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# Prevalence of foodborne bacteria isolated from fresh raw *ulam*

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**Abstract**

Although vegetables are considered to be an essential part of a healthy diet, studies have shown that they can also represent a hazard for human health as they are usually eaten raw and receive minimal treatment. In recent years, vegetables are among the food groups associated with higher rates of recurrence and are the leading cause of enteric diseases. There is a colossal amount of data available on fresh produce worldwide; however, limited data are available regarding the microbiological quality of *ulam* in Malaysia. In fact, cross -contamination that occurs during minimal processing of *ulam* has not yet been studied. Therefore, the aim of this study was to evaluate the microbiological quality and the occurrence of *Escherichia coli* and *Salmonella* in *ulam*. A total of 32 samples of *ulam* were randomly collected from wet markets and supermarkets in Kuala Terengganu, Malaysia. The samples were analysed for enumeration of aerobic mesophilic bacteria, coliforms, yeast and moulds, *Bacillus cereus*, *Listeria monocytogenes* and detection of *Escherichia coli* and *Salmonella*. In this study, the microbiological quality of *ulam* were in the range of 6.36-8.83; 4.14-7.48; 0-8.16; 3.94-6.45 $\log_{10}$ CFU/g for aerobic mesophilic bacteria, coliforms, *Salmonella* and yeast and moulds, respectively. *Escherichia coli* and *Salmonella* were detected in 3.13% and 9.4% of *ulam* samples, respectively. The findings from the study are intended to provide insight into the potential health risks associated with the consumption of *ulam*. The strong interdisciplinary approach by various agencies and development of safe agricultural systems will ensure the delivery of safe vegetables to the end-users.

## 1. Introduction

Herbs or *ulam* are important food intakes among the Malays because of their nutritional value. They provide proteins, fibre, sugar, minerals and many vitamins, which are essential to promote health for human life (Gruda, 2005; Bachok *et al*., 2014). Even though *ulam* can promote good health, *ulam* that is usually eaten raw and without enough heating process may be contaminated with a wide range of microorganisms, including foodborne bacteria. The contamination of vegetables may occur at any stage, including during pre and post-harvest handling (Chaturvedi *et al*., 2013). As a result, the foodborne disease outbreaks linked to fresh produce have also increased. The Center for Science in the Public Interest (CSPI) published a report stating that 363 outbreaks and 13,568 cases of illness were related to green leafy vegetables. These ranked them number one in the United States of America (USA) Food and Drug Administration (FDA) Top Ten (Center for Science in the Center for Science in the Public Interest., 2009). In Malaysia, the information of foodborne outbreaks related to the consumption of fresh produce was unknown. When an outbreak occurred, little attempt was made to discover the magnitude of the problem and source of the outbreak, likely due to the isolation and identification process of microorganisms that is time-consuming (Kuan *et al*., 2017; New *et al*., 2017).

Over the past decade, several foodborne bacteria commonly detected in fresh vegetables include *E. coli*, *Salmonella* spp., *Shigella*, *Bacillus* and *Listeria monocytogenes* (Mritunjay and Kumar, 2015). *Escherichia coli* and *Salmonella* are among the most frequent foodborne bacteria that cause outbreaks through the consumption of contaminated vegetables. The

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Centers for Diseases and Control and Prevention (CDC) revealed that many *E. coli* O157:H7 outbreaks were linked to contaminated vegetables, including romaine lettuce, leafy greens, alfalfa sprouts, salads and spinach (CDC, 2019). In September 2006, 205 people were infected with a virulent strain of *E. coli* O157:H7 in the United States of America (USA). Among the infected people, 103 individuals were hospitalised, 31 cases of hemolytic-uremic syndrome (HUS) and three deaths. The outbreak was related to the consumption of fresh bagged spinach (Seltzer *et al*., 2009).

Many cases of salmonellosis in human are related to the consumption of contaminated food products, especially those of animal origins such as eggs, beef, milk, poultry and pork. However, this disease has also been associated with fresh vegetables, and it has been recognised as a transmission vehicle for *Salmonella* (Bouchrif *et al*., 2009). For example, *Salmonella* has been found in alfalfa sprouts, cucumber, cilantro, celery, lettuce, tomato and parsley (Pui *et al*., 2011; Food and Drug Administration (FDA), 2016). In addition, several outbreaks of *Salmonella* attributed to contaminated fresh vegetables have been reported worldwide. In August 2014, an outbreak of *Salmonella enterica ser.* Newport caused by cucumber was identified in 29 states of the US and the district of Columbia. The outbreak resulted in 257 cases, 34% were hospitalised, and one death was recorded (Angelo *et al*., 2015). Interestingly, Bahri *et al*. (2020) demonstrated that *E. coli* and *Salmonella* isolated from *ulam* were able to form biofilms and reveal the ability of these isolates to persist on the fresh produce and become hosts for the transmission of disease to humans or/and animals. In addition, the presence of antibiotic-resistant *E. coli* in raw vegetables may pose health threats to consumers and indicate the role of fresh produce as a reservoir of resistant pathogenic bacteria (Bahri *et al*., 2019).

Microbiological risk assessment is an indicator tool for the evaluation of the safety of food and water supplies used during food production. The presence of microorganisms indicates improper treatment or post-disinfection contamination (Chaturvedi *et al*., 2013). This study aimed to evaluate the microbiological quality of minimally processed *ulam* sold in wet markets and supermarkets in Kuala Terengganu, Malaysia. The incidence level of aerobic mesophilic bacteria, *Salmonella* spp., coliforms, *Bacillus cereus*, yeast and mould and *Listeria monocytogenes* were assessed from the *ulam* samples. Moreover, the presence of *E. coli* and *Salmonella* spp. were also detected in the samples analysed. Up to now, this is the first study on the microbiological quality of *ulam* sold in Kuala Terengganu, Malaysia.

## 2. Material and methods

### *2.1 Microbiological quality of fresh raw ulam*

A total of 6 types of fresh *ulam* were selected for microbiological quality which consists of leafy vegetables and non-leafy vegetables. The leafy vegetables include *pegaga* (*Centella asiatica*), *ulam raja* (*Cosmos caudatus*) and *selom* (*Oenanthe javanica*). The non-leafy vegetables are bean sprout/*tauge* (*Vigna radiata*), winged bean (*Psophocarpus tetragonolobus*), and long bean (*Vigna unguiculata*) (Table 2).

#### *2.1.1 Experimental design*

The detailed experimental design used for the microbiological quality of fresh raw *ulam* in supermarkets and wet markets is described in Table 1.

#### *2.1.2 Method for microbiological quality*

A total of 25 g of each cut of fresh raw *ulam* (*pegaga, ulam raja*, *selom*, bean sprout/*tauge,* winged bean and long bean) were weighed into a sterile stomacher bag. Each type of *ulam* was transferred into 3 sterile stomacher bags to obtain triplicate results. The 225 mL of sterile buffered peptone water (BPW) (Merck, Germany) was added and then stomached for 2 mins using a stomacher (BagMixer 400, Interscience, Singapore) (Buyukunal *et al*., 2015). The homogenised solution was made a serial dilution until $10^{-7}$.

