# EVALUATION OF MICROBIAL LOAD IN CUT FRUITS SOLD BY VENDORS IN KOTA BHARU

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# Penilaian Tahap Mikrobial dalam Buah-Buahan Potong yang Dijual oleh Gerai-Gerai di Kota Bharu


## ABSTRAK

Produk segar yang dipotong seperti buah-buahan mendapat permintaan yang tinggi oleh pengguna. Buah-buahan segar memberi banyak kebaikan kepada kesihatan tetapi ia juga menggalakkan pertumbuhan bakteria. Buah-buahan segar yang telah dihiris mempunyai kadar respirasi yang tinggi, disebabkan oleh kerosakan tisu buah. Penularan penyakit yang disebabkan oleh bakteria telah dikaitkan dengan buah-buahan seperti tembikai, betik, nanas, tembikai susu dan jambu batu. Faktor yang mempengaruhi tahap mikrobial dalam buahan potong ialah penjual dan peralatan yang tidak bersih, pH buah-buahan, kandungan gula, luas permukaan dan jenis kaedah *plate*. 10 sampel telah dibeli dari dua jenis gerai, untuk menilai tahap mikrobial dalam buah-buahan di Kota Bharu. Kaedah *pour plate* dan *spread plate* telah digunakan untuk mengkultur bakteria. Untuk kaedah *pour plate*, tahap mikrobial adalah dari $5.08 \times 10^2$ hingga $9.00 \times 10^4$ cfu/ml dalam buah yang dijual oleh gerai di kafeteria dan dari $3.36 \times 10^2$ hingga $2.80 \times 10^3$ cfu /ml dalam buah yang dijual oleh gerai di pinggir jalan. Manakala, kaedah *spread plate* mencatatkan tahap mikrobial dari $1.27 \times 10^4$ hingga $3.24 \times 10^6$ cfu /ml dalam buah yang dijual oleh gerai di kafeteria dan dari $7.60 \times 10^3$ hingga $8.10 \times 10^6$ cfu /ml dalam buah yang dijual oleh gerai di pinggir jalan.

## Evaluation of Microbial Load in Cut Fruits Sold by Vendors in Kota Bharu


ABSTRACT

Fresh cut products such as fruits are highly demand by consumer nowadays. Fresh cut fruits promote good health but harbor a wide range of microbial contaminants. Slicing of the fruits may cause tissue disruption, which lead to high respiration rate. Higher respiration rates in fruits may cause metabolism to active, then cause fruits to deteriorate. Outbreaks have been linked with cut watermelon, papaya, pineapple, honeydew and guava. Factor influencing microbial load in cut fruits such as unclean seller and equipment, pH of fruits, sugar content, surface area and plating method. To evaluate the microbial load of cut fruits in Kota Bharu, 10 samples of different fruits were purchased from two vendors. Pour plate and spread plate method have been used to culture microbial load. For pour plate method, microbial load ranged from $5.08 \times 10^2$ to $9.00 \times 10^4$ cfu/ml in cut fruit sold by cafeteria vendor and from $3.36 \times 10^2$ to $2.80 \times 10^3$ cfu /ml in cut fruits sold by roadside vendor. As for spread plate method, microbial load varied from $1.27 \times 10^4$ to $3.24 \times 10^6$ cfu /ml in cut fruit sold by cafeteria vendor and $7.60 \times 10^3$ - $8.10 \times 10^6$ cfu /ml in cut fruit sold by roadside vendor.

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List of Abbreviations

| | |
|---|---|
| NAD | Nicotinamide Adenine Dinucleotide |
| FAO | Food and Agriculture Organization |
| WHO | World Health Organization |
| CDC | Centre for Disease Control |
| O2 | Oxygen |
| NS | Not Stated |
| NA | Not Applicable |
| SPC | Standard Plate Count |
| TNTC | Too Numerous To Count |

