Int.J.Curr.Microbiol.App.Sci **(2019) Special Issue-8: 1-13**

EXCELLENT PUBLISHERS

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Special Issue-8 pp. 1-13**
Journal homepage: http://www.ijcmas.com

Original Research Article

Analysis of Antimicrobial Treatment on Green Leafy Salad Vegetables

Arpita Sharma[1,2*] and Kalpna Chauhan[3]

[1]School of Agriculture, Career Point University, Kota, Rajasthan, India – 325003
[2]Institute of Hotel Management, Catering & Nutrition, Pusa, New Delhi-110012
[3]Institute of Hotel Management, Catering & Nutrition, Pusa, New Delhi-110012, India
**Corresponding author: arpita1985sharma@gmail.com*

A B S T R A C T

Green leafy salad vegetables are consumed without any heat treatment, sometimes without washing and peeling and therefore the possibility of food borne disease is more. Fresh and fresh-cut leafy green vegetables are nutrient-rich foods with high levels of minerals, vitamins, and phytochemicals. GLSV's are essential part of people diet all around the world and they are usually consumed raw and often without heat treatment or thorough washing. Hence they have been known to serve as vehicles for the transmission of pathogenic microorganism associated with human diseases. Keeping in mind the necessity for a strong agent which can be able to sterilized outer surface. The study was conducted at IHM, Pusa, to provide health benefits for society. Vegetable production in India accounts for 12 per cent of world's production, but the per capita vegetable consumption in India is only 135 g/day compared to the minimum requirement of 250 g/day. Washing with tap water is the mostly common practice of consumers to reduce microbial contamination of GLSV's, though tap water has limited or no effect on killing microorganisms. Water containing 50 to 200 ppm of chlorine is widely used to sanitize green leafy salad vegetables because of its low cost and efficacy against bacterial pathogens. However chlorine efficacy is affected by the organic loads in the washing solution as suggested by many research. GLSV's releases large amount of organic matter that negatively impact the chlorine efficacy and convert active chlorine into inactive form. Therefore, alternative sanitizers are needed, which are more effective than chlorine or ClO_2 for suppressing foodborne pathogenic bacteria in fresh cut GLSV's. Thus the current study was designed to identify the effect of different doses of sanitizer (washing treatments) in order to reduce contamination from the various sources for food safety that is the prime concern of today for consumers.

Keywords

Food Safety, Sanitizers, Pathogenic bacteria, Green leafy vegetables

Introduction

India is the fruit and vegetable basket of the world and it's the second largest producer of vegetables in the world next to China. More than 50 varieties of vegetable crops are grown in India and the estimated area under vegetable crops is 10.29 million hectares (NHB, 2017-2018).

The GLSV's are least expensive and are easily grown and can be made available throughout the year from a small plot of land. Hence, GLSV's must form a part of everybody's daily diet specially for the vulnerable group which includes children, adolescents, pregnant, nursing and aged, as their required for antioxidants, vitamins and minerals are more (Rao *et al.*, 2015). According to FAO/WHO all vegetables and herbs of a leafy nature and of which the leaf (and core) is intended to be consumed raw include lettuce (all varieties), spinach, cabbages, chicory, leafy herbs (e.g. cilantro, basil, parsley) and watercress (FAO/WHO, 2008). The eating of salads is perceived by all sections of the population as making a positive contribution to our health but it's a big challenge to feed large population in future (FAO, 2009). Regular daily consumption of GLSV's in sufficient amount can help prevent major diseases such as cardiovascular diseases and certain cancers (Hung *et al.*, 2004). Increasing one and one half servings of GLSV's per day could significantly reduce the risk of type 2 diabetes by 14% (Carter *et al.*, 2010). Although GLSV's are associated with good health, can cause food poisoning due to contamination with bacteria (Times of India, 2002). The Center for Disease Control and Prevention (CDC) considers GLSV's as one of the most important vehicles of food borne illness outbreaks caused by bacterial pathogens including Escherichia coli 0157:H7 (Lynch *et al.*, 2009). In India, food borne illness is a serious public health problem. Under the Integrated Disease Surveillance Project (IDSP), food poisoning outbreaks reported from all sections of age group over India in 2009 increased to more than double as compared to the previous year (120 outbreaks in 2009, as compared to 50 in the year 2008) (Sharma *et al.*, 2009). GLSV's grow in the natural environment can become contaminated with microorganisms and also during harvesting and transportation or once the food reaches from farm to home and capable of causing human diseases (Gupta and Prakash, 2008).Thus the current study is designed to identify the importance of effective washing treatment in order to reduce contamination from the sources mentioned above as food safety is the prime concern of today for consumers.

