Bacteriological quality of some common vegetables among two different markets

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Abstract

Vegetables are major components of a healthy diet, but eating fresh uncooked product is not risk free.Microbiological control is very important in food industry to prevent foodborne diseases. Therefore, the present investigation was undertaken to assess the bacteriological quality of fresh vegetables collected from two different markets, supermarkets and open air marketsin downtown area of Yangon.Presence of hygiene indicator organisms were investigated by using the manual for food quality control (FAO, 1992) and Guideline for determining the microbiological quality of ready-to-eat foods (FSANZ, 2016). A total of sixty vegetables samples were taken from selectedcommon vegetables namely cauliflower, cabbage, lettuce, tomato and radish.Contamination was mainly found in cauliflower, cabbage and lettuce. The microbial load in vegetables procured from open air markets was significantly higher in comparison to vegetables from supermarkets with the exception of lettuce and tomato. In open air markets, maximum total plate count was observed in cauliflower followed by cabbage and lettuce while in supermarkets it was recorded in cabbage followed by lettuce. Similarly, maximum coliform count was observed in cabbage from open air markets followed by lettuce. Maximum coliform count in supermarkets was recorded in cabbage followed by cauliflower. Faecal coliform and *E. coli*were detected only in cabbage procured from shops of open air markets. This study showed that vegetables sold in the open marketswere usually having higher microbial load that may represent a risk for human health.

Keywords___ Bacteriological quality, common vegetables, different markets, coliform bacteria

Introduction

Fresh vegetables are main dish of daily meals for Myanmar people served in homes, school and restaurants. They are naturally good and contain vitamins and minerals and that can help to keep healthy. They can also help protect against some diseases.A diet rich in fruits and vegetables reduces the risk of obesity, cancers and cardiovascular diseases that are considered to be the leading causes of death and disability in the world(Joshipura*et al.,* 2006).

Vegetable contamination can occur at any time in the food chain, from the farms to the consumer's plate, through transportation, distribution and markets with the presence or absence of pathogens (Adjrah *et al*.,2013). Being ubiquitous in distribution, microorganisms can gain entry into the food from various sources during different stages of their processing,storage and serving. Besides, providing a suitable nutritional and physical environment for growth and multiplication, the foods have an inherent capacity to sustain microorganisms in large numbers.

The possible sources of contamination include environmental sources such as soil, dust, air, water etc., biotic factors(e.g. Animals, insects, food handlers having illness or unhygienic practices) and other miscellaneous sources that include laundry facilities used by the food handlers, raw materials and ingredients used in preparing foods. All these factors contribute towards the contamination of foods by a variety of microorganisms and occasionally by pathogenic ones. When the microorganisms involved are pathogenic, their association with our foodis critical from a public health point of view. Serious health hazards due to presence of pathogenic microbes in foods can lead to food poisoning outbreaks(Aiyar, 2001).

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Vegetables have been associated with outbreaks of food-borne diseases. In many countries, organisms involved included bacteria, viruses and parasites. These outbreaks vary in size from a few persons affected to many thousands. Contamination of vegetables may take place at all stages during pre and post-harvest techniques(De Rover, 1998).Cultivation and operation or preparation the vegetables are responsible for this contamination (Sumner *et al.*,1997).Unsafe water used for rinsingthe vegetables and sprinkling to keep them fresh is also a source of contamination (Mensah*et al*., 2002).Other possible sources of microorganisms include soil,faeces(human and animal origin), water(irrigation, cleaning),animals(including insects and birds),handling of the products, harvesting and processing equipment and transport(Johannessetne*et al*., 2004).

Consumption of fruit and vegetable products is commonly viewed as a potential riskfactor for infection with enteropathogens such as *Salmonella* and *Escherichiacoli* O157, with recent outbreaks linked to lettuce, spinach and tomatoes. Routes ofcontamination are varied and include application of organic wastes to agricultural and as fertilizer,contamination of waters used for irrigation with faecalmaterial,direct contamination by livestock, wild animals and birds and postharvest issues suchas worker hygiene (Heaton*et al*.,2008).

