AEROBIC BACTERIAL, COLIFORM, *ESCHERICHIA COLI*, AND *STAPHYLOCOCCUS AUREUS* COUNTS OF RANDOMLY SELECTED STREET FOODS IN KAMPAR, PERAK

By

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ABSTRACT

AEROBIC BACTERIAL, COLIFORM, *ESCHERICHIA COLI*, AND *STAPHYLOCOCCUS AUREUS* COUNTS OF RANDOMLY SELECTED STREET FOODS IN KAMPAR, PERAK

CHEONG JUN SEE

Street foods are rapidly rising in number due to their cost, accessibility and variety of choices. However, the safety of foods are not tightly regulated by the government which might put public health at risk, leading to food-borne illness. The mobile shops used by the street sellers, food handlers without proper training; lacking of basic infrastructure; and the surrounding environment are factors contributing to unpredictable level of street food safety. In Malaysia, there is lacking of street-food associated disease information. Therefore, this study was undertaken to obtain data on the microbiological quality of randomly selected street foods in Kampar, Perak and to compare the distribution of microbial loads among different classes of the street foods. A total of 30 street food samples (18 pre-cooked, 4 freshly cooked, 5 raw, and 3 deep-fried) were collected and analyzed for aerobic bacterial, coliform, *Escherichia coli* and *Staphylococcus aureus* counts. The hygienic quality of the foods were evaluated by comparing to the standard permitted by the Ministry of Health Malaysia and other international

guidelines such as $\geq 10^5$ cfu/g for aerobic plate count, $>10^3$ cfu/g for coliform, $>10^2$ cfu/g for *E. coli*, and $\geq 10^3$ cfu/g for *S. aureus*. *E. coli* was detected in 5 food samples that had counts ranged from 10^3 to 10^5 cfu/g. Whereas, *S. aureus* was detected in 11 food samples mainly from the pre-prepared and raw food category. Pre-prepared street foods had the highest microbial loads, followed by raw street foods and freshly cooked foods. All the deep-fried foods tested in this study showed no indicator organisms and pathogenic organisms. In this study, 56.67% of the street foods were found to be unsuitable for consumption and the prevalence of *E. coli* and *S. aureus* significantly indicating a potential risk to consumers in Kampar.

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DECLARATION

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

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(CHEONG JUN SEE)

APPROVAL SHEET

This project report entitled "**AEROBIC BACTERIAL, COLIFORM, *ESCHERICHIA COLI*, AND *STAPHYLOCOCCUS AUREUS* COUNTS OF RANDOMLY SELECTED STREET FOODS IN KAMPAR, PERAK**" was prepared by Cheong Jun See and submitted as partial fulfilment of the requirements for the degree of Bachelor of Science (Hons) Microbiology at Universiti Tunku Abdul Rahman.

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PERMISSION SHEET

It is hereby certified that **CHEONG JUN SEE** (ID No: 13ADB07285) has completed this final year project entitled "**AEROBIC BACTERIAL, COLIFORM, *ESCHERICHIA COLI*, AND *STAPHYLOCOCCUS AUREUS* COUNTS OF RANDOMLY SELECTED STREET FOODS IN KAMPAR, PERAK**" supervised by Dr. Teh Yok Lan from the Department of Biological Science, Faculty of Science.

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

Yours truly,

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(CHEONG JUN SEE)

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LIST OF ABBREVIATIONS

APC	aerobic plate count
CDC	centers of disease control and prevention
EMB	eosin methylene blue
EPEC	enteropathogenic *escherichia coli*
FAO	food and agriculture organization of the United Nations
FoSIM	food safety information system of Malaysia
ICMSF	international commission on microbiological specifications for foods
IR	incident rate
MOH	ministry of health of Malaysia
MPN	most probable number
MSA	mannitol salt agar
NA	nutrient agar
PBS	phosphate buffered saline
SFD	staphylococcal food-borne disease
UTAR	universiti tunku abdul rahman
WHO	world health organization
^{o}C	degree celcius
%	percentage
cfu/g	colony-forming unit per gram
g	gram
L	liter
µg	microgram
ml	milliliter
mM	millimolar

CHAPTER 1

INTRODUCTION

Street foods are becoming common and rapidly rising in number especially in developed and developing countries with high population or urban areas due to their inexpensive, accessibility and variety choices (Manguiat and Fang, 2013). According to World Health Organization, WHO (1996), street foods are foods that are prepared by street sellers and distributed to consumers for consumption directly at the point of sales or at a later time. There are some factors which exposed street foods to public health risks. Firstly, the mobile and temporary shops used by the street food sellers are lacking of basic infrastructure, such as potable water supplies (WHO, 1996). Moreover, most of the street food business activities and the quality of foods are not tightly regulated by the government (Alimi, 2016).

Nowadays, the public has become more concerned about the food safety issues because according to Nkere, Ibe and Iroegbu (2011), contaminated food and water are reported as a major vehicle for the transmission of foodborne diseases or health threats. Therefore, this has caused the public to become more aware and distrustful toward the safety of the associated food product especially street foods (Juneja and Sofos, 2009). Street foods are often

exposed to unsanitary practices which cause unpredictable level of food pathogen that might put public health at risk (Burt, Volel and Finkel, 2003).

Food can become contaminated at any point of preparation. Foods that are improperly prepared or mishandled will lead to food-borne illness, which is defined as disease contracted by ingesting microbiologically contaminated food (Juneja and Sofos, 2009). Besides, foods might also be contaminated by environmental microorganisms from sewage, air, soil, water, equipment, and packages (Ray and Bhunia, 2008).

Many foods provide a favorable environment for microbial growth. Some bacteria may contaminate the food without showing visible changes to the food appearance, taste and smell. However, their overgrowth on foods is associated with food spoilage and may cause foodborne disease or illness (Ray and Bhunia, 2008).

Microbiological quality is a key factor in assessing the quality and safety of food products as well as the personal hygiene level of food handlers. For instance, high *Escherichia coli* count and the degree of coliform contamination in food products reflect the poor sanitation level of food handlers during food preparation and handling (Sangadkit et al., 2012).

Street-food associated disease information is lacking because most of the cases are sporadic and always not reported (Chye and Lim, 2002). This study was

undertaken to obtain data on the microbiological quality of randomly selected street foods in Kampar, Perak. It is impossible to monitor and detect food samples for every possible presence of pathogenic microorganism. Therefore, the street foods were assessed for the presence of aerobic bacteria; indicator microorganisms such as coliform and *Escherichia coli*; and pathogenic microorganism such as *Staphylococcus aureus*. The data provide information on the possible microbial hazards associated with the street foods sold around Kampar area.

Hence, the objectives of this study were:

- To generally assess the microbiological quality of randomly selected common street foods in Kampar, Perak;
- To compare the microbial load for different classes of street foods.

