wjpls, 2015, Vol. 1, Issue 1, 135-145 Research Article ISSN 2454-2229

World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org

ASSESSING BACTERIOLOGICAL QUALITY OF VEGETABLES SOLD AT NEKEMTE TOWN, ETHIOPIA

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Article Received on 20/04/2015 Article Revised on 10/05/2015 Article Accepted on 03/06/2015

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ABSTRACT

Vegetables are naturally delicious food products that are widely consumed throughout the world. But, fresh vegetables may be exposed to potential pathogenic bacteria during preparation, harvesting, storage, and improper handling to become contaminated this product. Therefore the study was done to identify and determine the bacteriological quality of some selected vegetables sold at Nekemte town. The study was conducted in Nekemte town between March 2015 to may 2015. Totally five vegetables were collected specifically and analyzed for bacteriological examination at wollega university, biology laboratory. As the result indicated these selected vegetables had high total bacterial count, this indicated that vegetables were contaminated by bacteria. Except potato, all vegetables had high bacterial load; this shows that there was high contamination level. Most of the time fruits and vegetables are easily contaminated if lacks proper handling. Therefore, to overcome this problem it needs further consideration. i.e. create awareness to the community about and how vegetables caused disease.

KEYWORDS: Total bacterial count, pathogens, vegetables, contamination.

1. INTRODUCTION

Vegetables are the tender plants part which is not sweet and may be flavored or spiced with condiments before consumption. These plants or plant parts may be eaten raw as salads or added to some cooked foods like rice. Vegetables are known to be reach in vitamins, iron, calcium, protein and minerals. Leafy green and yellow vegetables are helpful in neutralizing the acid substance produce in the course of digestion of meat, cheese and other foods as they are valued as roughages which promote digestion and helps to prevent constipation (Oyenuga and Fetuga, 1985).

Contamination of vegetables can be reduced depending on the use of good agricultural practices in growing the vegetables, practices during the harvesting, picking, transporting and marketing. The importance of washing vegetables properly especially those eaten raw is to effectively remove pathogens from the vegetables any pathogenic micro organism which may cause infections up on ingestion. Chlorine containing solutions rooter antibacterial compounds have been employed to reduce the number of contaminating microorganisms in such vegetables (Lund, 1983).

Pathogenic organisms can enter fruits and vegetables through damaged surfaces, such as punctures, wounds, cuts and splits that occur during growing or harvesting. Contamination from raw materials and equipments, additional processing conditions, improper handling, prevalence of unhygienic conditions contribute substantially to the entry of bacterial pathogens in juices prepared from these fruits or vegetables (Oliveira *et al.*, 2006; Nicolas *et al.*, 2007).

In countries, where street food vending is prevalent, there is commonly a lack of information on the incidence of food borne diseases related to the street vended foods. However, microbial studies on such foods in American, Asian and African countries have revealed increased bacterial pathogens in the food. However, the health of consumer can be adversely affected by consumption of microbiologically unsafe vegetables. Microbiological contamination of vegetables can occur directly or indirectly through contamination with soil, water, dust. During cultivation, packaging and marketing (Eni et al, 2010).

In 1995, unpasteurized fresh orange juice contaminated with *Salmonella* was linked to an outbreak in a Florida Theme Park, USA. More than 60 visitors were affected (Schmidt *et al.*, 1997). In Australia, 427 confirmed cases of salmonellosis were reported in 1999 after drinking unpasteurized orange juice (Victorian Government Department of Human Services). A cholera epidemic in Pune city, India, was related to street vended sugarcane juice containing ice that was contaminated with *Vibrio cholerae* (Mosupye *et al.*, 1999).

Appropriate dilutions were then enumerated for Total aerobic plate counts using Nutrient Agar, Coliforms using Violet Red Bile Agar, *Staphylococcus aureus* using Salt Mannitol Agar, *Vibrios* on Thiosulphate Citrate Bile Sucrose Agar. Xylose Lysine Deoxycholate Agar was used for enumeration of *Salmonella* & *Shigella* (Mosupye *et al.*, 1999; Mudgil *et al.*, 2004). All the selective media were obtained from Himedia Laboratories Ltd, Mumbai, India. All plates were incubated under aerobic conditions at 36±1°C for 24 hrs. The mean number of colonies counted was expressed as log colony forming units (cfu)/100 ml. The MPN of total coliforms was determined, following APHA recommendations. Three serial dilutions (0.1ml, 1 ml and 10 ml) were inoculated in MacConkey broth and incubated at 37°C for 24-48 hrs. Positive tubes (gas and acid production) were streaked on Eosin Methylene Blue Agar. Typical *E. coli* colonies were seeded into Tryptone broth and Indol test was done. For confirmation of the pathogens, typical colonies were checked using appropriate biochemical tests as per Collins & Lyne's Microbial Methods 6th edition.

