

**MICROBIOLOGICAL QUALITY OF SUSHI SAMPLED FROM
SELECTED FOOD OUTLETS IN KOTA BHARU, KELANTAN**

By

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LIST OF ABBREVIATION

-	To/ until
%	Percentage
/	Over to
=	Equal to
>	More than
^o C	Degree Celsius
TPC	Total plate count
CDC	Centre of Disease and Control
CFU	Colony forming unit
<i>E.coli</i>	<i>Escherichia coli</i>
EMB	Eosin methylene blue
G	gram
HUSM	Hospital Universiti Sains Malaysia
RTE	Ready-to-eat food
TCC	Total coliforms count
MKKM	Makmal Keselamatan dan Kualiti Makanan
MOH	Ministry of Health
USM	Universiti Sains Malaysia
WHO	World Health Organization

ABSTRACT

MICROBIOLOGICAL QUALITY OF SUSHI SAMPLED FROM SELECTED FOOD OUTLETS IN KOTA BHARU, KELANTAN

As a ready-to-eat food sushi is also prone to microbial contamination and it has been associated with food borne outbreaks. A study was conducted to evaluate the microbiological quality of 30 sushi which were randomly sampled from selected food outlets in Kota Bharu, Kelantan from November 2014 until February 2015. A total of 9 (30%) samples exceeded the proposed guidelines for total plate count and 8 (23.33%) samples for total coliforms count. *E.coli* was identified in 2 (6.67%) out of 30 samples analyzed. All the outlets showed unsatisfactory status in hygiene and food preparation. Two of the outlets showed presence of *E.coli* particularly in the salmon sushi. This indicates that the microbiological quality of sushi sold in the food outlets are far from satisfactory and their safety is doubtful.

Keywords: Sushi, Microbiological quality, Total coliform count, Total plate count and *E.coli*.

ABSTRAK

KUALITI MIKROBIAL SUSHI YANG DISAMPEL DARI PREMIS MAKANAN TERPILIH DI KOTA BHARU, KELANTAN.

Makanan siap untuk dimakan seperti sushi adalah makanan yang memerlukan persiapan yang minimum. Sushi adalah makanan yang mudah terdedah kepada mikroorganisma kerana makanan adalah punca penyebaran penyakit dan penyakit bawaan makanan semakin meningkat dari tahun ke tahun. Satu kajian telah dijalankan untuk menganalisis kualiti mikrobiologikal sushi yang disampel secara rawak dari premis makanan terpilih di Kota Bharu, Kelantan antara November 2014 hingga Februari 2015. Sebanyak 9 (30%) sampel mempamerkan keputusan yang tidak memuaskan bagi ujian total plate count manakala 8(23.33%) sampel menunjukkan keputusan tidak memuaskan untuk total coliforms. *E.coli* juga telah dikesan dalam 2 (6.67%) sampel daripada sejumlah 30 sampel. Semua pasaraya menunjukkan tidak memuaskan dalam kebersihan dan penyediaan makanan. Dua daripada cawangan menunjukkan kehadiran *E.coli* terutamanya dalam sushi salmon. Ini menunjukkan bahawa sushi yang dijual di premis makanan yang dikaji mempamerkan kualiti mikrobiologikal kurang memuaskan dan keselamatannya yang meragukan.

CHAPTER 1

INTRODUCTION

1.1 General overview

Ready to eat foods are defined as edible food without any additional preparation (Mol., *et al*, 2013). There are different types of ready to eat foods that include washed raw fruits and vegetable, cooked animal food, plant foods that require further washing, cooking or other processing, bakery items such as bread, cakes which does not require further cooking for food safety, sushi, sandwiches, kebab, takeaway foods and bakery products. Some products such as dry fermented sausages, salt cured meat and dried meat have been produced in accordance with USDA guidelines and have received a lethality treatment for pathogens (FDA 2009).

Some ready to eat foods are regarded as potentially hazardous. Such foods can support the growth of pathogenic or food poisoning bacteria and must be kept under certain temperatures to minimize the growth of any pathogens that may be present in the food or to prevent the formation of toxins in the food. Ready to eat foods usually include a number of ingredients which may or may not be cooked. Due to the variety of ready to eat foods, the interpretation of microbiological results obtained from testing must account for the method of processing and the individual components of the food. (NSW Food Authority, 2009).

Moreover, storage and cooking temperatures are essential in keeping foods safe from contamination. If the food is kept at temperatures that are suitable for pathogens to grow, it can be contaminated. Therefore food must be kept or cooked in temperature such as freezing or heating to prevent growth of pathogens (Minnesota Department of Health, 2013). Additionally, worker who handle the food products have to practice sanitary steps, such as proper hand washing before and after handling food, after returning from the restroom, eating or smoking.