The objectives of the current study:

- To analyze the antibacterial effect of washing treatments on green leafy salad vegetables.
- To compare the efficacy of a commercial 'Veg fru wash' with other washing treatments for reducing the total bacterial count on green leafy salad vegetables.
- To suggest suitable washing method to reduce the microbial load on green leafy salad vegetables.

Materials and Methods

The role of fresh GLSV's in nutrition and healthy diet is well recognized and many countries have undertaken various initiatives to encourage consumers to eat more of these products. The present study was conducted to analyze the antibacterial treatment on GLSV's. It was an analytic experimental study, conducted in 'Microbiology Lab', Institute of Hotel Management, Catering and Nutrition, Pusa, New Delhi.

Sample size and locale

Three sample each of Lettuce, Cabbage and Spinach was collected from vegetable vendor of local market of sector-37, Faridabad and stored at 5°C overnight, before use the following day and transported in a cold box prior to analysis. It was a purposive sampling to collect the GLSV's (Lettuce, Cabbage and Spinach). The sampling period of the study was of five months *i.e.*, November 2011 to March 2012.

Tools and techniques

All analytical techniques were thoroughly standardized to minimize variation due to experimental error before applying test samples.

Preparation of sample

Three samples each of GLSV's (Lettuce, Cabbage and Spinach) were trimmed to remove old and damaged leaves and cored before experiment purpose. The inner leaves were used for the experiment and each sample (Lettuce, Cabbage and Spinach), each sample was divided into quarter and 10 gm was weighed. Sample were homogenized individually with the help of 'mortar pestle', 10 gm of each sample was transferred into a conical flask containing 90 ml of diluents (Sterilized distilled water).The first quarter *i.e.*,10 gm which was the control (without being cleaned), the second quarter (10 gm) washed with tap water for 1-2 min. The third quarter (10 gm), washed with chlorine water. The fourth quarter (10 gm), washed with 'Veg fru wash'.

Preparation of washing treatments

Before washing (as a control), Tap water, chlorine water (100 ppm) and 'Veg Fru wash'. Running tap water was used from the college laboratory which was provided by the Delhi Jal board.

Preparation of chlorine water

Chlorine water was prepared by adding 1 tablet of 'Aqua pura chlorine' in 1 litre of 'tap water' and stir. Wait for 1 minute till it dissolves. (WHO/FAO, 2003)

Preparation of 'Veg fru wash'

'Veg fru wash' is a HACCP (Hazard Analysis and Critical Control Point) certified commercial fruit and vegetable wash which contain 100% food grade edible ingredients (sorbitol ester and purified aqua). Fruits and vegetables are coated with waxy/resinous layer which is insoluble in water; hence plain water doesn't remove this layer. This waxy layer is host for microorganisms like bacteria, fungus and virus. 'Veg fru wash' emulsifies the insoluble resinous/waxy layer, makes it water soluble and washes away all unwanted surface contaminants /toxicants and effectively removes surface chemical pesticide residues. It also effectively removes 99% bacteria from the surface of vegetables, salads and fruits. 15 ml of 'Veg Fru wash' was added in 500 ml of tap water. Sample were soaked in the prepared solution for 1 min and soaked GLSV's were transferred into a strainer and washed thoroughly under running tap water.

