MICROBIOLOGICAL QUALITY OF SELECTED MALAY SALADS FROM CHOSEN MARKET IN SHAH ALAM, SELANGOR

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Abstract- Vegetables such as Malay salad are important foods that can help to promote health. However, it is widely exposed to microbial contamination during pre-harvest and post-harvest. Malay salad usually consumes raw, thus increase the probability of food poisoning among consumers. In this study, bitter bean, Indian pennywort and king's salads were obtained from the wet market and mini market in Shah Alam. Each of the vegetables was divided into three treatments, which are unwashed, washed with tap water and washed with 2.5% vinegar. The selected Malay salads were analyzed to study the microorganism presence on the sample. The range of total bacteria counts obtained from this study was from 5.55 to 7.35 log CFU/g from both markets. While the range of fungi count obtained was from 4.36 to 6.24 log CFU/g. The present of microorganism on Malay salads was varied in each sample that collected from both markets. It is still in satisfactory, acceptable and unsatisfactory level, which means it is still safe to be consumed. However, these results shows that the necessity for awareness among consumers regarding the microbiological quality of Malay salads.

Keywords- Bacteria Count, Fungi Count, Food Safety, Malay Salad, Microbiological Quality.

I. INTRODUCTION

Vegetables are important to human since it is rich in fiber, water, vitamins, minerals, protein, sugar and phytochemical including tannin, saponin, anthocyanin and many more [1]. They are important to promote health for human life and able to prevent many diseases such as cancer, diabetes, heart disease and many more [2, 3]. Since vegetables play important roles for health, consumption of vegetables and fruits has increased drastically in many countries [4]. In Malaysia, vegetable is the main dietary weight loss strategies that has been used by some people especially women [5]. There was a study that revealed the pattern of fruits and vegetables that is consumed among adults from different ethnics in Selangor, Malaysia including Malay, Chinese and Indian [6]. They found that people consumed various types of vegetables including Malay salad or traditional vegetables, which also known as "ulam". Usually, people eat this type of vegetables without cooking and consumed raw as salad.

Same like other vegetables, Malay salad has a lot of benefits and rich in calcium, iron, flavonoids, carbohydrates, phenolics, minerals, protein and also vitamin [7]. Due to that, the salad has potential to protect consumers from the disease [8] such as from coronary heart disease, problems that related to liver and improve the level of blood glucose [9, 10], blood circulation, high blood pressure [11] and also promote the formation of healthy bones [12]. Other than that, it is being used as medicine to treat many health problems such as vomiting, scabies, anticancer [13], gastrointestinal disease, asthma and eczema [14]. Even though, vegetables can promote good health, vegetable that is eaten raw and without undergoing enough heating process, contains a wide range of microbial contaminants, including pathogens [15, 16] and thus can be transmitted to the consumer [17]. Therefore can increase the probability of food poisoning and will lead to food borne illness [18]. It is supported by the Centers for Disease Control and Prevention that recorded the highest number of fresh produce related food-borne disease outbreaks between 1995 and 2005 from around the world [19]. Since most of people consume vegetables based on its freshness, color and how appealing they look before and after cooking instead of knowing the level of contamination [20].

Nowadays, the rate of food-borne disease is high and might due to some reasons that occur during pre-harvest and post-harvest [21]. Usually, food-borne pathogens were detected in fresh and raw vegetable such as cucumber, bell pepper, carrot, tomato, cabbage and lettuce [22, 23, 24]. The common of microorganisms that were detected include Escherichia coli [23, 24], Salmonella [22, 25], Shigella [26, 27], Enterobacter aerogenes, Pseudomonas sp. [28] and Listeria sp. [14, 15, 16, 29, 30]. Beside bacteria, other microorganism that contaminated vegetable such as cucumber, carrot, tomato, cabbage, gotukola, lettuce and many more is fungal. Among the contamination fungal are such as Aspergillus sp., Penicillum sp., Rhizopus, Saccharomyces, Botrytis, Fusarium and Trichoderma [24, 31, 32]. Some of yeast and moulds for example Fusarium, Penicillium or Aspergillus species may produce mycotoxin. Mycotoxin is able to impair health, which, it can cause allergic reactions or illness to animal and human [3, 33, 34] and economic loss [35].