In developing countries, foodborne illnesses caused by contaminated fruits and vegetables are common and in some areas and they cause a high proportion of diseases.However, due to the lack of investigation and monitoring of foodborne diseases in most of these countries, a very high proportion of outbreaks are not detected, or very few are mentioned in scientific reports (Beuchat and Ryu, 1998).

The present investigation was undertaken to evaluate the contamination of fresh vegetables(cauliflower, cabbage, lettuce, tomato and radish),collected from two different markets of downtown area of Yangon City.The aims of present work arevto fill as in parts of the baseline information for local bacteriological quality of fresh vegetables from different markets of Yangon as well as in Myanmar and to promote awareness about the possible health hazards.

The main objective of the study was to evaluate the microbiological qualities of fresh vegetables, originated from different sources, and to provide the level of microbial contamination of fresh vegetables. Results generated in this study are expected to be useful for consumer’s health awareness as well as the authorized persons to implement appropriate food safety measures to minimize the risk factors associated.

Materials and Methods

Study area

The study was carried out in downtown area of Yangon City. In the study of present research work, two areas were selected: supermarkets (SM) such as City mart, Gamone Pwint and open air markets (OM) such as Bo Galay Zay, 38 street Zay.

Sample collection and processing

Random sampling procedure was adopted to collect the sample materials. Atotal offour vegetable shops were selected;two from supermarkets and two from open air markets respectively. Sampling details are given in Table 1. Five commonly used vegetables namely cauliflower, cabbage, lettuce, tomato and radishwere selected for the present investigation. Vegetables were procured and the purchasing individually. Sterile polythene zip bags were used for collection of samples. Samples were carried to the laboratory in aseptic condition. The polythene bags with vegetables were kept in an ice box maintained at 6–10°C and processed within 2–4 hrs.

Bacteriological analysis of vegetables

25 g of each vegetable sample were weighed and blended in 100 mL of 0.1% peptone solution for 2 min under sterile conditions. The blender was carefully disinfected to prevent any cross contamination. The homogenates were collected in sterile bottles and each homogenate was serially diluted in sterile distilled water.Presence of hygiene indicator organisms were investigated by using the Manual for food quality control (FAO, 1992) and Guideline for determining the microbiological quality of ready-to-eat foods (FSANZ, 2016).

Total Pate Count Method (Standard Plate Count)

The preparation of 1 ml of each dilution mixed with sterilized plate count agar medium by using pour plate method. The plates were incubated at room temperature in 24 to 48hr for the appearance of colonies. This procedure was repeated at least 3 times for each sample. Plates having counts between 30-300 colonies were chosen (Postgate*et al.*, 1961).

After incubation, colonies were calculated and the total of bacteria was calculated for Colony Forming Unit (CFU) by using following formula.Colony counts were converted into $\log_{10}$ $CFUg^{-1}$

$$CFU / g = \frac{\text{No.of colonies} \times \text{diluction factor}}{\text{volume of inoculum}}$$