# CHAPTER 1: INTRODUCTION

## 1.1 Title

Evaluation of microbial load in cut fruits sold by vendors in Kota Bharu

## 1.2 Background of the Study

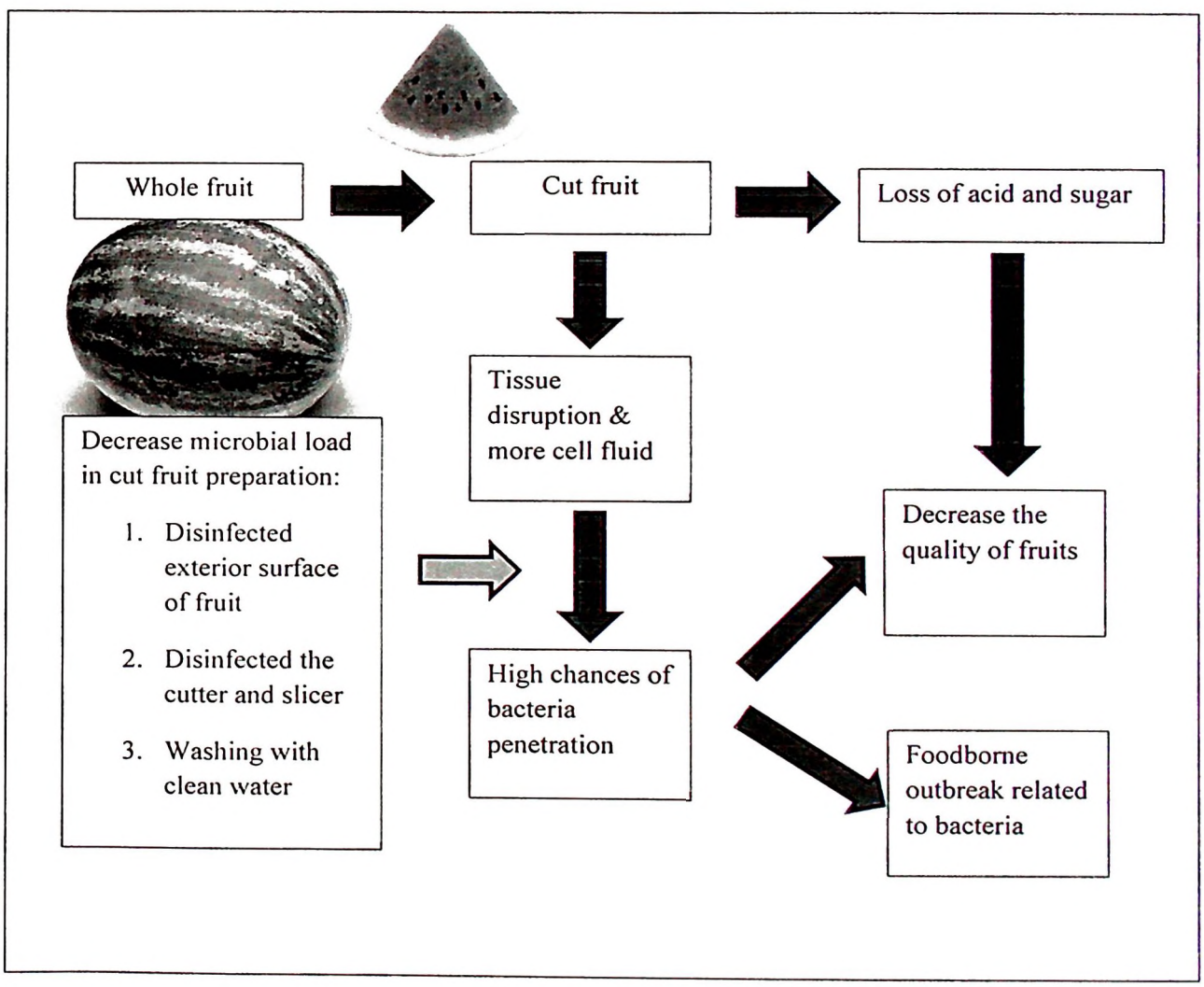
A study was conducted to investigate pattern of fruits and vegetables consumption among adults in Selangor, Malaysia. Study shows that median consumption of fruits among adults in Selangor is 160 g per day, meet WHO recommendation. During fruit season, seasoned fruits sold were cheaper than other fruits. But there is no increase in consumption even fruits sold in lower price (Nurul *et al.*, 2012). Around 2008, percentage of adults consuming fruits in Romania and Bulgaria is 45%, Switzerland is 84% and Italy is 75%. In Denmark, Germany and Mediterranean countries, women consume more fruits than men. Older people aged 65 and above consuming more fruit than young people. But in Romania and Bulgaria, young people consume more fruits. People who have higher educational level ate more fruits than those in lower educational level (OECD, 2012).

Fresh cut products such as fruits are highly demand by consumer nowadays. They prefer fresh cut products because they are fresh, additive free, healthy and nutritious. Tropical fresh cut fruits were consumed by young consumer as snacks. Fresh cut product also decrease wastage in household (James & Ngarmsak, 2010). People choose to buy fruit salad instead of whole fruit because the price was cheaper and more convenient (Brooks, 2014). Fruits contain vitamin and fiber which are essential for human health. Fruit salads consumed by individual were high nutritional quality due to mix of fruits (Brooks, 2014). Fruits also contain vitamin B6, Thiamine,

niacin and minerals. Fruits consumption was proved to reduce risk of cancer and heart disease (James & Ngarmsak, 2010).

The aim of this study was to evaluate microbial load in cut fruits sold by vendors in Kota Bharu, Kelantan, because cut fruit often been associated with bacteria contamination.

1.3 Conceptual Framework (Figure 1)



## 1.4 Problem Statement

Fresh cut product such as fruits have higher respiration rates that can accelerate deterioration and decrease the quality of fruits. Pieces of ripe cantaloupe melon have higher rates of ethylene production than pieces of less ripe fruits (Cantwell & Suslow, nd). It is a main concern because consumer demands for ripe fruits than unripe fruits. Hence, cut fruit will not fulfill demand of customers who are looking for healthy and nutritious food.

Cutting and slicing fruits by using dull knife may destroy more cell and tissue of fruits and may accelerate microbial spoilage (James & Ngarmsak, 2010). Contaminated equipment surface and water used when trimming, cutting, and washing may lead to higher chances of cross contamination (Doyle & Erickson, 2008).

## 1.5 Significance of the Study

The aim of this study was to evaluate microbial load in cut fruits sold by vendors in Kota Bharu, Kelantan. Cut fruits that usually sold by vendors including guava, watermelon, pineapple and papaya. People choose cut fruits because they are fresh and easily accessible. Cut fruits sold by vendors usually exposed to dust and bacteria. High microbial load present in cut fruits may cause foodborne illness. This research is important to increase knowledge of seller on how to decrease microbial load during cut fruits preparation. Knowledge about on how to choose fresh and quality fruits also will be discussed in this research. By doing this research, the quality of life would be better.