Foodborne diseases cause by microorganisms can be classified as either intoxication or infection. Food infection occurs whenever a person consume food that are contaminated with live microorganisms (bacteria, viruses, parasites), whereas food intoxication is caused by consuming foods contaminated with oral ingestion of microbial toxins of viable microorganisms or of the toxin in sufficient amount can lead to the development of pathology of the disease (Miliotis and Beer, 2003).

Foodborne diseases cause mild to serious disease in human depending on the health status of the individual. Common symptoms including vomiting, diarrhea, abdominal cramps, dysentery, dehydration and in more serious cases can result in death. Infant, young children, the elderly and pregnant women are more susceptible to foodborne diseases. The majority of foodborne diseases outbreak are caused by poor insanitary preparation and handling of foods as well as poor hygiene practices of food handlers which accounted for more than 50% of food poisoning incidences (Soon *et al.*, 2011).

Moreover, based on the incidence rates for food poisoning by state in Malaysia in 2010, Kelantan showed the highest incidence rate at 142.4 cases per 100 000 people, followed by Terengganu with 107.5 cases and Melaka with 64.2 cases. Meanwhile, Kuala Lumpur recorded the lowest incidence rate with 12.9 cases. In Kelantan, diseases spread through food remain a common and persistent problem that result in morbidity and mortality and one of the main disease concerned is food poisoning (Maizun, 2001). The Kelantan State Health Department had reported that outbreaks of food poisoning cases in Kelantan had risen over the last decade (from year 2000 to 2010) (MOH, 2010).

Sushi is one of the examples of ready to eat foods. Sushi is gaining popularity among Malaysians. It is a famous Japanese delicacy which consists of vinegar rice, raw seafood and other toppings. Sushi may pose high risk of microbial contamination as it contains perishable ingredients and short shelf life. This makes it susceptible to cross contamination as it involves raw and cooked food ingredients. Normally sushi is prepared by using hands or machine for wrapping the ingredients together. Thus this may pose risk of contamination if it is not handled hygienically. The cooking method of sushi does not involve heating or refrigeration which can affect the quality of the rice. This increases the risk of contamination leading to increase in food borne illnesses, (NSW Food Authority 2008). Therefore as these factors have an effect on the microbiological quality of sushi, sushi has to be properly prepared and kept under proper storage temperature.

A recent German study showed that sushi prepared by sushi bars had relatively high aerobic plate count levels as well as counts of *Escherichia coli* and *Staphylococcus aureus*. This could be a result of processing conditions or cooling and hygiene conditions during storage and preparation (Atanassova, *et al*, 2008). In August and November 2004, 2 clusters of diarrhea cases occurred among patrons of 2 affiliated sushi restaurants in Nevada. A stool sample from one ill sushi patron yielded enterotoxigenic [*Escherichia coli*] (ETEC) and a case of as diarrheal in a person who ate at the same sushi restaurant (Jain *et.al*,2004). Furthermore, there was report of the occurrence of *Campylobacter spp* in retailed ready to eat sushi in Kuala Lumpur (Tan, 2008) and the prevalence of *Salmonella* associated with sushi in Singapore. (J.Barralet.,*et.al*, 2004).

These food poisoning incidences have raised safety concern in the consumption of sushi among customers. It is doubtful whether the food purchased is safe for consumption and has been prepared in a hygienic manner as sushi is prepared in advance by the food handlers. Thus the objective of this study is to determine the microbiological quality of sushi and to identify whether the sushi is safe for consumption and free from contamination and food borne pathogens causing food borne illnesses. The results obtained are then compared with the microbiological quality guidelines, (Malaysian Food Guide, 2007).

1.2 Rational of study

This study seeks to determine the microbiological quality of sushi sampled from selected food outlets in Kota Bharu. Sushi which is a ready-to-eat food were chosen in this study as there were many cases of foodborne disease outbreaks due to consumption of the ready-to-eat foods. Based on previous study done in Kota Bharu on the hygienic standard of food premises, unsatisfactory coliform counts were found in 47.8% of total food sampled, that include both the staple and traditional snacks sampled (Zaliha and Muhamad Rusli, 2011).

In this study, several types of sushi were randomly sampled from the outlets. Although sushi is not consumed as part of the main dishes, they are served as snacks or dessert and during tea time and at other time. Usually, it is consumed in small amount. However, if the microbial load is high, it can cause serious health problem to the consumers. Sushi from food outlets may potentially be contaminated due to poor handling and the poor personal hygiene of the workers.

The microbiological analyses chosen for this study were total plate count, total coliforms count and *E.coli*. This is because the presence of high count of either aerobic colony count or total coliforms count can be an immediate concern as many diseases can spread through the environment. The aerobic colony count is the count of viable bacteria based on counting of colonies on nutrient agar plate. The aerobic colony count is usually performed to determine the sanitary quality of foods and the level of microorganism in the food (Food and Drug

Administration, 2013). Higher number of plate count implied low sanitary quality of the foods.