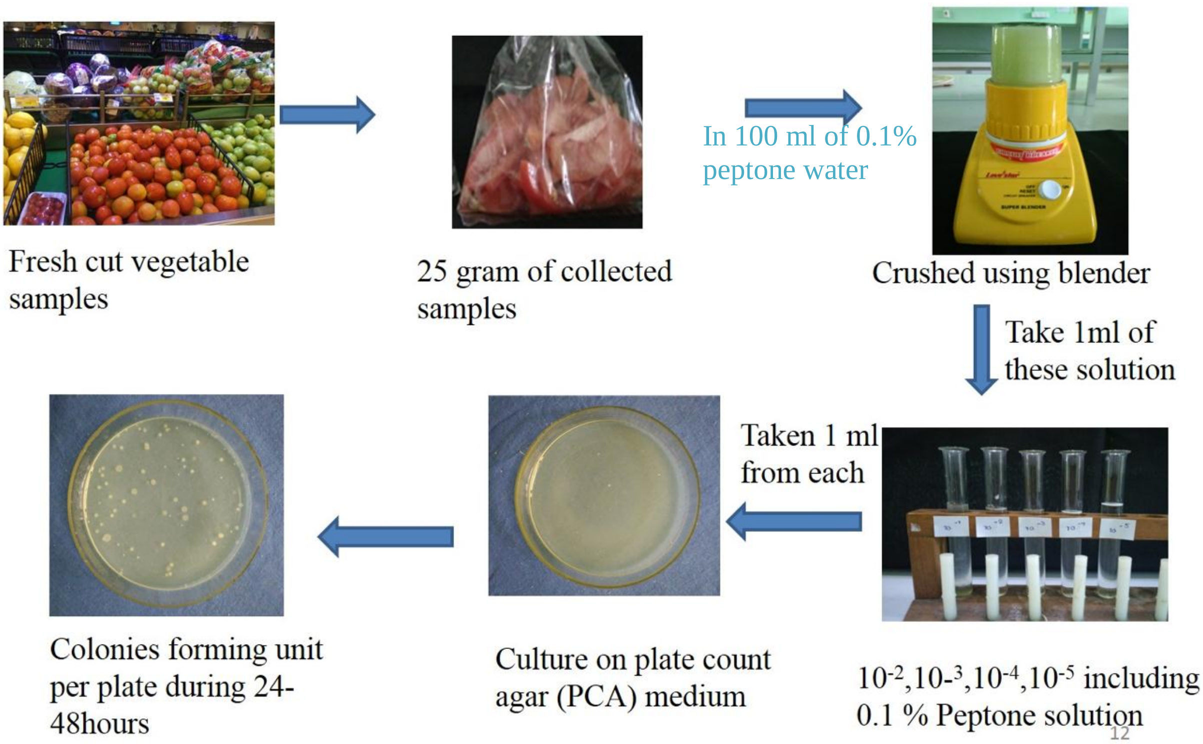


Figure 1 Procedure of bacteriological analysis from fresh cut vegetables (Harrigan*et al.*, 1996)

Most Probable Number (MPN) method for total coliform bacteria

In the MPN test, thesample to be tested is prepared in 10 fold dilutionseries, and then 1 ml samples of eachdilution areinoculated into triplicate broth culture tubes forincubation. Following incubation, all tubes are examined for collection of gases in inverted durham tubes thatare scored against a table of such values. The MPNtable from the US Food and Drug Administration'sBacteriological Analytical Manual (BAM)was used as references.

Presumptive test of total coliform

The test tube containing 10ml of lauryl sulphate tryptose broth (LST) (containing inverted durham tube) was mixed with 1ml of food homogenate sample. Each test tube was kept in incubator at 35 ºC for 24 hr. Record tubes showing gas production after 24hr and reincubate negative tubes for further 24hr. Record tubes showing gas production(Andrew *et al*., 2001).

Confirmed test of total coliform

A loopful from each gas positive tube of LST broth was transferred to a separate tube of Brilliant Green Lactose Bile broth (BGLB).Incubate the BGLB broth tubes at 35 ºC for 24 hr. The formation of gas formation confirms the presence of coliform bacteria. Record the number of positive tubes that were confirmed as positive results for coliform bacteria (Andrew *et al*., 2001).

Isolation of faecalcoliform

Aloopful from each gas positive tube of LST broth was transferred to a sterilize separate tube of 8ml *Escherichia coli* (EC)broth (containing inverted durham tube). Incubate at 45 ºC in water bath for 24hr. Record tubes showing gas production(Andrew *et al*., 2001).

Isolation of*E.coli*

The sterilized Levine's Eosin-Methylene Blue (L-EMB) medium was streaked with one loopful of each positive BGLB tube. Each platewas incubated inverted position of 35ºC for 24 hr. The appearance of plate observed typical nucleated dark centered colonies(Michael J.*et al*.,1996).If typical colonies were present, pick it from each L-EMB plate by using loop to the center of the colony and transfer to PCA slant(Andrew *et al*., 2001). Perform the gram stain in a smear prepared from 18 hours PCA slant. Presence of small red colour rods confirms *Escherichia coli*.