CHAPTER 2

LITERATURE REVIEW

2.1 Background of food safety legislation in Malaysia

Food safety in Malaysia is not only the responsibility of a single authority but required the effort for all relevant authority such as government, industries, producers, academia and consumers. Malaysia is continuously searching for strategies to improve the food safety and constantly formulating and revising food laws, regulations and standards in order to meet with the international requirements, strengthening law enforcement, promoting certification, and increasing participation in international activities related to food safety **(**FAO, 2004).

The Food Act 1983 and the Food Regulations 1985 are the Malaysian food legislations established to ensure food is safe at the time of human consumption (Department of Standards Malaysia, 2012). These two legislations replaced the Sale of Food and Drug Ordinance and Regulations 1952. The main objective of the Food Act 1983 and the Food Regulations 1985 is to make sure the health of the public is protected (FoSIM, 2000).

Other than the Food Act 1983 and the Food Regulations 1985, another food legislations known as Food Hygiene Regulations 2009 were established by the ministry of health of Malaysia to control the hygiene and safety of food sold to the public. These regulations state that, it is compulsory for all food handlers to undergo training program and obtain a training certificate (Rosnani et al., 2014).

According to World Health Organization (2015b), more than 200 diseases are spread through food. Every year, there are millions of people fall sick and die because of consuming contaminated food or drinking water. Diarrhea diseases caused by ingesting unsafe food alone kill around 1.5 million children annually. Therefore, continuous revision of the Food Regulations 1985 is conducted by the Food Quality Control Division, Ministry of Health Malaysia to ensure food safety system is tightly regulated (WHO, 2015a).

2.2 Foodborne diseases in Malaysia

The occurrence of foodborne illness in tropical countries such as Malaysia is not uncommon due to the warm temperature and humidity throughout the year. This weather condition support and encourage the growth of most bacteria and even pathogenic bacteria (Abdul-Mutalib et al., 2014). However, the number of foodborne illness incidence reported is lower compared to other countries such as Australia and United States due to the complexity of the reporting

system that results in not reporting for most of the foodborne illness cases in Malaysia (Soon, Singh and Baines, 2011).

Table 2.1 Number of Cases and Incidence Rate of Food and Water Borne Diseases in Malaysia from 2009-2013.

Year	Food Poisoning		Typhoid		Cholera		Dysentery		Hepatitis A	
	Case	IR	Case	IR	Case	IR	Case	IR	Case	IR
2009	10,238	36.2	303	1.1	276	1.0	154	0.5	40	0.1
2010	12,519	44.2	210	0.7	443	1.6	104	0.4	39	0.1
2011	16,292	56.3	242	0.8	586	2.0	44	0.2	496	1.7
2012	13,182	44.9	219	0.8	282	1.0	86	0.3	464	1.6
2013	14,202	47.8	218	0.7	171	0.6	83	0.3	121	0.4

Note: IR - Incidence rate per 100,000 populations

(Department of Statistic Malaysia, 2014)

Table 2.1 shows that food poisoning was the major cause of food and water-borne diseases reported in Malaysia from 2009-2013 as compared to the others such as typhoid, cholera, dysentery and hepatitis A. Food poisoning cases are on the rise with the incidence rate of 36.2 cases per 100,000 population in year 2009 and 47.8 incidence rate in year 2013. The food borne illness outbreak happened in Malaysia are mainly due to the unhygienic food handling practices that contribute to more than 50% of the food poisoning cases (Sharifa Ezat, Netty and Sangaran, 2013). Zulkifle (2007) reported that Selangor state had the highest cases of food poisoning, followed by Perak, Terengganu and Kelantan. In order to overcome this issue, the ministry had

held food safety roadshows and exhibitions to create awareness about the importance of food safety and proper way in food preparation.

The rapid rising of the incidence rate are due to the fast pace of living condition that changes the eating behaviour of the Malaysians. A study was carried out by observing the Malaysian's food consumption behaviour pattern and it was found that the eating habit was no longer attached in the household but shifted to the trend of eating outside (Ali and Abdullah, 2012).

According to Jaspal and Kumaran (2016), a food poisoning outbreak in Batu Gajah, Perak recently had caused 103 people admitted to the hospital with 39 people suffered from severe food poisoning and, a lady had died from food poisoning after she ate food that was contaminated by carbamate, a compound found in pesticides. Furthermore, a food poisoning incident happened at a school in Tapah due to *Salmonella* contamination of chicken curry that were not properly stored (Koris, 2016).

2.3 Risk factors

Small premises with licensed and illegal mobile stalls are more susceptible to food-borne disease outbreak because of the poor environmental cleanliness, food handlers without proper training, and lacking in safe water supply.

According to WHO (2015b), street food vendors are often poorly educated and lack of safe food handling training. Samapundo et al. (2016) reported that most of the street food vendors in Ho Chi Minh city, Vietnam had poor food safety knowledge level and they found that 95% of the street food vendors did not attend any food safety training. Omemu and Aderoju (2008) also revealed that out of 87 street vendors in Nigeria, only 12% of them received knowledge in food preparation through formal training. For instance, an outbreak of food-borne cholera in Penang in 1996 was related to unsanitary food handling practices of the black jelly, ice and nasi lemak sold along the street (Meftahuddin, 2002). A study also reported that food handlers in Putrajaya had limited basic knowledge about food safety, especially regarding the safe storage temperature for cooked foods (Rosnani et al., 2014).

The majority of the street foods is cooked well and served hot, there is less chance for food poisoning to occur. However, the environmental cleanliness around the street premises may contribute to episodes of food poisoning (Makelele et al., 2015). Since most street foods are sold at the roadside, they are exposed to the unfavorable surrounding conditions, such as the presence of domestic animals, and exposure to air or dust (Muyanja et al., 2011). According to Samapundo et al. (2016), dust may become a potential vector in transmission of pathogenic microorganism to the street foods when the utensils and foods were not covered properly.

Furthermore, the inadequacy of safe portable water supply had led to the higher occurrence of food poisoning in Malaysia (Meftahuddin, 2002). A study had reported that most of the street vendors carried their own water to their stalls, which lead to the shortage and contamination of the water (Muinde and Kuria, 2015).

2.4 Coliform

Coliforms are Gram negative rod which can ferment lactose with both gas and acid production. In food microbiology, coliform are indicator organisms that show the possible presence of more harmful pathogens in food, beverages and water. Although coliform are used as the universal food hygiene indicator, they are unable to indicate the presence of specific pathogenic microorganisms (Szita et al., 2003). According to Chye and Lim (2002), there was a significant correlation between coliform and *E. coli*. Their studies showed that tested food samples with higher number of coliform detected will also have higher *E. coli* counts.