As for total coliforms, it refers to a large group of Gram-negative, rod shaped bacteria that shared several characteristics. The group includes thermotolerant coliforms and also bacteria from fecal origins. Total coliform bacteria and *E.coli* are also used as indicators of microbial contamination because they are easily detected. Moreover, they are more tolerant to adverse environment compared to the disease causing microorganism. Hence, they can be used as bacteriological safety indicators (Hui *et al*, 2002). Thus, it is important to carry out all the detection of the indicator organisms to inspect the levels of microorganisms in sushi and to ensure it is free from hazardous microorganisms and safe to eat.

1.3 OBJECTIVES

The objectives of this project were:

General Objective:

- a) To determine the microbiological quality of sushi sold in selected food outlets in Kota Bharu, Kelantan.

Specific Objectives:

- b) To determine total plate count and total coliforms count in sushi samples.
- c) To determine the presence of *E.coli* in sushi samples.

1.4 Research conceptual framework

The research conceptual framework is shown in Figure 1. Sushi is a type of ready-to-eat (RTE) foods that are usually consumed by the locals. In this study, the various types of sushi such as tamago sushi, kani salad, kani sushi, ebiko sushi, tobiko sushi, octopus sushi, salmon sushi, unagi sushi, golden ball, corn mayo, egg mayo and ebi tempura were sampled from three food outlets in Kota Bharu, Kelantan. The samples were collected by simple random sampling. Thirty samples (n=30) were collected over a period of two months. Microbiological analyses by total plate count, total coliform count and *E.coli* were done. The result of the microbiological analyses were compared in compliance to the Microbiological Guidelines for Ready-To-Eat Food and Malaysia Food Act 1985 where total plate count should be below 100 000 colonies counts per 25 grams of sample, total coliforms below 100 colonies count per 25 grams of sample and lastly no presence of *E.coli* for ready-to-eat foods to be satisfactory and safe for consumption.