Table 1. Sampling details of vegetables from open air markets and supermarkets

Sr. No.	Samples	OM Samples*	SM Samples*	Frequency of observation	Total
1	Cabbage	2	2	3	12
2	Cauliflower	2	2	3	12
3	Lettuce	2	2	3	12
4	Tomato	2	2	3	12
5	Radish	2	2	3	12
					60

* A total of four shops (two from OM and two from SM) were selected for sampling of vegetables; OM, Open air markets, SM, Supermarkets

Statistical analysis

Mean of the vegetable samples and standard deviation was calculated using Microsoft Excel 2010 program.

Results

A total of five vegetables were selected for the study namely cauliflower, cabbage, lettuce, tomato and radish. Two samples of each vegetable were taken from two different shops in both supermarkets as well as open air markets. Sample collection and investigation were made three times for each sample during the study period, i.e., a total sixty samples were taken. Total plate count (TPC), total coliform, faecal coliform and*E. coli* bacteria were determined. Figure 2depicts the TPC count among the some common vegetables in open air markets and super market. The microbial load in three vegetables (cauliflower, cabbage and radish) procured from open air markets was significantly higher in comparison to vegetables from super market. Maximum TPC (5.36log_{10} cfu/g) was observed in cauliflower from open air market followed by cabbage 5.25 log_{10} cfu/g) and lettuce, tomato, and radish (4.872log_{10} cfu/g, 4.64 log_{10} cfu/g, 4.51 log_{10} cfu/g). Maximum TPC in supermarkets was recorded in cabbage(5.15log_{10} cfu/g) followed by cauliflower (4.87 log_{10} cfu/g) whereas minimum for tomato and radish were 4.81and 4.3 log_{10} cfu/g(Table4).

In the results, the highest total coliform count of bacteria in open air markets were revealed (1100 MPN/g) for OMCab.5 and lowest coliform count of (15 MPN/g) represent for OMRa.2 and 4. In Supermarkets (210 MPN/g) for SMCali.3,4,6, SMCab.1,2,3,5, and SMLet.3 were shown highest total coliform count and (15 MPN/g) for SMRa.1,4,5 and 6 were revealed lowest count of coliform bacteria as shown in Table 5.

Similarly, table 6shows the coliform bacteria count among the selected vegetables. The analysis of the statistic data reveals difference in coliform bacteria count between the two different markets.Maximum coliform bacteria count was observed in open air markets for cabbage (538.3±283.4), followed by lettuce (170.82±70) and cauliflower (167.5± 54). Maximum coliform count in supermarkets were recorded in cabbage (170.84±70.74), cauliflower (158.33 ±73.86) followed by lettuce (97.66 ± 82.2), tomato (66.0±65.1) and radish (18± 5.25).

In coliform test, all samples show as positive on LST broth (presumptive test) and similar results on BGLB broth (confirmed test) as shown in Figure 4 and Figure 5. However, in faecal coliform test, only one sample (OMCab.5) was shown the present of fecal coliform as positive in EC broth for open air markets (Figure.6).

In the result of identification for *E.coli*,positive tube of BGLB broth for OMCab 5 samples,grown on (L-EMB) medium were showed dark centered colony, gram negative, rod shape (Figure.7)

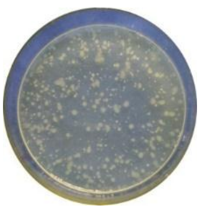
Table.2 Total plate count of collected vegetables samples from supermarkets

Sample. No	Cabbage		Cauliflower		Lettuce		Tomato		Radish	
	CFU/g	Log_{10} CFU/g	CFU/g	Log_{10} CFU/g	CFU/g	Log_{10} CFU/g	CFU/g	Log_{10} CFU/g	CFU/g	Log_{10} CFU/g
1	6.0 x 10^5	5.778	2.8 x 10^4	4.447	3.5 x 10^4	4.544	3.8 x 10^4	4.579	2.6 x 10^4	4.414
2	3.3 x 10^4	4.518	3.7 x 10^4	4.568	4.5 x 10^5	5.653	3.3 x 10^4	4.518	3.5 x 10^3	3.544
3	4.6 x 10^5	5.662	5.8 x 10^5	5.763	3.5 x 10^4	4.544	3.1 x 10^4	4.491	4.2 x 10^4	4.623

