Bacterial Assessments on Vegetables and 'Ulam' Sold in Jeli Wet Market, Kelantan

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DECLARATION

I declare that the work in this dissertation entitled **Bacterial Assessments on Vegetables and 'Ulam' Sold in Jeli Wet Market, Kelantan** has been composed solely by myself and that it has not been submitted, in any previous application for degree. Except the excerpts and summaries, each of which I have stated the source.

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Bacterial Assessment on Vegetables and 'Ulam' Sold in Jeli Wet Market, Kelantan

ABSTRACT

Jeli wet market is a famous wet market among local where people can find fresh vegetables and poultry for daily consumption. Vegetables and 'ulam' are important to supply high vitamins for human's health. 'Ulam' is Malaysian salad that are eaten raw or slightly processed. However, the consumption of contaminated fresh produce may result to foodborne illness. The impact of this disease associated with foodborne outbreaks that happened with symptoms such as diarrhoea. The common bacteria that able to cause this problem are *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi*. All of these bacteria can be found typically in food included in raw vegetables and 'ulam' which obtained either from the farm, transport or in the wet market. In this study, the presence of bacteria in vegetables and 'ulam' sold in Jeli wet market were evaluated and identified. Some of the fresh produce are not safe to consume which are cassava, Chinese okra, 'pucuk ubi kayu' and 'pucuk kaduk' while the rest are safe to consume which mean the level of CFU/ml are lower than 10^6 CFU/ml which as recommended by World Health Organisation (WHO). The presence of all the bacteria in the fresh produce were confirmed after done selective media, gram staining, catalase test and TSI agar test.

Keyword: vegetables, bacteria, wet market

Penilaian Bakteria ke atas Sayur-Sayuran dan Ulam-Ulaman yang Dijual di Pasar Basah Jeli, Kelantan

ABSTRAK

Pasar basah Jeli merupakan pasar yang terkenal di kalangan penduduk tempatan di mana mereka boleh mendapatkan sayur dan daging segar seperti ayam sebagai makanan harian. Sayur dan ulam adalah penting untuk membekalkan vitamin yang tinggi kepada manusia. Ulam merupakan salad rakyat Malaysia yang dimakan mentah atau diproses sedikit. Walaubagaimanapun, pengambilan hasil segar yang tercemar akan menyebabkan penyakit bawaan makanan. Impak daripada penyakit ini mempunyai kaitan dengan wabak bawaan makanan yang berlaku dengan gejala contohnya cirit-birit. Bakteria yang biasanya mampu menyebabkan masalah ini adalah *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi*. Kesemua bakteria ini boleh didapati kebiasaannya di dalam makanan termasuklah sayur dan ulam mentah sama ada dari ladang, semasa pengangkutan atau di pasar basah. Dalam kajian ini, kehadiran bakteria di dalam sayur dan ulam yang dijual di pasar basah Jeli telah dinilai dan dikenal pasti. Beberapa daripada hasil segar tersebut adalah tidak selamat untuk dimakan mentah iaitu ubi kayu, petola, pucuk ubi kayu dan pucuk kaduk manakala yang lain selamat dimakan, ini bermakna paras CFU/mL lebih rendah dari 10^6 CFU/ml seperti yang dicadangkan oleh World Health Organisation (WHO). Kehadiran semua bakteria di dalam sayur dan ulam tersebut telah dipastikan selepas media terpilih, pewarnaan gram, ujian katalase dan ujian agar TSI senget dijalankan.

Kata kunci: sayur, bakteria, pasar

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Symbol		Page
>	More than	3
˚C	Degree celcius	16
g	Gram	23
mL	Millilitre	23
atm	Atmospheric pressure	24
µL	Microliter	27

LIST OF ABBREVIATIONS

		Page
CFU/mL	Colony-forming unit per milligram	3
NCDs	Non-communicable disease	10
WHO	World Health Organization	10
MOH	Malaysia Ministry of Health	13
$2H_2O_2$	Hydrogen peroxide	20
H_2S	Hydrogen sulphide	20
TSI	Triple sugar iron	20
XLD	Xylose Lysine Deoxychocolate	24
APC	Aerobic plate count	27
TNTC	Too numerous to count	27
TPC	Total plate count	27

CHAPTER 1

INTRODUCTION

1.1 Research Background

This research is focusing on the vegetables and 'ulam' that available in Jeli wet market, Kelantan. Vegetables and 'ulam' referred as any type of plant that various parts of them from rhizhomes to the root can be eaten raw as salad or cooked in dishes. 'Ulam' is also known as traditional vegetable which has been important among multicultural cultures in Malaysia community and well known to improve health beside famous in beauty purpose such as anti-aging since a long time ago. There are more than 100 plants species come from various families were consumed as 'ulam' recorded by previous study (Awang et al., 2020).

Thus, the selling of this fresh produce in market have been one of source of income for the small-scale farmers in Malaysia since it is profitable. Grow vegetables at

the backyard have been a healthy habit among Malaysian and supply the demand need for the society at the same time. In Kelantan, sellers in the fresh market usually plant their own fresh produce. Small scale farmers usually use traditional planting method to their plants and their farm which located nearby their houses, that will ease them to take care of the plants such as to water the crop every day. They also need to decide types of fertilizer and pesticide to be used during planting. Other than that, these sellers also use traditional packaging method, they often use newspaper and rubber band to pack leafy vegetables. The quality of the vegetables cannot be improved after harvest. Proper packaging is needed to protect the vegetables from damages, to contain them and make sure they last longer.

1.2 Problem Statement

Every day, everyone must eat five to nine servings of vegetables and fruits as recommended by scientists in order to maintain good health. The increased choices and availability of fresh produce on the fresh market tables will assist us as consumer to achieve the consumption target. However, many contaminated ready-to-eat vegetables being consumed without undergo any treatment to destroy the pathogenic microorganisms. In recent years, the risk for food-handling errors has increased due to the increase of consumer demand for fresh “organically” and “natural” in cultivated produce. Even without any discernible loss of the products quality, contamination may still occur.

Microbes can be everywhere including in the air. They contribute to larger percentage of nonpathogenic such as epiphyticmicroflora and few pathogenic normal flora species. Thus, human activities from farm to fork included all stages of handling the vegetables cannot be separated completely from microbes' existence. Contaminated soil, raw manure, dirty water source and unhygienic farm tools are the possible causes of pathogen contamination at the farm.

A higher risk of contamination of pathogenic microbes may occur when a bigger production of vegetables within shortest possible time to fulfil the increasing demand once making the uncertainties of consumer safety. There are few ways that proven effective to control microbes' contamination in vegetables and 'ulam' that we bought from wet market. These include surveillance system establishment to observe the chain of production and clean the vegetables by washing them with water and vinegar or salt. Other washing techniques also may be effective but make sure one cycle of water is used to wash all the vegetables.

1.3 Hypothesis

H_o: The number of bacterial count on vegetables and 'ulam' sold in Jeli wet market, Kelantan is lower than the recommended maximum limit, 10^6 CFU/ml.

H_1: The number of bacterial count on vegetables and 'ulam' sold in Jeli wet market, Kelantan is higher than the recommended maximum limit, $>10^6$ CFU/ml.

1.4 Objectives

1. To determine the bacterial count on vegetables and 'ulam' sold in Jeli wet market.
2. To identify types of bacteria isolated from vegetables and 'ulam' sold in Jeli wet market.

1.5 Scope of Study

The focus of this study is to observe the bacterial count in the vegetables and 'ulam' whether consuming the fresh produce is safe or not. This research also important to identify types of bacteria in the vegetables and 'ulam' sold in Jeli wet market so that we can predict the source of the contamination and effect of consuming those veggies. Other than that, this research is made to find out the importance of clean vegetables to our health. Lastly, to avoid and reduce the occurrence of foodborne disease. Contaminated raw materials and cross contamination handling are the main factor which contribute to food poisoning.

1.6 Significant of Study

The significance of this research is to assess the quality of hygienic and the most common bacteria's prevalence in the vegetables and 'ulam' sold in Jeli wet market, Kelantan. The fresh market hygiene is influenced by the awareness of planters and sellers themselves. This is one of the factor of the absence of bacteria in the vegetables they sell once made food safety an important issue at fresh market. We as customers and consumers are expecting the vegetables we purchase to be grown and handled with clean. Sellers who are aware of vegetables' hygiene practices must be able to handle the food safety and sanitary practices successfully. It is to avoid potential threat of foodborne diseases and related issue if we consume the vegetables especially raw.

It is crucial to know the effects of biological hazards including microbes, bacteria and mold in the vegetables that we consumed towards our body. Toxins will be released when germs which causes foodborne diseases enter our body system that will cause diarrhea, vomiting are the common symptoms of foodborne illness that usually last for one to seven days.

CHAPTER 2

LITERATURE REVIEW

2.1 Site Sampling of Vegetables and 'Ulam'

Kelantan is located in the north-east coast of Malaysia which has adverse climate during November, December and January. Various types of crops for example paddy, palm oil, varieties of fresh produce included fruits, vegetables and 'ulam' can be found in Kelantan because it has fertile alluvial plain. Jeli district is the backbone of Peninsular Malaysia which located in the foot of the Main Range (Nazaruddin and D.Adriansyah, 2017).