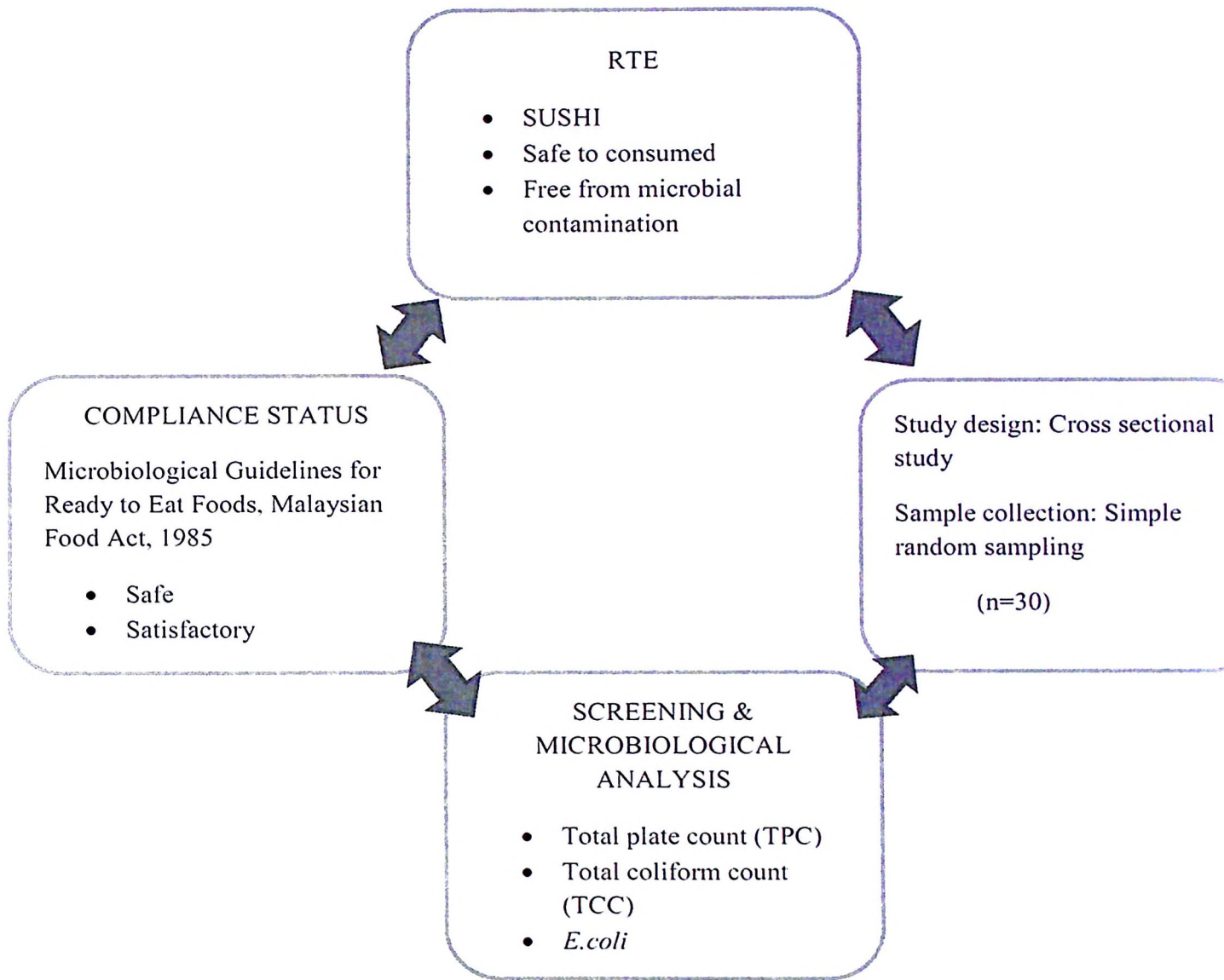


Figure 1: Research conceptual framework

CHAPTER 2

LITERATURE REVIEW

2.1 READY TO EAT FOODS

Ready to eat foods can be described as the status of foods being ready for immediate consumption at the point of sale. Different terms have been used to describe such ready to eat foods. These include convenient, ready, instant and fast foods. Examples of such ready to eat foods include pastries, meat pie, sausage, rolls, burger, moin-moin, salad or coleslaw, fried meat, fried chicken, refrigerated seafood product such as sushi, pre-packed raw vegetables or deli type salads such as coleslaw and tuna, milk and milk products. In other words, ready to eat foods are edible food without any preparation to achieve food safety and it could be raw or partially cooked (FDA 2013).

Ready to eat foods have increased in terms of consumption due to changes in people's lifestyle and global trade food distribution. These foods are popular and has acceptance among consumers in Malaysia because they are easily accessible and can be eaten right away or only require minimal heating process. Moreover, they can be bought at reasonable and affordable prices. Another reason why consumers prefer buying convenience food rather than making their own food is that they are also readily available and can be obtained almost everywhere

such as restaurants, hypermarkets, and stalls (Harvard School of Public Health 2014). Some of the foods also play vital role in the nutritional supply as it provides an opportunity for consumers to meet their daily requirements.

2.2 SUSHI

Sushi is an example of ready to eat food. Sushi, originates from Asia and is not only popular among the Japanese but has also gained popularity worldwide. It is rich in high quality protein and an excellent source of omega-3 fatty acids because of the seafood ingredients (Cysneiros *et al.*, 2009). The most common toppings are vegetables and seafood such as salmon, fish roe, tuna, crabstick and many more. Since sushi is often served raw, no cooking fat is introduced during its preparation. It is low in fat, concentrated with nutrients, and delicious to consume; therefore it became very popular all over the world (Simpson *et al.*, 2008). People also enjoy the fact that a typical serving of sushi, about 8-10 pieces, is around 350-400 calories (Cysneiros *et al.*, 2009).

The term “sushi” refers to food consisting of cooked rice flavored with vinegar, sugar and salt, and garnished with seafood, roe, and vegetable which may or may not be wrapped with seaweed (Anon, 2000). It is eaten cold, and most of the toppings are raw seafood. Nowadays, there are many different types of sushi in the market. One of them is nigiri which is small rice balls with fish, shellfish, tuna, shrimp and many more. Gunkan are small cups made of sushi rice

and dried seaweed filled with seafood. Norimaki is sushi rice and seafood ingredients rolled in dried seaweed sheets. Temaki are cones made of nori seaweed and filled with sushi rice, seafood and vegetables (Japanese Food 2009). Normally, sushi is eaten with sauces such as soy sauce and wasabi to enhance the taste of sushi.

Sushi exhibits distinct features that are associated with microbiological hazards. If storage temperature is not properly maintained, it could contribute to growth and persistence of microorganisms (Anon, 2000; Cysneiros *et al.*, 2009). Thus, microbiological assessment of sushi provides information regarding the product safety.

Sushi is usually prepared and served at the same time. However, it is necessary to consider industrial production due to the high consumer demand (Simpson *et al.*, 2008). In recent years, selling of sushi in supermarkets is easier through sushi machines. Sushi machines provide the mass production of sushi combining the delicate skills used by sushi chefs, therefore making and selling sushi more accessible to countries around the world (Cysneiros *et al.*, 2009). Pre-packed sushi and sashimi are available in the supermarkets, shops, or in shopping centers. Importers, food manufacturers, restaurant owners and even supermarket entrepreneurs have been attracted to participate in these varieties of business (Anon, 2000). In this context, keeping the quality of this product become important. (Mol *et al.*, 2014).