Despite this body of work, we will have a limited understanding of the diversity produce associated microbial communities. The factor that influence the composition of these communities and distribution of individual taxa across produce types. We expected the overall composition of microbial communities to vary across produce type for a variety of reasons. We know that arrange of environmental factors composition can shape microbial community composition including PH and moisture availability can vary across produce types (Nguyen.C and Carlin.F, 1994).

Vegetables are an extraordinary dietary source of nutrients, micronutrients, vitamins and fibre for humans and are thus vital for health and well being. Fruits and vegetables are widely exposed to microbial contamination through contact with soil, dust and water and by handling at harvest or during postharvest processing. They therefore harbour a diverse range of microorganisms including plant and human pathogens (Nguyen-the and Carlin, 1994; Dunn et al., 1995; Carmo et al., 2004). Differences in microbial profiles of various vegetables result largely from unrelated factors such as resident microflora in the soil, application of nonresident microflora via animal manures, sewage or irrigation water, transportation and handling by individual retailers (Ofor et al., 2009). In developing countries continued use of untreated waste water and manure as fertilizers for the production of fruits and vegetables is a major contributing factor to contaminations (Amoah et al., 2009).

Thus despite their nutritional and health benefits, outbreaks of human infections associated with the consumption of fresh or minimally processed fruits and vegetables have increased in recent years (Beuchat, 2002). Enteric pathogens such as *Escherichia coli* and *Salmonella* are among the greatest concerns during food-related outbreaks (Buck et al., 2003). Several cases of typhoid fever outbreak have been associated with eating contaminated vegetables grown in or fertilized with contaminated soil or sewage .These increases in fruits and vegetables-borne infections may have resulted from increased consumption of contaminated fruits and vegetables outside the home as most people spend long hours outside the home.

In Ethiopia for instance, street vending of handy ready-to-eat sliced fruit and vegetables has recently become very common and the market is thriving. Bacteriologically safe vegetables are essential to maximize the health benefits promised by adequate consumption of these produce. Proper washing of vegetables is essential for decontamination. Water supplemented with varying concentrations of organic acids, such as acetic, citric and sorbic acids, has been shown to reduce microbial populations on fruits and vegetables (Beuchat, 1998).

This study was undertaken to determine the microbial load of vegetables sold at Nekemte Town Ethiopia to determine the bacteriological load of Vegetables sold at Nekemte Town.

2. DESCRIPTION OF THE STUDY AREA

The study was conducted in Nekemte town, East Wollega, Ethiopia. Nekemte is found in east Wollega zone, Oromiya regional state, Ethiopia, which is 331 km from the capital city of the country (Addis Ababa).This town is bounded by Jiregna kebele in the north, Gari kebele in the north east, Dalo komto kebele in the east, Gete kebele in the south east, Negasa kebele in the south,Durakane kebele in the south west and Fayera kebele in the west. The total land area of the town is 52.63 km. According to CSA Nekemte branch office (1999), the town has an estimated total population of 72,219. At altitude and longitude of 90° 5'N and 36° 33'E respectively with an elevation of 2,088 meters above sea level. The minimum and maximum rain fall and daily temperature ranges are between 1450to2150 mm and 15 to 27c° respectively.

2.1. Study design

A cross sectional survey was conducted to assess bacteriological quality of vegetables sold at Nekmete Town (Cabbage, papaya and mango). The samples were regularly collected at three week interval during March 2015 and June 2015, and analyzed for **Aerobic mesophilic**

bacterial count, feacal coliform count and total coliforms at Wollega University, Biology laboratory.

2.2. Sample collection

A total of 36 samples comprising five types of fresh vegetables (cabbage, mango, papaya, tomato and potato,) were collected from Nekemte town using a random sampling technique method. All samples were collected aseptically in a sterilized universal container and plastic bags and transported to Wollega University, Biology laboratory for laboratory processing. The samples were cooled during transportation using a cooler box to keep the normal conditions of the microflora of vegetables. The analysis began immediately after the sample arrival at laboratory.

