Quality Assessment of Green Leafy Vegetables Collected from Different Market Sites of Shegaon and Akola

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Abstract: *Green leafy vegetables has great nutritional and therapeutical value and is used as main ingredient for consumption. The aim of this study was to identify the bacterial population in different area of green leafy vegetables obtained from local market sites of Shegaon and Akola. In order to identify them, bacteriological analysis was performed on five fresh green leafy vegetables (Spinach, Fenugreek, Coriander, Dill leaves, Chuka) were collected. All vegetables sample proceed to identify standard plate count SPC CFU/ ml. Highest no. of bacterial colony found in Fenugreek (5.1×10^3 CFU/ ml) followed by Coriander (4.0×10^3 CFU/ml), Spinach (2.1×10^3 CFU/ml), Chuka (3.0×10^3), Dill leaves (2.8×10^3 CFU/ ml) . E. coli, S. aureus, P. aeruginosa, S. enterica , K. pneumoniae were identified on the basis of morphology and Biochemical test. Then most probable number count in green leafy vegetables Spinach 18×10^2 , Fenugreek 90×10^2 , Chuka 45, Dill leaves 55, Coriander 18×10^2 MPN/ 100 ml. The NaCl treatment used for the reduced microbial load of green leafy vegetables. The bacterial load ranged after NaCl treatment in SPC method. Spinach 1.5×10^3 CFU/ ml, Fenugreek 4.8×10^3 CFU/ ml, Chuka 2.1×10^3 , Dill leaves 2.0×10^3 , Coriander 3.0×10^3 . Most probable number count after NaCl treatment. Spinach 18×10^2 MPN/ 100ml, Fenugreek 18×10^2 CFU/100 ml, Chuka 25 MPN/ 100ml, Dill leaves 20 MPN/ 100ml, Coriander 18×10^2 MPN/ 100ml. This study revealed that vegetables were contaminated different bacteria and the Microbial load can be reduced when properly washed especially with NaCl water.*

Keywords: Vegetables, Microbial load, SPC, MPN, NaCl treatment

1. Introduction

The term vegetable applies to edible part of the plant that stores food in stems or leaves. Vegetable are green and leafy- like in appearance bearing edible stems or leaves and roots of plants (Sharma, O. P., 2004).They are value mainly for their high carbohydrate, vitamin and mineral contents. Vegetables may be edible root, stems, leaves, fruits or seeds. Each group contributes to diet in its own way (Robinsonm, O. S., 1990.) Vegetables are one of an important part of a balanced diet. Recently, the consumption of fruits and vegetable have been increased significantly, because are dietary sources of nutrients, micro nutrients, vitamins and fiber for human and well- being (Eni *et al.,* 2010). In addition, therefore, the nutrition policies have strongly promoted the consumption of a diet containing more than 400 gm / day of fresh vegetables as a notional goal for health promotion (FAO/WHO, 2004).

The number of outbreaks of food- borne illness associated with consumption of fresh vegetables and fruits have increased due to the demand for fresh produce (Behrsing J, *et al.,* 2000; Sengun I.Y *et al.,* 2004). The presence of spoilage and pathogenic micro- organisms of leafy vegetables has long been recognized (Nascimento MS, *et al.,* 2003). *Salmonella* spp. *Escherichia* coli and *Staphylococcusaureus* (Viswanathan P., *et al.,* 2001, Abongo B. O., *et al.,* 2008., Soriano JM *et at.,* 2000.) have been isolated from vegetables which can become contaminated with these pathogens any of several points from the field through to the time of consumption (Szabo E. A. *et al.,* 2000; Beuchat L. R., 2002; Harris L, *et al.,* 2003.)**.** It was reported that the vegetables such as fenugreek, and spinach, Dhaniya, Sepu, Sourly leaf carries *Escherichiacoli*, *Staphylococcusaureus*, *Salmonellaenterica*, *Klebsiellaspp.*

(R. V. Sudershan, *et al.,* 2014). The incidence of food borne outbreaks caused by contaminated vegetables has increased in recent years (Mukherjee, A., *et al.,* 2006).Effective and feasible sanitation methods are required to remove pathogens and also to prevent food born diseases associated with consumption of fresh vegetables and fruits (Karapinar M , *et al.,* 2007). Although most processors and consumers assume that washing fresh vegetables and fruits will reduce the microbial load on their surface, studies have shown that water washing alone is not effective in reducing microbial population on the fresh vegetable (Beuchat LR *et al.,* 1998, Sapers G. M. 2001). The leafy vegetables are deep in the NaCl containing 15-20 min. The Back containing 10-20gm. In 100-150 liter is the most widely used sanitizer in fresh produce process. NaCl creates about 1 to 2 log microbial reduction on fresh vegetables at commonly used concentration. (Delaquis PJ, *et al.,* 2004, Allend A *et al.,* 2008).