Table 1. The experimental design for microbiological quality

| Objective | To quantify the occurrence of *Escherichia coli* and *Salmonella* in *ulam* in Terengganu |
|---|---|
| Number of samples | 6 samples of *ulam* |
| Factor (Independent variable) | 6 samples of *ulam* collected from wet market and supermarket (*selom, pegaga, ulam raja,* bean sprout, winged bean and long bean) |
| Factor level (Treatment) | F1: Plating samples on different types of selective media agar (PCA, MacConkey, XLD, MYP, PALCAM, DRBC agar)<br>F2: Dilution from $10^{-2}$ until $10^{-7}$ |
| Arrangement | Two-way arrangement |
| Replication | 3 replications |
| Experimental unit (EU) | (6 x 2 types of market) x 3 replications = 36 EU |
| Response (Dependent Variable) | Colony Forming units (CFU) |
| Statistical Analysis | Independent t-test and Mann- Whitney Test |

 

Table 2. Ulam samples examined in this study for microbiological quality

| Local name | Scientific Name | English name | Supermarket | Wet Market | Total |
|---|---|---|---|---|---|
| *Pegaga* | *Centella asiatica* | Indian pennywort | 1 | 1 | 2 |
| *Ulam Raja* | *Cosmos caudatus* | Wild parsley | 1 | 1 | 2 |
| *Selom* | *Oenanthe javanica* | Japanese parsley | 1 | 1 | 2 |
| *Kacang Panjang* | *Vigna unguiculata* | Long bean | 1 | 1 | 2 |
| *Kacang Botol* | *Psophocarpus tetragonolobus* | Winged bean | 1 | 1 | 2 |
| *Tauge* | *Vigna radiata* | Bean sprout | 1 | 1 | 2 |
| Total | | | 6 | 6 | 12 |

For the total plate count, 100 µL of the serial dilutions were inoculated on the Plate count agar (PCA) (Oxoid, UK). The inoculum was spread rapidly over the entire agar surface using a glass spreader. Prior, the glass spreader was sterilised by placing it in 95% ethanol and then flaming it until all the alcohol has evaporated. Then, the plates were incubated at 35°C for 24 hrs. After incubation, the number of colonies was counted and recorded (Da Silva *et al*., 2007).

For coliform count, the sample preparation was carried out as mentioned above. Then, 100 µL of the prepared sample was spread plated on MacConkey agar (Oxoid, UK) and incubated at 35°C for 24 hrs. Total coliform counts on MacConkey agar were determined by counting the red or pink, round, medium-sized colonies. The number of typical colonies was recorded for each plate (Thunberg *et al*., 2002).

The method used for the enumeration of *Bacillus cereus* was described in the Bacteriological Analytical Manual (Tallent *et al*., 2019). Approximately 100 µL of the prepared sample was spread plate on Mannitol Egg Yolk Polymyxin agar (MYP) agar (Oxoid, UK) with sterile glass spreader and incubated at 35°C for 24 hrs. *B. cereus* colonies are typically pink on MYP agar surrounded by a precipitate zone, indicating that lecithinase is produced. The typical colonies of *B. cereus* were counted and recorded.

Yeast and moulds counts were conducted by spread plate method on Dichloran Rose Bengal Chloramphenicol (DRBC) agar (Oxoid, UK). Approximately 100 µL of each dilution was inoculated on DRBC agar and incubated at 25°C. The colonies of yeast and moulds were counted and recorded after 3 and 5 days in plates containing up to 150 colonies to avoid overgrowth (ISO 21527-1, 2008). The average of the counts was from triplicate determinations, recorded and converted into $\log_{10}$ CFU/g.

The method used for enumeration of *Salmonella* spp. is based on the Bacteriological Analytical Manual (Andrews *et al*., 2011). Each type of *ulam* was transferred into 3 sterile stomacher bags to obtain triplicate results. A total of 25 g of sample was homogenised with 225 mL of buffered peptone water (BPW) (Merck, Germany) and incubated at 35°C for 24 hrs. Then, the culture was inoculated into Rappaport Vassiliadis soya peptone (RVS) broth (Oxoid, UK) and Tetrathionate (TT) broth (Merck, Germany) and incubated at 35°C for 24 hrs. After that, the culture was serially diluted until $10^{-7}$. Then, 100 µl of each diluted sample was inoculated onto Xylose Lysine Desoxycholate (XLD) agar (Oxoid, UK) by spread plate method. Then, the plates were incubated at 35°C for 24 hrs. *Salmonella* spp. colonies usually grow as red colonies with a black centre. The typical colonies of *Salmonella* spp. were counted and recorded.

The method used for enumeration of *Listeria monocytogenes* is based on the United States Department of Agriculture (United States Department of Agriculture-Food Safety And Inspection Service [USDA-FSIS], 2013). Each type of fresh raw *ulam* was transferred into 3 sterile stomacher bags to obtain triplicate results. A total of 25 g of sample was homogenised with 225 mL of sterile Listeria selective enrichment broth (UVM1) (Oxoid, UK) and stomached for 2 mins using a stomacher. The enrichment bag was incubated at 30°C for 24 hrs. Then, the culture was inoculated into Fraser Broth (Oxoid, UK) and incubated at 35°C for 24 hrs. After being incubated, the culture was serially diluted until $10^{-7}$. Then, 100 µL of the diluted sample was inoculated onto PALCAM agar (Oxoid, UK) by the spread plate method and incubated at 35°C for 24 hrs. *Listeria monocytogenes* colonies grow as a grey-green coloured colony with a black zone. The typical colonies of *Listeria monocytogenes* were counted and recorded.

### *2.2 Isolation and detection of Escherichia coli and Salmonella spp.*

#### *2.2.1 Sample selection*

A total of 32 samples of *ulam* were randomly purchased from wet markets and supermarkets based on their availability from 2016 to 2017 in Kuala Terengganu, Terengganu, Malaysia (Table 3). The fresh vegetables consisted of *pegaga* (*Centella asiatica*), *ulam* raja (*Cosmos caudatus*), *ketumbar* (*Coriandrum sativum*), *kangkung* (*Ipomoea aquatica*), *daun sup*/ parsley (*Petroselinum crispum*), Vietnamese coriander/ *kesum* (*Persicaria odorata*), lettuce/*salad kampung* (*Lactuca sativa*), *pucuk putat* (*Barringtonia racemosa*),

 

Table 3. *Ulam* samples examined in this study for isolation of *E. coli* and *Salmonella*

| Local name | Scientific Name | English name | Total |
|---|---|---|---|
| *Pegaga* | *Centella asiatica* | Indian pennywort | 4 |
| *Tauge* | *Vigna radiata* | Bean sprout | 4 |
| *Kacang Panjang* | *Vigna unguiculata* | Long bean | 4 |
| *Ulam Raja* | *Cosmos caudatus* | Wild cosmos | 3 |
| *Kacang Botol* | *Psophocarpus tetragonolobus* | Winged bean | 3 |
| *Salad Kampung* | *Lactuca sativa* | Lettuce | 2 |
| *Daun Sup* | *Petroselinum crispum* | Parsley | 2 |
| Cucumber | *Cucumis sativus* | Cucumber | 2 |
| *Kangkung* | *Ipomoea aquatica* | Water spinach | 2 |
| *Selom* | *Oenanthe javanica* | Japanese parsley | 2 |
| *Kesum* | *Persicaria odorata* | Vietnamese coriander | 1 |
| *Ketumbar* | *Coriandrum sativum* | Coriander | 1 |
| *Bayam* | *Amaranthus* | Spinach | 1 |
| *Pucuk Putat* | *Barringtonia racemosa* | Freshwater mangrove | 1 |
| Total | | | 32 |

bean sprout/*tauge* (*Vigna radiata*), long bean/*kacang panjang* (*Vigna unguiculata*), winged bean/*kacang botol* (*Psophocarpus tetragonolobus*), *selom* (*Oenanthe javanica*), spinach/*bayam* (*Amaranthus*) and cucumber *(Cucumis sativus).*

*2.2.2 Sample preparation for isolation of Escherichia coli and Salmonella*

The samples were purchased fresh in the morning and placed in an icebox with ice packs. Then, they were transported to the laboratory immediately and processed within 2 hrs of collection. No additional washing steps were applied to the samples after collection as this would represent the actual microflora present in the *ulam* samples (Hassan and Purwani, 2016). Then, 25 g of each cut of fresh raw *ulam* were aseptically weighed in a sterile stomacher bag and homogenised with 225 ml of sterile buffered peptone water (BPW) (Merck, Germany) for two minutes using a stomacher (BagMixer 400, Interscience, Singapore) (Buyukunal *et al*., 2015).