In Jeli, Kelantan, the famous wet market is Pasar Besar Jeli. The sellers at the fresh market usually sell produce of village farmers making it the focus of the residents around Jeli to get vegetables or traditional food of Kelantan community. The demand for supplies vegetables has grown asynchronies with growing population of the district. The rural area farmers rarely have access to expend their produce to bigger market, thus they

usually sell to wet market. Due to poor storage facilities, they need to sell the vegetables even at peak times when the prices are low (Shariff et. al., 2019).

2.2 Types of Fresh Produce

The lively environment of Jeli wet market is because of the crowd of visitors who come to buy local produce as their ingredients for cooking or readymade food that available there. The sellers of the traditional wet market have prepared varieties of unique vegetables and 'ulam' which usually subjected as ready-to-eat vegetables, they were consumed either by minimal or no process at all. Some of the fresh produce commonly available in the market are root and tubers, leafy vegetables, fruit vegetables, stems, brassica and varieties of 'ulam'.

2.2.1 Vegetables

The vegetables used in this experiment are cassava (*Manihot esculenta Crantz*) which mainly cultivated by small farmers with limited resources for their starchy roots, they are used as human food either in fresh or processed form such as flour and pasta. Cassava is a tuber crop that can adapt in stressful environments and requires minimal care

(Polthanee and Anan, 2018). Secondly is root vegetables, the top-ten most economically important food crop in the world which is carrot (*Daucus carota subsp*. *Sativus*), our daily vitamin A can be supplied by consuming one medium-sized carrot (Char and Cielo D, 2018). The third vegetable is a famous fruit vegetable among local because it is low in calories and fat, chinese okra (*Luffa acutangula*), it is less bitter cultivars during immature and usually cooked as soup or fried (Al-Snafi and Ali Esmawi, 2019). Fourth, tomato (*Solanum lypersicum*) which is the second most important fruit vegetables, contains a lot of health-promoting compound and commonly consumed fresh. The last vegetable is cabbage (*Brassica oleraceae*), a leafy vegetable commonly used as dishes either cooked or raw. Cabbage rich in protein and phytochemical content (Pavlovic et. al., 2019).

2.2.2 'Ulam'

The 'ulam' used as samples in this research are callerya atropurpurea (*Milletia atropurpurea*), its common name is 'pucuk jenereh'. Pucuk jenereh is commonly found in Sothern part of Thailand (Bhat et al., 2020), the leaves contain high possess anti-lipid peroxidation and protein level which usually eaten with chilli paste. Secondly, king's salad (*Cosmos caudatus*) popuarly known as 'ulam raja'in Malaysia. Locals like to consume this 'ulam' because it acts as anti-aging agent, body heat reducer, strengthening bone marrow and improve blood circulation (You et. al., 2021). Thirdly, tapioca shoot (*Manihot Esculenta*) or 'pucuk ubi kayu', beside consumed raw, it is one of herbal

medicine to relieve fever, headache and diarrhoea. The fourth 'ulam' is wild pepper (*Piper sarmentosum*) with local name 'pucuk kaduk' which commonly used treat cough and asthma, flu and headache, it is also act as antimalarial and antiseptic (Feng et. al., 2019). The final 'ulam' in this research is gotu kola (*Centella asiatica*) or known as 'pegaga' among local. This 'ulam' grows well in shady and moist places especially in subtropical regions (Prakash et. al., 2017).

2.3 Importance of Bacterial Assessment on Food Product

Food sellers also known as food vendors are those who handle food or item that we can consume and any connection with food like the utensil used to eat or drink. They can be vector to spread harmful microorganisms through cross contamination. The contaminated object made the transmission of intestinal enteropathgenic bacteria directly or indirectly effected the consumers. It has been shown that a number of outbreaks of bacterial infections caused by unhygienic food product (Nasrolahei et. al, 2017). Therefore, the bacterial assessment on food product regularly is extremely important to prevent probable morbidity and protect consumers' health.

2.4 Foodborne Diseases Caused by Fresh Produce

Illness and death due to contaminated food have been a constant threat to our community and a significant impediment to the development of socio-economic worldwide. Each year, 600 million cases of foodborne diseases were recorded with 420, 000 deaths worldwide recorded by WHO. Annually, there are 9.4 million cases which causes 12, 800 hospitalizations and 3000 deaths due to foodborne diseases in United States. Whereas foodborne outbreaks are common in Taiwan (Lai et al., 2020). Foodborne contaminants are numerous, that include bacteria and viruses, chemicals, parasites and toxins which usually a wide range of outbreak occurred. Due to limited data, many pathogens that caused tropical diseases neglected. Usually, outbreaks go unreported, uninvestigated and unrecognized, they may only be visible if the public health or economic affected (Faour et. al., 2020).

In worldwide, fresh produce like vegetables and 'ulam' has become popular due to its importance as essential components of source of minerals, dietary fibre, healthy diets and nutrients for human body (Wallace et al., 2020). Diets rich in vegetables was recommended by World Health Organization (WHO), low vegetables intake will lead to poor health and increase the risk of non-communicable disease (NCDs) (WHO, 2017). Globally, about 5 million of death related due to low vegetables intake (Afshin et al., 2019). Even though vegetables and 'ulam' are important to human health, several critical steps may affect their microbial safety. These fresh produce can be contaminated at any point in the food chain either from the farmers, sellers or consumers. A hazard can exist during any system of production such as water, fertilizer, local environment, postharvest

practices and hygiene (Macieira, 2021). Besides, the growth of many microorganisms can be supported by most produce. Non-foodborne routes can transmit foodborne pathogen.

Every year, 1 in 10 people are afflict with foodborne disease which about 33 million lives per year worldwide. There are 20-40% of intestinal distress in our country Malaysia, related to foodborne pathogens. Pathogenic viruses or bacteria causing foodborne diseases and illness, toxins caused intoxication produced by pathogens like *S. aureus* and *Bacillus cereus* (Sharif et al., 2018).

2.4.1 Listeria

Listeria disease is caused by a zoonotic pathogen, *Listeria monocytogenes* (*L. monocytogenes*) which is one of the most recent recognized and least understood infectious bacteria. It is a Gram-positive foodborne bacterium that causes *listeriosis*. Human diseases ranging from gastroenteritis to septicaemia, abortion among females, mature birth, meningitis among new-born child and meningoecephalitis, are the symptoms of this ubiquitous bacterium. It can tolerate ordinary disinfectant, sanitizer and antimicrobial that will causes contamination in food contact surface (Halbedel et al., 2018). It also has ability to multiply in refrigeration temperature which contaminate the environment of food processing and the equipment like chilling, packing and cutting. While most bacteria retard at 4 ˚C, *L. monocytogenes* can still survive in temperature from below -7 ˚C to body temperature, but at -18 ˚C to 10 ˚C it grows the best (Saldivar et. al.2018). Therefore, even though the fresh produce has been kept properly in refrigerator,

L. monocytogenes may still can be transmitted. Its unique growth capabilities make it frequently overlooked as a possible cause of illness.

In some cases, *L. monocytogenes* has been responsible for major literiosis outbreaks. The large and protracted outbreaks believed to come from primary food product including dairy products, fresh produce, vegetables and fruits and meats that ready to be eaten. Cabbage that use fertiliser from sheep manure and subsequently stored under 4 ˚C was also implicated in one outbreak. Other recorded outbreak is because of contaminated cheese and milk (Saldivar et. al., 2018). The fatality rate can be as high as 30 % among risky people which are people with disease such as cancer, leukaemia and also pregnant women. Listeria has been estimated causing annually 1600 cases of listeriosis, resulting in 400 to 500 deaths by raw or contaminated food (Shamloo et. al., 2019). The increased availability and consumption of fresh vegetables may contribute to the increases of foodborne illness (Mustapha et. al., 2020).

2.4.2 Diarrhoea

The leading cause of global mortality in 2018 is diarrhoea disease and it causes over 1 million deaths (Troeger et al., 2018). The major factor of diarrhoea is the presence of *E. coli* that usually transmitted by multiple contaminated environmental pathways which are hands, soil, flies, food and water. However, the health hazard may not have possessed by *E. coli* until it reaches elevated numbers, foodborne illness possibly will occur (Khan et al., 2019). The emerging pathogens, *E. coli* most commonly associated

with persistent paediatric diarrhoea which caused retardation in developing countries. Once there was a big bloody diarrhoea outbreak in few European countries caused by contaminated ready-to-eat salads with diarrheagenic *E. coli* (Roth et al., 2018).

Other than that, another microorganism that can cause diarrhoea is *Salmonella spp*. *Salmonella* is a diverse genus of Gram-negative bacilli and one of major foodborne pathogen that form no spore. *Salmonella* capable to cause fever, severe illness such as high fever with abdominal pain and not typical diarrhoea or vomit which is general malaise. The usual infection of *Salmonella* caused by contaminated food such as poultry product, animal product or raw vegetables. The preparation of vegetables by food vendors especially street vendors have higher chances to be contaminated by these faecal materials and harmful bacteria. Malaysia Ministry of Health (MOH) suggested to eat cooked food within four hours after preparation to avoid food poisoning. The foodborne case in Baling, Kedah suspected caused by the consumption of laksa, a type of noodle soup. The laksa noodles was made and processed by using unclean noodle and the processing temperature was insufficient to kill bacteria resulted the present of *Salmonella* with diarrhea, abdominal pain, fever and sometimes vomit as symptoms (Abatcha et al., 2018).

