 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 2. Siew Fei Kie 3. Wong Siew Ching	Dr Chang Sui Kiat 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
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
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Appendix B

Questionnaire

Section 1: Sociodemographic

1. Gender
   - ☐ Women
   - ☐ Men

2. Ethnicity
   - ☐ Malay
   - ☐ Indian
   - ☐ Chinese
   - ☐ Other

3. Household income
   - ☐ B40 (Less than RM 4, 849)
   - ☐ M40 (RM 4,850 to RM 10, 959)
   - ☐ T20 (More than RM 10, 960)

4. I learned about safe food preparation …….
   - ☐ from family
   - ☐ at school
   - ☐ from books, magazine
   - ☐ from the internet
   - ☐ at work

Section 2: Knowledge, self-reported practices and awareness on controlling the microbiological pathogens in PBM Items

1. I would check for freshness before purchasing any plant-based meat items.
   - ☐ Yes
   - ☐ No

2. Improper home raw plant-based meat items preparation presents a health risk.
   - ☐ Yes
   - ☐ No

3. I pay attention to sufficient heat treatment of plant-based meat items.
   - ☐ Yes
   - ☐ No

4. I always store plant-based meat items in the same place in a refrigerator.
   - ☐ Yes
   - ☐ No

5. I defrost plant-based meat ……
   - ☐ on the kitchen surfaces at room temperature
   - ☐ in a refrigerator
   - ☐ under tap water
   - ☐ in a microwave

6. Sometimes, I would freeze the previously defrosted plant-based meat items.
   - ☐ Yes
   - ☐ No

Section 3: Food Microbiology Knowledge

1. All microorganisms are harmful to humans.
   - ☐ Yes
   - ☐ No
   - ☐ I don't know

2. All bacteria are harmful to humans.
   - ☐ Yes
   - ☐ No
   - ☐ I don't know

3. All yeast fungi are harmless to humans.
   - ☐ Yes
   - ☐ No
   - ☐ I don't know

4. Plant-based meat items in retail can be contaminated with harmful microorganisms.
   - ☐ Yes
   - ☐ No
   - ☐ I don't know

5. Microorganisms can multiply in a fridge.
   - ☐ Yes
   - ☐ No
   - ☐ I don't know

6. Microorganisms can multiply in prepared, heat-treated foods that left on a kitchen counter.
   - ☐ Yes
   - ☐ No
   - ☐ I don't know

Appendix C

FM-IAD-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)	Wong Siew Ching
ID Number(s)	19ADB02999
Programme / Course	Bachelor of Science (Hons) Dietetics
Title of Final Year Project	Microbiological analyses and undergraduate student's 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: 9 **%** **Similarity by source** Internet Sources: 6 % Publications: 4 % Student Papers: 3 %	
Number of individual sources listed of more than 3% similarity: **0**	

Parameters of originality required and limits approved by UTAR are as follows:

(i) Overall similarity index is 20% and below, and
(ii) Matching of individual sources listed must be less than 3% each, and
(iii) Matching texts in continuous block must not exceed 8 words

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: 16/10/2023

Signature of Co-Supervisor
Name: Dr. Lam Ming Quan
Date: 16/10/2023

Appendix D

Summary page of the Turnitin Originally Report

Turnitin Originality Report

Processed on: 07-Sep-2023 15:18 +08
ID: 2159703652
Word Count: 16428
Submitted: 1

Microbiological analyses of plant based foods
1 By Siew Ching Wong

Similarity Index	Similarity by Source	
9%	Internet Sources:	6%
	Publications:	4%
	Student Papers:	3%

1% match (student papers from 01-Sep-2022)
Submitted to Universiti Tunku Abdul Rahman on 2022-09-01

1% match (Internet from 27-Oct-2019)
https://link.springer.com/article/10.1007%2Fs11033-018-4370-x

1% match (Internet from 03-Nov-2022)
https://moam.info/food-safety-knowledge-self-reported-practices-and_5c3a87fb097c47785b8b458b.html

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

< 1% match (Internet from 06-Apr-2023)
https://link.springer.com/article/10.1007/s10876-022-02265-y?code=4b20c4fc-b770-4a3a-babe-5e02d236deda&error=cookies_not_supported

< 1% match ()
Suhaili M.,, Nor-Khaizura M.A.R.,, Nur Hanani Z.A.,, M.R. Ismail-Fitry,, N.I.P. Samsudin,, N.N. Jambari,. "Assessment of microbiological safety and physicochemical changes of grey oyster mushroom (Pleurotus sajor-caju) during storage at 4 °C and 25 °C", 'Penerbit Universiti Kebangsaan Malaysia (UKM Press)', 2021

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Fatma Sag˘ırlı. "Chemical and microbial stability of high moisture dried apricots during storage", Journal of the Science of Food and Agriculture, 01/17/2008

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Submitted to MAHSA University on 2021-12-03

< 1% match (student papers from 18-Jul-2023)
Submitted to University of Wales Institute, Cardiff on 2023-07-18

< 1% match ("Supplement A, July 2019", Journal of Food Protection, 2019)
"Supplement A, July 2019", Journal of Food Protection, 2019

< 1% match (Internet from 07-Jul-2023)
https://WWW.MDPI.COM/2073-445X/9/1/13

< 1% match (Internet from 28-Nov-2022)
https://www.mdpi.com/1999-4923/14/12/2585/html

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Andreja Špernjak, Anja Jug Puhmeister, Andrej Šorgo. "Public opinions and knowledge about microorganisms", Research in Science & Technological Education, 2021

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