2.3. Microbiological analysis

For microbiological analysis, 25 g of leafy material was aseptically removed from each vegetable sample using a sterile scalpel and blended in 225 ml of sterile 0.1% (w/v) bacteriological peptone water for 1-3 min. The samples were homogenized using stomacher blender. The homogenate was used as a source of microbial source for determining the aerobic mesophilic bacteria counts, total coliform counts and feacal coliform counts following the standard methods of APHA (1998).

2.4. Data analysis

In this study, all statistical analyses were computed using SAS software version 9.1 for microbiological analyses to analysis and determine the bacteriological quality of fresh vegetables sold at Nekemte Town. The data were subjected to analysis of variance (ANOVA) to assess the effect of Vegetable type and site of production on the concentrations of microbial contaminants in the vegetables tested. As the level of microbial contamination might vary with sample collection site and vegetable type, ANOVA was used to test the existence of significant difference between means. In all statistical analyses, confidence level was held at 95% and $P<0.05$ (at 5% level of significance) was considered as significant.

3. RESULTS AND DISCUSSION

This study was prepared to examine the bacteriological quality of vegetables sold at Nekemte town. As the result shown that from tables below, there is high contamination of vegetables with bacteria count the colony.

3.1. Bacteriological analysis of leafy vegetables

This study attempted to determine the percentage of vegetable contamination (Bacteriological quality analysis) with **aerobic mesophilic bacteria** (AMB), **coliform bacteria** (TCC) and **faecal coliforms** (FC) as well as their microbial loads through aerobic mesophilic bacteria counts (AMB), total coliform counts (TCC) and fecal coliforms (FC). The highest percentage was obtained for aerobic mesophilic bacteria (100%) as demonstrated by its occurrence in all vegetable samples analyzed. On the other hand, 100, 83.3, and 83.3 of the samples of Vegetables collected from local market were found contaminated with coliform bacteria.

As of the data in Table 1, it can be understood that there was an improper pre harvest handling of the vegetables in selected study area. The high percentage of vegetables contaminated with coliform bacteria and fecal coliforms may suggest high risk of acquiring infectious diseases through the consumption of these vegetables. The occurrence of such indicator microorganisms is an indication of the contamination of the vegetables with faecal matter derived from humans and other animals (Cornish et al., 1999).

Table 1: Bacteriological Quality analysis of vegetables at Nekemte Town, 2015

Indicator organism	Samples (positive samples)	Vegetables					
		Cabbages		Mango		Papaya	
		%	F	%	F	%	F
Aerobic Mesophilic count	12(100%)	12	100	12	100	12	100
Total coliform	12(100%)	12	100	10	83.3	10	83.3
Fecal coliform	12(100%)	8	66.6	7	58.3	9	75

The results of this investigation additionally showed that the vegetable samples collected from both farms were heavily contaminated by aerobic mesophilic bacteria counts ranging from **$10x^{-7}$-$10x^{-8}$ CFU/g**. The mean values of aerobic mesophilic bacteria counts were in the order of spinach > lettuce > cabbage for both sites as shown in Table 2. The data further showed that all the bacterial counts recorded in this study exceeded the recommended levels by WHO and International Commission on Microbiological Specifications for Food (ICMSF) standards (10 to 100 coliforms g/1, 10 faecal coliformg/1and 4.9×10^{6} aerobic count g/1) wet weight vegetables.

Table 2: Bacteriological Vegetables Mean Quality Analysis, 2015 Nekmete

No. of examined samples	Indicator organisms (CFU/g)	Vegetable types			
		Cabbage	Mango	Papaya	Mean
12	Aerobic Mesophilic count	9.3 × 107 ± 8.8	1.7 × 108 ± 8.8	2.2 × 108 ± 8.8	1.6 × 108 ± 5.1
12	Total coliform	5.0 × 106 ± 0.8	6.6 × 106 ± 0.8	5.2 × 106 ± 0.8	5.6 × 106 ± 0.5 d
12	Fecal coliform	5.2 × 105 ± 0.7	3.1 × 105 ± 0.7	3.7 × 105 ± 0.7	4.0 × 105 ± 0.4

The data showed that there was a highly significant difference in the average counts of AMB amongst the vegetable types, but there was no significant difference between local markets. On the other hand with respect to the mean TCCs, the ANOVA did not show significant difference amongst vegetable types, but there was significant difference between sites. However, as can be seen from the same tables, there was a significant difference between the two sites. Similarly, the results of the analysis of variance for FC counts showed that in both farms, there was a significant difference amongst vegetable types but not between sites.