2. Material and Methods

Vegetables are important protective food and highly beneficial for the maintenance of health and prevention of diseases. They contain valuable food ingredients which are essential for the proper function of the body. This research was carried out to investigate the microbiological quality of some green leafy vegetables and total microbial load determination according to standardize method to control the microbial population i.e. NaCl method. For this study the following material was used and methods were adopted :-
- **Collection of sample**: Five types of fresh leafy vegetables were purchased from local market of Shegaon and Akola. All samples were collected in a clean and sterile polythene bags and transported to the laboratory analysis.

- **Material used:** During this research work on " Coliform contamination in green leafy vegetables " following materials were used for the study.

All the media used were procured from Hi-media. Nutrientagar, EMB agar (Eosin methylene blue), Macconkey agar, Macconkey broth, Lactose broth, Mannitol salt agar, Psedomonas isolation agar, Bismuth sulphite agar, Deoxycholate citrate agar, Xylose lysin Deoxycholate agar, MR- VP medium, Coser citrate broth, sugar fermentation broth, Reagents and staining solutions.

3. Methodology

1) **Study Zone:** The study was carried out with green leafy vegetable collected from local market of Akola and Shegaon. Spinach, Dill leaves, Chuka, Fenugreek, Coriander leaves were analysed during the course of study at regular time intervals.
2) **Collection of samples:** Total 25 samples of the above mentioned green leafy vegetables were collected and further processed for microbial analysis. Samples were collected in sterile polythene zip bag to avoid any external contamination during handling and transported to Microbiology Laboratory for further analysis.
3) **Sample processing:** Twenty five gram of each collected vegetables was weighed in sterile condition and homogenized in sterile saline water using Pestle and Morter for five minutes. All the sterile conditions were maintained throughout the process. The homogenates were collected in sterile tubes and stored at -20°C for further used (Uzeh, R. E. *et al.,* 2009).
4) **Isolation of Bactertia:** One ml of each sample was serially four fold diluted in sterile water upto 10^{-2} dilution. The amount of 0.1 ml at 10^{-2} dilution was spreaded over Nutrient agar media using sterile spreaders. The plates were incubated at 37°C for 12 – 24 hours for the appearance of colonies. Discrete colonies were subcultured in Nutrient Broth and streaked over differential media agar plates i.e. MacConkey Agar,

EMB agar and mannitol salt agar, Pseudomonas isolation Agar were incubated at 37°C for 12 – 24 hours. The pure bacterial colonies obtained were primary identified using a Morphological analysis. Each isolated pure culture was maintained at 4°C for further analysis (Ray Bibek, 2004).

Total plate count of Bacteria (CFU/ml) :-
Microbial load in each vegetable sample was determined as CFU/ml and was calculated using formula (Prescott L. M., *et al.,* 2009).

Total coliform number is countedby most probable number method (MPN) .

Presumptive Test
In MPN method the Presumptive test performed using with Lactose broth and incubated at 37°C for 24 hrs.

Confirmed test:
This test is done by using MacCokey Agar Medium to check the growth of coliform bacteria.

Completed test:
The completed test performed also the Nutrient agar plate. Coliformed bacteria isolated by presumptive test, confirmed test and completed test (Olivera M., 2010). The confirmed bacteria were *Escherichia coli, Staphylococcus aureus, Psedomonas aeruginosa*, *Salmonella enterica* and *Klebsiella pneumonia.* All the colonies of this bacteria were then analyzed for their biochemical characteristics. Identification was confirmed by morphological, Biochemical and Cultural characteristics on the basis of Bergay's Manual of determinative bacteriology (Buchanan R. E. and Gibbons N. E., 1974).