4	6.2 x 10^5	5.792	2.5 x 10^5	5.397	4.2 x 10^4	4.623	3.3 x 10^4	4.518	3.5 x 10^4	4.544
5	4.3 x 10^4	4.633	3.5 x 10^4	4.544	3.9 x 10^4	4.591	4.2 x 10^5	5.623	2.8 x 10^4	4.447
6	3.5 x 10^4	4.544	3.2 x 10^4	4.505	4.2 x 10^5	5.623	1.5 x 10^5	5.176	6.4 x 10^4	4.806

Table.3 Total plate count of collected vegetables samples from open air markets

Sample. No	Cabbage		Cauliflower		Lettuce		Tomato		Radish	
	CFU/g	Log_{10} CFU/g	CFU/g	Log_{10} CFU/g	CFU/g	Log_{10} CFU/g	CFU/g	Log_{10} CFU/g	CFU/g	Log_{10} CFU/g
1	3.5 x 10^4	4.544	3.0 x 10^4	4.477	4.3 x 10^5	5.633	3.2 x 10^4	4.505	2.9 x 10^4	4.462
2	6.3 x 10^4	4.799	9.8 x 10^5	5.991	3.3 x 10^5	5.518	1.3 x 10^4	4.133	3.2 x 10^4	4.505
3	5.7 x 10^5	5.755	4.5 x 10^5	5.653	3.5 x 10^4	4.544	2.5 x 10^5	5.397	3.9 x 10^4	4.591
4	4.5 x 10^5	5.653	6.5 x 10^5	5.812	4.8 x 10^4	4.681	3.5 x 10^4	4.544	2.5 x 10^4	4.397
5	3.3 x 10^4	4.518	3.7 x 10^4	4.568	2.9 x 10^4	4.462	4.4 x 10^4	4.643	3.5 x 10^4	4.544
6	1.8 x 10^5	5.255	4.8 x 10^5	5.681	2.5 x 10^4	4.397	4.2 x 10^4	4.623	3.8 x 10^4	4.579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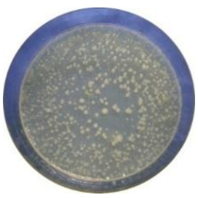
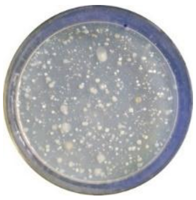
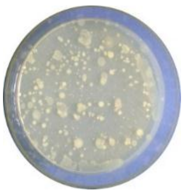
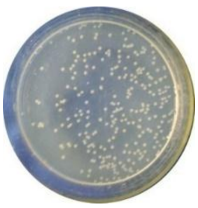

OMCauli.2

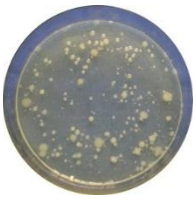

OMLet.1

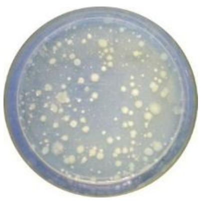

OMTo.3


OMRa.3


SMCab.4


SMCauli.3


SMLet.2


SMTo.5


SMRa.6

Figure 2 Total plate count of vegetables samples from different markets

Table.4 Mean and deviation of total plate count for collected vegetable samples from two different markets

Name of vegetables	Mean of Log_{10} CFU/g	
	Samples from Open Market	Samples from Super Market
Cauliflower	5.363±0.66	4.87±0.56
Cabbage	5.255±0.54	5.154±0.64
Lettuce	4.872±0.55	4.829±0.54
Tomato	4.64±0.41	4.81±0.47
Radish	4.51±0.07	4.396±0.44