*2.2.3 Escherichia coli*

The isolation of *E. coli* was done by following the method described in the Bacteriological Analytical Manual (Feng *et al*., 2011). The culture from the stomaching bag containing BPW was inoculated onto MacConkey (Oxoid, UK), Violet Red Bile Agar (VRBA) (BD, France) and Eosin Methylene Blue (EMB) (Lab M, UK) agar using the streaking method. The plates were incubated at 35°C for 24 hrs in an incubator (Memmert, Germany). *Escherichia coli* ATCC 25922 was used as a positive control and *Staphylococcus aureus* ATCC 25923 as a negative control.

*2.2.4 Salmonella spp.*

The isolation of *Salmonella* spp. was done by referring to the method based on the Bacteriological Analytical Manual (Andrews *et al*., 2011). The homogenised buffered peptone water (BPW) (Merck, Germany) were incubated at 35°C for 24 hrs. Then, the BPW culture was inoculated into Rappaport Vassiliadis soya peptone (RVS) broth (Oxoid, UK) and Tetrathionate (TT) broth (Merck, Germany). The broth was incubated at 35°C for 24 hrs. The culture from RVS and TT enrichment were then inoculated to Xylose Lysine Desoxycholate (XLD) agar, Bismuth Sulphite (BS) agar (Merck, Germany) and Hektoen Enteric (HE) agar (Merck, Germany) using the streaking method. Those plates were incubated at 35°C for 24 hrs and examined for typical colonies. *Salmonella* ATCC 14028 was used as a positive control, and *Enterococcus faecalis* ATCC 29212 as a negative control.

*2.3 Biochemical test*

*2.3.1 Triple sugar iron (TSI) and lysine iron (LI) agar test*

The presumptive *Salmonella* spp. were selected and screened biochemically using triple sugar iron (TSI) agar (Oxoid, UK) or lysine iron (LI) agar (Merck, Germany) slopes in conjunction with urease and sucrose/lactose media. First, the colonies were inoculated on nutrient agar and incubated at 35°C for 24 hrs. The well-isolated colony was inoculated on the TSI and LI agar. The agar medium was first stabbed through the middle to the bottom of the tube then streaked along the surface of the agar slant. After that, the TSI and LI agar were incubated at 35°C for 24 hrs (Acharya, 2013). The typical strains of *Salmonella* produce an acid (yellow) butt and an alkaline (red) slope in TSI agar and an alkaline (purple) reaction throughout the LI medium, both with blackening due to hydrogen sulphide production, are urease negative and do not ferment sucrose or lactose (Neogen Coorporation, 2017). The *Salmonella* ATCC 14028 were used as positive control and *Enterobacter cloacae* ATCC 23355 as a negative control. The negative control produces acid

 

butt, alkaline slant and no production of hydrogen sulphide in LI agar.

*2.3.2 Gram staining*

The standard protocol of Gram staining was done by referring to the method described by Cappuccino and Sherman (2014). First, the bacterial smear was air-dried and heat-fixed on a slide. Then, the smear was flooded with crystal violet (Merck, Germany) for 1 min and rinsed with distilled water. After that, the smear was flooded with Gram's iodine (R and M Chemicals, UK) for 1 min, rinsed and decolourised by using 95% ethanol (R and M Chemicals, UK) for 5-10 s. Then, the smear was immediately rinsed with distilled water, flooded with safranin (Sigma Aldrich Inc., Germany) for 1 min, rinsed with distilled water, blot dried and observed under oil immersion using a light microscope with a total magnification of 1000x (Leica DME, Matrix Optics (M) Sdn Bhd, Malaysia).

*2.3.3 Oxidase test*

A strip of filter paper was soaked with a little freshly made Kovacs oxidase reagent. Then, a large mass of pure culture was rubbed on it by using a toothpick. The filter paper was observed for 60 s. The area of inoculation indicated a positive reaction turned from dark blue to almost black. If the colour changes did not occur, the result was considered a negative result (PHE, 2019). Members of the family *Enterobacteriaceae* such as *E. coli* and *Salmonella* were classified as oxidase negative.

*2.3.4 Analytical profile index (API20E)*

The API20E test was carried out according to Holmes *et al*. (1978). First, three-quarters of water was added to fill all the honeycombed wells of the tray. Then, a single isolated colony was selected and emulsified in an ampule of API NaCl 0.85% medium. The isolate was then transferred to the well by using a sterile pipette. For (CIT, VP and GEL tests), both tubes and cupules were filled with the isolate solution. While only the tubes were filled for the remaining tests. For (ADH, LDC, ODC, $H_2S$ and URE) tests, the tubes were overlaid with mineral oil to achieve anaerobiosis. The strips were incubated at a temperature of 37°C for 18-24 hrs. After the incubation period, the strip was read by referring to the Reading Table. If three or more tests (GLU test + or -) were positive, the strip should require the addition of a reagent. One drop of TDA reagent was added to the TDA test. One drop of JAMES reagent was added to the IND test. One drop each of VP 1 and VP 2 was added to the VP test. After 10 mins, the colours against the chart were compared, and the API record sheet was recorded. If the number of positive results (including the GLU test) was less than 3, the strip was re-incubated for another 24 hrs without any reagent being added. The identification of unknown isolates was performed by using *apiweb*™ identification software online.

*2.4 Statistical analysis*

Colony counts were converted into $\log_{10}$ CFU/g. The data were analysed by an independent sample *t*-test to determine mean values, standard deviation, and any statistically significant difference among all samples from wet markets and supermarkets using IBM SPSS Statistic Version 20 (SPSS Inc. Chicago, USA).

## 3. Results and discussion

*3.1 Microbiological quality of fresh raw ulam*

A total of twelve samples of *ulam* were selected for this study, namely *pegaga* (*Centella asiatica*), *ulam raja* (*Cosmos caudatus*), *selom* (*Oenanthe javanica*), bean sprout/*tauge* (*Vigna radiata*), winged bean (*Psophocarpus tetragonolobus*), and long bean (*Vigna unguiculata*). The samples were taken from two different types of markets which were wet markets and supermarkets. Overall, this study reveals that all twelve fresh raw *ulam* were contaminated with bacteria. The results present that the microbial load varied with the location of sampling and the type of *ulam*.

According to Nyenje *et al*. (2012), one of the microbiological indicators is the presence of aerobic mesophilic bacteria in food, and it reflects the exposure of the sample to any contamination. This parameter is useful to confirm if cleaning, disinfection and temperature control during processing, transportation and storage, have been carried out properly. Figure 1 shows the aerobic mesophilic count in the samples analysed, respectively. The mean aerobic mesophilic count for all tested samples was 7.38 $\log_{10}$ CFU/g, ranging from 6.36 to 8.83 $\log_{10}$ CFU/g (Table 4). Similar to the present study, Mritunjay and Kumar (2017) reported that the majority of raw salad vegetables collected from the retail market in India were also in the range of 6.0 to 8.0 $\log_{10}$ CFU/g. Another study by Abadias *et al*. (2008) which examined fresh-cut and whole vegetables from supermarkets in Spain also found similar high loads of aerobic mesophilic bacteria up to 8.9 and 8.0 $\log_{10}$ CFU/g, respectively. According to the Hazard Analysis and Critical Control Points-Total Quality Management (HACCP-TQM) Technical Guide, raw foods containing less than 4 $\log_{10}$ CFU/g, 4-6.69 $\log_{10}$ CFU/g, 6.69-7.69 $\log_{10}$ CFU/g and more than 7.69 $\log_{10}$ CFU/g (aerobic mesophilic count) are classified as good, average, poor and spoiled food, respectively (Aycicek *et al*., 2006). In this study, 42% of *ulam* samples were considered as spoiled food, whereas 42% as poor and 16% as average.