## 1.6 Objectives

2.1 General objective: To evaluate microbial load in cut fruits sold by vendors

2.2 Specific objective:

1. To determine whether cut fruits sold by 2 different type of vendors (cafeteria and roadside) having different in microbial load.
2. To observe whether different type of fruits sold by vendors having different number of microbial load.
3. To determine whether microbial load in cut fruit is increasing with pH of fruit
4. To observe whether different type of plate count method affecting microbial load in media

## 1.7 Hypothesis

1. $H_{A1}$: There is a mean difference in number of microbial load between cafeteria vendors and roadside vendor.
2. $H_{A2}$: There is a mean difference in number of microbial load between 5 types of fruits (guava, pineapple, papaya, honeydew and watermelon)
3. $H_{A3}$: Microbial load in cut fruits is increasing with pH of cut fruits
4. HA4: There is a mean difference in number of microbial load between two type of plate count method (pour plate and spread plate method)

# CHAPTER 2: LITERATURE REVIEW

## 2.1 Fruit consumption by Malaysian and Other Countries

A study was conducted to determine pattern fruits and vegetables consumption in Malaysia. Fruit and vegetables consumption in Malaysia was lower compared with other countries (Khairunnisa *et al.*, 2013). Study conducted in Malaysia revealed that 78% respondents consumed less than five servings of fruit and vegetables (Hall *et al.*, 2009). Research also conducted by collecting data from 52 countries in order to study the prevalence of low fruits and vegetables intake. Research shows that 78% of the participants involved consumed less than 5 daily fruits and vegetables servings. Women had a higher prevalence of low fruits and vegetables intake compared to men in Dominican Republic, Comoros, Morocco, Paraguay and Guatemala. The risk of low fruits and vegetables intake reduced with increasing income (Hall *et al.*, 2009).

Fruits and vegetables consumption among Malaysian were influenced by many factors including age, income, ethnicity, education, health conditions and smoking status. The result shows that individuals below 30 years of age consume less fruits and vegetables than those between 41 and 58 years of age (Steven & Andrew, 2011). Other study found that prevalence of low fruit and vegetables consumption increases with age (Hall *et al.*, 2009). Individuals who come from low-income individuals consume low fruits and vegetables than those who come from high-income (Steven & Andrew, 2011; Hall *et al.*, 2009). Some of families with low-income who live in rural area in Selangor consume fruits only from their fruit trees near their house and they were less accessible to varieties of fruits sold by market. They can consume fruits only when fruit planted near their house are available. This point can support that individuals who come from low-income consume less fruits than those who come from high income (Nurul *et al.*, 2012). Chinese ethnic eat more fruits than other ethnic, Malay and Indian. Those who have

primary school education consume less serving compared with individuals who have at least high school education. For health conditions, diabetic patients consume less fruit compared to healthy people. While, patients with hypercholesterolemia consume more fruit compared to healthy people (Steven & Andrew, 2011).

## 2.2 Health benefit and nutritional composition of fruit

Fruits such as watermelon, papaya, guava, honeydew and pineapple contain vitamin and fiber which are essential for human health, in which reduce risk of cancers, heart disease and stroke (Brooks, 2014; Shashirekha *et al.*, 2015). All type of fruit involved in this study contains fiber, vitamin C, carotene, vitamin B1, vitamin B2 and niacin (Table 1).

Dietary fiber has unique characteristics such as high viscosity and high water holding capacity. High viscosity of fiber can delayed gastric emptying that can reduce person's food intake and increased transit time of small intestine. While, high water holding capacity of fiber can affects the bulk and volume of the intestinal content (Tungland & Meyer, 2002).

Vitamin C or ascorbic acid is an important micronutrient to prevent scurvy in human. Vitamin C has an ability to act against reactive oxygen species (ROS), and prevent neurodegenerative disease and heart disease. Vitamin C also serves as a cofactor in vital enzymatic reactions and have role in collagen production, a protein that serves so many connective functions in the body (Poiroux-Gonord *et al.*, 2010). Vitamin C consumption has been inversely associated with lung, breast, pancreas, stomach and rectum cancer (Simon *et al.*, 2001). Vitamin C also can lowers blood pressure among elderly (Walingo, 2005). Vitamin C in guava is higher than other fruit (Table 1).

Carotene or vitamin A is an important component for human diet to prevent night blindness and premature death. Carotene can synthesize hormone, provide immune respond to human body and involve in cell growth and differentiation. Adequate consumption of carotene-rich food can reduce the risk of getting cancers, coronary heart disease and degenerative diseases (Shashirekha *et al.*, 2015)

Vitamin B1 or Thiamin have role in synthesis of hemoglobin, red blood cell and acetylcholine, which are important in nervous system, heart and muscle. This vitamin should be taken adequately to prevent disease such as Karsacoffs syndrome, Beri-beri and optic neuro pathy (Lakaye *et al.*, 2004). Vitamin B2 is a water soluble vitamin that is essential for human health, to prevent deficiency such as cheilosis, angular stomatitis and glossitis (McCormick, 1997). Niacin deficiency will result in pellagra (McCormick, 1997). Niacin is important to synthesize nicotinamide adenine dinucleotide (NAD), which function in dehydrogenase–reductase systems (McCornick, 1996)

Table 1: Nutrient composition of fruit

| Portion=100g | Fiber/g | Vitamin C/mg | Carotene/µg | Vitamin B1/mg | Vitamin B2/mg | Niacin/mg |
|---|---|---|---|---|---|---|
| Watermelon | 0.13 | 3.07 | 229.83 | 0.02 | 0.03 | 0.13 |
| Papaya | 0.50 | 70.94 | 1159.10 | 0.03 | 0.07 | 0.13 |
| Guava | 6.70 | 151.40 | 59.30 | 0.10 | 0.05 | 1.02 |
| Honeydew | 0.75 | 25.00 | 53.00 | 0.04 | 0.02 | 0.50 |
| Pineapple | 0.62 | 15.23 | 270.00 | 0.07 | 0.08 | 0.08 |

Note. From "Nutrient Composition of Malaysian Foods" by Tee *et al.*, 1997 and "Honeydew melon, raw" (n.d).