Commonly, there are 3 microbiological methods to detect the degree of coliform contamination such as most probable number, lactose fermentation count and *Escherichia coli* count. A study has done to compare the two broad techniques that were commonly applied to detect coliforms in foods and water. Most probable number (MPN) and plate count technique such as lactose fermentation count were carried out on the same food sample. The results

show that there are no significant differences between these two methods (Nkere, Ibe and Iroegbu, 2011).

2.4.1 *Escherichia coli*

Escherichia coli is the indicator bacterium that suggest either direct or indirect fecal contamination. The presence of *E. coli* might not possess health hazards but when they reach an elevated numbers or the presence of certain enteropathogenic or toxigenic *E. coli* strains such as *E. coli* O:157:H7, foodborne illness is more likely to occur (Mhone, Matope and Saidi, 2011). In Bangladesh, enteropathogenic *E. coli* (EPEC) is the major cause of diarrhea and it is easily transmitted through contaminated food and water (Ali, Khan and Saha, 2012). There was a large outbreak of bloody diarrhea in some European countries due to the consumption of ready-to-eat salads that were contaminated with diarrheagenic *E. coli* O104:H4 (Castro-Rosas et al., 2012).

Several studies had showed that most of the street vendors handled and served the foods with their bare hands (Muinde and Kuria, 2015; Samapundo et al., 2016; Omemu and Aderoju, 2008). Hands are the important vehicles in cross contamination and spreading of fecal-oral bacteria such as *E. coli* (Cogan et al., 2002). Harakeh et al. (2005) reported that street foods acted as a suitable medium for the dissemination of antimicrobial-resistant *E. coli* such as Shiga toxin producing *E. coli.*

2.5 *Staphylococcus aureus*

2.5.1 Staphylococcal food-borne disease (SFD)

Staphylococcus aureus is a cluster-liked Gram-positive bacterium that is able to tolerate high salt concentration and grow at minimum nutrient condition (Normanno et al., 2005). *S. aureus* is commonly found on the skin of street vendors and environmental or food contact surfaces. *S. aureus* causes several human illnesses, which include food-transmitted disease. Therefore, *S. aureus* is the common pathogenic microorganism that is often chosen to be detected in order to determine the potential health hazard in ready-to-eat food (Aycicek, Cakiroglu and Stevenson, 2005).

In the Unites States, food-borne disease caused by *S. aureus* was estimated around 250,000 cases and 3000 deaths per year. It was reported that 93% of the cases were caused by errors in food preparation such as insufficient cooking, prolonged exposure of foods to ambient temperature and unclean kitchen utensils (Normanno et al., 2005; Kadariya, Smith and Thapaliya, 2014).

The presence of *S. aureus* or its enterotoxins in foods indicate the lack of sanitation during the preparation of food (Alarcon, Vicedo and Aznar, 2006). Several studies have reported the prevalence of *S. aureus* in various food products such as ready-to-eat meals and meat based products. This indicates that consumers are exposed to risk of Staphylococcal intoxication (Kadariya,

Smith and Thapaliya, 2014). Merson (1973 cited in Aycicek, Cakiroglu and Stevenson, 2005) reviewed that Staphylococcal intoxication had caused 4.4% of fatality rate and 14% of hospitalization rates.

Staphylococcal food-borne disease is commonly caused by heat-stable enterotoxins which are produced by *S. aureus* (Kérouanton et al., 2007). Staphylococcal enterotoxins are produced when *S. aureus* proliferate to more than 10^5 cfu/g. Although heat treatment can remove *S. aureus* in foods, staphylococcal enterotoxins are still able to survive on foods due to their heat resistant characteristic (Huong et al., 2010). A study has shown that most of the *S. aureus* strains can produce one or more than one type of enterotoxins (Normanno et al., 2005). A small dose of enterotoxin ranging from 0.1 to 1 µg can cause illness with gastrointestinal symptoms such as vomiting, nausea and diarrhea (Corry, Curtis and Baird, 2012). For instance, there was an outbreak of staphylococcal food poisoning in United States in July 2012 after a military unit lunch party. Thirteen persons were hospitalized due to gastrointestinal illness after consuming perlo (a chicken, sausage, and rice dish) that contained staphylococcal enterotoxin A (CDC, 2013).

2.5.2 Retention and biofilm formation on food contact surfaces

The ability of *S. aureus* to retain on inert surface such as food contact surface leads to cross contamination of food products. The most commonly food contact surfaces used are stainless steel and polystyrene. Even though food

contact surfaces are usually dry, *S. aureus* are able to tolerate the dry condition of the surface and remain viable for at least 4 days (Kusumaningrum et al., 2003). According to Scott and Bloomfield (1990), *S. aureus* can survive on kitchen utensils, cloths and hands for a few days after initial contact with *S. aureus*.

When *S. aureus* adhere and colonize on food processing surface, they will form an organized community known as biofilm. Formation of biofilms on food processing surfaces may become a continuous source of contamination (Di Ciccio et al., 2015). A study has shown that *S. aureus* found on food processing plants are responsible for foodborne outbreaks related to the consumption of these contaminated foods (Marques et al., 2007).

CHAPTER 3

MATERIALS AND METHODS

3.1 Experimental Design

The overview of this study is shown in Figure 3.1.