Microbiological analysis

Preparation of Nutrient agar medium

Nutrient agar medium was prepared (Hi-media) in 1000 ml of distilled water in a conical flask and the pH was set 7.0. Media was autoclaved at 15 psi for 15-20 min. Once the agar had been autoclaved, it was cooled to room temperature and media was poured into petri plates in Laminar air flow chamber aseptically. All plates were poured aseptically. Agar plates were allowed to solidify and then seal with parafilm. Six petriplates were prepared for each washing treatment and 'control'. The procedure was repeated three times (Microbiology, A Laboratory Manual, VII Edition by Cappuccino & Sherman).

For serial dilution

The UV light of laminar flow hood switched on about 15-20 minutes prior. The diluents were labelled from 10^{-1} through 10^{-6}. 1 ml of dilution with a sterile micropipette from conical flask (containing 90 ml diluents with homogenate mixture of samples) was transferred to a test tube marked 10^{-1}. This gives 1:10 dilution of each sample. Aseptic conditions were maintained during experiment and whole procedure was done in laminar. From 10^{-1} dilutions, further dilutions were prepared in a series from 10^{-2} to 10^{-6}. This was done by adding 1ml of 1:10 dilution into diluents tube marked 10^{-2} and shake vigorously. 1ml of 10^{-2} (1:100) was then transferred to a tube marked 10^{-3} (1:1000) and so on. After each transfer, shake suspension vigorously. Six dilution test-tubes were prepared for each washing treatment and 'control'. The procedure was repeated three times for each washing treatment given to every sample to get a consistent value (Microbiology, A Laboratory Manual, VII Edition by Cappuccino & Sherman).

Standard plate count

Nutrient agar plates were labelled from 10^{-1} through 10^{-6}. 0.1 ml of dilution was transferred onto the centre of the plate. A sterilized glass spreader was sterilized over a bunsen burner to spread bacteria evenly on agar plate. The whole procedure was done in the laminar flow to maintain aseptic conditions. Plates were placed in a incubator at 37°C or 24 hours in an invert position. Observed the plates for colony count in Quebec Colony Counter and determined the CFU/g of the sample by using only those plates that fit the criteria of the counting rules (*i.e.*, colony count between 30-300). This procedure was also repeated three times for each treatment (Microbiology, A Laboratory Manual, VII Edition by Cappuccino & Sherman).

Calculation and data analysis

$$\text{CFU/gm} = \frac{\text{Number of colonies formed} \times \text{dilution factor}}{\text{Volume sample}}$$

The data was analysed using various statistical tests like Mean, Standard error (S.E.), Log_{10}, Analysis of variance (ANOVA).

Results and Discussions

Quality and safety of fresh produce depend on their microbiological flora. Microbiological content keep on increasing from the point of farm till fork. The aim of this study was "Analysis of effect of antibacterial treatments (Tap water, Chlorine water, 'Veg fru wash') on green leafy salad vegetables (GLSV's). A total of nine GLSV's *i.e.*, 3 sample each of lettuce, cabbage and spinach were used for present research as given in table 1.

Analysis and comparison of antibacterial treatments on green leafy salad vegetables by microbiological analysis

Each sample of GLSV's was treated in triplicates with three washing treatment (Tap water, Chlorine water and 'veg fru wash') and Unwashed kept as a control as shown in Table 2 below.

Total bacterial count was estimated in triplicates using the Standard Plate Count (SPC) technique. Using sterile distilled water as a diluents, serial dilution (10^{-1} to 10^{-6}) were prepared for each washing treatment given to every sample and incubated for 24 hrs at 37°C. Total bacterial counts were determined on nutrient agar medium. Plate with a total count less than 30 or greater than 300 were rejected as they lead to high degree of error and not acceptable for statistical analysis. In current study, it was found that colonies were greater than 300 (above the counting criteria limit) in unwashed and tap water sample of all GLSV's with dilution 10^{-1}. Thus, 10^{-1} dilution readings were not recorded in because it was TNTC. Microbiological assessment of GLSV's, lettuce was performed as given in table 3.