Recently, the food-borne illness associated with vegetables has been reported increase drastically. Thus, this has raised concern regarding the safety of the products. Since Malay salads are usually eaten raw, it is crucial to wash it such as with water [22,36,37] or vinegar in order to reduce the number of contamination [18,24]. The objectives of the this

study were to determine the number of total bacteria count and total fungi count in selected Malay salads including bitter bean (Parkia speciosa), Indian pennywort (Centella asiatica) and king's salad (Cosmos caudatus). All the samples were purchased from two different types of market, which is a wet market (market A) and mini market (market B) in Shah Alam, Selangor. Besides that, the aim of this study is to determine the count of microorganism for unwashed, washed with tap water and washed with 2.5% vinegar on selected Malay salads.

II. MATERIALS AND METHODS

1.1. Sample Collection

The samples were collected during a period of seven months from March to September 2015, from wet market (market A) and retail or mini market (market B) located in Shah Alam, Selangor. All samples were purchased fresh in the morning and transported to the laboratory in separate sterilized polyethylene bags.

1.2. Sample Preparation

All equipments such as chopping board and knife were wiped with 70% ethanol. Three sets of 25 grams of each vegetable were weighed. Each set was treated differently. The first set was unwashed (UW), second set was washed three times separately with tap water (WT) and another set was washed with 2.5% vinegar (WV) for 10 minutes [24]. Then, each sample was placed in a sterile stomacher bag containing 225 ml of sterile 0.1% buffered peptone water (BPW) and rubbed by hand to homogenized and suspended the surface microbes [38, 39].

Subsequently, 1 ml of each sample suspension was serially diluted (10 fold) and 0.01 ml of the dilution was placed on plate count agar (Difco). Then, it was followed by spread plate techniques and incubated at 37 °C for 24 hours for bacteria counts. Meanwhile, for fungi count, 0.1 ml of the dilution was spread on Potato dextrose agar (Difco). The plates were incubated at 28 °C for 72 hours up to 14 days. The average of the counts were from triplicate determinations, recorded and converted into log $_{10}$ CFU/g.

1.3. Statistical Analysis

All of the samples were tested in triplicate. Statistical analyses were estimated using SPSS statistic version 21. In order to analyze the data, standard deviation, mean and standard error were calculated. The two-way ANOVA was used to determine any statistical significant different ($p<0.05$) among all the samples and treatments from both markets.

III. RESULTS AND DISCUSSION

The average of total bacteria counts (TBC) for bitter bean, Indian pennywort and King's salad from two different markets of each treatment is as shown in Table 1. The mean bacteria count ranged from 5.55 to 6.47 log CFU/g for wet market and 5.67 to 7.35 CFU/g for mini market. The results of the average total fungi count are represented in Table 2. The count recorded was ranged from 4.36 to 6.24 log CFU/g for Malay salad from wet market and 5.51 to 6.22 log CFU/g for samples from mini market. The result shows the highest numbers of bacteria and fungi count obtained are 7.35 log CFU/g and 6.24 log CFU/g respectively. These results were recorded for Malay salad samples of unwashed sample, which is a bitter bean (mini market) and King's salad (wet market).