In addition, for potentially hazardous foods that have been cooked and cooled, such as some of the ingredients used in making sushi, businesses that wish to employ the 4-hour/2-hour rule must also be able to demonstrate that the food is cooled safely, in accordance with Standard 3.2.2 and record the time at which the food is removed from refrigeration and the time at which it must be discarded. (NSW, 2008). The 4 hour/2 hour rule is known as any ready-to-eat potentially hazardous food, if it has been at temperatures between 5°C and 60°C: for a total of less than 2 hours, must be refrigerated or used immediately, and for a total of longer than 2 hours but less than 4 hours, must be consumed immediately; or and for a total of 4 hours or longer, must be discarded (FSANZ, 2001).

The safety of sushi is largely dependent on the initial microbiological quality of the ingredients used in preparing the sushi, and the implementation of good hygienic practice and process control to ensure that contamination does not occur during preparation. A lack of control over any of these factors may contribute to an increase in risk of contamination of the finished product. (NSW, 2008). Therefore, proper hygiene practices and handling of food should be practiced to prevent contamination of food. It is necessary to practice food safety programs such as Good Manufacturing Practices (GMP) and Hazard Analysis Critical Control Point (HACCP) by the manufacturers before and during food processes.

2.2.1 Common microorganisms found in sushi

A food safety hazard refers to any agent with the potential to cause adverse health consequences for consumers. Food safety hazards occur when food is exposed to hazardous agents which result in contamination of that food. Food hazards include biological, chemical and physical hazard. Hazards may be introduced into the food supply at any time during harvesting, formulation and processing, packaging and labeling, transportation, storage, preparation, and serving. (Health Canada, 2014). These hazards make food undesirable for consumption.

Biological hazards occur when hazardous or pathogenic organisms are introduced to food and thus pose food safety concern to consumers. Biological hazards include bacteria, viruses and parasites of public health significance. Biological hazards can be introduced to food from the environment (e.g. soil bacteria, agricultural run-off) or from inadequate sanitation practices and cross contamination during transportation, handling, processing, and storage (e.g., poor food hygiene practices). The type and magnitude of microbial growth is determined in part by the nature of the food, package conditions and storage environment. The bacteria associated with food borne illnesses included *Escherichia coli*, *Staphylococcus aureus*, *Shigella*, *Clostridium perfringens*, *Clostridium botulinum* and *Salmonella spp.*

Chemical hazards occur when chemicals are present in foods at levels that can be hazardous to humans. Contamination may occur through various pathways

including the environment (e.g., air, soil and water), intentional use of chemicals, such as pesticides and veterinary drugs, manufacturing processes, and addition of food additives. Physical hazards are any additional or foreign matter normally not present in food which could cause injury, psychological trauma and harm to human health. Examples of physical hazards are metal, glass and wood.

2.2.2 Sources of Sushi Contamination

Potential sources of contamination should be considered. In particular, primary food production should not be carried on in areas where the presence of potentially harmful microorganisms would lead to an unacceptable level of such microorganisms in food. Sources of food safety hazards can be cause from many sites including food handlers, raw materials, packaging materials, processing methods, rodents and insects, garbage and sewage, air, water and soil contamination and also utensils that have been used. So, if food is contaminated by one of these factors it can lead to food poisoning, infection or diseases. One of the major causes that plays important role in food safety is food handlers. The person who is in charged in preparing food must follow proper hygiene in order to handle the food hygienically. They should wear proper attire, gloves and aprons, hand washing before and after toilet breaks. Workers must wash their hands between handling raw food and cooked or ready-to-eat foods. If workers wear gloves, they must remember that if the gloves become contaminated from handling raw food or other sources of contamination, they must change the gloves

and wash their hands before putting on new gloves. Furthermore, if foods are not properly handled, the microorganisms from hands of food handlers tend to be transferred to food. So this situation will cause the food to be unsafe for consumption (Caerphilly Food Safety, 2014).

In addition, improper processing method can also cause cross contamination. Cross contamination occurs when bacteria and viruses are transferred from a contaminated surface to one that is not contaminated. The bacteria and viruses can come from people, work surfaces or equipment, and other foods. For example, it can happen when bacteria from the surface of raw meat, poultry and raw vegetables with visible dirt (such as unwashed potatoes), are transferred onto ready to eat food, such as green salads, rice or pasta salads, cooked meats or poultry or even fruit. The bacteria on the raw food are killed when the food is cooked, but ready to eat food is eaten without further cooking. (Food Safety Knowledge Network 2009).