## 2.3 Procedure to prepare fresh cut fruit

First of all, exterior surface of fruits were disinfected before peeled. After fruits peeled, fruits will be cut using cutter or slicer. Some seller or worker used manual method to slice fruits by using their hand. Some fruits will be washed after slicing and some were not. Development of modified atmosphere in container is important for the storage of soft fresh-cut fruits. Therefore, it is important to pack soft fresh-cut fruits in rigid containers equipped with plastic film lid. Modified atmosphere packaging also have role to prevent deterioration and maintain the quality of fresh-cut fruits. During storage and handling, juicy fresh-cut fruits often loss fluid. Hence, workers need to take measures to handle this problem although it is a common problem (Cantwell & Suslow, nd).

## 2.4 Physiology of fresh cut fruit

Fresh-cut fruits means tissue of the fruits have been cut and sliced. Slicing of the fruits may cause tissue disruption, which lead to high respiration rate (James & Ngarmsak, 2010). Higher respiration rates in fruits may cause metabolism to active, then cause fruits to deteriorate. Loss of acids and sugars may cause fruits be in low quality. Loss of sugar cause fruits loss their sweetness and usually not prefer by consumer. Bruising and wounding of fruits also will cause high production of ethylene (Cantwell & Suslow, nd). Ethylene may initiate the metabolism of phenolic (James & Ngarmsak, 2010). In consequences, other biochemical reactions rate will be increase and cause side effects to appear such as browning, changes in flavor, texture and nutritional quality (Cantwell & Suslow, nd).

Blade sharpness also affects wounding in fruits (Cantwell & Suslow, nd). Cutting and slicing fruits by using dull knife may destroy more cell and tissue of fruits. The most important thing should be avoided are excessive peeling and cutting. Fruits quality can be improved by minimizing mechanical damage during cutting. Shelf life of fruits can be prolonging by using sharpest cutting tools instead of dull utensils (James & Ngarmsak, 2010). Fresh cut fruits are more complicated in physiology than fresh cut vegetables. Pieces of ripe cantaloupe melon have higher rates of ethylene production than pieces of less ripe fruits. Comparing with the size, cantaloupe sliced into large pieces has lower production of ethylene than small pieces (Cantwell & Suslow, nd).

## 2.5 Outbreak of bacteria in fruit

### 2.5.1 Melons

Fruits pulp of melon contains carbohydrate that may enhance the survival of *Salmonella Enteritidis* spp. (Penteado & Leitao, 2004). *Salmonella* spp. outbreaks have been linked with cut cantaloupe and watermelon consumption. Cut cantaloupe has low acidity (pH 5.2 to 6.7) that may favor the growth of the bacteria (Harris *et al.*, 2003). Watermelon also has lower acidity (6 to 7) (Andersen, nd). *Salmonella* spp. was found in 11 of 1440 cantaloupe in 1990. While in 1991, the percentage of cantaloupe positive with *Salmonella* spp. has increase. Rind of melon has been assumed to be contaminated with *Salmonella* spp. during washing, which be the cause of outbreak. Outbreak can happen when there are improper storage of fruits and condition of fruits surface that favor bacterial growth (Harris *et al.*, 2003). After 168h, there is more than 5 log unit of *Salmonella* spp. population in melon and watermelon pulp (Penteado & Leitao, 2004).

2.5.2 Papaya

Papaya has pH ranged from 5.96 to 6.01 at maturation stage (Luthfunnesa *et al.*, 2006). The study reported that papaya in Calcutta was prepared in less hygiene condition. *Escherichia coli* spp. was present in cut papaya because it was sold at roadside and uncovered. While, *Staphylococcus Aureus* spp. were present in cut papaya due to hand peeled and poor human hygiene (Mukhopadhyay *et al.*, 2002). Compared to watermelon, papaya pulp has lower growth of *Salmonella Enteritidis* spp., which only has 1.8 log units after 168h (Penteado & Leitao, 2004).

2.5.3 Pineapple

10 pieces of pineapple were analyzed in one study. The study found that 8 samples contain *Salmonella typhi* spp. and *Escherichia coli* spp. 2 samples contain *Staphylococcus aureus* spp., *Klebsiella aerogenes* spp. and *Leuconostoc mesenteriodes* spp. High load of *Escherichia coli* spp. and *Klebsiella aerogenes* spp. are due to contamination of water used to process and rinse fruits (Jolaoso *et al.*, 2010).

2.5.4 Guava

Guava has pH from 4.3 to 4.7(Ma *et al.*, 2016). Although pH is low, *Escherichia coli* spp. can still survive in it for 3 days, Leite *et al.* (as cited in Strawn *et al.*, 2011). Study has been conducted to determine the isolation of bacteria in guava. There are total 59 bacteria were isolated from guava, comprises of *staphylococcus aureus* spp. (5), *Klebsiella* spp. (32), *Proteus* spp. (9) and *Escherichia coli* spp. (13). Higher number of bacteria isolated was due to lack of hygiene practice among sellers and buyers at the market, hawkers and road-side marketers (Dangana *et al.*, 2013).