 

Table 4. The distribution of contaminated samples according to the types of medium agar used

| Microorganisms | Number of samples | Microbial load ($log_{10}$ CFU/g) | | | Percentage of contaminated samples in the indicated interval (%) | | | |
|---|---|---|---|---|---|---|---|---|
| | | Min | Max | Mean | <3 | 3-<6 | 6-<9 | >9 |
| Aerobic mesophilic bacteria | 12 | 6.36 | 8.83 | 7.38 | 0 | 0 | 12(100) | 0 |
| *Salmonella* spp. | 12 | 0 | 8.16 | 4.3 | 5 (42) | 0 | 7(58) | 0 |
| Coliforms | 12 | 4.14 | 7.48 | 6.02 | 0 | 5(42) | 7(58) | 0 |
| *Bacillus cereus* | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Yeast and Molds | 12 | 3.94 | 6.45 | 5.42 | 0 | 8(67) | 4(33) | 0 |
| *Listeria* spp. | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

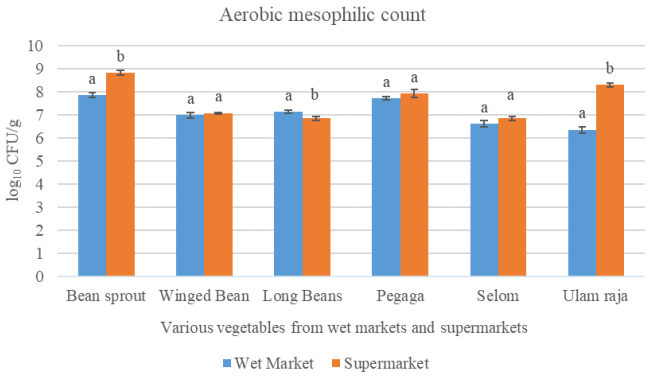


Figure 1. The aerobic mesophilic count of various vegetables from wet markets and supermarkets. Bars are mean±standard deviation of triplicates. Bars with different notations indicate significant difference ($p<0.05$) between samples taken from supermarkets and wet markets.

The highest aerobic mesophilic plate count was observed in bean sprouts (8.83±0.09 $log_{10}$ CFU/g) from supermarkets, followed by *ulam raja* (8.31±0.09 $log_{10}$ CFU/g) and *pegaga* (7.93±0.17 $log_{10}$ CFU/g). Maximum TPC in wet markets was recorded in bean sprout (7.88±0.09 $log_{10}$ CFU/g) followed by *pegaga* (7.73±0.07 $log_{10}$ CFU/g) whereas minimum for *ulam raja* (6.36±0.14 $log_{10}$ CFU/g) and *selom* (6.63±0.13 $log_{10}$ CFU/g). These results are in line with Thunberg *et al*. (2002) who reported that the aerobic mesophilic count in sprouts at retail markets in Washington D.C. was 8.7 $log_{10}$ CFU/g. Similarly, Abadias *et al*. (2008) conducted a survey on sprouts in Spain and found that the aerobic mesophilic bacteria was 7.9 $log_{10}$ CFU/g. High microbial loads in bean sprouts might be due to the contaminated water and soil, improper handling and favourable conditions during germination such as suitable temperature, pH, moisture and nutrients. Soaking bean sprouts overnight in water during the seed germination process was found to increase their aerobic microbial counts by ten folds (Seow *et al*., 2012).

T-tests were used to analyse the significant difference between two markets, supermarket and wet market. Statistically, the colony count of aerobic mesophilic bacteria of bean sprout and *ulam raja* purchased from the supermarket were significantly higher than the wet market ($p < 0.05$). Interestingly, the bacterial count of long beans purchased from the wet market was significantly higher than the supermarket ($p < 0.05$). However, no significant of these two markets was observed for winged bean and *selom* samples ($p > 0.05$).

Coliforms are common inhabitants of animal and human guts and are considered to be a hygiene indicator, especially for faecal contamination (Chaturvedi *et al*., 2013; Mritunjay and Kumar, 2017). In this study, the mean coliform counts for all *ulam* samples was 6.02 $log_{10}$ CFU/g, ranging from 4.14 to 7.48 $log_{10}$ CFU/g (Table 4). These results are in agreement with Mritunjay and Kumar (2017), where all raw salad vegetables collected in India had coliform counts from 3.0 to 7.8 $log_{10}$ CFU/g. According to Food Standards Australia New Zealand (2016), total coliforms in ready-to-eat food was categorised as unsatisfactory if higher than 4.0 $log_{10}$ CFU/g. Surprisingly, these results revealed that all *ulam* purchased from both wet market and supermarket surpassed the limits for the total coliform count, signifying the raw vegetables were not fit to be eaten.

As presented in Figure 2, the highest coliform counts in supermarkets were observed in bean sprout (7.48±0.09 $log_{10}$ CFU/g), followed by *ulam* raja (7.11±0.03 $log_{10}$ CFU/g) and *pegaga* (6.46±0.38 $log_{10}$ CFU/g). For wet markets, the highest coliform counts were recorded in the bean sprout (6.96±0.04 $log_{10}$ CFU/g), followed by *pegaga* (6.40±0.16 $log_{10}$ CFU/g) and *selom* (5.47±0.05 $log_{10}$ CFU/g). The microorganism was also detected in the long bean, winged bean and *ulam raja*. These results are similar to the previous study, where most of the leafy vegetables showed coliform counts of more than 4.0 $log_{10}$ CFU/g. Sair *et al*. (2017) reported that lettuce from local distributors and retailers in Pakistan had a high coliform count of 6.2 $log_{10}$ CFU/g. Another study conducted by Mritunjay and Kumar (2017) also found that the coliform counts in spinach and coriander from the retail market in India were 5.8 and 4.7 $log_{10}$ CFU/g, respectively. The possible explanation related to high coliform counts in leafy vegetables is that they have open leaves with large surface areas and folds. These open leaves often touch the soil and irrigation water, making them more vulnerable to bacterial

 

contaminations and adhesion (Seow *et al.*, 2012; Mritunjay and Kumar, 2017).

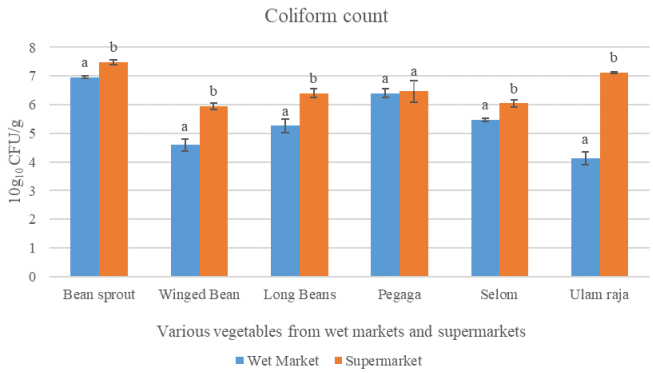


Figure 2. Total coliform count of various vegetables from wet markets and supermarkets. Bars are mean±standard deviation of triplicates. Bars with different notations indicate significant difference ($p<0.05$) between samples taken from supermarkets and wet markets.