2.5 Contaminants on Fresh Produce

In human food supply, fresh produce occupies an increasingly important role because it promotes various properties of nutritional health. Most fresh produce is consumed raw or with minimal process such as cleaning and washing, the contamination

of pathogen can represent a serious health risk. Pathogenic bacteria not only survive, they also can grow on fresh produce under proper condition (Alegbeleye et. al., 2018). Due to contaminants on fresh produce, there has been an increase cases and outbreaks of pathogen, however the literature data collected of the contaminated food are uneven in Malaysia due to no such cost analysis study done pertaining foodborne disease. The flows of some environmental pollutants into fresh produce threaten food safety and human health. The consumption of fresh produce effect the increase of outbreaks of foodborne diseases in frequency.

2.6 Factors Causing Fresh Produce Contamination

The health benefits and nutritional of consuming vegetables and 'ulam' have been widely recognized and publicized. There are numerous possible sources and routes of fresh produce contamination. Pathogens may contaminate the fresh produce 'on-field' by various way included groundwater, uptake from contaminated soil and atmospheric deposition (Alegbeleye et. al., 2018). The preparation of fresh produce requires minimal washing and processing before it can be consumed. By using suitable knives, it needs to be cut or shredded into preferred shapes and sizes and serve as one of the menu in daily meals. Due to poor environmental hygiene, improper food handlers training and lack of clean water supply, small stall at fresh market are tend to have food-borne illness outbreak more than bigger shop or market. Most of the sellers in the fresh market in Kelantan are elder people who have poor food safety knowledge. Some of the vegetables and 'ulam'

are eaten raw which increase chance for food poisoning to occur. Plus, the cleanliness of the environmental around the fresh market, exposed to unfavourable surrounding like mixed with live chicken and dust may also contribute to foodborne disease. A potential vector in transmission of pathogenic microorganism in vegetables is dust from surrounding. It usually happened because of the vegetables are not packed and covered properly. Also, important vehicles to transmit and spread faecal-oral bacteria is hands. Other than that, environmental factors along the entire food chain like conditions of weather and practices of postharvest handling may also effect the contamination of fresh produce. During any steps in the farm to table, microbial contamination may occur either from the farmers themselves or environment such as animals and soil (Machado-Moreira et al., 2019).

2.6.1 Manure Application

Livestock excreta, abattoir waste, slurries, sewage sludge as organic materials that used as soil amendments is already widespread since a long time ago (Alegbeleye et. al., 2018). This is one of the most cost effective routes to supply nutrients to plant. However, research has demonstrated that the raw manure possibly as well as contaminated and may be untreated properly that lead to a significant risk of pathogenic contamination for the fresh produce. Through direct interaction of the surface of the vegetables with the manure via rainfall or overhead irrigation, pathogens can be spread easily (Muhammad et. al., 2020). The application of manure can be by broadcast as solid, semi-solid and liquid, it

depends on the livestock faeces or the field at distinct locations. The survival of the pathogens in the manure is depending on the source of the manure, process of production, technique of treatment application and physicochemical factors such as relative humidity, pH, condition of water and type of soil (Zhang et. al., 2020). The apparent regrowth and recontamination of pathogen after treatment shows that many bacteria are capable to withstand the process of manure treatment, they constituting a major risk of contamination. A popular manure treatment is composting; this treatment exceeds 55 ˚C for 3 days which consider sufficient to kill most bacteria. However, few studies showed that the heat-induced death of pathogen in composted materials is a complex phenomenon (Awasthi et. al., 2020).

2.6.2 Postharvest Process

In agriculture sector, postharvest is one of important process need to take precaution and care to maintain the quality of the fresh produce. The stage of postharvest included harvest, cooling, storage, packaging, processing and transportation. Human pathogen can be hosted by fresh produce without showing any spoilage symptoms, the concerns regarding food safety must be raised. The control of foodborne diseases need to be started from field. Thus, for a long time ago, agronomic practices to control phytopathogens from spread to the crops have been used during pre and postharvest. Microorganism can be found as epiphytes on composition part of vegetables' tissue. There are a lot of postharvest diseases such as blue mold, gray mold and speck rot, caused

by fungal growth in the vegetables which can threat human health at the same time bring massive economic losses (Moraes Bazioli, 2019). Therefore, it is important for the farmer to deal with these fungi properly such as by using suitable fungicide for the crops.

2.6.3 Storage and Transportation

During storage, most vegetables require low temperature and high humidity but few need low both temperature and humidity. Proper storage for vegetables and 'ulam' will make them last until a month or longer. The proper storage includes cold and humid, cold and dry, cool and dry and cool and humid. Temperature is one of important things that need to be maintained during postharvest. By refrigerating, vegetables will age slowly, reduce undesirable growth, moisture loss and spoilage. It is also use to control the respiration rate of vegetables which cause loss of flavour, weight and value. To postpone deterioration effectively during refrigeration, the temperature must be in cold storage room constantly (Chen et. al., 2019). The exposure of warm and cold temperature may cause accumulation of moisture on the surface of vegetables and hasten the decay. The small-scale producers have challenge for on-farm cooling facilities which is set-up cost. Coolers are used for root crops such as tuber, ginger and sweet potatoes. Other than that, any spoilage of vegetables is going to induce the other produce. Thus, rub off soil and clean the vegetables by washing then let them dry before put them in storage. It is important for onion, pumpkin and garlic to be cured thoroughly before storage to avoid spoilage (Shrestha et. al., 2017).

Transportation of fresh market produce is usually from farm to farmers' houses for postharvest process and their houses to the fresh market. During postharvest transportation, vegetables may suffer few considerable damage which caused by vibration and shock (Perkins-Veazie P, 2017). The vibration will lead to bruises and punctures on the vegetables and contributed to higher amount of farmers' losses. Thus, it is important for the farmers and fresh market sellers to minimize vibration intensity that occur during the transportation. This is because contamination of the vegetables may occur due to fungal disease that enter through the bruise or puncture tissue. Few ways to reduce the risk of vibration such as avoid unmaintained road or road segments which have high amplitude of vibrations. Besides, the seller also can use suitable container and packaging for the vegetables to lower the fresh produce to hit each other (Sibanda et. al., 2020).

2.7 Bacterial Identification

2.7.1 Selective Media

During nineteenth of century, scientist discovered that culture media allowed the growth of microbes and bacteria was the first cultured to study human microbiota, artificial medium was used to allow the growth and isolate the bacteria (J.C Lagier et. al., 2015). In order for the bacteria to grow, they need minimum amount of nutrients which are water, source of carbon, nitrogen and mineral salt. The minimal media used in selective media does not let certain kind of bacteria to grow. However, growth factors, elements which the bacteria are not allowed to synthesize from nutrients available is

sometimes necessary to be added to culture media in small quantities to boost the bacteria multiplication.

The growth factor determined by the absence or blocking of metabolic pathway in the bacterium. A selective culture media is used to isolate a particular genus or species of bacteria. The objective of selective media is to eliminate unwanted microbial flora after the addition of inhibitors to the medium culture. This medium is made of basic medium with additional of antibiotics, dyes, antiseptic, phages or chemicals based on their needs (Bonnet et. al., 2020).

2.7.2 Morphological Characteristic

Morphological characteristic of bacterial species has been maintained since countless generations. The morphological test was used to examine colour, shape, margin and transparency of the colonies formed. The well-known shapes included rod and cocci, but there are also varieties of exotic shapes such as serpentines, moustaches and even undefined shapes (van Teeseling et. al., 2017). These characteristics were observed under microscope for all the isolates.

The gram stain is the most common staining method in bacteriology which used to differentiate organism of the domain bacteria based on the structure of bacterial cell walls. Gram positive bacteria have thick peptidoglycan layer and will have blue or purple stain after staining while gram negative bacteria have thin peptidoglycan which will result

in pink stain after staining. The technique used in this procedure is important to make sure the bacteria can be seen clearly under microscope. For example, result of the stain is affected by thickness of the smear. Young and fresh growing cultures is recommended to be used during gram stain (Ann C.S and Marise A.H., 2021).

2.7.3 Biochemical Test

Biochemical test is a test which shorten the time needed to identify unknown bacteria. This test low in cost and improve the accuracy of bacterial identification through the reactions. The bacterial isolates were characterized biochemically to evaluate their chemical nature. Catalase test used to detect the enzyme catalase in bacteria and differentiate catalase-positive or catalase-negative (Wu et. al., 2020). Catalase test detect the formation of bubbles within 10 seconds after hydrogen peroxide, $2H_2O_2$ solution added to the purified bacterial. If gas bubbles detected, it considered as positive catalase and vice versa (Al-Dhabaan and Fahad A, 2019). The catalase enzyme serves to neutralize the effect of bactericidal of the hydrogen peroxide. It breakdown the $2H_2O_2$ into water and oxygen which produce rapid bubbles (Reiner and Karen, 2010).

Triple Sugar Iron (TSI) agar test conducted to examine the fermentation of glucose, lactose, sucrose, hydrogen sulphide, H_2S and gas formation. Some of the bacteria can ferment sugars particularly and some of them cannot ferment any. Therefore, bacterium fermentation of sugar is an important criterion for identification. However, this test is not as sensitive to detect H_2S compared to other medium which contain iron. Weak

production of H_2S may show little trace of H_2S activity. Some species of bacteria family may delay the reactions or do not react at all to the fermentation (Sarker et. al., 2021).