Comparative analysis:
Further the study has focused on decreasing the load of microbial population from vegetable sample for that alternative NaCl treatment to green leafy vegetable has been carried out.

4. Results and Discussion

Observation Table

Table 1: Collection of samples Green leafy vegetables from different area

Sr. No	Botanical name of sample	Area	Shegaon	Area	Akola
1	*Spinacea oleracea* (Palak)	Bus stop side	4	Tower chowk	4
2	*Foenum graecum* (Methi)	Bhagat sing chowk	3	Khadki	2
3	*Rumex Vesicarius* (Ambat chuka)	Phule nager	2	Jatharpeth	3
4	*Peucedanum graveolence* (Sepu)	Nagzari road	2	Janta market	2
5	*Coriandrum sativum* (Kothimbir)	Station road	2	Ramdaspeth	1
	Total		13		12
	Total Samples				25

Table 2: Number of isolates and their percentage

Sr. No.	Name of isolates	No. of isolates	% of isolates
1	*E. coli*	5	26. 31%
2	*S. aureus*	5	26. 31%
3	*S. enteric*	3	15. 78%
4	*P. aeruginosa*	2	10. 52%
5	*K. pneumoniae*	4	21.05%
	Total isolates	19	
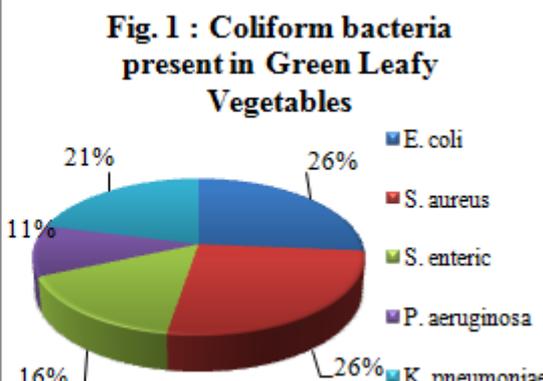
Fig. 1 : Coliform bacteria present in Green Leafy Vegetables

Table 3: Total coliform present in Green Leafy Vegetables by SPC method

Sr. No	Botanical name of samples	SPC
1	*Spinacea oleracea*	2.1×10^3 CFU/ ml
2	*Foenum graecum*	5.1×10^3 CFU/ ml
3	*Rumex Vesicarius*	3.0×10^3 CFU/ ml
4	*Peucedanum graveolence*	2.8×10^3 CFU/ ml
5	*Coriandrum sativum*	4.0×10^3 CFU/ ml
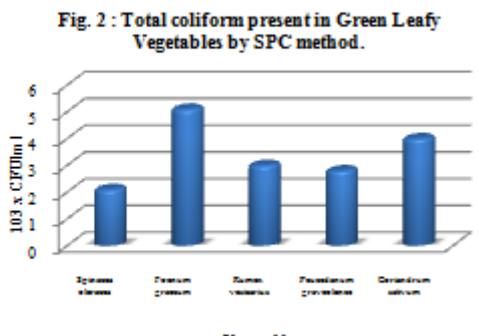
Fig. 2 : Total coliform present in Green Leafy Vegetables by SPC method.

Table 4: Total Coliform present in Green leafy vegetable by SPC method after NaCl treatment solution

Sr. No	Botanical name of samples	SPC
1	*Spinacea oleracea*	1.5×10^3 CFU/ ml
2	*Foenum graecum*	4.8×10^3 CFU/ ml
3	*Rumex Vesicarius*	2.1×10^3 CFU/ ml
4	*Peucedanum graveolence*	2.0×10^3 CFU/ ml
5	*Coriandrum sativum*	3.0×10^3 CFU/ ml
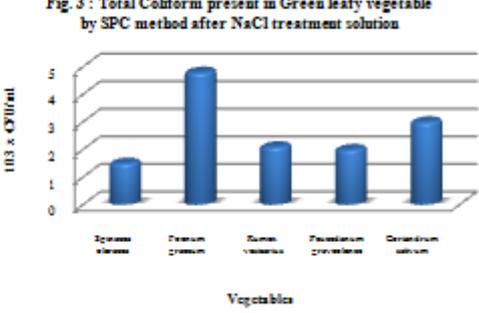
Fig. 3 : Total Coliform present in Green leafy vegetable by SPC method after NaCl treatment solution