There is a significant difference in the number of total coliforms between wet markets and supermarkets. Total coliforms count in bean sprout, winged bean, long bean, *selom* and *ulam raja* from supermarkets were significantly higher compared to wet market ($p < 0.05$). No significant difference was found in *pegaga* isolated from the wet market and supermarket ($p > 0.05$).

Figure 3 present the results of *Salmonella* spp. counts of all *ulam* samples analysed. In this analysis, the level of *Salmonella* spp. in all *ulam* samples ranged from 0 to 8.16 $\log_{10}$ CFU/g, with a mean of 4.30 $\log_{10}$ CFU/g. The *Salmonella* spp. counts in the present study were relatively higher than in previous research. Hassan and Purwani (2016) reported that *Salmonella* spp. counts for fresh vegetables in West Java, Indonesia ranged from 1.30 to 3.95 $\log_{10}$ CFU/g. Similarly, Abakari *et al.* (2018) assessed the microbial quality of salad samples (cabbage, lettuce, onions and tomato) in Tamale, Ghana and found a lower *Salmonella* spp. counts ranged from 0 to 4.54 $\log_{10}$ CFU/g. According to Food Standards Australia New Zealand (2016) and NSW Food Authority (2009), the *Salmonella* spp. should not be detected in 25 g ready to eat foods for human consumption otherwise it should be categorized as potentially hazardous. Based on these guidelines, 58.3% of *ulam* samples were identified as unsatisfactory for human consumption.

In the supermarket, the contamination of *Salmonella* spp. was observed to be higher in leafy vegetables than in non-leafy vegetables. Among the leafy vegetables, *selom* (8.16±0.13 $\log_{10}$ CFU/g) had the highest colony count, followed by *pegaga* (8.01±0.12 $\log_{10}$ CFU/g) and *ulam raja* (7.85±0.24 $\log_{10}$ CFU/g). These results further support the statement of FAO/WHO (2008), which ranked leafy green vegetables as the highest level priority in terms of fresh produce safety and has been linked to high numbers of diseases. The *Salmonella* spp. can contaminate leafy vegetables at any stage of the production process, but large numbers of multistate outbreaks suggest that contamination occurred early in production. The leafy vegetables can be contaminated through animal manure used for fertilisers, irrigation water, and feral animals that invaded the vegetable fields (Herman *et al.*, 2015). For the wet market, *Salmonella* spp. were not detected in mostly raw vegetables and only present in long bean (6.71±0.08 $\log_{10}$ CFU/g).

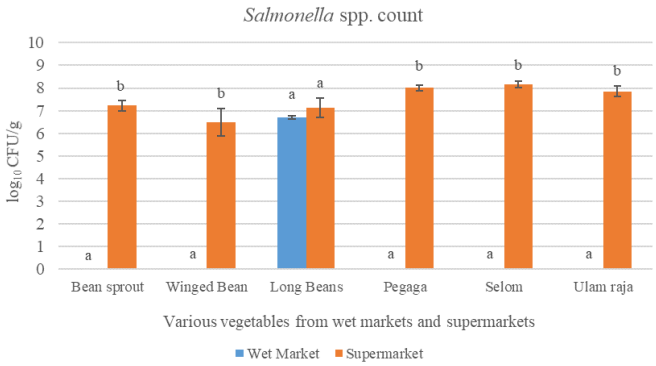


Figure 3. *Salmonella* spp. count of various vegetables from wet markets and supermarkets. Bars with different notations indicate significant difference ($p<0.05$) between samples taken from supermarkets and wet markets.

The data highlighted that the colony count of *Salmonella* spp. in bean sprout, winged bean, *pegaga, selom* and *ulam raja* from supermarkets was significantly higher compared to wet markets ($p < 0.05$). There are several explanations attributed to the contamination, such as a longer holding time (the period between harvesting and selling) of *ulam* in the supermarket, thus giving more time for microorganisms to grow and multiply. During the raw vegetables selection process, unhygienic handling practices by the customers at the display unit will result in cross-contamination in the supermarket. Moreover, plastic containers used for storage and transportation of fresh produce may contribute to cross-contamination and foodborne infection (Hassan and Purwani, 2016). However, no significant difference was examined between the wet market and supermarket with regard to long bean samples ($p > 0.05$).

The results of yeast and mould counts are shown in Figure 4. In this study, the *ulam* samples had a mean yeast and mould count of 5.42 $\log_{10}$ CFU/g, ranging from 3.94 to 6.45 $\log_{10}$ CFU/g. The mean value for yeast and moulds in all *ulam* samples is lower than aerobic mesophilic bacteria. Similar to the present study, Najafi and Bahreini (2012) reported that the mean count of yeast and mould for mixed fresh-cut vegetable salads was 5.68 $\log_{10}$ CFU/g, ranging from 3.85 to 6.7 $\log_{10}$

 

CFU/g. Abadias *et al*. (2008) revealed that the yeast and mould count for fresh-cut vegetables was 5.2 $\log_{10}$ CFU/g, with a range of 2.0 to 7.8 $\log_{10}$ CFU/g. These findings are further supported by a study conducted in Malaysia. Kuan *et al*. (2017) reported that the yeast and mould counts of most organic and conventional vegetables varied from 3 to 6 $\log_{10}$ CFU/g. According to James *et al*. (2019), the contamination of vegetables occurs because they have high moisture content, and fungi usually grow well under this condition. The presence of moulds in vegetables may pose a health risk to the consumer, as some of them may produce harmful mycotoxins or cause allergic reactions (Mritunjay and Kumar, 2017).

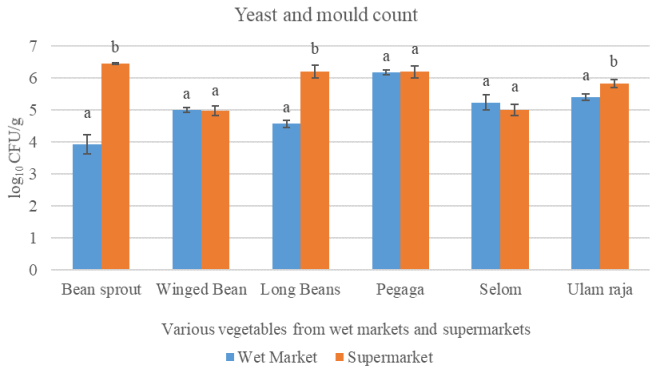


Figure 4. Yeast and moulds of various vegetables from wet markets and supermarkets. Bars with different notations indicate significant difference ($p<0.05$) between samples taken from supermarkets and wet markets.

The highest number of yeast and mould in *ulam* purchased from the supermarkets was recorded for bean sprout (6.45±0.03 $\log_{10}$ CFU/g), followed by long bean (6.20±0.20 $\log_{10}$ CFU/g) and pegaga (6.20±0.19 $\log_{10}$ CFU/g). These results are in agreement with Jeddi *et al*. (2014), who found that the yeast and mould count in mung bean and wheat sprout were 6.9 and 6.8 $\log_{10}$ CFU/g, respectively. Further analysis demonstrated that the highest number of yeast and mould in *ulam* obtained from the wet market was reported for *pegaga* (6.18±0.07 $\log_{10}$ CFU/g), followed by *ulam raja* (5.41±0.09 $\log_{10}$ CFU/g), *selom* (5.24±0.23 $\log_{10}$ CFU/g) and winged bean (5.01±0.08 $\log_{10}$ CFU/g). These findings are consistent with Kuan *et al*. (2017), who detected yeast and mould in the levels of 5.61 $\log_{10}$ CFU/g. in winged bean. The minimum colony count of yeast and mould purchased from the wet market was detected in bean sprout (3.94±0.30 $\log_{10}$ CFU/g), followed by long bean (4.57±0.11 $\log_{10}$ CFU/g). From Figure 4, it can be seen that the colony count of yeast and moulds in bean sprout, long bean and *ulam raja* purchased from the supermarket was significantly higher than the wet market ($p < 0.05$). No significant difference was observed between the supermarket and wet market with respect to winged bean, *pegaga* and *selom* ($p > 0.05$).