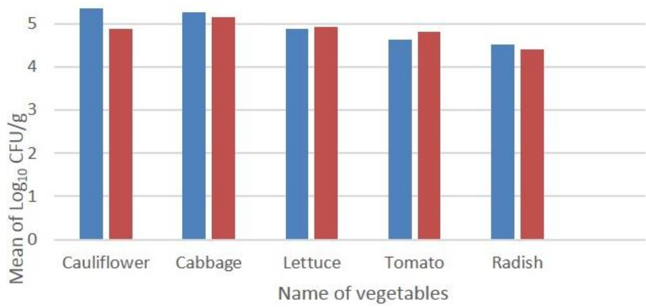


Figure.3 Comparison of total plate count for vegetable samples from two different

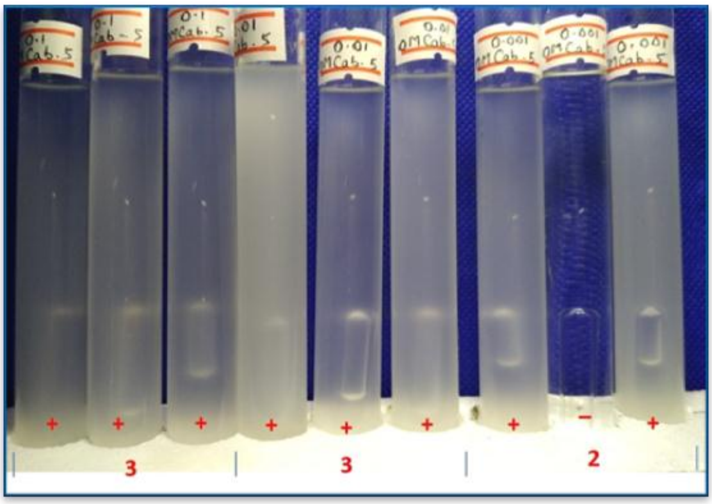


Figure. 4 Presumptive test for total coliform on LST broth

Figure.5 Confirmed test for total coliform on BGLB

Figure.6 Determination of faecal coliform on EC broth (Detected in OMCab.5)

E.coli on L-EMB medium plate

Dark centered *E.coli* colony on L-EMB medium plate

E.coli on PCA slant

Gram staining of *E.coli* bacteria (Gram negative, rod) 400X

Figure. 7 Pure culture and cell morphology of *E.coli*isolated from OMCab.5 vegetable samples

Table.5 Most Probable Number (MPN) of total coliform bacteria in vegetables samples of different markets

Samples from open air market					Samples from super market				
Sample Name	Positive Tube number			MPN/g	Sample Name	Positive Tube number			MPN/g
	0.1	0.01	0.001			0.1	0.01	0.001	
OMCauli-1	3	2	2	210	SMCauli-1	3	2	1	150
OMCauli-2	3	2	1	150	SMCauli-2	2	1	1	20
OMCauli-3	3	2	2	210	SMCauli-3	3	2	2	210
OMCauli-4	3	1	1	75	SMCauli-4	2	2	2	210
OMCauli-5	3	2	1	150	SMCauli-5	3	2	1	150
OMCauli-6	3	2	2	210	SMCauli-6	2	2	2	210
OMCab-1	3	3	1	460	SMCab-1	3	2	2	210
OMCab-2	3	3	1	460	SMCab-2	2	2	2	210
OMCab-3	3	3	1	460	SMCab-3	3	2	2	210
OMCab-4	3	2	3	290	SMCab-4	3	2	1	150
OMCab-5	3	3	2	1100	SMCab-5	3	2	2	210
OMCab-6	3	3	1	460	SMCab-6	2	2	2	35
OMlet-1	3	2	2	210	SMlet-1	3	2	1	150
OMlet-2	3	2	2	210	SMlet-2	2	2	1	28
OMlet-3	3	2	1	150	SMlet-3	3	2	2	210
OMlet-4	2	2	2	35	SMlet-4	3	2	1	150
OMlet-5	3	2	2	210	SMlet-5	2	2	1	28
OMlet-6	3	2	2	210	SMlet-6	2	1	1	20
OMTo-1	2	1	1	20	SMTo-1	2	2	1	28
OMTo-2	2	2	1	28	SMTo-2	3	2	1	150
OMTo-3	2	2	1	28	SMTo-3	2	1	1	20
OMTo-4	3	2	1	150	SMTo-4	2	2	1	28
OMTo-5	2	2	1	28	SMTo-5	2	1	1	20
OMTo-6	3	2	1	150	SMTo-6	3	2	1	150
OMRa-1	2	2	1	28	SMRa-1	2	1	0	15
OMRa-2	2	1	0	15	SMRa-2	2	1	1	20
OMRa-3	2	2	1	28	SMRa-3	2	2	1	28
OMRa-4	2	1	0	15	SMRa-4	2	1	0	15
OMRa-5	3	1	1	75	SMRa-5	2	1	0	15
OMRa-6	2	2	1	28	SMRa-6	2	1	0	15