Table 5: MPN count of Coliform organism using MacConkey Broth

Sample	MPN/100 ml
Spinacea oleracea	18×10^2
Foenum graecum	90×10^2
Rumex Vesicarius	45
Peucedanum graveolence	55
Coriandrum sativum	18×10^2
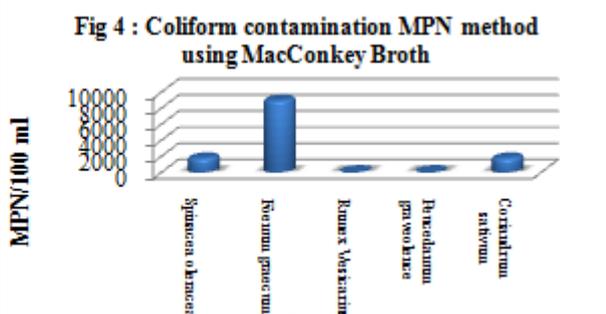
Fig 4 : Coliform contamination MPN method using MacConkey Broth

Table 6: MPN count of Coliform organism using MacConkey Broth after NaCl treatment

Sample	MPN/100 ml
Spinacea oleracea	18×10^2
Foenum graecum	18×10^2
Rumex Vesicarius	25
Peucedanum graveolence	20
Coriandrum sativum	18×10^2

Fig 5 : Coliform contamination NaCl/ MPN method using MacConkey Broth
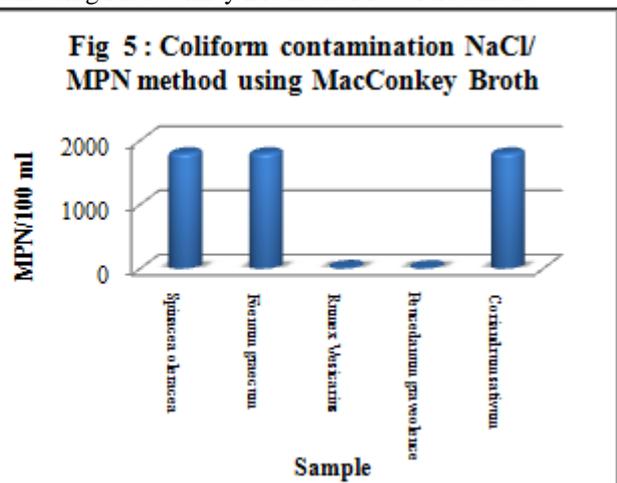
5. Discussion

Vegetables are consumed widely as an energy source by human beings. They are rich in vitamins and minerals. Green leafy vegetables are popular around the world. Heavy metal contamination of vegetables cannot be underestimated as these foodstuffs are important components of human diet. There are many pathogenic microorganism reside over them, which can cause many food born infection such as diarrhea and Salmonellasis.

All the vegetable samples in this study were microbial contaminated. However the total viable count of Green leafy vegetables varied with type of samples (Table.1).

A total of 25 sample of fresh Green leafy vegetables comprising five types Spinach, Dill leaves, Coriander, Chuka, Fenugreek. All the vegetables samples were collected from local market of Shegaon and Akola. The samples were collected in sterile plastic bags and were transported to the laboratory for microbial analysis.

According to the cultural, morphological and biochemical characteristics of the organisms isolated a five bacterial genera was isolated i.e. *E. coli, K. pneumoniae, S. aureus, S. enterica, P. aeruginosa*. (Table No. 2). *E. coli* (26.31%) and *S. aureus* (26.31%) was the most frequently isolated a followed by *K. pneumoniae* (21.05%), *S. enterica* (15.78%) and *P. aeruginosa* (10.52%) (Fig. 2) was least frequently isolated. Our result are in accordance with the result of Nwachukwu E. *et al.,* (2013) they reported that the isolates *Staphylococcus, Streptococcus, Bacillus, Klebsiella, Escherichia, Psedomonas, Salmonella, Saccharomyces, Aspergillus, Rhizopus* were obtained from vegetables and fruit samples.