Interestingly, there was no evidence of *Bacillus cereus* in any of the *ulam* samples. This finding is consistent with other previous studies. For example, Nguz *et al*. (2005) conducted a survey on fresh organic mixed vegetables and green beans obtained in Zambia and these samples were found to be free of *B. cereus*. This is maybe attributed to postharvest treatments such as washing and disinfection that are effective in killing pathogenic bacteria (Thunberg *et al*., 2002). In contrast to earlier studies, Kim *et al*. (2016) reported a high incidence of *B. cereus* in organic (70%) and conventional (30%) vegetables purchased from the retail market in South Korea. Moreover, six samples of organic vegetables had a high level of *B. cereus* over 4 $\log_{10}$ CFU/g, which is considered to be unsatisfactory. In China, 50% of vegetable samples (coriander, lettuce, cucumber and tomato) were contaminated with *B. cereus*.

In this study, *Listeria monocytogenes* was not detected in any *ulam* samples analysed. Similarly, the *L. monocytogenes* was absent in a survey conducted on fresh vegetables in Istanbul, Turkey (Buyukunal *et al*., 2015). However, these findings differ from Ponniah *et al*. (2010), who surveyed the occurrence of *L. monocytogenes* in raw vegetables (carrot, sweet potatoes, Indian pennywort, cabbage, Japanese parsley, wild parsley, winged bean, yardlong bean, tomato and cucumber) purchased from markets in Selangor, Malaysia. They found that 22.5% of the raw vegetables were contaminated with *L. monocytogenes*. Moreover, Sant'Ana *et al*. (2012) reported that *L. monocytogenes* was found in 3.1% of ready-to-eat vegetables sold in Sao Paulo, Brazil. In their study, *L. monocytogenes* was detected in five samples, which ranged from 1 to 2.41 $\log_{10}$ CFU/g. Although *L. monocytogenes* was absent in this study, the humid and warm environment may favourable the growth of *L. monocytogenes* in fresh vegetables (Kuan *et al*., 2017). Therefore, it is essential to continuously monitor the prevalence of *L. monocytogenes* sold in our market.

### *3.2 Isolation and biochemical test of Escherichia coli*

A total of 87 presumptive *E. coli* were isolated after incubation in the selective media. *E. coli* colonies growing on Violet Red Bile Agar (VRBA) were appeared pink to red colonies with a red precipitate around colonies. While for MacConkey agar, *E. coli* was seemed to be pink to red with bile salt precipitate surrounding colonies. The *E. coli* colonies appeared blue -black centred colonies with green metallic sheen when grown on Eosin Methylene Blue (EMB) agar. Those

 

isolates were further subjected to the pre-identification test, which includes Gram staining, oxidase test and API20E.

Gram staining is a common technique used to distinguish bacteria into two large groups of bacteria (Gram-positive and Gram-negative bacteria) based on their cell wall compositions. Members of the family *Enterobacteriaceae* such as *E. coli* and *Salmonella* were grouped in Gram-negative rod-shaped bacteria (Wardani *et al*., 2019). Moreover, the oxidase test was performed on the *E. coli* isolates. The oxidase test is used to determine bacteria that contain cytochrome c oxidase, a large transmembrane protein complex in the respiratory electron transport chain. If present, the cytochrome c oxidase would oxidize the reagent to dark blue, then the result is positive. However, if no colour changes occurred within three minutes, the result is considered to be negative. This test is helpful in screening colonies of suspected *Enterobacteriaceae* since they are generally lacking this enzyme and characterized as oxidase negative (Acharya, 2012). As shown in Table 5, only 23 *E. coli* isolates were classified as Gram-negative rod-shaped bacteria and oxidase negative.

The presumptive isolates (23) were further tested with the API20E, and all of them were identified as *E. coli* (Table 6). Table 7 represents the incidence of *E. coli* in the 32 vegetable samples. In this study, *E. coli* was detected in 31.3% of samples (*pegaga*, bean sprout, *ulam raja*, *salad kampung*, *daun sup*, *kangkung*, *kesum*, *ketumbar* and *pucuk putat*). This result is in agreement with Hassan and Purwani (2016), which reported the incidence level of *E. coli* in fresh vegetables was 34%. Similarly, in Pakistan, *E. coli* was found in 30.1% of vegetable samples (Sair *et al*., 2017). In this study, the incidence of *E. coli* isolates was higher than that found in previous reports. In India, Mritunjay and Kumar (2017) reported that *E. coli* was found in 16.7% of raw salad vegetables collected from the local and retail market. In addition, Abadias *et al*. (2008) also reported that 11.4% of fresh-cut vegetables contained *E. coli*. The *E. coli* was observed in arugula, lettuce, spinach, and mixed salad sold in Spain. The levels of *E. coli* is used as an indicator of faecal contamination and to monitor the sanitary conditions of food (Nguz *et al*., 2005; Mritunjay and Kumar, 2017). Table 8 shows the distribution of *E. coli* varied with the types of vegetables. Among them, the *E. coli* isolates were found to be predominant on *ketumbar* (n = 5), followed by *kangkung* (n = 4), *salad kampung* (n = 4) and *daun sup* (n = 4). No *E. coli* was detected in long bean, winged bean, cucumber and *selom*.

### *3.3 Isolation and biochemical test of Salmonella spp.*

A total of 118 presumptive *Salmonella* were isolated after incubation in selective media. *Salmonella* colonies growing on XLD agar appeared red colonies with black centres. While for BS agar, *Salmonella* colonies seemed to be black or greenish-grey may or may not have a

Table 5. The results of Gram staining and oxidase test for presumptive *E. coli*

| Market | Sample | Coding | Gram Staining | Oxidase Test |
|---|---|---|---|---|
| Wet Market | *Daun Sup* | WMDS | Negative | Negative |
| | *Salad Kampung* | WMSK1 | Negative | Negative |
| | | WMSK4 | Negative | Negative |
| | | WMSK6 | Negative | Negative |
| | | WMSK7 | Negative | Negative |
| | *Pucuk Putat* | WMPP2 | Negative | Negative |
| Supermarket | *Pegaga* | SMP1 | Negative | Negative |
| | *Daun Sup* | SMDS1 | Negative | Negative |
| | | SMDS2 | Negative | Negative |
| | | SMDS4 | Negative | Negative |
| | *Kangkung* | SMKG1 | Negative | Negative |
| | | SMKG2 | Negative | Negative |
| | | SMKG3 | Negative | Negative |
| | | SMKG4 | Negative | Negative |
| | *Ketumbar* | SMKB2 | Negative | Negative |
| | | SMKB3 | Negative | Negative |
| | | SMKB4 | Negative | Negative |
| | | SMKB5 | Negative | Negative |
| | | SMKB8 | Negative | Negative |
| | *Kesum* | SMK2 | Negative | Negative |
| | | SMK3 | Negative | Negative |
| | *Ulam Raja* | SMU2 | Negative | Negative |
| | Bean Sprout/*Tauge* | SMT4 | Negative | Negative |