Table 2: Foodborne disease from fruit in United State from 1998 to 2014

| | Total outbreak | *Escherichia coli* | *Salmonella* | Other sources |
|---|---|---|---|---|
| Honeydew | 18 | 0 | 6 | 12 |
| Watermelon | 26 | 2 | 11 | 13 |
| Papaya | 2 | 0 | 2 | 0 |
| Pineapple | 21 | 0 | 0 | 21 |
| Guava | NS | NS | NS | NS |

*Note. From "Foodborne Outbreak Outline Database (FOOD Tool)" by CDC, 2015*

*NS= Not Stated*

2.6 Contamination and survival of bacteria

In order for outbreak of illness happen, bacteria or pathogen must first contaminate the fruits. Some bacteria can cause illness only in small amount and does not need to replicate and reproduce. But they have to survive in fruits before consumed by individuals. While, some other bacteria needs to come in a higher amount by reproducing in fruits to cause illness, for example *Clostridium perfringens* spp. *Staphylococcus aureus* spp. that produce toxin in contaminated fruits. Pathogens that cause illness normally came from human intestinal tract and fecal material. Soil, water and decaying plant usually are a shelter to *Clostridium botulinum* spp (Harris *et al.*, 2003).

Non-pathogenic bacteria differ from pathogenic bacteria. Non pathogenic bacteria cause fruits to spoil but not for pathogenic bacteria. People will avoid consume fruits that spoil. Cut fruits did not show any spoilage even high load of pathogenic bacteria present in fruits, which

may cause harm to consumers (Harris *et al.*, 2003). 544 foodborne disease outbreaks that linked to fresh fruits and vegetables have been reported in US between 1990 and 2003 (Bassett & McClure, 2008).

Fruits that do not damaged or sliced have natural protective barrier and make bacteria or pathogens unable to penetrate the tissue. Bacteria does not have enzyme that can break natural protective barrier of the fruits. Different with cut fruits that have been cutting or slicing, bacteria have higher chances to penetrate the tissue because the protective barrier of fruits are damage and tissue of fruits are left opened. For example, pathogens have been found to replicate on the surface of cut melons. Cut fruit provide cell fluids that may favor bacteria survival. Hence, cut fruits will enhance and promote survival and multiplication of bacteria (Harris *et al.*, 2003). Alteration of ecological environment and pathogens behavior may happen when there is fecal material on the surface of fresh produce and enters tissue of cut fruits. Alteration of ecological environment may cause mold growth, which will increase pH of fruits. Bacteria growth will be enhanced when fruits have higher in pH. Yeast and mold also present naturally on the skin of fruits when fruits tissue are damaged or sliced (Beuchat, 2002).

Many bacteria cannot survive in acidic fruits because acid in fruits have an ability to inactivate the activity of bacteria. Fruits such as melons have lower acidity. Therefore, melon can support the growth of pathogen because bacteria may survive there. Outbreak of human disease, have been linked to consumption of fruits of higher pH or low acidity. It has been concluded that there is a relationship between pH of fruits and the presence of bacteria in fruits. A study found that climatic fruits provide better environment for pathogen growth than non-climatic fruits. Examples of climatic fruits are apple, banana, plum, and pear. Examples of non-climatic fruits are pineapple, cherry, citrus, strawberry and grape (Bassett & McClure, 2008).

## 2.7 Source of contamination

### 2.7.1 Pre-harvest factor

Pre-harvest factors such as climatic factor play role in affecting quality of fresh produce. Climate and environment that involve around crops can give effect on fresh produce especially quality and shelf-life. Nutritional quality of fruits depends on the intensity of light and temperature. Levels of riboflavin, carotene, and ascorbate also can be influenced by location of crop. Increase in mechanical damage might happen among crops that were planted in high level of rainfall. Texture and firmness of produce that are expose to high temperature or radiation of sun may be affected, especially in fruits (James & Ngarmsak, 2010).

Animal manure from wild and domestic animals that have not been composted were used during pre-harvesting of fruits. Animal manure contains microorganisms that can increase risk of contamination. Untreated sewage or irrigation water, soil, feces, dust and human handling also leads to contamination (Beuchat, 2002). *Escherichia coli* spp. and *Salmonella* spp. can survive in soil for extended period of time. Higher population of bacteria in soil usually comes from contaminated source of irrigated water. Bacteria in soil then will contaminated and attach the produce, and may cause higher bacterial load (Harris *et al.*, 2003). Organic waste used as fertilizer for plant contains pathogen. Pathogen may incorporate into soil. From soil, pathogen will contaminate crops when there was heavy rain happened or water irrigation used (Heaton & Jones, 2008).

### 2.7.2 Harvest factor

Harvest factor can also influence produce quality. Overripe fruits should be avoided because it can increase susceptibility of fruit to damage during slicing and cutting. Guava and papaya can

be harvest when they are almost mature, because they can be ripened after harvest. But not for pineapple, it needs to be harvested when they are mature (James & Ngarmsak, 2010).

2.7.3 Post-harvest factor

Post-harvest factors such as handling, humidity and temperature condition can gives impact on fresh produce quality. Unclean worker can serve as source of contamination (Kader, 2002). Processing equipment, transport container, human handling, insect, rinse water, ice and transport vehicles also can be the source of microbial contamination during post-harvesting (Beuchat, 2002). Favorable temperature and humidity are needed to maintain fresh produce quality (James & Ngarmsak, 2010).

Factor such as physical damage can reduce produce quality of produce. Cutting shape plays an important role in order to reduce physical damage. Cross-contamination of bacteria can happen during processing. Trimming, cutting, washing and packaging are the process involved in fresh-cut processing. During this process, cross-contamination such as direct contact between uncontaminated and contaminated foods can happen. Contaminated equipment surface and water used when trimming, cutting, and washing may lead to higher chances of cross contamination to occur (Doyle & Erickson, 2008). Cross contamination of fruits through knives, chopping board and hand often happened during final preparation (Harris *et al.*, 2003).