The AMB for Vegetables were in the range of $2.0\times10^{8-}$ 9.3×10^{9} CFU/g, respectively. This high aerobic mesophilic bacterial count might be due to pollution by humans, animals or irrigation water. In agreement with this result, Thunberg et al. (2004), reported total viable count as 5.0×10^{8}, 4.0×10^{8}, and 3.1×10^{7}CFU/g for Cabbage, papaya and mango respectively. The high aerobic mesophilic bacteria counts in cabbage and papaya could be due to the wide surface area of vegetable leaves which is suitable for water contact and microbial contamination (Anonymus, 2002). The mean aerobic mesophilic bacteria counts of the papaya in this study are 1.7 $\times10^{8}$ CFU/g. In agreement with this result, Viswanatha and Kaur (2001) from in India indicated that total aerobic plate count for cabbage and lettuce was found to be 2.8×10^{6} - 1.2×10^{8} and 1.3×10^{7} - 2.3×10^{7}CFU/g, respectively.

The total coliform levels recorded under this study were high in all the vegetable samples analyzed. Total coliform levels ranged from 4.1×106 - 5. 0×106 CFU/g for cabbage, 4.1×106 -6.6×106 CFU/g papaya and 3.1×106 -5.2×106 CFU/g for mango. Similar findings are reported by Nguz et al. (2005) in Zambia which found a range of total coliform counts on vegetable products between 1.6×102 and 7.9×105 CFU/g. According to Nguz et al. (2005), fecal coliform counts are efficient indicators of sanitization, but the presence of fecal coliforms does not necessarily indicate the presence of a pathogen. In this study, the fecal coliform counts of vegetable samples collected from sites ranged between 5.2×10^5 to 5.7×10^5, 2.3×10^5 to 3.1×10^5 and 2.2×10^5 to 3.7×10^5 CFU/g, respectively. The mean fecal coliform values of all the three vegetable samples exceed the ICMSF recommended level of 10 fecal coliform g-1 fresh weights. This may be due improper use of irrigation. In addition to this, application of organic manures is common practices of farmer for production of crops in that area.

However, sources of fecal coliform contamination of mango may include overhead irrigation of mango with already contaminated water, planting in contaminated soils and frequent application of poultry manure which was not well composted (Amoah et al., 2005). Of the three vegetables, cabbage shows significant difference among crop types of both farms by contamination of fecal coliform counts. This shows cabbage was more contaminated by fecal coliforms than other leafy vegetables.

These results correlate with the probability of the vegetable samples to be more in contact with the source of contamination during growth (Heaton and Jones, 2008). Moreover, application of fresh poultry manure without sufficient drying used for vegetable production registers high fecal coliform counts (Drechsel et al., 2000). Generally, variation in the AMB, TCC and FC values of the present study and previous works may be either due to differences in the geographical location of the cultivation area, or due to the difference in contamination load at different sections of the drainage canal and different pre-harvest handling practices.

4. CONCLUSION

This current study revealed that there was bacterial contamination of fresh vegetables (papaya, cabbage and mango) sold at Nekemte Tow vendors from different farm land. Bacterial numbers recorded in this study range from 2.2×10^5 to 2.22×10^8CFU g-1 which is above the ICMSF (1998) limit of 10^3 to 10^5 coliforms 100g/l wet weight of vegetables usually eaten raw. Mango was found to be the most contaminated vegetable by aerobic mesophilic bacterial count (2.03×10^8 to 2.22×10^8, CFU/ g). This might be due to the fact that mango have wide leaves surface in contact with wastewater, soil and dust. In contrast cabbage was the least in aerobic mesophilic bacterial count (8×10^7 to 9.3×10^7 CFU/g).

In this study, high total coliform counts (6.6×10^6 CFU/g) were observed on papaya collected from local market. Likewise, the fecal coliform counts of vegetable samples collected from both site ranged between 2.2×10^5 and 5.7×10^5 CFU/g. However, cabbage showed high fecal coliform counts among the three analyzed vegetable samples. Therefore, great attention should be paid in using contaminated water for production of vegetables for the public health perspective.

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