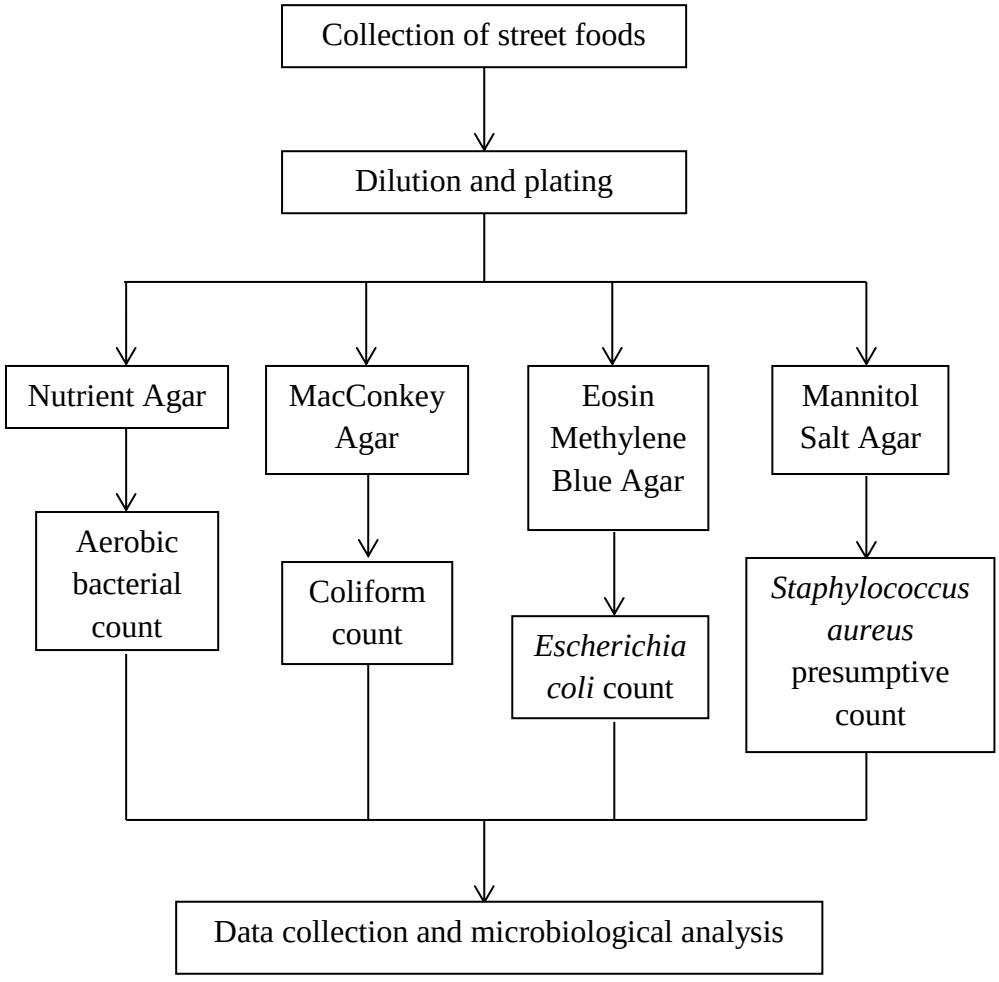

Figure 3.1: Experimental design of this study.

3.2 Apparatus and Equipment

The apparatus and equipment used in this study and their respective manufacturers are listed in Table A1 in the Appendix.

3.3 Chemicals and Media

The chemicals and media used in this study and their respective manufacturers are summarized in Table A2 in the Appendix.

3.4 Preparation of Media and Solution

All media and solutions were autoclaved at 121˚C for 15 minutes unless otherwise stated.

3.4.1 Eosin methylene blue agar

Eosin Methylene Blue Agar is both a selective and differential culture medium. It is selective culture medium for Gram-negative bacteria and against Gram-positive bacteria (Hall, Brown and Lewis, 1967). In addition, EMB agar is commonly used for the isolation and differentiation of coliforms and fecal coliforms. The bacteria which do not ferment lactose appear as colorless colonies while those that ferment lactose will give dark purple colonies. EMB medium assist in visual distinction of *Escherichia coli* that grow with a metallic sheen with a dark center (Lal and Cheeptham, 2007).

Eosin methylene blue agar (EMB) was prepared by suspending 36 g of the EMB agar powder in 1 L of distilled water. After autoclave, when the medium was cooled to 45-50ºC, it was dispensed into petri dishes. All the plates were allowed to cool and solidify at room temperature.

3.4.2 MacConkey agar

MacConkey agar is a selective culture medium for the detection of coliform organisms and enteric pathogens. The selective agents such as crystal violet and bile salt inhibit the growth of Gram-positive microorganisms. Besides, it also provides differentiation between lactose fermenter and non-lactose fermenter. Lactose fermenter will appear as pink colonies on MacConkey agar (Nkere, Ibe and Iroegbu, 2011).

MacConkey agar was prepared by using the same steps as describe for EMB agar but 52 g of the MacConkey agar powder was used instead of EMB agar powder.

3.4.3 Mannitol salt agar

Mannitol salt agar (MSA) is both a selective and differential medium used for the isolation of presumptive staphylococci. Most of the other bacteria are inhibited by the high concentration of 7.5% sodium chloride but *Staphylococcus* species can tolerate high salt concentrations (Kateete et al.,

2010). MSA is a differential medium because it distinguishes bacteria based on the ability to ferment mannitol, the only carbohydrate energy source in the medium. Phenol red acts as the pH indicator for MSA which give an appearance of yellow color when mannitol is fermented and acid is produced which lower the pH of agar (Anderson et al., 2006).

MSA was prepared by using the same steps as described for EMB agar but 111 g of the MSA powder was used instead of EMB agar powder.

3.4.4 Nutrient agar

Nutrient Agar is a general culture medium that contains many nutrients needed for the growth of a wide range of non-fastidious microbes.

Nutrient agar **(**NA) was prepared by using the same steps as described for EMB agar but 28 g of the NA powder was used instead of EMB agar powder.

3.4.5 Phosphate buffered saline (9.57 mM)

Phosphate buffered saline of 9.57 mM was prepared by dissolving 10 tablets of phosphate buffered salt into 1 L of distilled water. The medium can be used immediately after cooling.

3.5 Bacterial Samples

Standard reference strains of Gram-positive bacteria *Staphylococcus aureus* and *Staphylococcus epidermidis* were obtained from the Faculty of Science, UTAR. The reference strains were cultured and maintained in mannitol salt agar (MSA).

3.6 Collection of street food samples

Ready-to-eat street food samples were selected and purchased randomly from street food vendors along the streets and public places around Kampar area in Perak. The samples were collected from the street food vendors at the point-of-sale using the vendors' own packages and utensils. The foods were delivered to the laboratory within two hours under temperature-controlled condition in a cold-box containing ice-blocks. On the other hand, food samples which were collected at night were stored overnight at 4^{o}C and were analyzed the next day with the holding time not more than 16 hours. All the food samples were kept in ice-box until they were analyzed for their microbiological quality.

3.7 Food sample preparation

Food sample was handled and opened aseptically and around 20 g of food sample was transferred into a sterile stomacher bag using a sterile spatula. The food sample was diluted by adding 180 ml of sterile phosphate buffered saline (PBS) to make a ten fold dilution. The mixture was then homogenized in a

stomacher for 2 minutes. Additional ten fold serial dilutions were prepared as required by transferring 1 ml of the homogenate into 9 ml of PBS. The mixture was mixed well with vortex mixer.

3.8 Microbiological analysis

Microbiological examinations were carried out on the collected food samples, which consisted of aerobic colony count, coliform bacteria count, detection of indicator *Escherichia coli* and presumptive examination for pathogenic *Staphylococcus aureus*. The microbiological evaluation of each food sample was carried out twice with an interval of about two to three weeks.

3.8.1 Aerobic colony count

The serial dilutions prepared from 10^{-1} to 10^{-3} dilutions or higher were mixed well and then 0.1 ml of various dilution levels was spread-plated in duplicate on Nutrient agar. The Nutrient agar plates were then allowed to dry before incubated at 37^{o}C for 24-48 hours. Aerobic colony forming units were determined by using a colony counter after incubation and the average aerobic colony count was obtained from the two duplicated plates. The highest dilution that yielded about 25-250 CFUs was used to determine the colony forming units per gram CFU/g of the test sample.