According to 10^{-2} dilution the initial load (unwashed) in lettuce was found to be 240± 9.41 which was reduced to 214.6± 12.2, 77.3± 3.92 and 41.3± 1.08 with application of tap water, chlorine water and 'veg fru wash' respectively. In 10^{-3} dilution initial load was 220± 9.41 which was reduced to 186.6± 12.2, 62.6± 3.92 and 32± 0 with application of tap water, chlorine water and 'veg fru wash' respectively. In 10^{-4} – 10^{-6} dilution initial load was 209.3± 8.48 - 165.3± 6.58 which was reduced to 160± 9.41- 136± 8.6 by tap water and there was no growth of bacteria in chlorine water at and 'veg fru wash' in dilution 10^{-4}-10^{-6}. Therefore, it can be concluded that chlorine water and 'veg fru wash' were effective antimicrobial treatment to reduce microbial load than washing with tap water. In a similar study conducted by Ibrahim, 1995 on 'efficacy of water rinsing and chlorine treatment in reducing the microbial contamination of some fresh vegetables (lettuce, parsley, cucumber and carrot)' and found that the chlorine dip (20 ppm) was more effective than water rinsing in reducing both total aerobes and *coliform* than water. Smith *et al.*, 2003 'compared the efficacy of a commercial produce wash with that of water for reducing the total bacterial population on lettuce' and the result revealed that 'Victory' produce wash was more effective in reducing indigenous flora on lettuce in a food service setting than water (Fig. 1).

In dilution 10^{-2} – 10^{-6}, the value of unwashed lettuce varied from 2.37- 2.19 log_{10} CFU/g. Minimal decreases were seen after lettuce was washed with tap water (2.32-2.13 log_{10} CFU/g), chlorine water (1.88 log_{10} CFU/g- no growth of bacteria) and 'veg fru wash' (resulting in decreases of 1.61 log_{10} CFU/g to no growth of bacteria). Hence, according to this study it can be concluded that chlorine water and 'veg fru wash' were effective antibacterial treatment than tap water. Adams *et al.*, 1989 conducted a study on the 'factors affecting the efficacy of washing procedures used in the production of prepared salads' and found that inclusion of 100 ppm of free chlorine in water used to wash lettuce leaves has been reported to reduce populations of aerobic mesophiles by more than 98% as compared to a 93% reduction using tap water without chlorine. The Table 4 and Figure 2 depict the mean microbial load in CFU/g and effect of antibacterial treatment on microbial load of cabbage in Log_{10} CFU/g.

According to dilution 10^{-2} the initial load in cabbage was found to be 272±12.01 which was reduced to 245.3± 16.0, 84± 5.3 and 56± 3.7 with application of tap water, chlorine water and 'veg fru wash' respectively. In 10^{-3} dilution, initial load was 249.3± 14.16 which was reduced to 209.3± 21.7, 68± 0 with application of tap water, chlorine water respectively. In 10^{-4} – 10^{-6} dilution, initial load varied from 233.3± 12.27 - 165.3± 34.92 which was reduced to 173.3± 12.2 - 121.3± 19.8 by tap water and there was no growth of bacteria in chlorine water in dilution 10^{-4}-10^{-6} and 'veg fru wash' in dilution 10^{-3}-10^{-6} as well. Therefore, it can be concluded that chlorine water and 'veg fru wash' were effective antibacterial treatment to reduce microbial load in cabbage than tap water.

The above Figure 2 shows that in dilution 10^{-2} to 10^{-6} maximum value of unwashed cabbage varied from 2.4 – 2.18 log_{10} CFU/g. Minimal decreases were seen after cabbage was washed with tap water (resulting in decreases of 2.38 -2.06 log_{10} CFU/g), chlorine water (resulting in decreases of

1.91 log_{10} CFU/g – no growth of bacteria) and 'veg fru wash' (resulting in decreases of 1.74 log_{10} CFU/g to no growth of bacteria). Hence, according to this study it can be concluded that chlorine water and 'veg fru wash' were effective antibacterial treatment than tap water. Microbiological assessment of GLSV's was performed and The Table 5 depicts the mean microbial load in spinach in CFU/g.