Temperature abuse may cause bacteria easily penetrate into the cell of fruit. It happens when the temperature of a water suspension of cells is lower than the temperature of fruit (Beuchat, 2002). Bacteria can be found in dust that surrounds fruits, especially among street vendors. Street vendors that sold fruits mainly placed along street of the city and close to bus stations may cause fruits susceptible to foodborne chemical (Proietti *et al.*, 2014). Melons that

were cut into cylinder shape have firmer texture than slices or trapezoidal shape, when stored up to 10 days (Aguayo *et al.*, 2004). But slice papaya have longer shelf life than cube papaya, when stored at 5 and 10°C (Rivera-Lopez *et al.*, 2005)

## 2.8 Method to improve quality of fresh fruit

Cutting and slicing the fruits may cause browning due to ethylene production. It is important for the workers to have knowledge on how to prevent or retard browning. Antioxidants, acidifying and chelating agents are useful to decrease browning in fruits such as apple and guava. Examples of antioxidants are ascorbic acid and erythorbic acid. Solution of hypoclorite and sulfites can be used to retard maillard reaction or browning (Cantwell & Suslow, nd). Firmness of fruits can be maintained and enhanced by using sulfites and calcium chloride. By using agents mentioned, seller can maintain and enhance the quality of fruits. Sanitizing agent usually used to reduce or destroy microorganism. Sanitizing agents used must not have effect on consumer safety and produce quality (James & Ngarmsak, 2010).

Farmers and fruits handlers should receive education about procedures of clean handling, cross-contamination control and personal hygiene (FAO/WHO, 2008). Farmer should use chemical fertilizer instead of animal manure, in order to reduce contamination (Beuchat, 2002). Higher respiration rate, microbial growth and deterioration in fruits can decrease the quality of fruits sold (Cantwell & Suslow, nd). Those problems can be handled by storing fresh-cut fruits in low temperature (0°C to 5°C) (Cantwell & Suslow, nd). It was reported that, the use of low temperature will reduce the differences in the rate of respiration and ethylene production

between fresh cut fruits and whole fruits. So, microbial contamination on cut surface of fruits can be handled by using low temperature (Cantwell & Suslow, nd).

Every seller should ensure all knives and chopping boards are cleaned and sanitized properly before use (James & Ngarmsak, 2010). Sellers that sold foods such as fruits in vendor should wash their hand after using toilet or after handling dirty material such as rubbish. They are also should ensure that their nails are short and clean. Long and dirty nails may keep microbes. Handler or sellers are advised to wear apron during service. They should also stop their selling activities for a while when they are having diarrhea, vomiting or having skin wound. They are avoided from talking and sputtering over the fruits when serving it. They are also prohibited to blow their nose near the fruits. Saliva and liquid from nose may cause contamination of bacteria. Cut fruits that prepared too long will give bacteria times to grow. Hence, seller should ensure that fruits were kept at right temperature and only prepare them when customer comes to buy (FAO, 2009).

Old utensils such as knife and chopping board that have damaged surface should not be used. Those utensils may become breeding grounds for bacteria and are difficult to clean. Sellers are recommended to wear gloves during service. Using gloves are better than hand because they are easy to clean, while hand can harbor bacteria under nails. Gloves should be washed because seller might touch money when buying process happened. Money from customer can be the source of bacterial contamination. Seller can using fork or tong instead of hand when cutting fruits. Fruits should be washed with clean water and contaminants that attach to surface of fruits should be removed. Displayed fruits should be kept in closed container and protected from dust. Flies and other insects can transmit microbes and parasites if fruits were not closed properly.

Fruits that have been cut should be wrapped in plastic or other appropriate wrapper before taken away by customers (FAO, 2009).