3.8.2 Coliform count

After spread-plated and incubation, MacConkey agar plates with colonies ranging from 25 to 250 with pale pink to dark pink colour were counted using a colony counter. The average coliform count was obtained from the two duplicated plates of the same dilution. Then, the colony count was converted to colony forming units per gram (CFU/g).

3.8.3 *Escherichia coli* detection

After spread-plated and incubation, *E. coli* colonies with green metallic sheen on EMB agar plates were counted visually (Nkere, Ibe and Iroegbu, 2011). Lastly, the colony count was converted to colony forming units per gram (CFU/g).

3.8.4 Presumptive *Staphylococcus aureus* count

After spread-plated and incubation, golden yellow colonies on MSA were counted as presumptive *S. aureus* counts and converted to CFU/g.

3.9 Data collection and analysis

The microbiological tests results collected were compared with available microbiological standard guidelines for ready-to-eat food (Manguiat and Fang, 2013). The microbiological quality of the street foods was compared to the

local microbiological standard guidelines permitted by the Ministry of Health Malaysia and other international guidelines.

CHAPTER 4

RESULTS

4.1 Classification of street food samples

A total of 30 types of randomly selected street food samples were classified into 4 different classes, namely pre-cooked/prepared, freshly cooked/prepared, raw, and deep fried food.

4.2 Aerobic plate count and coliform count of street-food samples

4.2.1 Pre-cooked/prepared street food samples

Eighteen out of thirty of the street food samples were classified into the class of pre-cooked food samples. Foods that were prepared earlier before selling to the consumers were categorized in this class. Table 4.1 summarizes the results of aerobic plate count and coliform count of all the pre-cooked food samples.

All the pre-cooked foods showed the presence of aerobic count and coliform count after 24-48 hours of incubation except for egg tart, kaya puff, kueh cara manis and sugar doughnut. The highest aerobic count was detected in cendol with 1.21 $x10^8$ CFU/g followed by kueh lopes with 2.12 $x10^6$ CFU/g. Whereas, cendol showed the highest coliform count compared to the other pre-cooked

food samples. Figure 4.1 shows the coliform colonies formed on the MacConkey agar for the 10^{-5} dilution of cendol sample.

Table 4.1 Aerobic plate and coliform counts for pre-cooked food sample.

Food samples (n=18)	Mean Aerobic Colony Count ($x10^4$ CFU/g)	Mean Coliform Count ($x10^4$ CFU/g)
Egg tart	0	0
Steamed yam cake	86 ± 59.39	2.70 ± 0.14
Sandwich with raw vegetables	15.8 ± 2.26	2.70 ± 0.85
Sandwich	0.4 ± 0.04	0.56 ± 0.07
Kaya puff	0	0
Nasi lemak	1.82 ± 0.17	1.48 ± 0.28
Nasi lemak (warm during collection)	0.28 ± 0.02	0.26 ± 0.01
Fried bee hoon	82.5 ± 0.08	66 ± 2.83
Kueh talam sagu	142 ± 26.16	115 ± 15.56
Kueh Lopes	212 ± 8.49	19 ± 5.66
Kueh dadar	7.6 ± 0.28	3.2 ± 0.28
Kueh cara manis	0	0
Sugar doughnut	0	0
Herbal jelly	1.17 ± 0.28	0.22 ± 0.04
Steamed layer cake	12.4 ± 0.78	0.7 ± 0.03
Steamed soft cake with red bean	8.7 ± 3.04	5.7 ± 2.19
Pickled vegetable	5.9 ± 0.64	0.29 ± 0.01
Cendol	12100 ± 565.69	6900 ± 141.42

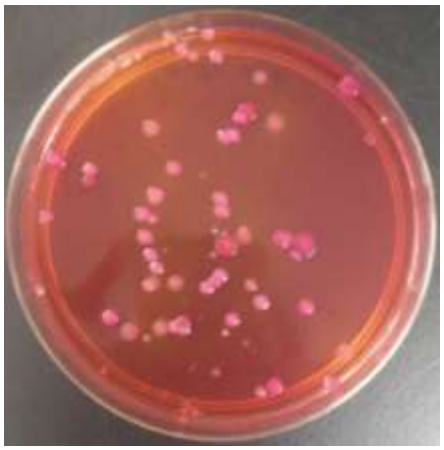

Figure 4.1: Coliforms' growth on MacConkey agar. A mixed culture of coliforms ranging from light pink to dark pink colonies was detected in cendol sample.

4.2.2 Freshly cooked/prepared street food samples

Burger, turnover pancake, pan-fried dumpling and chicken satay were prepared or cooked by the street vendors upon order. Table 4.2 summarizes the aerobic count and coliform count for each food sample in this category. Pan-fried dumpling was the only food sample with the absence of aerobic count and coliform count. Whereas, turnover pancake had the highest aerobic count and coliform count compared to the others.

Table 4.2 Aerobic plate counts and coliform counts of freshly cooked food samples

Food samples (n=4)	Mean Aerobic Colony Count ($x10^4$ CFU/g)	Mean Coliform Count ($x10^4$ CFU/g)
Burger	6.1 ± 0.57	4.9 ± 0.35
Turnover pancake	52 ± 19.80	43 ± 14.14
Pan-fried dumpling	0	0
Chicken satay	0.17 ± 0.06	0

4.2.3 Raw street food samples

Five kinds of raw street foods were collected and their microbial loads are summarized in Table 4.3. All the foods showed the presence of aerobic plate count ranging from 10^4 to 10^7 CFU/g and coliform count ranging from 10^4 to 10^6 CFU/g. The highest aerobic bacterial count and coliform count were found in rojak with 1.03 x10^7 CFU/g and 2.8 x10^6 respectively. The second highest microbial load was found in vegetable spring roll with aerobic bacteria count of 1.56 x10^6 CFU/g of and coliform count of 9.6 x10^5 CFU/g.

Table 4.3 Aerobic plate counts and coliform counts of raw street food samples

Food samples (n=5)	Mean Aerobic Colony Count (x10^4 CFU/g)	Mean Coliform Count (x10^4 CFU/g)
Fresh cut honeydew	43.2 ± 2.83	7.2 ± 6.36
Fresh cut watermelon	6.9 ± 1.34	4.3 ± 1.27
Fresh cut water apple	126 ± 7.78	94 ± 2.83
Vegetable spring roll	156 ± 7.78	96 ± 5.66
Rojak	1030 ± 70.71	280 ± 28.28

4.2.4 Deep-fried street food samples

Three types of deep-fried foods, fried banana, curry puff, and fried spring roll were tested and none of them showed growth of aerobic bacteria and coliform.