CHAPTER 3

METHODOLOGY

3.1 Sample Collection

Five types of vegetables and five different ‘ulam’ were bought from Jeli Wet Market in Kelantan (Table 3.1). Some of the samples sold by the sellers were from local farmers and some were from Pasir Puteh wet market. The samples were freshly bought from the wet market within 1-2 days before the experiment and placed in different zip lock bags A4 size based on their types. Then, all the samples were stored at 1-8 ˚C in the Food Laboratory in Universiti Malaysia Kelantan, Jeli campus to maintain the quality and to avoid the presence of extraneous materials.

Table 3.1:Name of vegetables and 'ulam' used in research

Type	Name
Vegetable	Cassava, carrot, Chiese okra, tomato, cabbage
‘Ulam’	Pucuk jenereh, ulam raja, pucuk ubi kayu, pucuk kaduk, pucuk pegaga

3.2 Equipment

Cutter, cutting board, sterile stomach bag, zip lock bag A4 size, zip lock bag A5 size plastic petri dish (90 x 15 mm), 15 mL falcon tube, gloves, label tagging, incubator, laminar flow hood cabinet, weighing balance, spatula, aluminium foil, beaker 250 mL, beaker 500 mL, beaker 1000 mL, media bottle 500 mL, media bottle 1000 mL, yellow pipette tip, blue pipette tip, bunsen burner, wire loop, retort stand.

3.3 Appliance

Autoclave machine (Hirayama HVE-50), laminar flow (Azteclab), BagMixer® 400 CC lab blender (Interscience), colony counter (Funke Gerber), incubator (Lab Companion) and brightfield microscope (Leica DM750).

3.4 Sample Preparation

The weighing balance, cutter and cutting board were sterilized. All the samples were cut and weighed at 25 g. Then the samples were put into zip lock bags A5 sized.

3.5 Chemical Phosphate Buffer Solution Preparation

33.33 mL of sodium hydroxide (R&M, United Kingdom), 56.25 mL of potassium dihydrogen phosphate (HmbG) and 135.42 mL of distilled water were mixed together to produce 225 mL of phosphate buffer solution.

3.6 Growth Media

MacConkey agar (Oxoid Ltd, England), Baird Parker (Merck KGaA, Germany) and Nutrient agar (Oxoid Ltd, England), Xylose lysine deoxycholate (XLD) agar (Merck KGaA, Germany) and Triple Sugar Iron (TSI) agar (Merck KGaA, Germany).

3.6.1 MacConkey Agar Preparation

24.77 g of growth medium was suspended in 300 mL distilled water in media bottle and was shake gently. Distilled water added until the level reached 500 mL Then, the dissolved mixture was autoclaved at 121 °C, 1 atm for 15 minutes. The medium was poured into petri dishes in laminar flow and left to solidify for 30 minutes.

3.6.2 XLD Agar Preparation

27.5 g of the growth media was suspended in 300 mL distilled water in media bottle and was shake gently. Distilled water added until the level reached 500 mL Then, water bath was prepared at 50 °C in beaker using bunsen burner and retort stand. The media bottle was put in the water bath with frequent agitation until the suspension boiled. The medium was poured into petri dishes in laminar flow and left to solidify for 30 minutes.

3.6.3 Baird Parker Agar Preparation

30.0 g of growth medium was suspended in 300 mL distilled water in media bottle and was shake gently. Distilled water added until the level reached 500 mL Then, dissolved mixture was autoclaved at 121 °C, 1 atm for 15 minutes. The medium was poured into petri dishes in laminar flow and left to solidify for 30 minutes.

3.6.4 Nutrient Agar Preparation

28 g of nutrient agar powder was suspended in 800 mL distilled water in media bottle and was shake gently. Distilled water added until the level reached 1 L. Then, the dissolved mixture was autoclaved at 121 °C, 1 atm for 15 minutes. The medium was poured into petri dishes in laminar flow and left to solidify for 30 minutes.

3.6.5 TSI Slant Agar Preparation

3.25 g of agar media was suspended in 50 mL of distilled water in a beaker. The suspension was stirred in a water bath until boiled and dispersed into capped test tubes.

The media was then autoclaved at 121 °C, 1 atm for 15 minutes. When the agar still hot, the rack used to hold the test tubes was tilted on a solid surface to make sure the medium inside the tubes is at slanted position. The media was left to solidify at room temperature before ready to be used.

3.7 Bacteriological Analysis

Each of the vegetables and 'ulam' samples were weighed for 25 g and put into sterile stomacher bags separately. Inside a laminar flow hood, each of the samples filled with 225 mL phosphate buffer solution and homogenized using BagMixer for 2 minutes

3.7.1 Aerobic Plate Count

9 mL of Bacto Protease Pepton (BD Biosciences, United States) solution was put into each falcon tube. Serial dilutions were performed from 10^{-} to 10^{-9} using sterile pipets. Spread plate method was used to determine the total plate count (TPC). 100 µL (10^{-10}) of diluted samples were inoculated onto nutrient agar plates. Then, the isolated plates were incubated at 37 ˚C for 24 hours.

3.7.2 Colony Counting

Result were recorded as colony forming unit per millilitre (CFU/mL). Colonies on culture plates were counted by using colony counter. Results were reported following the BAM Chapter 3: Aerobic Plate Count (APC) by FDA guidelines:

1. Normal plates (25-250): Spreader-free plates were selected. All the colony forming units (CFU) including those of pinpoint size, on selected plates were counted. The dilutions used and total number of colonies counted were recorded.
2. Plates with more than 250 colonies: When number of CFU per plate exceeds 250, for all dilutions, the counts recorded as too numerous to count (TNTC) for all but the plate closest to 250, and CFU was counted in those portions of plate that were representative of colony distribution counted.
3. Spreaders (SPR): Colonies were usually spread in 3 distinct types: 1) a chain of colonies, not too distinctly separated; 2) one that develops in film of water between agar and bottom of dish; and 3) one that forms in film of water at edge or on surface of agar. If plates prepared from sample have excessive spreader growth so that (a) area covered by spreaders, including total area of repressed growth, exceeds 50% of plate area, or (b) area of repressed growth exceeds 25% of plate area, report plates as spreaders. When it is necessary to count plates containing spreaders not eliminated by (a) or (b) above, each of the 3 distinct spreader types were counted as one source. For the first type, the colony was counted as single colony if only one chain exists. Each source counted as one colony if one or more chains appear to originate from separate sources. Each

individual growth in such chains as a separate colony were not counted. Types 2 and 3 usually result in distinct colonies and are counted as such. The spreader and the colony were count combined to compute the APC.

4. Plates with no CFU: When plates from all dilutions have no colonies, APC reported as less than 1 times the corresponding lowest dilution used. The calculated APC were marked with asterisk to denote that it was estimated from counts outside the 25-250 per plate range. When plates from a sample are known to be contaminated or otherwise unsatisfactory, result were recorded as laboratory accident (LA).

The equation used to calculate plates with 25-250 single colonies:

$$\frac{CFU}{mL} = \frac{(No.\,of\ colonies\ x\ Total\ dilution\ factor)}{Volume\ of\ culture\ plated\ in\ mL}$$

3.8 Bacterial identification

The isolated bacteria from vegetable and 'ulam' samples were identified using selective media, Gram staining and biochemical test. 37 ˚C for 24 hours.

3.8.1 Identification by Selective Media

Bacterial isolates were identified using selective media; MacConkey agar for presence of *E. coli*, XLD agar for presence of *Salmonella spp*. and Baird Parker agar for presence of *S. aureus*. .100 µL of samples prepared in Section 3.6. was inoculated onto selective media. Plate were incubated 37 ˚C for 24 hours. Results were recorded based on the colonies appearances on the culture plates; pink colonies on MacConkey agar indicates presence of *E. coli*; yellow colonies on XLD agar indicates the presence of *Salmonella spp*.; while pale yellow colonies on Baird Parker agar indicates the presence of *S. aureus*.

3.8.2 Identification by Gram Staining

A wire loop was heated to sterilize and used to transfer small drop of sterile distilled water onto a clean glass slide. The wire loop then sterilized again, cooled and used to transfer small amount of bacterial cells onto the water drop. The bacteria cells were dispersed into a thin layer smear and spread.

The smear was heat-fixed with crystal violet reagent for 1 minute. The slide was then washed with distilled water for 2 seconds. Iodine was used to flood the slide for 1 minute. Then, the slide was washed using distilled water for 2 seconds. Decolorizing agent, acetone was used to flood the slide for 15 seconds and washed with distilled water.

The slide was then flooded with counterstain, safranin for 30 seconds before washed again with distilled water. Results were observed using brightfield microscope under 100X magnification. Gram negative bacteria stained pink whereas gram positive bacteria stained purple.

3.8.3 Identification by Catalase Test

A small amount of bacterial cells was collected from a well isolated colony by using sterilized wire loop and placed onto a clean glass slide. A few drops of 30 % $2H_2O_2$ were put onto the bacterial cells. The formation of bubbles was observed against a dark background and recorded as presence of catalase enzyme.

3.8.4 Identification by Triple Sugar Iron (TSI) Agar Test

Triple Sugar Iron (TSI) agar (Merck KGaA, Germany) was used in this test. A well-isolated colony from isolation plates were inoculated onto the TSI agar slant by using sterilized wire loop. It was first stabbed to the centre of the medium to the bottom of the

tube followed by streaking on the surface of the agar slant. Results were recorded based on the following observation:

- The phenol red turns into yellow both in the butt and in the slant indicates a large amount of acid is produced by fermentation of lactose.
- The phenol red turns into yellow in the butt while the slant remains red. This indicates lactose was not fermented but the small amount of glucose is produced.
- The phenol red does not turns into yellow both in the butt and slant. This indicates both lactose and glucose were not fermented.
- The phenol red slant turns into black colour indicates H_2S is produced by the organism.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Aerobic Plate Count On Vegetable Samples

Table 4.1 shows the number of colonies formed on nutrient agar from vegetable samples after 24 hours incubation at 37 ºC. Some of the samples produced more than 250 colonies which reported as TNTC.