 

Table 6. The results of confirmation of *E. coli* by API20E

| Market | Sample | Coding | Confirmation (API20E) | No. ID |
|---|---|---|---|---|
| Wet Market | *Daun Sup* | WMDS | *Escherichia coli* | 5144572 |
| | *Salad Kampung* | WMSK1 | *Escherichia coli* | 5044552 |
| | | WMSK4 | *Escherichia coli* | 5144572 |
| | | WMSK6 | *Escherichia coli* | 5144572 |
| | | WMSK7 | *Escherichia coli* | 5044552 |
| | *Pucuk Putat* | WMPP2 | *Escherichia coli* | 5144572 |
| Supermarket | *Pegaga* | SMP1 | *Escherichia coli* | 5144552 |
| | *Daun Sup* | SMDS1 | *Escherichia coli* | 5144572 |
| | | SMDS2 | *Escherichia coli* | 5144552 |
| | | SMDS4 | *Escherichia coli* | 5144552 |
| | *Kangkung* | SMKG1 | *Escherichia coli* | 5144572 |
| | | SMKG2 | *Escherichia coli* | 5144572 |
| | | SMKG3 | *Escherichia coli* | 5144572 |
| | | SMKG4 | *Escherichia coli* | 5144572 |
| | *Ketumbar* | SMKB2 | *Escherichia coli* | 5044572 |
| | | SMKB3 | *Escherichia coli* | 5044572 |
| | | SMKB4 | *Escherichia coli* | 5044572 |
| | | SMKB5 | *Escherichia coli* | 5044552 |
| | | SMKB8 | *Escherichia coli* | 5044572 |
| | *Kesum* | SMK2 | *Escherichia coli* | 5544572 |
| | | SMK3 | *Escherichia coli* | 5544572 |
| | *Ulam Raja* | SMU2 | *Escherichia coli* | 5544572 |
| | Bean Sprout/*Tauge* | SMT4 | *Escherichia coli* | 5144572 |

Table 7. The incidence of positive samples for *E. coli* and *Salmonella* in vegetables samples

| Vegetables | n | Percentage (%) of positive samples | |
|---|---|---|---|
| | | *E. coli* | *Salmonella* |
| *Pegaga* | 4 | 1 | ND |
| Bean sprout/*Tauge* | 4 | 1 | 1 |
| Long bean/*Kacang Panjang* | 4 | ND[a] | 1 |
| *Ulam Raja* | 3 | 1 | ND |
| Winged bean/*Kacang Botol* | 3 | ND | ND |
| *Salad Kampung* | 2 | 1 | ND |
| *Daun Sup* | 2 | 2 | ND |
| Cucumber | 2 | ND | ND |
| *Kangkung* | 2 | 1 | ND |
| *Selom* | 2 | ND | 1 |
| *Kesum* | 1 | 1 | ND |
| *Ketumbar* | 1 | 1 | ND |
| *Bayam* | 1 | ND | ND |
| *Pucuk Putat* | 1 | 1 | ND |
| Total | 32 | 10/32 (31.3) | 3/32 (9.4%) |

[a] ND: not detected

sheen. Colonies were appeared blue-green with or without black centres when grown on HE agar. Those isolates were further subjected to the pre-identification test, which includes triple sugar iron (TSI) and lysine iron (LI) agar test, Gram staining, oxidase test and API20E.

Table 9 shows that only 25 presumptive *Salmonella* spp. isolates showed positive results on TSI agar. The *Salmonella* spp. isolates produced an alkaline (red) slope and acid (yellow) butt with blackening due to hydrogen sulphide production (Neogen Coorporation, 2017). The *Salmonella* spp. isolates were further subjected to Gram staining and oxidase test. All of the *Salmonella* spp. isolates were classified as Gram-negative rod-shaped and oxidase negative (Table 10).

 

Table 8. The distribution of 23 *E. coli* isolates by type of samples and location

| Location | Types of sample | Total number of isolates (%) | Isolates coding |
|---|---|---|---|
| Supermarkets | *Ketumbar* | 5 (21.74) | SMKB2, SMKB3, SMKB4, SMKB5, SMKB8 |
| | *Kangkung* | 4 (17.39) | SMKG1, SMKG2,SMKG3, SMKG4 |
| | *Kesum* | 2 (8.69) | SMK2, SMK3 |
| | *Daun Sup* | 3(13.04) | SMDS1, SMDS2, SMDS4 |
| | *Pegaga* | 1 (4.35) | SMP1 |
| | *Ulam Raja* | 1 (4.35) | SMU2 |
| | Bean sprout/*Tauge* | 1 (4.35) | SMT4 |
| Wet Markets | *Salad Kampung* | 4 (17.39) | WMSK1, WMSK4, WMSK6, WMSK7 |
| | *Daun Sup* | 1 (4.35) | WMDS |
| | *Pucuk Putat* | 1 (4.35) | WMPP2 |
| Total | | 23 (100) | |

Table 9. The results of triple sugar iron (TSI) test of presumptive *Salmonella* spp.

| Market | Sample | Coding | Butt | Slant Surface | $H_2$ |
|---|---|---|---|---|---|
| Wet Market | *Salmonella* ATCC 14028 | +SM | Yellow+Black | Red | + |
| | *Pegaga* | WMP.BS.TT 2 | Yellow+Black | Yellow | + |
| | *Selom* | WMS.HE.TT 2 | Yellow+Black | Red | + |
| | | WMS.HE.TT 3 | Yellow+Black | Red | + |
| | Long Bean/*Kacang Panjang* | WKP.XLD 2 | Yellow+Black | Red | + |
| | | WKP.XLD.RVS 1 | Yellow+Black | Red | + |
| | | WKP.HE.TT 1 | Yellow+Black | Red | + |
| Supermarket | *Ulam Raja* | SUR.XLD 1 | Yellow+Black | Red | + |
| | | SUR.XLD 3 | Yellow+Black | Red | + |
| | | SUR.XLD 9 | Yellow+Black | Red | + |
| | | SUR.XLD 10 | Yellow+Black | Red | + |
| | *Pegaga* | SP.XLD 3 | Yellow+Black | Red | + |
| | | SP.XLD 4 | Yellow+Black | Red | + |
| | | SP.XLD 5 | Yellow+Black | Red | + |
| | | SP.XLD 6 | Yellow+Black | Red | + |
| | Long Bean/*Kacang Panjang* | SMKP.XLD 1 | Yellow+Black | Red | + |
| | | SMKP.XLD 3 | Yellow+Black | Red | + |
| | | SMKP.XLD 4 | Yellow+Black | Red | + |
| | Bean sprout/*Tauge* | SMT.XLD 1 | Yellow+Black | Red | + |
| | | SMT.XLD 2 | Yellow+Black | Red | + |
| | | SMT.XLD 3 | Yellow+Black | Red | + |
| | | SMT.XLD.RVS 1 | Yellow+Black | Red | + |
| | *Selom* | SMS.XLD 1 | Yellow+Black | Red | + |
| | | SMS.XLD 2 | Yellow+Black | Red | + |
| | | SMS.XLD 4 | Yellow+Black | Red | + |
| | | SMS.XLD 5 | Yellow+Black | Red | + |

As presented in Table 11, all of the presumptive *Salmonella* spp. isolates were identified using API20E. Out of 25 isolates, only six showed a high percentage of similarity (99%) to *Salmonella enterica* ser. Arizonae. In this study, 9.4% of samples were found contaminated with *Salmonella* spp (Table 7). The incidence of *Salmonella* in this study is low compared to previous results from Selangor. Salleh *et al*. (2003) reported that *Salmonella* spp. was detected in 35% of vegetable samples collected from wet markets. However, another study conducted in the United Kingdom showed a lower incidence of *Salmonella* (Sagoo *et al*., 2003). They found that 0.2% of ready-to-eat salad vegetables were contaminated with *Salmonella*. Abadias *et al*. (2008) also reported that 1.3% of fruits and vegetable samples contained *Salmonella* spp. The *Salmonella* spp. was isolated from corn salad, lettuce, spinach and mixed salad. Table 12 presents the distribution of *Salmonella* spp. in the samples analyzed. The *Salmonella* spp. was only detected in three samples, which include bean sprout, *selom* and long bean. Most of the *Salmonella* spp. were isolated from bean sprout (four out of six isolates), while only one *Salmonella* spp. was found in *selom* and long bean. In contrast to this finding, no *Salmonella* spp.