Apart from that, utensils and equipment should be washed and clean before and after use. If fail to do so it will cause risk in food safety. The person in charged must avoid using the same work surfaces, area and equipment if possible. They should use separate equipment and work surfaces for handling raw food and cooked or ready-to-eat food and raw food. Next, they also must make sure that all equipment is thoroughly washed and disinfected after using it for raw foods. It is important for them to check that cleansing is taking place effectively. Besides, cloths also can carry bacteria so it is essential that they are clean and changed regularly. Dirty cloths will spread contamination from one area to another and

can easily cause cross contamination. As wiping cloths often remain wet and warm for long periods of time, they can transfer high levels of harmful bacteria. It is best to use disposal cloth or paper during any food preparation. If they use any clothes during food preparing sessions they should clean and disinfect them by washing them at a high temperature (boil wash or machine wash at 65°C or above) or by using chemical disinfectants.

2.2.3 Sushi and its Safety

NSW Food Authority has conducted a study to investigate the effect of non-refrigeration display of sushi has on the product safety. The study has concluded that the product needs to be displayed for longer than 4 hours at 25°C or higher for bacteria to grow. Therefore, the business that sells sushi has to display sushi at temperature greater than 5°C for no more than 4 hours followed by following guidelines:

- a) Requirements of Food Standards Code have to be followed during the receipt, preparation and storage of raw materials and sushi.
- b) Acidification of rice to a pH less than or equal to 4.6 to inhibit growth of food poisoning bacteria and the pH must be recorded.
- c) Implementation of a system to monitor the length of time sushi can be displayed at greater than 5°C.
- d) Sushi is never displayed at temperatures above 25°C.

According to the study which have been conducted in Hong Kong, where 1020 samples of sushi and 906 samples of sashimi (slices of raw fish with seafood without rice and with a dipping sauce) from 1997 through 1999 were analysed for microbiological assessment. Among these samples, 13.8% of sushi and 11.1% of sashimi were unsatisfactory in terms of their hygienic quality and 0.26% of all samples yielded specific pathogens including one *Vibrio parahaemolyticus*, two *Staphylococcus aureus*, one *Salmonella* species, and one *Listeria Monocytogenes*. Both the product quality as well as safety could be improved by incorporating safety measures throughout the manufacturing process, such as by adopting the Good Manufacturing Practice and Hazard Analysis Critical Control Point system. (Food and Environmental Hygiene Department, 2000).

2.3 Food Poisoning related to Sushi

According to World Health Organisation (WHO), foodborne diseases (FBD) are an important cause of morbidity and mortality worldwide but the full extent and cost of unsafe food, and especially the burden arising from chemical and parasitic contaminants in food, is currently still unknown. Precise information on the burden of FBD is, however, needed to adequately inform policy-makers and allocate appropriate resources for food safety control and intervention efforts.

Food borne diseases like cholera, typhoid fever, hepatitis A, dysentery and food poisoning occur as the results of ingestion of foodstuffs contaminated with microorganisms or chemical. There are many different kinds of microorganisms that cause these diseases such as salmonellosis is caused by *Salmonella*, cholera is caused by *Vibrio cholera* and enterotoxigenic is caused by *E.coli*. The true incidence of food borne disease in Malaysia is not known, however the incidence is low ranging from 1.56 to 0.14 cases per 100,000 population. However, the food poisoning cases is on the rise as evidence by the incident rate of 62.47 cases per 100,000 population in 2008 and 36.17 in 2009 (Sharifa, *et.al*, 2013).

The rapid population growth and demographic shift toward ageing population, changing eating habit such as consumption of raw or lightly cooked food, long storage of such food, lack of education on basic rules of hygienic food preparation and food trading without appropriate microbiological safety procedure become contributing factors for food borne diseases. Food borne disease in Malaysia is in the rise and the direct and indirect cost management of FBD will become one of the most common issues to face by the government. The world is spending millions and millions in cost of treatment due to food borne diseases. (Sharifa Ezat W.P., *et.al*, 2013)

In Malaysia, the reported food and waterborne diseases in 2009, e.g. cholera, dysentery, typhoid and Hepatitis A were low, ranging from 0.14 to 1.07 cases per 100,000 populations. In Malaysia, the main contributing factor was identified as insanitary food handling procedures which accounted for more than 50% of the poisoning episodes (MOH, 2007a). For example, in January 2008

alone, thirty incidents of food poisoning and one food intoxication were reported. Most of the implicated food settings occurred in schools and academic institutions' food preparation premises and inappropriate food handling practices, meals prepared too early and kept at ambient temperature until served and unhygienic practices were the causes of food poisoning cases. A total of 997 food poisoning cases were reported throughout the country from 1st January to 2nd February 2008.