Table 4.1: Number of colonies formed in vegetable samples

Dilution	Vegetable Name				
	Cassava	Carrot	Chinese Okra	Tomato	Cabbage
10^{-2}	TNTC	TNTC	TNTC	0	237
10^{-3}	TNTC	63	100/ SPR	0	61/ SPR
10^{-4}	69	20	54/ SPR	0	53/ SPR
10^{-5}	5	0	29	0	0
10^{-6}	0	0	0	0	0
10^{-7}	0	0	0	0	0
10^{-8}	0	0	0	0	0
10^{-9}	0	0	0	0	0
10^{-10}	0	0	0	0	0

The highest value of CFU/mL belongs to cassava $6.9x10^6$ CFU/mL, followed by Chinese okra $1x10^6$ CFU/mL, carrot $6.3x10^5$ CFU/mL and cabbage $2.37x10^5$ CFU/mL while the lowest is tomato 0 CFU/mL (Table 4.2).

Table 4.2: CFU/mL in vegetable samples

Vegetable Name	**Cassava**	**Carrot**	**Chinese Okra**	**Tomato**	**Cabbage**
CFU/ml	6.9×10^7	6.3×10^5	1×10^6	0	2.37×10^5

The CFU/ml value of cassava and Chinese okra were exceeded the acceptable amount as in BAM Chapter 3: Aerobic Plate Count (APC) by FDA guidelines which is 10^6 CFU/ml. Cassava is a tuber vegetable which usually be planted by local people with traditional techniques including the use of animal manure during plantation. The cassava samples also were sold without proper process of cleaning. The soil was still on the vegetable during the experiment. Even though carrot is a tuber plant too, there was no soil stuck at the skin of the carrot sample. It seems like the sample has been cleaned before sold to the wet market. Soil contains a lot of bacteria that might come during manuring or origin from the soil itself. Study shows that bacteria survive in alkaline pH better than acidic pH (Eni et. al., 2010) and manure usually come in alkaline state. While Chinese okra has rough skin texture, it is easier for the bacteria to be trapped and stuck to the skin. No colonies were isolated from tomato sample, indicating that it is not contaminated and safe to consume. This fruit vegetable might be well processed. Tomato have their natural protective epidermal layer that effectively protect against most bacteria and plant spoilage (Bello et. al., 2016). Another difference of tomatoes from other plants is they have nature

of exocarp as well as the range of antagonistic microorganisms that may cause this protection.

All the bacteria isolated from those vegetable samples show the amount of contamination that were obtained from the sellers or handlers. The high value of CFU/ml affected by high the contamination, the food that contain more than 10^6 CFU/ml is not safe to be consumed. Despite the high bacterial counts obtained in this experiment, it is important to know that the outward appearance of these vegetables is not one of the factor to measure the presence of bacteria (Jaeger et. al., 2018). Thus, the appearance of the vegetables may not be good criteria to judge the bacterial quality in vegetables, even though the vegetables did not show any sign of spoilage, it does not mean that the fresh produce is safe to consume. All the vegetables should be washed well before consumption either by consumer or the seller, decontaminant such as vinegar is a must during washing (Erhirhie et. al., 2020)

4.2 Aerobic Plate Count on 'Ulam' Samples

Table 4.3: Number of colonies formed in 'ulam' samples

Dilution	**Ulam's Name**				
	Pucuk Jenereh	**Ulam Raja**	**Pucuk ubi kayu**	**Pucuk kaduk**	**Pegaga**
10^{-2}	151/ SPR	TNTC	TNTC	TNTC	115
10^{-3}	54/ SPR	70/ SPR	147/ SPR	102/ SPR	7/ SPR
10^{-4}	16/ SPR	32/ SPR	56/ SPR	39/ SPR	3
10^{-5}	0	28/ SPR	3	3/ SPR	0
10^{-6}	0	2/ SPR	2	0	0
10^{-7}	0	1	0	0	0
10^{-8}	0	1	0	0	0
10^{-9}	0	0	0	0	0

The table 4.4 shows the value of CFU/mL of 'ulam' in this experiment where 'pucuk ubi kayu' is the highest $1.47x10^6$ CFU/mL followed by 'pucuk kaduk' 1.02×10^6 CFU/mL, 'ulam raja' 7×10^5 CFU/mL, 'pucuk jenereh' 1.51×10^5 CFU/mL and lastly 'pegaga' 1.15×10^5 CFU/mL.

Table 4.4: CFU/mL in 'ulam' samples

'Ulam' Name	**Pucuk Jenereh**	**Ulam Raja**	**Pucuk Ubi Kayu**	**Pucuk Kaduk**	**Pegaga**
CFU/ml	1.51×10^5	7×10^5	1.47×10^6	1.02×10^6	1.15×10^5

The level of CFU/mL of 'Pucuk ubi kayu' and 'pucuk kaduk' exceed the acceptable level, which they are not suggested to be consumed raw or else the consumer may get infected. The other 'ulam' are safe to consume raw.

All the ‘ulam’ used in this experiment are leafy and leafy green are the most frequently linked to bacterial infections (Luna-Guevera et. al., 2019). Moreover, ‘ulam’ are usually grow by themselves because they fit easily in any kind of soil and need minimal care, they can be planted around house and picked for sale. At wet market, ‘ulam’ usually wrapped in newspaper before put in plastic to sell to customer which contribute to exposal of bacteria. Besides, the arrangement of the vegetables display area in the wet market was next to wet section where chicken, seafood and meat were sold, likelihood the cross-contamination might be affected by this proximity (Bahri et. al., 2019). Moreover, ‘ulam’ usually consumed raw which increase the probability of food poisoning among consumers.

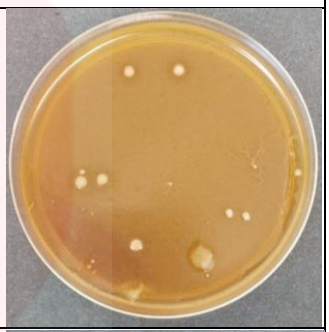
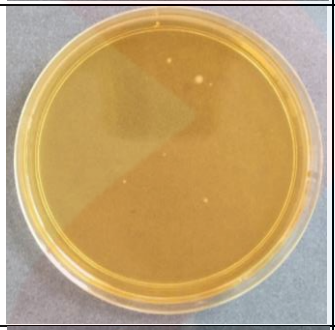
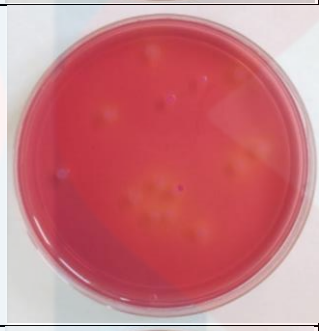
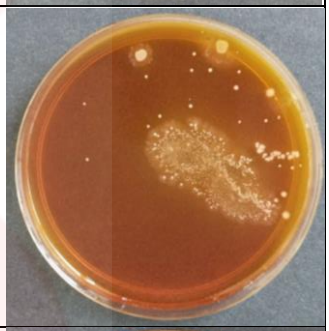
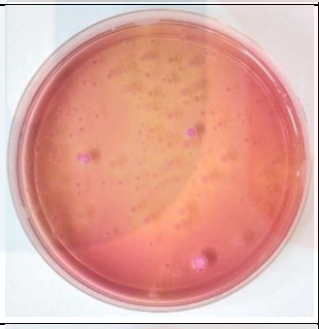
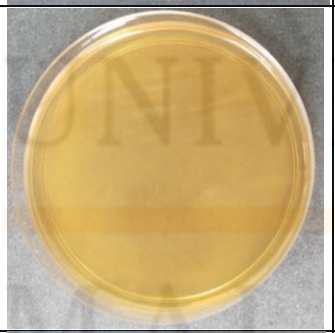
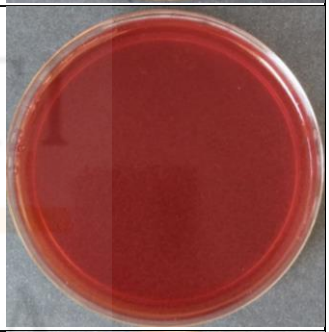
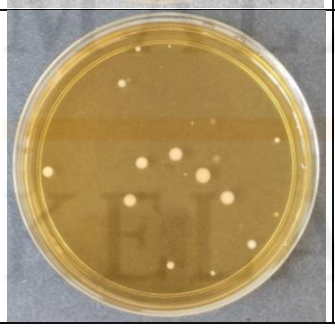
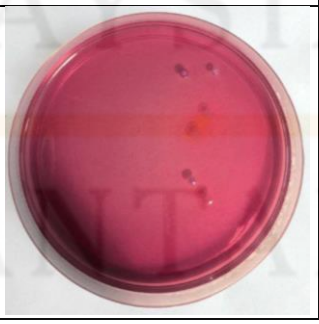
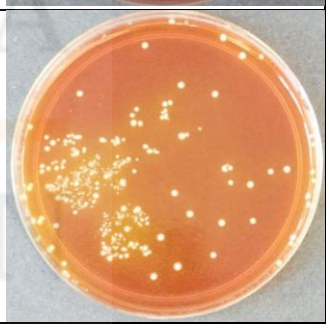
‘Pucuk ubi kayu’ has the highest amount of CFU/mL value. It is a medium tall plant which found commonly in tropical country. The growth of this plant is easily affected by pest such as rat and boar. This is because the pests want to eat the cassava at the plant’s root. However, it disturbs the hygiene of the plant included the leaves. Pesticide is needed to control beside good hygiene practices to prevent the arrival of those pests. ‘Pucuk kaduk’ is a shrub type of herb plant that grows on the top of the soil. Soil contain lots of bacterial that can cause foodborne illness. The application of poultry manure on the soil also contribute to higher value of CFU/mL value (Kong et. al, 2018). Although ‘pegaga’ is also a shrub plant that grows by creeping on soil, the sample seemed like has been washed or cleaned because there was no soil stuck at the sample like ‘pucuk kaduk’ sample. ‘Pucuk jenereh’ come from a tall and big plant that commonly used as roadside shade tree especially in the northern states. It was different in Kelantan where this plant being sold widely and most commonly found in wet market. This plant can always be pruned about 2 feet to ensure it does not grow tall and can be picked easily. With that distance, bacteria from pest and soil cannot reach the leaves part easily.