 

Table 10. The results of Gram staining and oxidase test for presumptive *Salmonella* spp.

| Market | Sample | Coding | Gram Staining | Oxidase Test |
|---|---|---|---|---|
| Wet Market | *Pegaga* | WMP.BS.TT 2 | Negative | Negative |
| | *Selom* | WMS.HE.TT 2 | Negative | Negative |
| | | WMS.HE.TT 3 | Negative | Negative |
| | Long Bean/*Kacang Panjang* | WKP.XLD 2 | Negative | Negative |
| | | WKP.XLD.RVS 1 | Negative | Negative |
| | | WKP.HE.TT 1 | Negative | Negative |
| Supermarket | *Ulam Raja* | SUR.XLD 1 | Negative | Negative |
| | | SUR.XLD 3 | Negative | Negative |
| | | SUR.XLD 9 | Negative | Negative |
| | | SUR.XLD 10 | Negative | Negative |
| | *Pegaga* | SP.XLD 3 | Negative | Negative |
| | | SP.XLD 4 | Negative | Negative |
| | | SP.XLD 5 | Negative | Negative |
| | | SP.XLD 6 | Negative | Negative |
| | Long Bean/*Kacang Panjang* | SMKP.XLD 1 | Negative | Negative |
| | | SMKP.XLD 3 | Negative | Negative |
| | | SMKP.XLD 4 | Negative | Negative |
| | Bean sprout/*Tauge* | SMT.XLD 1 | Negative | Negative |
| | | SMT.XLD 2 | Negative | Negative |
| | | SMT.XLD 3 | Negative | Negative |
| | | SMT.XLD.RVS 1 | Negative | Negative |
| | *Selom* | SMS.XLD 1 | Negative | Negative |
| | | SMS.XLD 2 | Negative | Negative |
| | | SMS.XLD 4 | Negative | Negative |
| | | SMS.XLD 5 | Negative | Negative |

Table 11. The results of confirmation of *Salmonella* spp. by API20E

| Market | Sample | Coding | Confirmation (API20E) | No. ID |
|---|---|---|---|---|
| Wet Market | *Pegaga* | WMP.BS.TT 2 | 93.6% *Citrobacter freundii* | 1604532 |
| | *Selom* | WMS.HE.TT 2 | 99.8% *Salmonella enterica* ssp Arizonae | 7705512 |
| | | WMS.HE.TT 3 | 99.8% *Citrobacter youngae* | 3604513 |
| | Long Bean/*Kacang Panjang* | WKP.XLD 2 | 99.8% *Citrobacter youngae* | 3604512 |
| | | WKP.XLD.RVS 1 | 99.8% *Citrobacter youngae* | 3604512 |
| | | WKP.HE.TT 1 | 99.8% *Citrobacter youngae* | 3604512 |
| Supermarket | *Ulam Raja* | SUR.XLD 1 | 99.9% *Citrobacter youngae* | 3604112 |
| | | SUR.XLD 3 | 99.8% *Citrobacter youngae* | 3604512 |
| | | SUR.XLD 9 | 99.8% *Citrobacter youngae* | 3604512 |
| | | SUR.XLD 10 | 99.8% *Citrobacter youngae* | 3604512 |
| | *Pegaga* | SP.XLD 3 | 99.8% *Citrobacter youngae* | 3604512 |
| | | SP.XLD 4 | 99.8% *Citrobacter youngae* | 3604512 |
| | | SP.XLD 5 | 99.0% *Citrobacter youngae* | 3605512 |
| | | SP.XLD 6 | 99.8% *Citrobacter youngae* | 3604512 |
| | Long Bean/*Kacang Panjang* | SMKP.XLD 1 | 99.8% *Citrobacter youngae* | 3604512 |
| | | SMKP.XLD 3 | 99.0% *Citrobacter youngae* | 3605512 |
| | | SMKP.XLD 4 | 99.8% *Salmonella enterica* ssp Arizonae | 7704512 |
| | Bean sprout/*Tauge* | SMT.XLD 1 | 99.8% *Salmonella enterica* ssp Arizonae | 7704572 |
| | | SMT.XLD 2 | 99.8% *Salmonella enterica* ssp Arizonae | 7704512 |
| | | SMT.XLD 3 | 99.8% *Salmonella enterica* ssp Arizonae | 7704512 |
| | | SMT.XLD.RVS 1 | 99.8% *Salmonella enterica* ssp Arizonae | 7704512 |
| | *Selom* | SMS.XLD 1 | 99.8% *Citrobacter youngae* | 3604512 |
| | | SMS.XLD 2 | 99.8% *Citrobacter youngae* | 3604113 |
| | | SMS.XLD 4 | 99.8% *Citrobacter youngae* | 3604113 |
| | | SMS.XLD 5 | 99.8% *Citrobacter youngae* | 3604113 |

 

Table 12. The distribution of 6 *Salmonella* spp. isolates by type of samples and location

| Location | Sample | Total number of isolates (%) | Isolates coding |
|---|---|---|---|
| Supermarket | *Tauge* | 4 (66.6) | SMT.XLD1, SMT.XLD2, SMT.XLD3.SMT.XLD.RVS1 |
| | *Kacang panjang* | 1 (16.7) | SMKP.XLD4 |
| Wet Market | *Selom* | 1 (16.7) | WMS.HE.TT2 |
| Total | | 6 (100) | |

was detected in 600 samples of bean sprout produced in Italy (Lucilla *et al*., 2017).

## 4. Conclusion

This study provides information on the microbiological quality of *ulam* sold in supermarkets and wet markets in Kuala Terengganu, Terengganu, Malaysia. The findings showed that all *ulam* samples were contaminated with bacteria. The microbial load of *ulam* samples were in the range of 6.36-8.83; 4.14-7.48; 0-8.16; 3.94-6.45 $\log_{10}$ CFU/g for aerobic mesophilic bacteria, coliforms, *Salmonella* spp. and yeast and moulds, respectively. A total of 23 *E. coli* and 6 *Salmonella enterica* ser. Arizonae were identified by API20E. All presumptive *E. coli* and *Salmonella* isolates were also confirmed as Gram-negative rod-shaped bacteria and oxidase negative. In this study, *E. coli* was detected in 31.3% of *ulam* samples, whereas 9.4% of samples were found contaminated with *Salmonella*. The contamination of *ulam* samples could occur during growth, harvesting, packaging, transportation and distribution. Therefore, good hygiene practices must be implemented by the producers in order to minimise the risk of transmission of foodborne bacteria. Statistically, the total number of coliforms in bean sprout, winged bean, long bean, *selom* and *ulam raja* from supermarkets was significantly higher compared to wet market ($p<0.05$). Poor handling along the supply chain could have contributed to the high microbial load in the supermarket. Therefore, the employees and customers should always implement a good handling practice to avoid cross-contamination from occurring.

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