Table.6Statistical analysis of total coliform bacteria in vegetables samples of different markets

Name of vegetables	**Mean and standard deviation of coliform bacteria MPN**	
	Samples from Open Market	**Samples from Super Market**
Cauliflower	167.5± 54	158.33± 73.86
Cabbage	538.3± 283.4	170.84± 70.74
Lettuce	170.82± 70	97.66± 82.2
Tomato	67.33± 64.1	66.0± 65.1
Radish	31.5± 22.4	18± 5.25

Discussion and Conclusion

In the present study, wide range of total plate count was found between cauliflower and radish as log_{10} CFU value of 5.991 to 3.544 (Table 2 and Table 3). Bacteria plate count found in food is one of the microbiological indicators for food quality (Aycicek *et al*., 2004). These microorganisms reflect the exposure of the sample to any contamination and in particular, the existence of favorable conditions for the multiplication of microorganisms. For many reasons, this parameter is useful to indicate whether cleaning, disinfection and temperature control during processing, transportation and storage, have been performed sufficiently (Tortora, 1995). Most of the vegetables with higher microbial load grow near to soil. This may be responsible for their higher count. Besides, other sources of contamination are improper handling and improper storage and transportation conditions. Radish grow under the soil but still microbial count in this vegetables was found to be very low. This may be attributed to the antimicrobial activity of these vegetables.

Inthe present research, total coliform counts varied from 15 MPN/g to 1100 MPN/g for selected vegetables samples. The highest total coliform counts of bacteria in open air market was revealed (1100 MPN/g) for cabbage and lowest was revealed (15 MPN/g) for radish from supermarket (Table 5).High loading of coliform countwas mainly found in cauliflower, cabbage, and lettuce from open air market and as well as in samples of cabbage and cauliflower from supermarket (table 5 and table 6).

The vegetables on display for sale are often touched by many hands of the customers and by the vendors. The customers pick and drop as many vegetables as are available, to enable them make a choice. Frequent handling by unhygienic hands, dusty environments of the roads and busy roads were major factors contributing to the high microbial load.

Using unhygienic water for rinsing and sprinkling the vegetables to keep them fresh is also another potential source of contamination. One time detection of *E. coli* bacteria was observed in cabbage sample from open air market (Figure 6 and Figure 7). The results of the present study are also in accordance with the findings of Halablab *et al.* (2011). They reported presence of microbial contamination in vegetables. Other possible sources of microorganisms in fresh agricultural produce may be soil, faeces (human and animal origin), water (irrigation, cleaning), animals (including insects and birds), handling of the product, harvesting and processing equipment and transport. Johannessen *et al.* (2004) also opined that these may be potential source of microbial contamination in fruits and vegetables.

Present study are in concurrence with those of Mundhada and Tambekar (2006). They reported the presence of various food-borne bacterial pathogens in fresh vegetables. They reported that coliform bacteria, *E. coli*, *Staphylococcus aureus* and *Salmonella* species were commonly detected in fresh vegetables.Therefore,finding of *E. coli* strains in present study was not serious. However, prevalence of atypical *E. coli* was needed to aware and take care of food handler and consumers.

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