According to dilution 10^{-2} the initial load in spinach was found to be 285± 0 which was reduced to 280±0, 80±6.70 and 40±0 with application of tap water, chlorine water and 'veg fru wash'. In 10^{-3} dilution, initial load was 278±12.7 which was reduced to 264± 9.24, 61.3±11.3 with application of tap water, chlorine water. In 10^{-4}–10^{-6} dilution, initial load varied from 246.6±7.5-209.3±17.5 which was reduced to 240±0-164±21.7 by tap water and there was no growth of bacteria in chlorine water in dilution 10^{-4}-10^{-6} and 'veg fru wash' in dilution 10^{-3}-10^{-6} as well. Therefore, according to this study, it was concluded that chlorine water and 'veg fru wash' were effective antibacterial treatment than tap water as depicted in Figure 3 and Table 5.

The Figure 3 shows that in dilution 10^{-2} to 10^{-6} maximum value of unwashed spinach varied from 2.45 – 2.31 log_{10} CFU/g. Minimal decreases were seen after spinach was washed with tap water (2.44 -2.21 log_{10} CFU/g), chlorine water (resulting in decreases of 1.89 log_{10} CFU/g – no growth of bacteria) and 'veg fru wash' (resulting in decreases of 1.57 log_{10} CFU/g to no growth of bacteria). Hence, it can be concluded that chlorine water and 'veg fru wash' were effective antibacterial treatment than tap water. Researchers (Xu and Leskovar, 2015; Yadav, 2018) revealed that spinach were traditionally used in various folklore medicines like antipyretic, diuretic, maturate, flatulence and febrile and some other researcher explored nutritive value of Spinach, it is a suitable food for obese and diabetic people. It is also a good source of chlorophyll, which is known to aidin digestion (Verma, 2018).

Suitable antibacterial washing treatment to reduce the microbial load on green leafy salad vegetables

From the above Table 6, it can be seen that 'veg fru wash' had the least mean value (14.66± 9.0, 11.20±11.2, 8.00±8.0) of bacterial count followed by chlorine water (27.88±17.22, 30.40±18.7, 28.26±17.5) and tap water (167.96±14.5, 177.84±22.5, 231.20±20.7) on GLSV's respectively. Hence, it can be concluded that 'veg fru wash' was the most effective antibacterial washing treatment to reduce microbial count on GLSV's than other treatment (tap water and chlorine water) at 1% level of significance.

Table.1 Number of sample analysed

Green leafy salad vegetables	Sample=9
Lettuce	3
Cabbage	3
Spinach	3

Table.2 Sample treated with treatment

Sample	Unwashed (as a control)	Washing treatment		
		Tap water	Chlorine water	Veg fru wash
Lettuce	✓	✓	✓	✓
Cabbage	✓	✓	✓	✓
Spinach	✓	✓	✓	✓

Table.3 Mean microbial load in Lettuce in CFU/g

Dilutions	MEAN ± SE			
	Unwashed	Tap water	Chlorine water	Veg fru wash
10^{-1}	TNTC	TNTC	112.6± 3.5	62.6± 2.01
10^{-2}	240± 9.41	214.6± 12.2	77.3± 3.92	41.3± 1.08
10^{-3}	220± 9.41	186.6± 12.2	62.6± 3.92	32± 0
10^{-4}	209.3± 8.48	160± 9.41	*	*
10^{-5}	181.3± 8.48	142.6± 9.6	*	*
10^{-6}	165.3± 6.58	136± 8.6	*	*