The result of two-way ANOVA recorded in Table 3. For market A, indicated that sample (bitter bean, Indian pennywort and king's salad) and treatment (UW, WT and WV) had significance different ($p<0.05$) on the bacteria count, and their interaction (sample x treatment) was significance different ($p<0.05$). For market A, indicated that sample (bitter bean, Indian pennywort and king's salad) and treatment (UW, WT and WV) had significance different ($p<0.05$) on the fungi count, and their interaction (sample x treatment) was significance different ($p<0.05$). For market B, indicated that sample (bitter bean, Indian pennywort and king's salad) and treatment (UW, WT and WV) had significance different ($p<0.05$) on the bacteria count, and their interaction (sample x treatment) was significance different ($p<0.05$). For market B, indicated that sample (bitter bean, Indian pennywort and king's salad) and treatment (UW, WT and WV) had significance ($p<0.05$) effect on the fungi count, and their interaction (sample x treatment) was significance ($p<0.05$) too.

Table 1: Average total bacteria count for Malay salads from Market A (Wet market) and B (Mini market) in Shah Alam

Sample	Market A (log CFU/g)			Market B (log CFU/g)		
Treatment	UW	WT	WV	UW	WT	WV
Bitter bean	6.41±0.01	6.32±0.03	5.84±0.11	7.35±0.09	6.22±0.04	5.69±0.09
Indian pennywort	6.32±0.14	5.81±0.13	5.55±0.06	7.25±0.06	6.09±0.13	5.67± 0.03
King's salad	6.47±0.01	6.40±0.07	6.04±0.04	7.14±0.01	6.46± 0.01	5.80± 0.08

Note: UW=unwashed, WT=washed with tap water, WV=washed with 2.5% vinegar

Table 2: Average total fungi count for Malay salads from Market A (Wet market) and B (Mini market) in Shah Alam

Sample	Market A (log CFU/g)			Market B (log CFU/g)		
Treatment	UW	WT	WV	UW	WT	WV
Bitter bean	5.74± 0.09	5.51± 0.03	4.36±0.32	5.92±0.06	5.74± 0.05	5.51±0.05
Indian pennywort	6.21±0.03	6.07± 0.02	5.48±0.04	6.22±0.03	6.19±0.03	5.59±0.09
King's salad	6.24 ± 0.01	6.06 ±0.05	5.47 ±0.03	6.19±0.06	6.04±0.04	5.49±0.03

Note: UW=unwashed, WT=washed with tap water, WV=washed with 2.5% vinegar

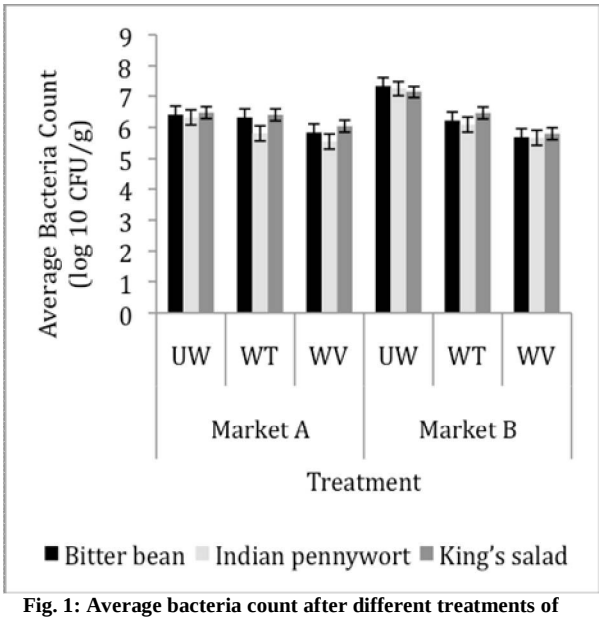


Fig. 1: Average bacteria count after different treatments of selected Malay salad collected from market A and market B. Data are mean ± standard error.