Sushi is traditional Japanese food, mostly consisting of raw seafood alone or in combination with rice. Eating sushi has become very popular not only in Japan but among many countries. Sushi is prepared with cold cooked rice acidified with vinegar, and it is shaped into bite-sized pieces and topped with raw or cooked fish, or formed into a roll with fish, eggs or vegetables and wrapped in seaweed. (Atanassova *et al.*, 2008). Sushi that contains fish such as sardine, mackerel and herring will be high in omega-3 fatty acids which help in the prevention of heart disease and stroke. The rice used in sushi is high in carbohydrates and gluten-free. Furthermore, sushi is also a good source of protein (Institute of Food Technologies 2011).

Sushi is ready-to-eat (RTE) food and regarded as a potentially hazardous. Presence of foodborne diseases related to sushi consumption have been reported (Adams *et al.*, 1994; Atanassova *et al.*, 2008; Barralet *et al.*, 2004; Food and Environmental Hygiene Department HKSAR, 2000; MAsotti *et al.*, 2004; Millard and Rockliff, 2003; NSW Food Authority, 2006). Pathogenic bacteria such as *Salmonella spp.*, *Staphylococcus aureus*, *Vibrio parahaemolyticus*, *Vibrio*

cholera, *Listeria monocytogenes*, *Bacillus cereus* can occur in materials used for their preparation such as fish, seafood products, vegetables and rice.

Food poisoning cases in sushi has been reported around the world but rarely in Malaysia. There was an occurrence of *Campylobacter spp* in retailed ready to eat sushi in Kuala Lumpur (Tan, 2008). Furthermore, an outbreak of enterotoxigenic *Escherichia coli* has been found to be associated with sushi restaurant in Nevada, 2004. This was due to poor food handling practices and infected food handlers (Seema, 2008). Another case that was reported is outbreak in Sushi King in Bentonville in 2006 due to *Salmonella typhimurium* and the restaurant was closed. The symptoms were gastroenteritis. It was then found out that the outbreak was due to unsafe food handling, temperature abuse and improper storage and sanitation (Clark, 2006). A recent German study showed that sushi prepared by sushi bars had relatively high aerobic plate count levels as well as counts of *Escherichia coli* and *Staphylococcus aureus*. This could be a result of processing conditions or cooling and hygiene conditions during storage and preparation (Atanassova, *et al*, 2008).

CHAPTER 3

METHODOLOGY

3.1 Study Design

This is a cross sectional study. Thirty (30) sushi were sampled by convenience random sampling at selected food outlets in Kota Bharu, Kelantan. This section of the dissertation described the list of materials and instruments used in the study as well as the sampling method and the microbiological analyses.

3.2 Apparatus, Materials and Equipments

There are a few apparatus and materials used in this study such as bunsen burner, wire loop, petri dish, hockey stick, stomacher bag, pipette and 70% alcohol. Stomacher was used in sample preparation as a sample homogenizer. The plastic bags or sampling bags were sterilized using 70% alcohol. Besides, an enclosed, ventilated laboratory workspace requiring a Biosafety Cabinet (BSC) chamber for safety working with materials that are suspected to be contaminated with pathogens. It is essential that the apparatus used should be in sterile condition to reduce risk of contamination. Therefore apparatus such as Duran bottles, measuring cylinder, beakers, and micropipette tips were sterilized using the autoclave machine before conducting the experiment. Moreover the lab bench and laminar flow was wiped with 70% ethanol for sterilization. Media such as nutrient agar, eosin methylene blue agar and Mac Conkey agar were prepared according to standard procedures and were used to culture the selected

microorganisms. Incubators with various temperatures were used to incubate the culture media.