* No growth
SE- Standard error, TNTC – Too numerous to count

Table.4 Mean microbial load in Cabbage in CFU/g

Dilutions	MEAN ± SE			
	Unwashed	Tap water	Chlorine water	Veg fru wash
10^{-1}	TNTC	TNTC	120± 3.1	62.6± 2.05
10^{-2}	272± 12.01	245.3± 16.0	84± 5.3	56± 3.7
10^{-3}	249.3± 14.16	209.3± 21.7	68± 0	*
10^{-4}	233.3± 12.27	173.3± 12.2	*	*
10^{-5}	178.6± 33.14	140± 18.8	*	*
10^{-6}	165.3± 34.92	121.3± 19.8	*	*

Table.5 Mean microbial loads in Spinach

Dilutions	MEAN ± SE			
	Unwashed	Tap water	Chlorine water	Veg fru wash
10^{-1}	TNTC	TNTC	114.6± 4.2	66.6± 1.0
10^{-2}	285± 0	280± 0	80± 6.70	40± 0
10^{-3}	278± 12.7	264± 9.24	61.3± 11.3	*
10^{-4}	246.6± 7.5	240± 0	*	*
10^{-5}	232± 19.6	208± 16.7	*	*
10^{-6}	209.3± 17.5	164± 21.7	*	*

Table.6 Statistical analysis between the various treatments

GROUPS/ TREATMENT	LETTUCE Mean± S.E	CABBAGE Mean± S.E	SPINACH Mean± S.E
Unwashed	203.18± 13.3	219.70± 20.5	250.8± 14.1
Tap water	167.96± 14.5	177.84± 22.5	231.20± 20.7
Chlorine water	27.98± 17.2	30.40± 18.7	28.26± 17.5
Veg fru wash	14.66± 9.0	11.20± 11.2	8.00± 8.0
F value	47.71*	30.95*	66.44*

* $p \leq 0.01$

Figure.1 Effect of antibacterial treatment on microbial load of lettuce sample in Log_{10} CFU/g

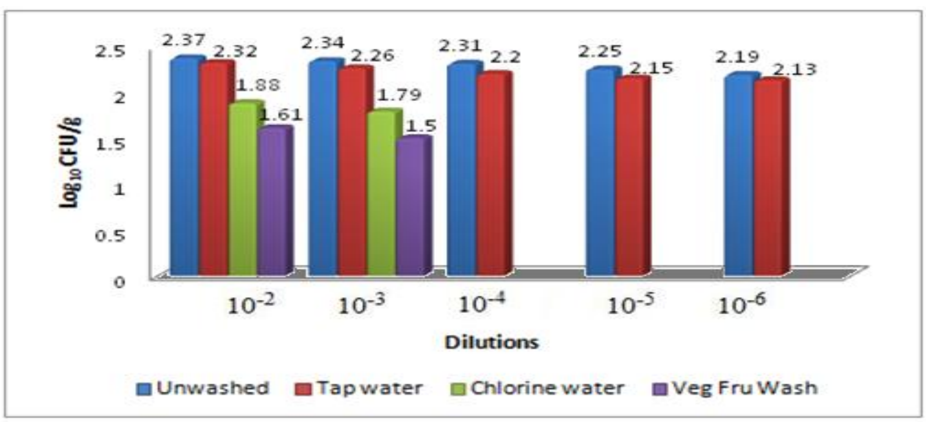


Figure.2 Effect of antibacterial treatment on microbial load of cabbage in Log_{10} CFU/g