## 2.9 Pour plate and spread plate method

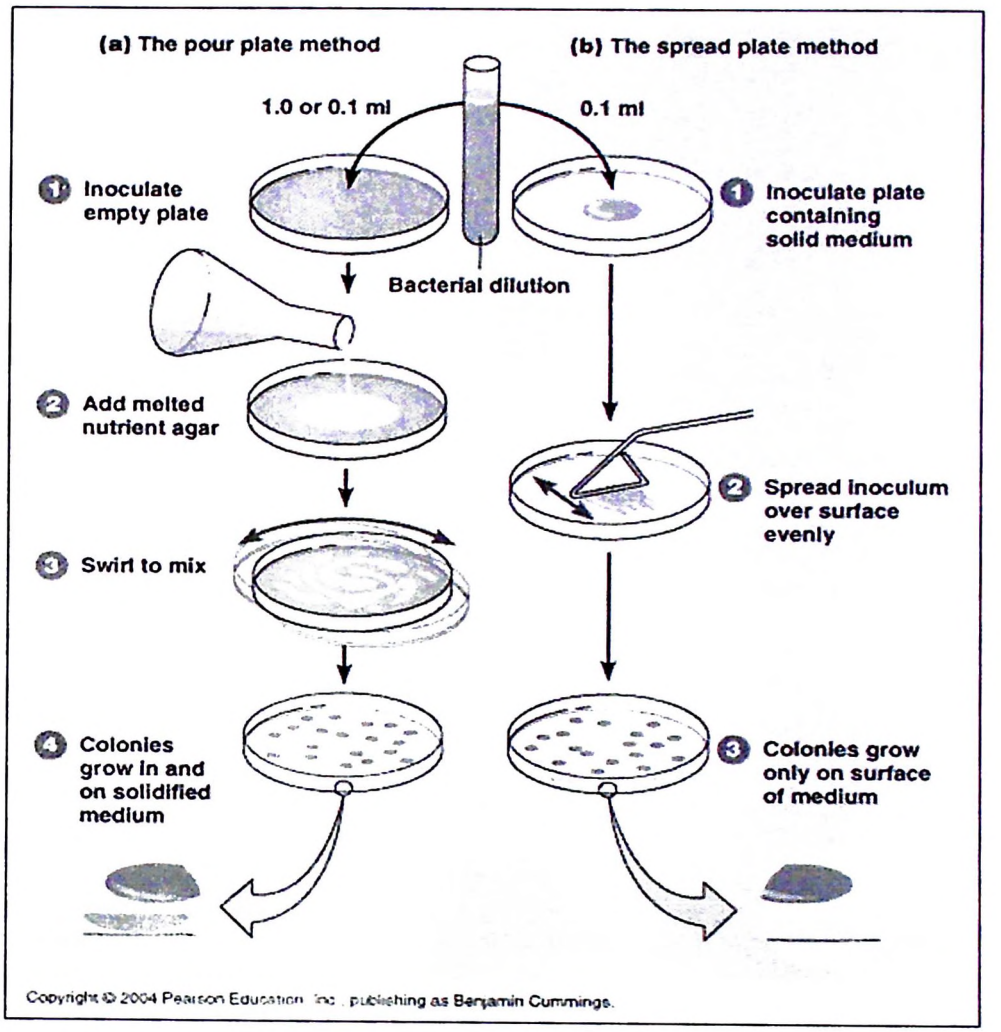
### 2.9.1 Pour plate method

Pour plate is a simple and easy method to culture microbial load. It is also low in cost and can be used with variety selective media. Pour plate method culture the sample of bacteria beneath agar medium, in which bacteria colonies tend to grow in small densities and do not show any morphological characteristic. Bacteria may not be at optimal growth because they are subjected to microaerobic environment. The usefulness of the procedure to analyzed larger sample volume will be limiting because the maximum volume of sample is only 1.0ml (Reasoner, 2004).

### 2.9.2 Spread plate method

Spread plate method is easier and tedious than pour plate because nutrient agar medium can be prepared prior to culture technique. This method require glass rod, since bacteria sample need to be spread and distribute over the surface of the agar. Glass rod need to be sterile with alcohol before used to avoid bacteria contamination. Besides glass rod, nutrient agar medium also need to be checked for contamination before used since it have been prepared in advance. Nutrient agar medium that have been solidified must not too moist or too dry (Clark, 1971).

Figure 2: Pour plate and spread plate method




Note. From "Microbiology vurses Food Microbiology," by Shariza, A.R., 2015

## 2.10 Microbial growth curve

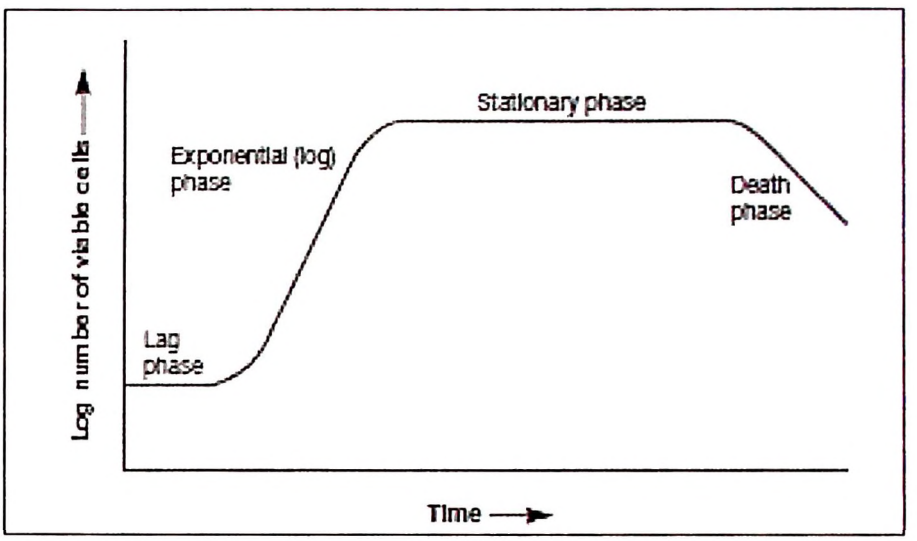
There are 4 phases of microbial growth, which are lag phase, exponential phase, stationary phase, and death phase. Lag phase begin when microorganisms are transferred or introduced into medium such as nutrient agar medium. During this phase, number of cell is not increase because they are trying to adapt with new environment that are different with previous environment. Microorganism also needs to secrete new enzymes in order to survive. End of this phase, cell begin to increase their cell mass and multiply themselves (Ingraham *et al.*, nd).

Then at the exponential phase, microorganisms are growing and multiplying themselves at higher rate. The rate of growth is increasing with available nutrient. After achieve maximum level, microorganisms will have constant growth rate, indicates that cell cycle is complete. It also indicates that the transport system of microorganism is saturated and there is no more increase in growth rate (Ingraham *et al.*, nd).

Stationary phase occur when growth curve become horizontal and density of microorganisms have achieved around $10^9$ cells per ml. There are several factors that cause microbial population to enter this phase. Firstly, there is limitation of nutrient that cause microorganism to compete with each other. Population growth is decrease when nutrient is not sufficient. Second, low $O_2$ availability will affect aerobic microorganism growth. Only the surface culture (Spread plate method) will have adequate $O_2$ concentration for growth. But, cells that are culture beneath the surface (pour plate method) will not able to grow unless there are anaerobic bacteria. Stationary phase also occur when toxic waste products exceed nutrient availability (Ingraham *et al.*, nd).