3.3 Sample collection

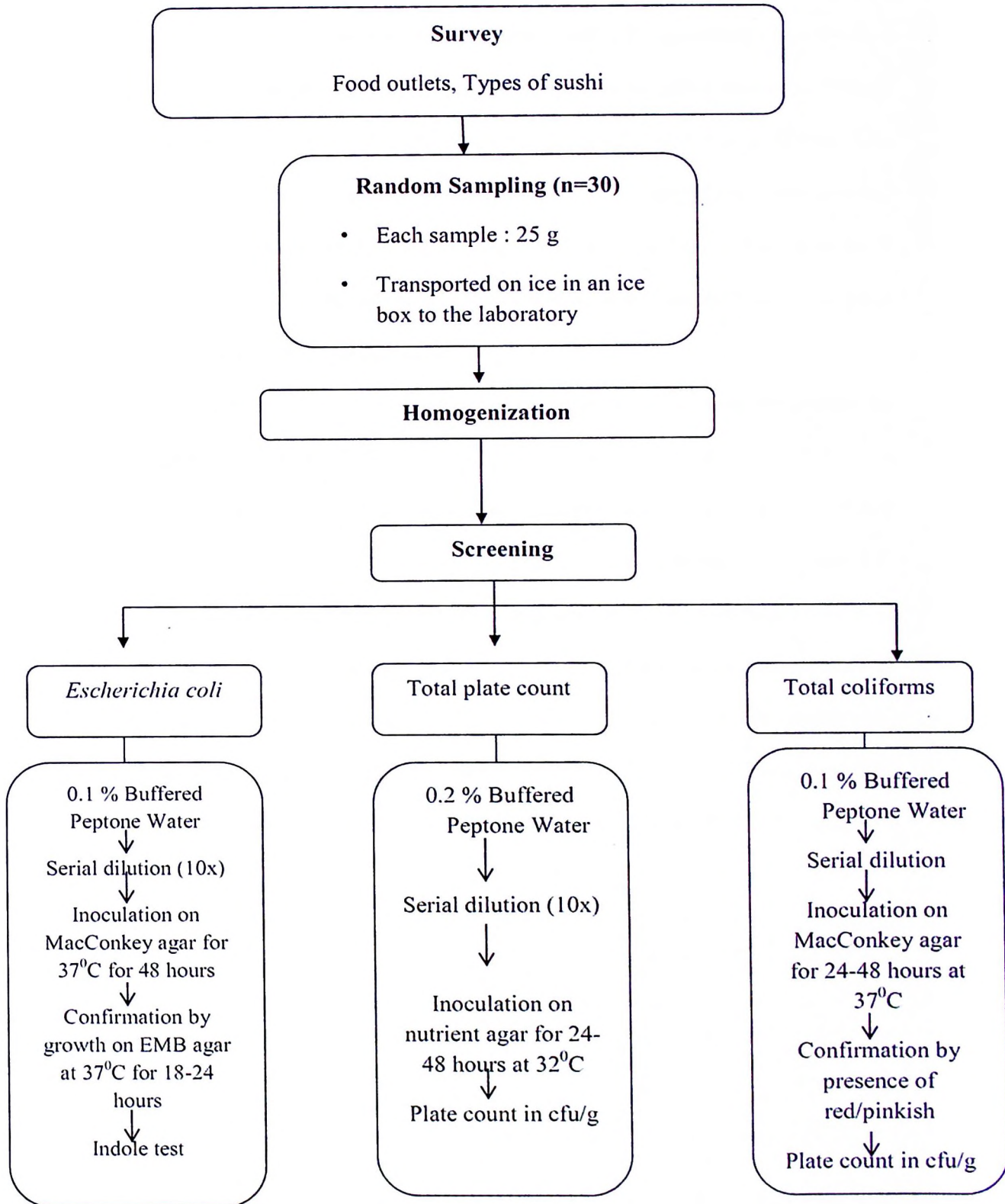
The samples used in this study consisted of sushi. The samples were purchased from food outlets in Kota Bharu and put into sterile plastic bags and sealed it. They were all stored in an ice box while it is transported to USM laboratory for maintenance of samples condition and quality and to prevent spoilage.

3.4 Media and chemical preparations

The preparation of the media use in this study is prescribed according to manufacturer`s instruction. These media solutions were prepared and there were also some chemicals prepared which is buffered peptone water for serial dilution in proportions shown in the table in appendix.

The media and chemical powders were added into the bottles and water was added slowly and gradually. The solution was shaken vigorously to obtain a well-mixed solution and to prevent clumping of powder. Once prepared, they were then autoclaved. When sterilisation was done, they were then kept in water bath at 50°C before pour into petri dishes.

3.5 Flow chart



3.6 Sample preparation

Twenty five gram of each sample was taken for processing. The food homogenate was prepared for serial dilutions and pH adjustment. Twenty five grams (25g) of sample was weighed and transferred into sterile stomacher plastic bag that contains 225ml of 0.1% of buffered peptone water as a diluent. The plastic bag was then inserted into the stomacher. The sample was homogenized by using stomacher to produce the homogenized solution. Next serial dilutions of 10^{-2} until 10^{-6} were prepared for the determination and enumeration of total plate count, total coliform count and *E.coli*.

The spread plate was used for the enumeration of microorganism by counting the colonies which grew in the plate. Prior to culturing, the plates will were labeled with sample code number and dilution factor. Then 100 μ l of diluted sample solution was pipetted onto three plates for each sample; 10^{-2} until 10^{-6} dilutions. A hockey stick was used to spread evenly the diluted suspension over the surface on the appropriate medium plate. The plates were then incubated. After the incubation period, all the colonies that grew on the plate were considered for the enumeration. Plates with less than 50 or more than 250 colonies will not be counted. Each colony forming units (CFU) represents a bacterium that was present in the diluted sample.