4.3 Bacterial Isolated on Selective Media

4.3.1 Bacterial Isolated on Selective Media from Vegetable Samples

Table 4.5 shows bacterial colonies of vegetables samples formed on selective media, used to distinguish different type of bacteria. The colonies formed a lot on Baird Parker medium from cassava sample but no colonies formed in tomato sample. While the colonies formed on MacConkey and XLD medium can be seen a lot from Chinese okra but none in tomato.

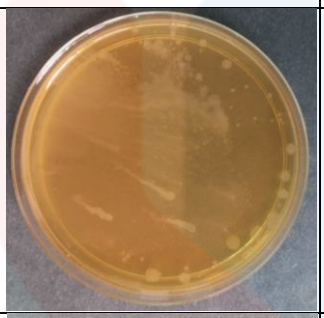
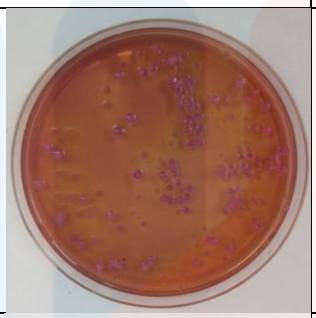
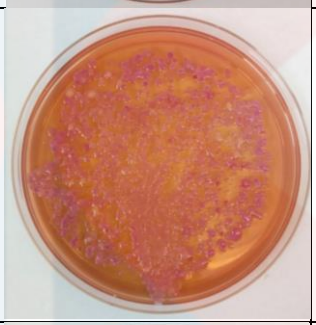
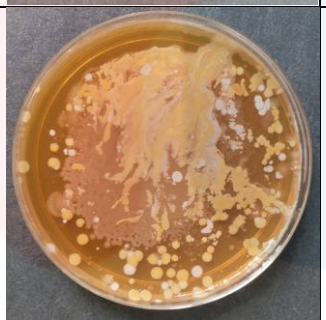
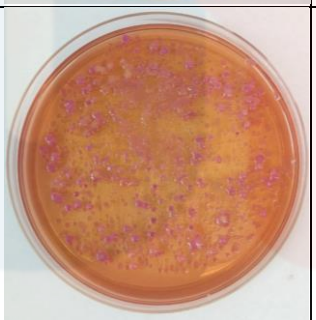
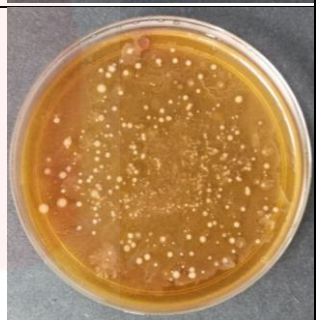
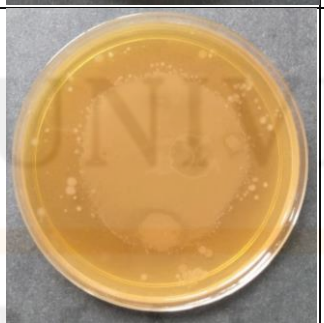
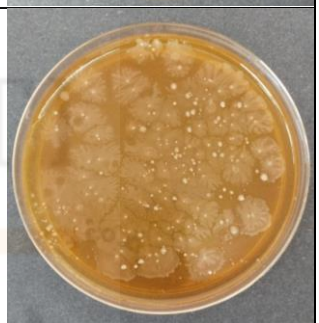
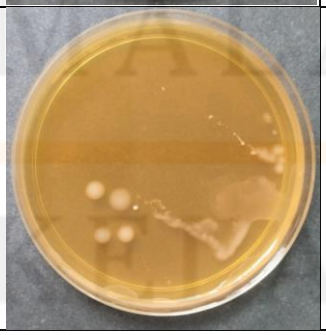
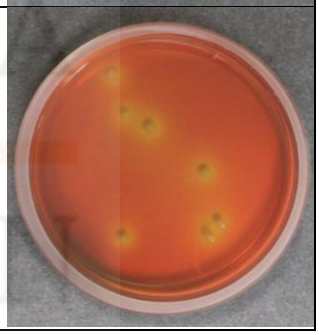
Table 4.5: Bacterial isolated from vegetable samples on selective media

Samples	Selective Media		
	Baird Parker	MacConkey	XLD
Cassava			
Carrot			
Chinese Okra			
Tomato			
Cabbage			

4.3.2 Bacterial Isolated on Selective Media from 'Ulam' Samples

Table 4.6 shows the formation of colonies on selective agar for 'ulam' samples. For Baird Parker medium, the colonies can be seen a lot on 'pucuk ubi kayu' while the least on 'pegaga'. Colonies on MacConkey formed a lot on all samples except 'pegaga'. On XLD medium, colonies can be seen a lot at all 'ulam' samples except on 'pegaga'.

Table 4.6: Bacterial isolated from 'ulam' samples on selective media

Sample	Selective Media		
	Baird Parker	MacConkey	XLD
Pucuk Jenereh			
Ulam Raja			
Pucuk Ubi Kayu			
Pucuk Kaduk			
Pagaga			

In this study, the bacteriological contamination of vegetables and 'ulam' sold in Jeli wet market, Kelantan determined. There is no study on contamination or food safety of these fresh produce published. Thus, the results obtained could not be compared in this research with any previous local data. Three types of selective agar, Baird Parker, MacConkey and XLD were used to identify the presence of three different bacteria which are *S. aureus, E. coli* and *Salmonella sp*. in all those samples. However, all those media can also grow other type of bacteria, thus morphological characteristics test was done by gram staining. The morphological characteristic of these bacteria were showed in Table 4.7.

In this study, the presence of the *S. aureus* which detected on Baird Parker medium is an indication of poor hygienic practices mainly by carriage in nasal passages of the farmers, handlers during transporting or wet market sellers. The extensive handling also usually associated with the packaging itself lends to the fresh produce contamination. Besides, this pathogenic microorganism in ready-to-eat vegetables and 'ulam' may also come from contaminated environmental surfaces and equipment (Wu et. al., 2018).

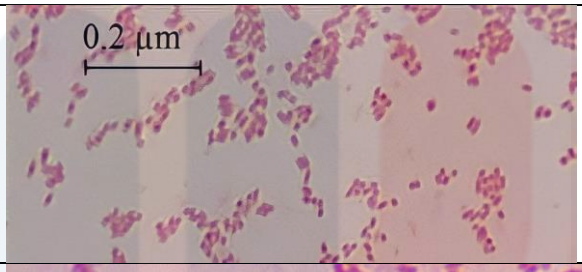
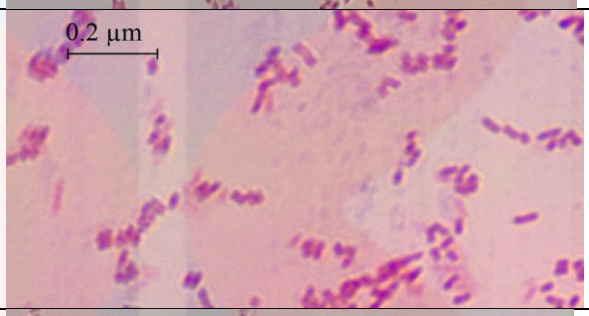
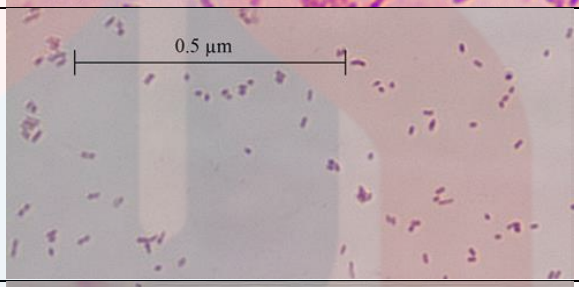
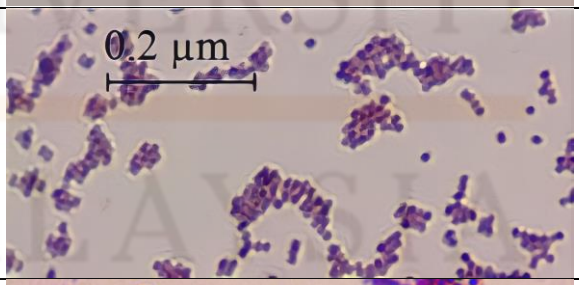
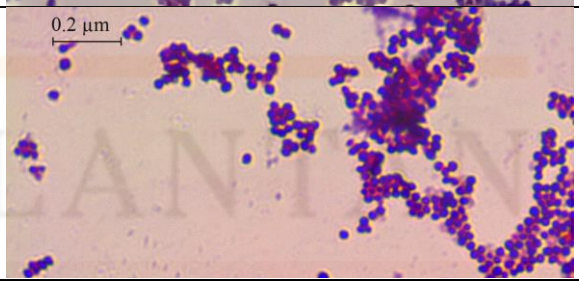
The presence of *E. coli* which detected on MacConkey medium in the samples is an indicator of faecal contamination either directly or indirectly, where usually come from the farm, which could be the manure in the soil or contaminated irrigation. Generally, low presence of *E. coli* persisted for more than 100 days in manure-fertilized soil and spotted in 132 to 168 days after the application or manure (Ingham et. al., 2004;Muhammad et. al.,2020). High presence of *E. coli* in samples may due to these contaminations; faecal from animal manures, irrigated water, poor hygiene by food handlers or utensils used. *E. coli* can retain by several part of plants even after washed vigorously (Luna-Guevara et. al., 2019). The recent contamination of faecal item showed in the presence of *E. coli* and any other pathogens in the samples are known to cause foodborne diseases.