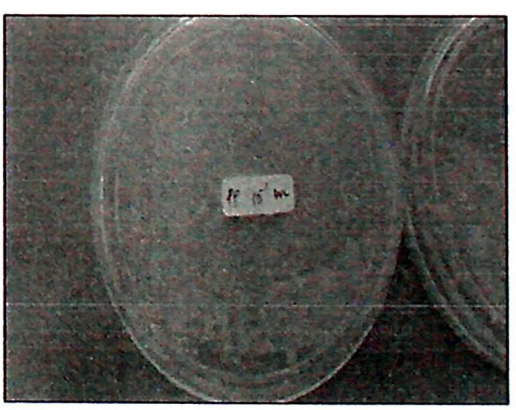
Lastly, death phase happen when there is irreparable harm and loss of viability, in which there is no cellular growth was observed even bacteria are transferred into fresh medium. In this phase cells are died (Ingraham *et al.*, nd).

Figure 3: Microbial Growth Curve in a Closed System.




Note. From "Microbial growth" by Ingraham *et al.*, n.d

Figure 4: White spot of microbial load in cut watermelon



2.11 Guideline levels for determining the microbiological quality of ready-to-eat foods

Table 3: Standard Plate Count according to level

| **Standard Plate Count** | **Microbiological Quality (CFU/g)** | | |
|---|---|---|---|
| | **Satisfactory** | **Marginal** | **Unsatisfactory** |
| Level 1 | $<10^4$ | $<10^5$ | $\geq 10^5$ |
| Level 2 | $<10^6$ | $<10^7$ | $\geq 10^7$ |
| Level 3 | N/A | N/A | N/A |

N/A= Not Applicable

**Level 1**: Applied to ready-to-eat foods in which all components of the food have been cooked in the manufacturing process/preparation of the final food product and, as such, microbial counts should be low.

**Level 2**: Applied to ready-to-eat foods which contain some components that have been cooked and then further handled (stored, sliced or mixed) prior to preparation of the final food or where no cooking process has been used.

**Level 3** - SPCs not applicable: This applies to foods such as fresh fruits and vegetables, fermented foods. It would be expected that these foods would have an inherent high plate count because of the normal microbial flora present (Food Standards Australia New Zealand, 2015).

# CHAPTER 3: METHODOLOGY

## 3.1 Research Design

The research design is a quantitative research where the data is collected through the experimental study. It is classified as a quantitative research because this research is based on quantitative measurement of microbial load in cut fruits sold by vendors.

## 3.2 Study Setting

The study setting of this research would be experimental study. The study will be conducted in Kota Bharu area where the samples of cut fruit were collected from cafeteria and roadside vendors.

## 3.3 Sampling Design

Random sampling was used to collect cut fruits from vendors to make results of this study more representative. Samples of cut fruits were collected from 2 types of vendors, cafeteria and roadside vendor. 5 different types of cut fruits (guava, watermelon, papaya, honeydew and pineapple) were collected randomly from each type vendor.

## 3.4 Data Collection

Data will be collected from 2 types of vendors. Samples of cut fruit were collected from 2 types of vendors, cafeteria and roadside vendor. 5 different types of cut fruits (guava, watermelon, papaya, honeydew and pineapple) were collected from each type of vendor, cafeteria and roadside vendors.

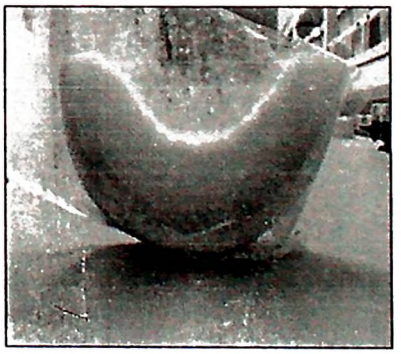
3.5 Data Analysis

3.5.1 Laboratory Analysis

Samples of cut fruit were purchased from 2 types of vendors, cafeteria and roadside vendor. Five different types of cut fruits (guava, watermelon, honeydew, papaya and pineapple) were collected randomly from each type vendor. Samples will be taken randomly from two types of vendors to make results of this study more representative. Samples with their original package were transported to the laboratory in a cooler box for processing.

Guava cafeteria

Guava roadside

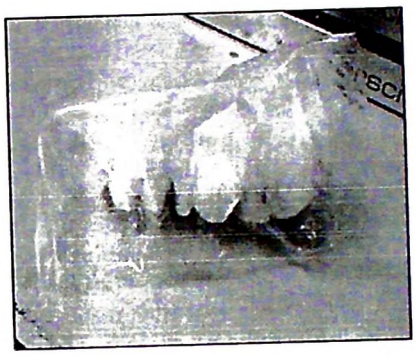


Honeydew cafeteria

Honeydew roadside

Papaya cafeteria

Papaya roadside



Pineapple cafeteria

Pineapple roadside

Watermelon cafeteria

Watermelon roadside

Figure 5: 10 samples of cut fruit

3.5.2 Media

Materials:

Culture media, petri dish, autoclave, 500ml duran bottle and distilled water.

Procedure:

There were 10 samples of cut fruit analyzed in this study. Each sample of cut fruit (25g) needs 800ml distilled water and 22.4g nutrient agar for pour plate and spread plate method.

For example, cut papaya sample. Cut papaya sample were taken and weighed for 25g only. The preparation for solution (distilled water + nutrient agar) was done first before weighing the sample to avoid further bacteria contamination. 11.2g of nutrient agar was used for 400ml distilled water in pour plate method. While, remaining 11.2g nutrient agar was used for 400 ml distilled water in spread plate method. Nutrient agar was mixed with distilled water in 500ml duran bottle both pour plate and spread plate method. Then two 500ml duran bottles with solution were heated and stirred above heating plate. 400ml solution used for 15 petri dishes of pour plate method, and other 400ml solution used for 15 petri dishes of spread plate method. The solutions have been sent to laboratory for autoclave at 100°C. Autoclave is important to avoid bacteria contamination in media.