4.3 Detection of fecal contamination by *E. coli* in street-food samples

As summarized in Table 4.4, 5 out of 30 types of street-food samples showed the presence of *E. coli*. The 5 food samples were sandwich with raw vegetable, kueh lopes, turnover pancake, cendol and rojak. Cendol had the highest *E. coli* count with 1.88 x 10^5 CFU/g, followed by rojak with 1.57 x 10^4 CFU/g. Figure 4.2 shows *E. coli* colonies with a metallic green sheen on the eosin-methylene blue agar.

Table 4.4 Street-food samples with *E. coli* contamination

Food samples	**Mean *E. coli* count (x10^4 CFU/g)**
Sandwich with raw vegetable	0.2 ± 0.28
Kueh lopes	0.66 ± 0.25
Turnover pancake	0.56 ± 0.79
Cendol	18.8 ± 9.55
Rojak	1.57 ± 0.76

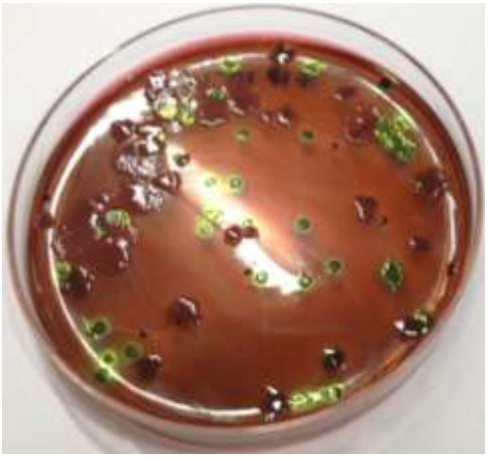

Figure 4.2: Typical metallic green sheen colonies found in sandwich with raw vegetable sample.

4.4 Detection of *Staphylococcus aureus* presumptive count in street-food samples

The foods listed in Table 4.5 were foods tested positive for the presence of *Staphylococcus aureus.* These foods were mainly from pre-cooked and raw street food samples. Figure 4.3(a) shows the presumptive *S. aureus* that grew as yellow colonies on mannitol salt agar and Figure 4.3(b) shows a mixture of *S. aureus* and *S. epidermidis.*

Table 4.5 Presumptive *S. aureus* count on street-food samples

Food samples	Presumptive *S. aureus* count ($x10^4$ CFU/g)
Steamed yam cake	2.65 ± 3.75
Sandwich with raw vegetable	1.45 ± 2.05
Kueh lopes	15.5 ± 2.19
Nasi lemak	0.13 ± 0.18
Kueh talam sagu	21.0 ± 29.70
Kueh lapis	1.70 ± 2.40
Fried bee hoon	1.35 ± 1.91
Cendol	36.0 ± 11.31
Fresh cut honeydew	0.18 ± 0.25
Vegetable spring roll	13.5 ± 1.56
Rojak	2.64 ± 0.48

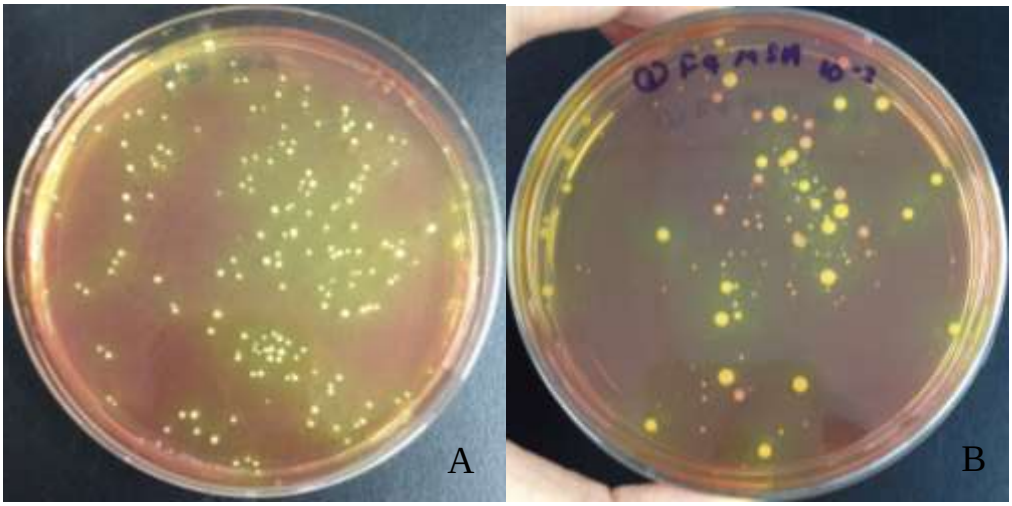

Figure 4.3: Golden yellow colonies of *S. aureus* on MSA (a) *S. aureus* colonies on 10^{-2} dilution plate for vegetable spring roll food sample. (b) Mixture of *S. aureus* and *S. epidermidis* were detected for sandwich with raw vegetable food sample.

4.5 Mean aerobic, coliform, *E. coli* and *S. aureus* count on different classes of street foods

Among the four classes of street foods, pre-prepared street foods had the highest microbial loads for aerobic, coliform, *E. coli* and *S. aureus* count. The class of food with the second highest microbial loads detected was raw foods, followed by freshly cooked foods. Whereas, there was no aerobic bacterial, coliform, *E. coli* and *S. aureus* detected in deep-fried foods. *S. aureus* was only detected in pre-cooked and raw street food samples as shown in Figure 4.4.

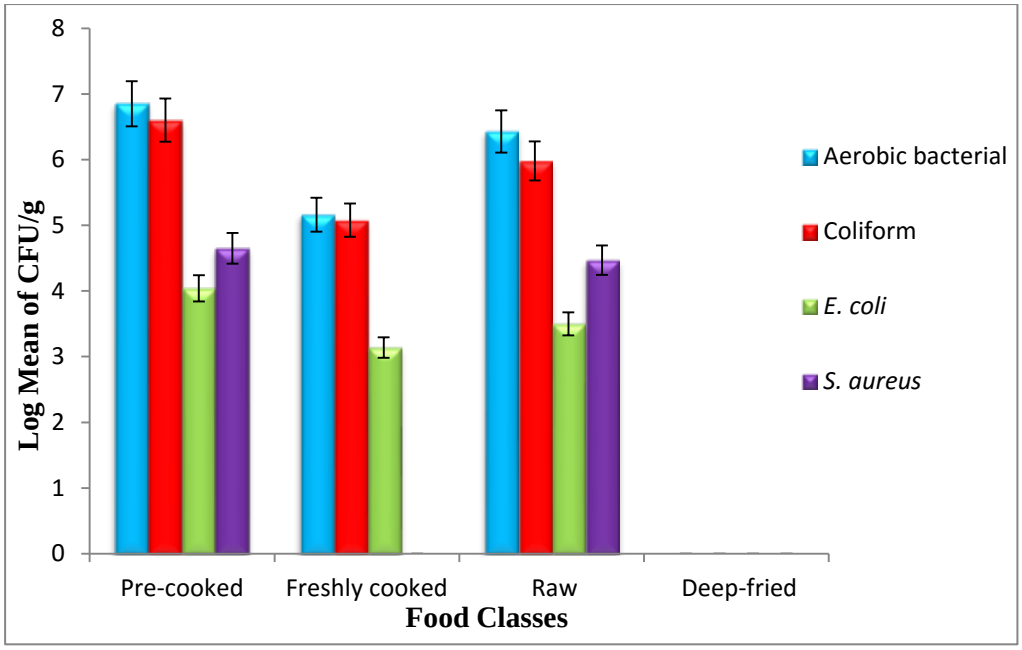

Figure 4.4: The distribution of microbial loads among the different classes of street food.