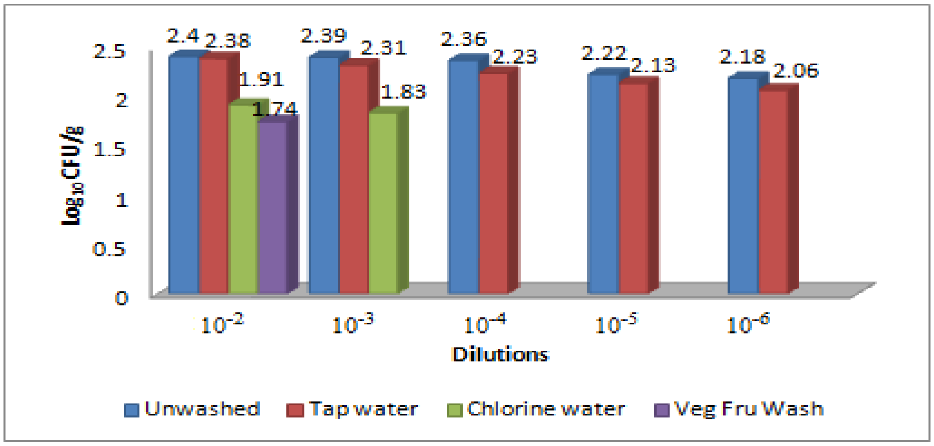
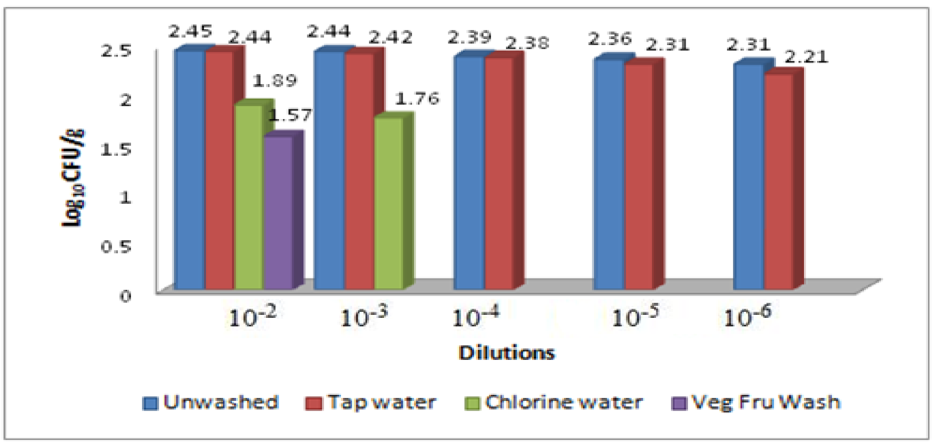


Figure.3 Effect of antibacterial treatment on microbial load of spinach in Log_{10} CFU/g