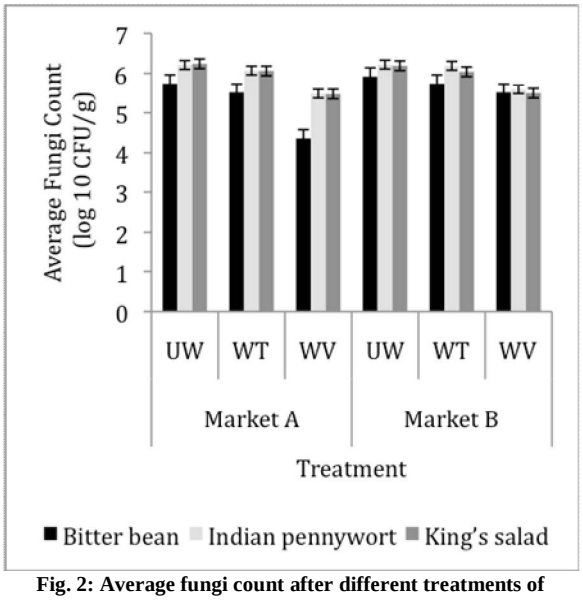


Fig. 2: Average fungi count after different treatments of selected Malay salad from market A and market B. Data are mean ± standard error.

Table 3: The result of two ways ANOVA based on two different markets (wet market and mini market), selected Malay salad (bitter bean, Indian pennywort and King's salad) and different treatments (unwashed, washed with tap water and washed with 2.5% vinegar).

Univariate market	Source of variation	*F*-ratio	*P*
Market A (Bacteria)	Sample	59.876	0.000*
	Treatment	117.980	0.000*
	Sample x treatment	7.542	0.001*
Market B (Bacteria)	Sample	83.074	0.000*
	Treatment	1090.396	0.000*
	Sample x treatment	66.024	0.000*
Market A (Fungi)	Sample	118.987	0.000*
	Treatment	182.139	0.000*
	Sample x treatment	9.308	0.000*
Market B (Fungi)	Sample	65.494	0.000*
	Treatment	306.347	0.000*
	Sample x treatment	12.784	0.000*

Note: * Significant at the 0.05 level.

The result of average total bacteria of both selected markets has shown that the microorganisms are abundant on each Malay salad. The unwashed Malay salad shows highest in the number of bacteria and fungi compare to samples that was washed with tap water and washed with 2.5% vinegar. This result was supported by another study, proved that unwashed vegetables such as tomato, cabbage and many other samples collected from Kandy district, Sri Lanka that was in the range of 6 log CFU/g [36]. Another study recorded the value of bacteria count on untreated vegetables was about 3 to 7.8 log CFU/g [40]. Most studies conducted by other researchers, stated that counts for total plate count was ranged between 4 to 8 log CFU/g [41]. Even though the plate count does not relate to food poisoning and infections, it can act as an indicator of food quality and safety [15,42,43]. Based on Figure 1 and Figure 2, it was shown that the average of bacteria and fungi count present in each selected

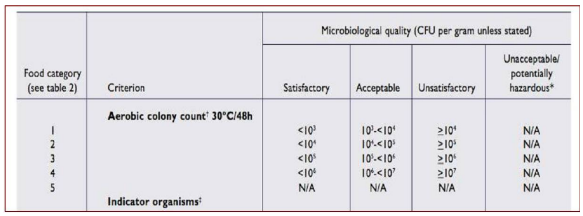


Fig. 3: Guidelines for the Microbiological Quality of Various Ready-to-eat Foods [55].

Malay salad from both markets, was reduced in number after being applied with different treatment.

In this study, the average of total plate count of bacteria and fungi on each Malay salad is not depending on the types of market. This was proved by another study, which stated that the amount of contamination equally high in most of studied vegetables that were purchased from different markets, including retail or mini market, roadside market and supermarket [36]. In addition, another study that conducted in hypermarket in Malaysia found that food-borne pathogen such as Listeria monocytogenes had been reported to be isolated in a large number from raw leafy vegetables [30]. However, there was a study conducted in Nigeria, found that the total viable count was higher in salad samples from open market (6.77 log CFU/g) and has lower number of bacteria from fast food outlets (4.41 log CFU/g) [44]. Another study reported that all microorganism counts were no significant in fresh open air markets and supermarkets in Philippine [45].