4.6 Non-compliance of tested street food samples in Kampar

The results collected on the tested foods were compared with the microbiological guidelines or standards imposed by the Ministry of Health of Malaysia (MOH) and the other international standards. As summarized in Figure 4.5, raw street food samples showed the highest percentage of non-compliance to the acceptable limit. Eighty percent of raw food samples showed unacceptable level of total aerobic count. Furthermore, 100% of raw street foods exceeded the acceptable coliform standard. Lastly, 20% and 60% of raw street foods also showed unacceptable level of contamination by *E. coli* and *S. aureus* respectively.

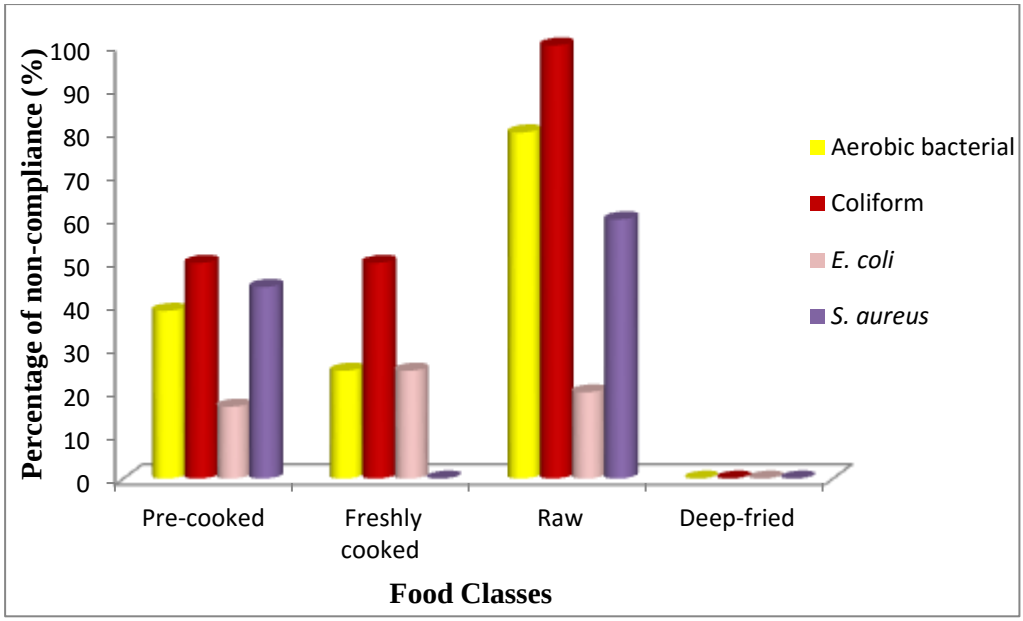

Figure 4.5: The percentage of non-compliance for different classes of street food randomly selected in Kampar.

CHAPTER 5

DISCUSSION

5.1 Overall microbiological quality of street foods in Kampar, Perak.

In order to prevent the occurrence of food-borne disease, it is necessary to ensure that the foods sold to the consumers are hygienic and safe for consumption. The microbial load and the presence of pathogenic microorganisms in food will reflect the food hygienic quality and the associated potential health hazards (Hoque et al., 2015). This study was carried out to evaluate the street foods' hygienic status based on microbiological detection of specific indicator and pathogenic organisms such as aerobic plate count, coliform, *E. coli* and *S. aureus.* They are common tests to examine the microbiological safety of foods. For example, they were tested in several studies to examine the microbiological safety of ready-to-eat foods in Kota Kinabalu, Sabah (Chye and Lim, 2002), Pahang (Jeyaletchumi et al., 2006), Kelantan (Rosmawati et al., 2014), Taiwan (Fang et al., 2003), and the Philippines (Manguiat and Fang, 2013).

In this study, 30 street food samples were tested and 56.67% were found to be unsuitable for consumption because they showed unsatisfactory for either indicator organisms or pathogenic organisms. The remaining 43.33% of food samples were considered to be satisfactory.

According to the results of this study, there were marked differences in the loads of viable bacteria, coliform, *E. coli* and *S. aureus* in different classes of food. Besides, the microbial loads also varied among the individual food samples within the same class.

5.1.1 Aerobic plate count and coliform count

Aerobic plate count, also referred as the total viable count, is one of the most common tests employed to indicate the sanitary quality of the foods (Hall, Brown and Lewis, 1967). According to the aerobic plate count (APC) standard permitted by the Food Act 1983 and Food Regulation 1985 of Malaysia, aerobic plate count above the maximum limit ($\geq 10^5$ cfu/g) is considered unacceptable (FoSIM, 2000). Moreover, based on International Commission on Microbiological Specifications for Foods, ICMSF (1986) coliform counts greater than 10^3 cfu/g is also considered as unacceptable.

In this study, 40% of tested food samples showed unacceptable aerobic plate count that exceeded the permitted limit ($\geq 10^5$ cfu/g). This could be comparable to the findings done in Dhaka city, Bangladesh where 33% of street foods were heavily contaminated with aerobic bacteria (Hoque et al., 2015). The mean APC detected in this study ranged from 0-1.21 x 10^8 cfu/g. Although high APC was detected in some of the street food samples, it does not directly link to health risk (Jeyaletchumi et al., 2006).

The coliform counts detected in this study ranged from 0-6.9 x10^7 CFU/g and more than half (53.33%) of the tested foods did not meet the permitted microbiological standard. This result reflects that most of the street foods in Kampar are in poor microbiological quality and poses a potential health hazard to consumers.

5.1.2 *Escherichia coli* contamination

Escherichia coli counts exceed 10^2 cfu/g is considered unacceptable according to the standard permitted by the Malaysian Ministry of Health (FoSIM, 2000). Out of the 30 food samples tested, 5 of the foods had exceeded the limit permitted and the other 25 foods were free of *E. coli*. Sandwiches contain vegetables that are normally prepared by hands showed the presence of *E. coli*. This result was comparable to that reported by Lopašovský et al. (2016) which showed that most of the sandwiches sold along the street were highly contaminated with *E. coli* due to the insufficient hand washing of the sandwich makers, microflora of the vegetables, and recontamination during cutting (Chye and Lim, 2002).