The XLD agar used to identify the presence of *Salmonella spp*. from the samples which the presence of bacteria may cause by polluted irrigation such as stream water while well water is an indication of faecal contamination (Afolabi O and Oloyede A, 2010; Alegbeleye O et. al., 2020). *Salmonella spp*. can enter part of plants by contaminated soil or irrigation water, wild animals living in the wet market especially during rainy season (Nguyen et. al., 2021). The presence of this bacteria in fresh produce has been linked to serious outbreak and the effect now is a worldwide major public health concern.

4.4 Gram Staining

The colonies taken from one of the MacConkey agar plate produce pink stain, under the microscope, rod shape bacteria seen. It is the same with *E. coli* taken from control plate under the 100X magnification. *Salmonella spp*. which taken randomly from XLD agar plate also appeared with pink stain and rod shape bacteria can be seen (Table 4.4) under the microscope. The pink stain produced by *E. coli* and *Salmonella spp*. because they do not retain the crystal violet stain. They are Gram-negative bacteria which their cell enveloped. Purple stain produced by colonies taken from random sample of Baird Parker agar plate. Same stain formed on *S. aureus* taken from control plate. The bacteria have same spherical shape means that the sample is *S. aureus* which is a Gram-postive bacteria. Gram-positive bacteria took up crystal violet stain (Sharma et. al., 2015).

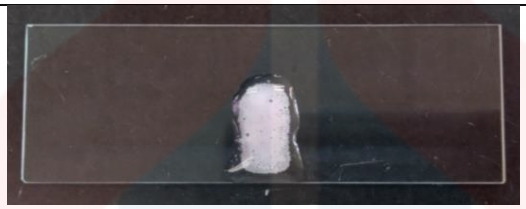
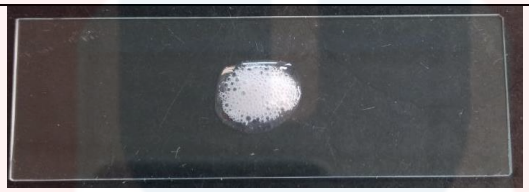
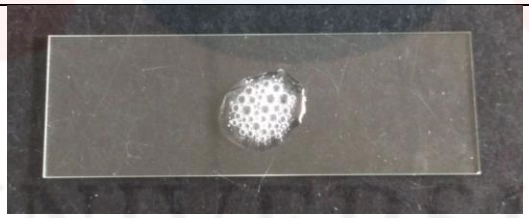
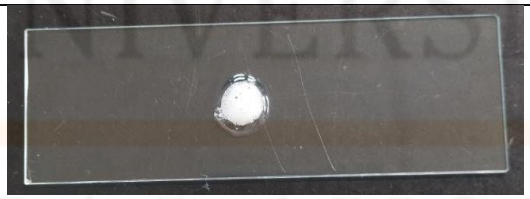
Table 4.7: Bacteria under x100 microscope after gram staining

Types of bacteria	**Colour formed**	**Bacteria under Microscope 100x**	**Gram**
Control(*E .coli* from ATCC8739) on MacConkey media	Pink		Negative
Isolate 1 on MacConkey media	Pink		Negative
Control(*S. typhi* ATCC14028) on XLD media	Pink		Negative
Isolate 2 on XLD media	Pink		Negative
Control(*S. Aureus* ATCC6538) on Baird Parker media	Purple		Positive
Isolate 3 on Baird Parker media	Purple		Positive

4.5 Catalase Test

Table 4.8 shows the bubble formed on slide after catalase test were done. All of the colonies taken from the isolated strains samples produced bubbles. The colonies taken from control plate which are *E. coli*, *Salmonella and S. aureus* were also producing bubbles.

Table 4.8: Formation of bubbles after few drops of $2H_2O_2$

Type of Bacteria	Catalase Test Result		Enzyme
Control *(E. coli* ATCC8739) on MacConkey media		Bubble	Positive
Isolate 1 on MacConkey media		Bubble	Positive
Control (*S. typhi* ATCC14028) on XLD media		Bubble	Positive
Isolate 2 on XLD media		Bubble	Positive
Control (*S. aureus* ATCC6538) on Baird Parker media		Bubble	Positive
Isolate 3 on Baird Parker media		Bubble	Positive

Catalase test is one of biochemical test with immediate result to facilitate the detection of catalase of enzyme in bacteria. The catalase test result showed all type of bacteria which are *E. coli, S. aureus and Salmonella spp*. where all of the bacteria produced bubble. That means all of the colonies taken from the isolated media are positive-catalase. They all capable to respire using oxygen as a terminal electron acceptor. The catalase enzyme breakdown the $2H_2O_2$ into oxygen and water during the formation of bubbles. The low production of bubbles showed the lack of catalase. Thereby, bacteria defend themselves from fatal effect of hydrogen peroxide which accumulated as final product of aerobic carbohydrate metabolism (Kaushal et. al., 2018).

4.6 TSI Slant Agar

Figure 4.1 and 4.2 show the colour of TSI slant agar from isolated strains after 24 hours of incubation. Seven slant were made in this reconfirmation test. Four agar turn yellow, one turns black at butt while red on slant, one turn yellow on the slant only and one unchanged. The colonies were taken from MacConkey, XLD and Baird Parker media. Figure 4.1 shows TSI slant agar which the colonies taken from control *(E. coli* ATCC8739) on MacConkey media and control (*S. aureus* ATCC6538) on Baird Parker media tubes turn yellow. However, the control (*S. typhi* ATCC14028) on XLD media turns black at the bottom and remain red at the slant. The un-inoculated tube remains red. Figure 4.2 shows the colonies taken from MacConkey and Baird Parker media from samples changed the TSI slant agar from red phenol to yellow while the colonies taken from XLD media from samples turn yellow on the slant and remain red at the bottom.