The number of microorganism present differences from each market, may be due to some factors that occur during pre-harvest and post-harvest [21,24]. For the pre-harvest factor can occur during use of manure as a fertilizer [46], the animal waste may contain pathogenic bacteria, including Salmonella [47]. Besides that, farmers also must know and practice a good way to handle fresh produce. This is because cracks and cavities on vegetables can support the survival of microorganism including the pathogenic bacteria [48], growing location and soil [49], type of irrigation system [3,26,50], regions, climatically conditions [3] and less individual hygiene of food handlers [20, 51]. Meanwhile, the vegetables may be contaminated during post-harvest, including incorrect sanitary conditions, unsuitable storage system [52], unhygienic preparation areas and use of unclean utensils [53] and environment during transportation [50,54].

According to Gilbert [55], the guidelines for the microbiological quality of ready-to-eat foods, this raw vegetable is in category 5. However, there was no

guideline for raw vegetable. Since there was no standard limit for raw Malay salad, the suitable and closest category for Malay salad is category 4. This is because, normally people eat this type of vegetables without cooking and consumed raw as salad. Malay salad also can be defined as a fresh green salad that usually tossed in fermented sauce, aromatic herbs or spices as side dished with rice. Sometimes, people serve it with crudités or condiments such as "budu" or shrimp paste [56]. Based on this study, the average of bacteria and fungi were 5.55 to 7.35 log CFU/g and 4.36 to 6.24 log CFU/g. Fig. 3 shows, the result for this study is "satisfactory" (<6 log CFU/g), "acceptable" (6 to 7 log CFU/g) and "unsatisfactory" (≥7 log CFU/g). The "satisfactory" category shows the results foods indicate a good microbial quality. While, "acceptable" reflect a borderline and "unsatisfactory" category shown that further inspection of the markets or places need to be done in order to determine whether the vendors practice enough hygiene handling or not. Based on this guideline, all selected Malay salad is safe to be consumed. Since the microbial quality was not "unacceptable or potentially hazardous" which mean it is potentially hazard to those who consume such foods.

However, the microorganism that presence on washed with tap water and washed with 2.5% vinegar Malay salad shown reduced in number. It is important to wash raw vegetable, treat it with sanitizers or using antimicrobial agents [57]. Based on another study, the viable bacteria count is reduced as a result of washing for three times [37]. Since water normally contains chlorine or other antibacterial compound which can help to reduce the number of microorganism [50]. However, it was also confirmed that through washing was not able to reduce pathogen levels to a safe limit in vegetables [37]. This contamination might due to use of treated wastewater for irrigation [53] or improper irrigation with untreated water [58,59,60] and may be due to the ability of some microorganism that able to attach to the vegetable surface [61].

The lowest number of bacteria and fungi recorded for this study may be due to the vinegar. This is because, vinegar is bactericidal [18] and an acidic since it composed with mainly 4% to 18% of acetic acid [62]. There was a study that detects the decreased in microbial load to the safer limit when samples were exposed to 2.5% vinegar solution [18,37]. Another study also revealed that acetic acid contains in vinegar, made it effective in reducing the number of microorganisms presence in foods, including bacteria and fungi contaminated on vegetables [63]. This was supported by another study, that proved vegetables that were treated with vinegar was relatively effective compare to potassium sorbate and sodium benzoate [24,64] while reducing the level of contamination. Thus, use of 2.5% vinegar as the rinsing agent for fresh vegetables can play an important role in food safety.

CONCLUSION

Most of unwashed samples carry high bacteria and fungi count. Since Malay salads usually consumed raw, it must be washed properly with tap water or washed with 2.5 % vinegar. This will help to reduce the number of bacteria and fungi present and improve better quality and safety of Malay salads to be consumed.

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