Besides that, rojak that requires fruit cutting contributed to higher *E. coli* count due to the utensils used such as cutting board and knife that might be contaminated with coliform or *E. coli* (Jeyaletchumi et al., 2006). Moreover, insufficient of portable water supply caused food handlers to reduce the washing of their utensils (Muinde and Kuria, 2015).

5.1.3 *Staphylococcus aureus* detection

Food-borne pathogen, *Staphylococcus aureus* should not be detected in ready-to-eat foods. However the presence of *S. aureus* is considered as unacceptable when it reach a limit of $\geq 10^3$ cfu/g (ICMSF, 1986). In this study, 36.67% of the foods contained unacceptable level of *S. aureus* ranging from 10^3 to 10^5. The presence of *S. aureus* in some pre-cooked and raw foods tested indicated poor hygienic practices of food handlers which caused cross-contamination during food preparation and storage (Öz et al., 2014). Although *S. aureus* is a normal microbiota found on the skin and mucous membrane of food handlers and is normally not harmful, high recovery of *S. aureus* from some of the foods may present a health hazards because it might indicate the possible presence of some pathogenic or toxigenic strains of *S. aureus* (Mhone, Matope and Saidi, 2011)

5.2 Distribution of microbial loads for different classes of street food

5.2.1 Pre-cooked foods

As compared to the other classes of street food, pre-cooked foods had the highest microbial count for all four tested parameters. Pre-cooked foods had mean APC of 7.04×10^6 cfu/g, mean coliform count of 3.96×10^6 cfu/g, mean *E. coli* count of 1.09×10^4 cfu/g and 4.44×10^4 cfu/g for presumptive *S. aureus* count. It could be due to these pre-cooked foods were prepared or cooked earlier by the street vendors in their home before selling to the consumers (Manguiat and Fang, 2013). Some researchers had reported that the

overall hygienic quality of pre-prepared street foods were unsatisfactory in Vietnam (Samapundo et al., 2016) and South Africa (von Holy and Makhoane, 2006). In addition, several studies revealed that pre-prepared foods were more susceptible to the growth of mesophilic microorganisms and most pathogenic microorganisms due to the prolonged hold time between preparation and consumption (Lopašovský et al., 2016), and the pre-prepared foods were left uncovered in plastic containers used by the food vendors (Muinde and Kuria, 2015). During the collection of pre-prepared food samples, it was observed that most of the foods were not handled properly by the food handlers. For instance, the same utensil was used continuously for picking up different types of food without changing or washing. Besides, majority of the pre-prepared foods were not warm during the collection. This indicated that the pre-prepared foods had prepared much earlier before sold to consumers.

5.2.2 Raw street foods

Raw street foods had lower mean APC, coliform, *E. coli* and *S. aureus* counts as compared to pre-cooked foods but had higher mean of microbial loads when compared to freshly cooked foods. Raw foods are most likely to contain more total bacteria since these foods do not undergo any cooking process. The results of this study corresponds with that of a study done by Chye and Lim (2002) which showed that raw vegetables and other raw foods were commonly contaminated by a larger number of microorganisms compared to cooked food. Among the tested raw foods in this study, rojak was the most contaminated followed by vegetable spring roll with a mean APC of 1.03 $x10^7$ and 1.56 x

10^6 respectively. These two foods showed extremely high microbial loads because they were not kept under adequate cold temperature during storage and some were just exposed to the surrounding temperature (Muinde and Kuria, 2015).

5.2.3 Freshly cooked foods

Freshly cooked foods should have lower or absence of microbial loads because most of the microorganisms present in the foods will be killed after cooking (Hoque et al., 2015). However, microbial loads were detected for some freshly cooked foods in this study such as burger and turnover pancake. Detection of APC and coliforms in burger and turnover pancake might due to the usage of raw, processed and pre-prepared sauces or ingredients as toppings (Lopašovský et al., 2016). On the other hand, pan-fried dumpling without the addition of sauces showed absence of aerobic bacterial, coliform, *E. coli* and *S. aureus*. This revealed that sauces or ingredients added had contributed to the presence of microbial loads. Manguiat and Fang (2013) reported that freshly cooked foods may be contaminated by raw or processed sauces such as chili sauces.

5.2.4 Deep-fried foods

Deep-fried food samples in this study had 100% satisfactory rating and were safe for consumption because no indicator organisms and pathogenic microorganisms were detected. The extreme heat treatment applied on deep-

fried food was sufficient to reduce and eliminate the microorganisms in the food (Manguiat and Fang, 2013). However, the results obtained from this study did not correspond with a study done by Acaylar et al. (2013) that showed the presence of microorganisms in deep fried chicken skin that were harmful to human health. It was believe that sometimes deep-frying was inadequate to eliminate the microorganisms from the foods. This difference might be due to several reasons such as different cooking or processing method of the vendors, storage temperature, and the origin of the associated food products.

5.3 Future Studies

A better risk assessment for the street foods could be done by taking samples from the environment where the preparation of food is done because it is closely related to the safety of the food product. Face to face interview of the street sellers about their food preparation process may help in identifying the major possibility of food contamination point during the process. In order to have a better accuracy in microbiological quality analysis of food products, the detection of more food-borne pathogens should be included and the number of food samples collected should be increased.

CHAPTER 6

CONCLUSION

The present study revealed that the majority of the street foods in Kampar are unsuitable for consumption and pose a potential risk of food-borne illness to consumers especially pre-prepared and raw street foods even though there have not been any report of outbreaks related to the consumption of street foods in Kampar. The results of this study suggested that street foods should be tightly regulated and good hygienic practices should be carried out in order to protect the health of consumers and to minimize the microbial contamination of the street foods.

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APPENDIX

The lists of apparatus and chemicals used in this project are as follows:

Table A1: Apparatus and consumables used and their respective manufacturers.

Apparatus and Consumables	Manufacturers
Colonies counter	Stuart
Incubator	Memmert, Germany
Laminar flow cabinet	Esco
Petri dish (90 x 15 mm)	Nest
Stomacher	BagMixer, Copens Scientific
Stomacher bag	BagFilter
Vortex mixer	Stuart
Weighing balance	Kern, Germany

Table A2: Chemicals and media used and their respective manufacturers.

Chemicals and media	Manufacturers
Eosin methylene blue agar	Laboratorious CONDA, Madrid
MacConkey agar	Oxoid Ltd, England
Mannitol salt agar	Laboratorious CONDA, Madrid
Nutrient agar	Oxoid Ltd, England
Phosphate buffered saline	Takara, Japan