Arfat Mohammed Goja et al. (2013) reported five different genera isolated from vegetables includes staphylococcus (33%) was the most frequently isolated followed by Enterobacteriaceae (25%), Bacillus (17%), Streptococcus (17%) and Micrococcus (8%). Bacteria of belonging to the same genera were also isolated and identified by other researchers from fruits and vegetables in different countries similarly Osamwonyi *et al.,* 2013; Eni *et al.,* 2010;

Rajvanshi, 2010; Tambekar and Mundhada, 2006; Uzeh *et al,* 2009; Adebolu and Ifesan, 2001.

All the isolates obtained were appeared at different frequencies in the vegetable sample. During study it was noticed that *S. aureus* and *E. coli* present in all leafy vegetables whereas very less percentage of *P. aeruginosa* was present.

The result reveled from Arfat Mohammed Goja (2013) shown that bacterial, coliform and fecal coliform counts observe from vegetables are recorded higher counts. The total viable count was ranged from 1.2×10^5 to 2.8×10^7 CFU ml^{-1}. This high load CFU mL^{-1} of viable count of vegetables could be attributed to the unhygienic practices right from the farm to the market and exposed to potential microbial contamination at every step, including cultivation, harvesting, transporting, packaging, storage and selling to the final consumers.

The predominant bacterial colonies were isolated from plate count agar by pour plate method (Harrigan 1998) Microbial count ranged from SPC method for Spinach 2.1×10^3 CFU/ ml, Fenugreek 5.1×10^3 CFU/ ml, Chuka 3.0×10^3 CFU/ml, Dill leaves 2.8×10^3 CFU/ ml, Coriander 4.0×10^3 CFU/ ml. (Table No. 3 and Fig 2). Similar finding were obtained from Arfat Mohammed Goja *et al.,* (2013) Microbial count range from 1.2×10^5 - 2.8×10^{-1} CFU/ ml for Mloukhia; 3.4×10^5 - 4.8×10^5 CFU/ ml for Tomato and 2.3×10 - 8.0×10 CFU/ ml^{-1} for green paper.

The presence of feceal and non feceal coliform organisms in green leafy vegetables indicates the contamination from different sources so it was obvious that eating these vegetables can exposed the consumer to many risk. Therefore, it recommended that these vegetables should be thoroughly washed before consumption. Hence in the present study with prefer NaCl treatment to green leafy vegetables to reduced the microbial population.

It was noticed that microbial count was reduced to some extent after NaCl treatment for 20 min. The microbial count ranged from SPC method Spinach 1.5×102, Fenugreek 4.8×10^2, Chuka 2.1×10^2, Dill leaves 2.0×10^2, Coriander 3.0×10^2.(Table No. 4 and Fig. 3). These results are in accordance

Paper ID: ART20172968

with the result of Nwachukwu E. *et al.,* (2013). According to them bacterial load range from 1.3×10^4 - 1.8×10^6 CFU/g. reduced by the chemical treatment.

The result form Nwachukwu E. *et al* (2013) reported the effect of chemical treatment on microbial load of fruits and vegetables reveled that each of the chemical reduced microbial count to various degrees. Similarly various scientist reported the used of different chemicals vinegar, Shalaby and El- Raliman (2006) reported used of potassium sorbate but was not found effective against many bacteria. However Frazier and Westhoff (1998) reported used of potassium sorbate, sodium benzoate and vinegar and they recognize it as a safe chemical.

Result from the study shows presence of total coliform and feacal coliform from the samples and the maximum levels for Spinach 18×10^2 MPN/ 100ml, Fenugreek 90×10^2 MPN /100ml, Chuka 45 MPN/ 100ml, Dill leaves 55 MPN/100ml, Coriander 18×10^2 MPN/100ml (Table No.5). Similar findings were obtained from Arfat Mohammed Goja *et al*., (2013) also reported bacterial count range for MPN from100^{-1}; (28.11) MPN/100ml^{-1} for Mloukhia (75.15) MPN /100 ml^{-1} for Tomato and (150, 20) MPN/100^{-1}ml for green pepper.

This study revealed that the green leafy vegetables were contaminated with microorganisms which could be of public health importance. Proper sanitary major should be adopted while handling green leafy vegetables to limit the level of Microbial contamination, so we recommended the use of NaCl treatment in washing the vegetables which is easily available, cheap and easy method for adoption.

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