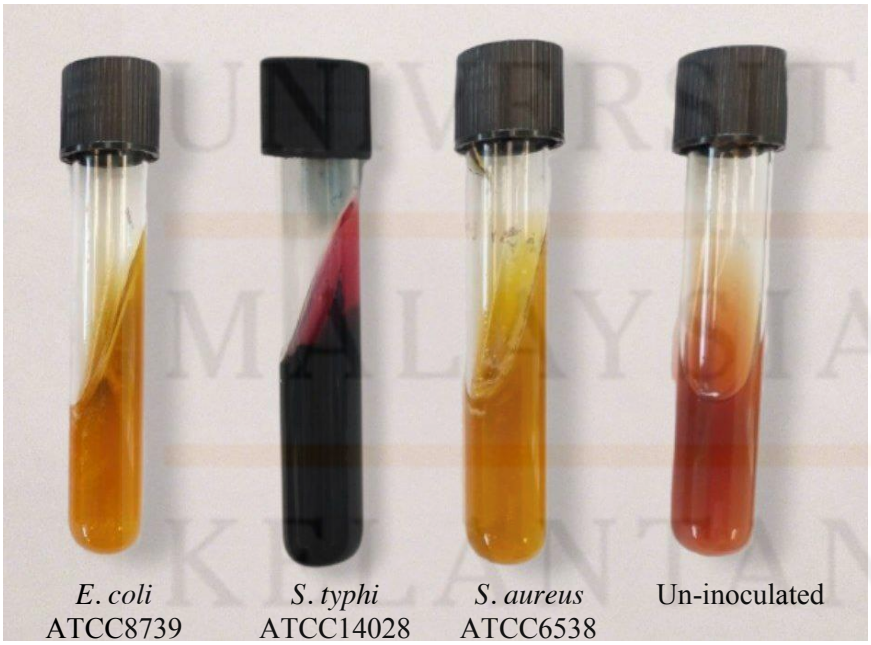


Figure 4.1: TSI agar from control bacteria after 24 hours of incubation

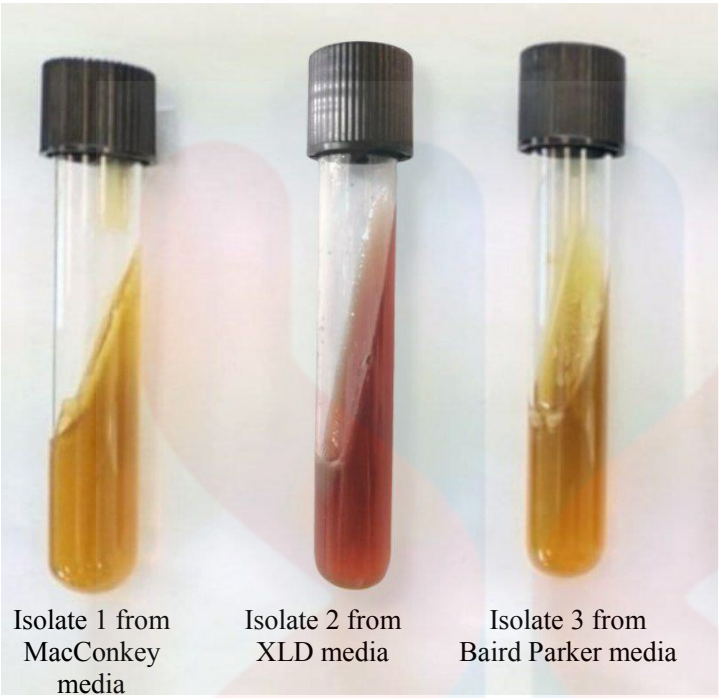


Figure 4.2: TSI agar from bacteria isolated from samples after 24 hours of incubation

TSI slant in isolate 1 from MacConkey media, isolate 3 from Baird Parker media, control *E. coli* and control *S. aureus* turned the TSI slant agars from red phenol to yellow. This means there are acidic reaction which indicate all types of sugar, dextrose, glucose and lactose fermented (Ahmad et. al., 2018). Isolate 2 taken from XLD media has changed the TSI agar into red slant and blackening medium. Fermentation of glucose occurred and production of H_2S happened. Slow growth of bacteria also effects the colour changes of the agar thus it still got more red. Compared to *S. typhi* in control tube which turned the TSI slant agar into red slant with blackening medium. This is because of glucose fermented and H_2S produced (Boatemaa et. al., 2019). The un-inoculated TSI agar remain red due to there was no fermentation of sugar and production of gas happened. No crack on the agar in this experiment which showed no gas produced.

CHAPTER 5

CONCLUSION

In conclusion, this study examined the presence of bacteria in fresh produces randomly bought from Jeli wet market and their CFU value calculated. Vegetables and 'ulam' are well recognized to have potential to harbour bacteria and one of vehicle to spread foodborne diseases. There are lot of research to find out the variety and complex source for the pathogen to enter vegetables and 'ulam'. The factor of contamination of these fresh produce may come from many routes from food chain to fork included field such as contaminated manure, cut from grower or contaminated planting equipment and postharvest process. There are abundant of information regarding the contamination factors that can be used as shield before consuming raw fresh produce. Results clearly indicate that not all vegetables and 'ulam' are safe to be consumed raw with the CFU/mL are higher than the recommended value given by WHO. Three types of bacteria which are *Escherichia coli, Salmonella typhi*. and *Staphylococcus aureus* were confirmed to be found in those fresh produces. Thus, it is important for consumers to not only wash the fresh produce with running water but also use decontaminant to kill the bacteria before consuming them to avoid foodborne illness.

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APPENDIX

Calculation of CFU/ml for each vegetable and 'ulam' samples

Cassava

$$\frac{CFU}{ml} = \frac{69\,x10^{4}}{0.1}$$

= 6.9 x 10^7 CFU/ml

Carrot

$$\frac{CFU}{ml} = \frac{63x10^{3}}{0.1}$$

= 6.3 x 10^5 CFU/ml

Chinese okra

$$\frac{CFU}{ml} = \frac{100x10^{3}}{0.1}$$

= 1 x 10^6 CFU/ml

Tomato

$$\frac{CFU}{ml} = \frac{0\,x\,10^{2}}{0.1}$$

=0

Cabbage

$$\frac{CFU}{ml} = \frac{237\,x\,10^{2}}{0.1}$$

=2.37 x 10^5 CFU/ml

Pucuk Jenereh

$$\frac{CFU}{ml} = \frac{151\,x\,10^{2}}{0.1}$$

= 1.51 x 10^5 CFU/ml

Ulam Raja

$$\frac{CFU}{ml} = \frac{70\ x\ 10^3}{0.1}$$

=7 x 10^5 CFU/ml

Pucuk Ubi Kayu

$$\frac{CFU}{ml} = \frac{147\ x\ 10^3}{0.1}$$

= 1.47 x 10^6 CFU/ml

Pucuk Kaduk

$$\frac{CFU}{ml} = \frac{102\ x\ 10^3}{0.1}$$

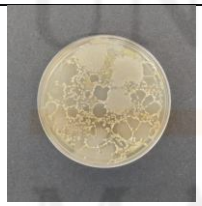
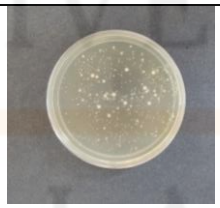
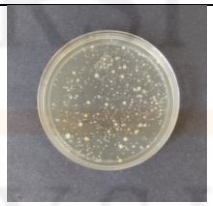
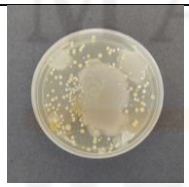
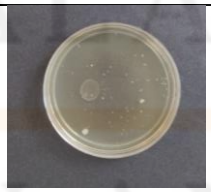
=1.02 x 10^6 CFU/ml

Pegaga

$$\frac{CFU}{ml} = \frac{115\ x\ 10^2}{0.1}$$

=1.15 x 10^5 CFU/ml

Table A.1: Colonies formed on nutrient agar for vegetable samples

Dilution	Cassava	Carrot	Chinese Okra	Tomato	Cabbage
10^{-2}	 TNTC	 TNTC	 TNTC	0	 237
10^{-3}	 TNTC	 63	 100/ SPR	0	 61/ SPR

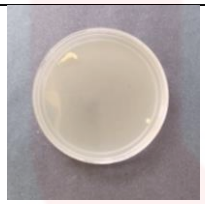
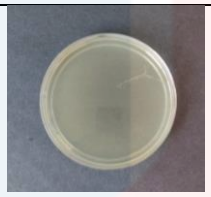
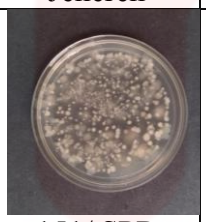
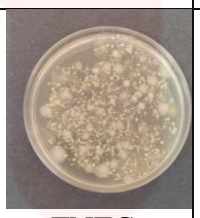
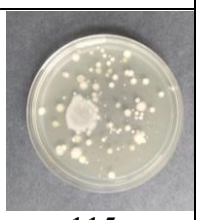
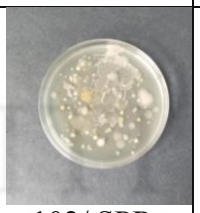
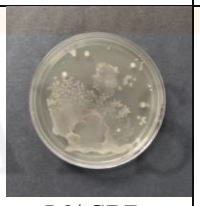
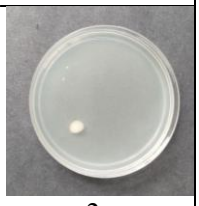
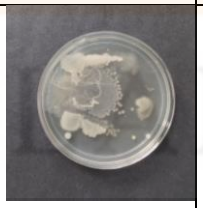
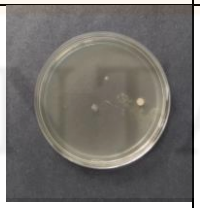
10^{-4}	 69	 20	 54/ SPR	0	 53/ SPR
10^{-5}	 5	0	 29	0	0
10^{-6}	0	0	0	0	0
10^{-7}	0	0	0	0	0
10^{-8}	0	0	0	0	0
10^{-9}	0	0	0	0	0
10^{-10}	0	0	0	0	0

Table A.2: Colonies formed on nutrient agar for 'ulam' samples

Dilution	Pucuk Jenereh	Ulam Raja	Pucuk ubi kayu	Pucuk kaduk	Pegaga
10^{-2}	 151/ SPR	 TNTC	 TNTC	 TNTC	 115
10^{-3}	 54/ SPR	 70/ SPR	 147/ SPR	 102/ SPR	 7/ SPR
10^{-4}	 16/ SPR	 32/ SPR	 56/ SPR	 39/ SPR	 3
10^{-5}	0	 28/ SPR	 3	 3/ SPR	0

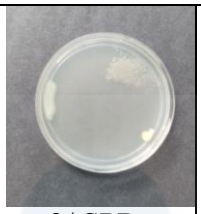
10^{-6}	0	 2/ SPR	 2	0	0
10^{-7}	0	 1	0	0	0
10^{-8}	0	 1	0	0	0
10^{-9}	0	0	0	0	0

Figure A.1: Pucuk jenereh

Figure A.2: Pucuk kaduk

Figure A.3: Pucuk ubi kayu

Figure A.4: Pucuk pegaga

FigureA.5: Ulam raja


Figure A.6: 25 g tomato


Figure A.7: 25 g Chinese okra


Figure A.8: 25 g cassava


Figure A.9: 25 g carrot

Figure A.10: 25 g cabbage