Brackett, 1992 reported that washing with tap water cannot completely remove pathogenic bacteria on fresh produce like spinach because it is an important green leafy vegetable and medicinal and part of diet (Miri and Roughani, 2019). Gonzalez *et al.*, (2004) revealed that chlorine is widely used to sanitize GLSV's but its efficacy is affected by the organic loads present in the washing solution. Freshly cut GLSV's release large amount of organic matter that negatively impact the chlorine washing efficacy. Chlorine also rapidly loses activity on contact with organic matter or exposure to air, light or metals and people who use chlorinated water as a disinfectant for prolonged period; it can cause irritation to the skin and respiratory tract (WHO/FAO 2003). Beuchat *et al.*, (1998), Zhang and Farber (1996) reported that the development of sanitizers are needed which are more efficacious than Chlorine or ClO_2 for suppressing foodborne pathogenic bacteria on GLSV's. On the other hand, 'FIT' was approximately as effective as 200 and 20000 ppm chlorine in reducing populations of *Salmonella* and *E. coli* O157:H7 on alfalfa seeds, but without the human health and environmental hazards associated with such a high level of chlorine. In a similar study done by Smith *et al.*, (2003) on “comparison of efficacy of a commercial produce wash with that of water for reducing the total bacterial population on lettuce’. The result indicate that ‘victory’ produce wash was effective than water in reducing indigenous flora on lettuce during food service preparation. Reductions obtained with ‘victory’ produce wash were significantly larger ($p = 0.0006$) than those obtained with water. Pezzuto *et al*., (2016) also analyzed if commercial fruit and vegetable washes were effective at removing bacteria specially *Salmonella spp.* and *Listeria spp.* from the surface of raw vegetables and found that commercial washes were not significantly different from one-another, except water compared to ‘FIT’ wash and ‘Veggie Wash’. The various routes of contamination are contaminated irrigation water, human handling, contaminated containers, animal waste fertilizers, wild and domestic animals, postharvest washing, improper cooking and /or improper holding temperature after cooking, improper storage, improper packaging and contamination from other foods in food preparation area (Sehgal, 2009). Hence, by the time the GLSV’s arrives on shelf at the market, it could be covered by a number of microorganisms and cause food borne diseases (Sapers *et al*, 2001). To prevent food borne illness, it is necessary to control contamination. However, when the exclusion of contamination is not feasible, decontamination processes become necessary. Research efforts have focused primarily on assessing the effectiveness of sanitizers conventionally used in the food industry or in the home (Vijayakumar and Wolf-hall, 2002). Washing with tap water is the mostly common practice of consumers to reduce microbial contamination of GLSV’s, though tap water has limited or no effect on killing microorganisms. Water containing 50 to 200 ppm of chlorine is widely used to sanitize green leafy salad vegetables because of its low cost and efficacy against bacterial pathogens (Adam *et al.*, 1989). However chlorine efficacy is affected by the organic loads in the washing solution (Gozalez *et al.*, 2004). GLSV’s releases large amount of organic matter that negatively impact the chlorine efficacy and convert active chlorine into inactive form. Therefore, alternative sanitizers are needed, which are more effective than chlorine or ClO_2 for suppressing food borne pathogenic bacteria in fresh cut GLSV’s

In conclusion, fresh and fresh-cut leafy green vegetables are nutrient-rich foods with high levels of antioxidant, minerals, vitamins and photochemical. GLSV’s are essential part of people diet all around the world and they are usually consumed raw and often without heat treatment or thorough washing. Hence they have been known to serve as vehicles for the transmission of pathogenic microorganism associated with human diseases. The result showed that in dilution 10^{-2} to 10^{-6} the highest microbial load was found in unwashed (control) lettuce ranged from (240± 9.41 - 165.3± 6.58 CFU/g, 2.37- 2.19 $\log_{10}$ CFU/g) with the application of tap water minimal reduction were seen (214.6± 12.2 - 136± 8.6 CFU/g, 2.32 -2.13 $\log_{10}$ CFU/g), chlorine water reduces (77.3± 3.92- 62.6± 3.92 CFU/g, 1.88- 1.79 $\log_{10}$ CFU/g) and ‘veg fru wash’ reduces 41.3± 1.08- 32± 0 CFU/g, 1.61- 1.5 $\log_{10}$ CFU/ g and there was no growth of bacteria in chlorine water and ‘veg fru wash’ in dilution 10^{-4}-10^{-6}.

The study also found that ‘Veg fru wash’ was most effective antibacterial washing treatment to reduce microbial load on GLSV’s at 1% level of significance than tap water and chlorine water. The current study concludes that use of commercial antibacterial washing treatment should become part of daily kitchen chores as even chlorine water is not acting as a full- proof method of reducing the bacterial count. Earlier studies have also reported that chlorine rapidly loses activity on contact with organic matter or exposure to air, light or metals and people who use chlorinated water as a disinfectant for prolonged period; it can cause irritation to the skin and respiratory tract. Commercial fruit and vegetable washes (veg fru wash, sunshine wash, armoaz vegetable wash etc.) are made from pure natural food grade edible ingredients and remove all harmful pesticides and disease causing parasites, bacteria from fresh fruits and vegetables without human health hazards. Hence, they are best suitable washing treatment to be used at household level. Government should plan effective measures to raise popularity and awareness to reduce microbial load from the surface of food and prevent food borne disease.

For future, this kind of research should be done with a larger sample size of salad vegetables and fruits in order to get more reliable and meaningful results. The future researchers could include other factors such as inoculation of pathogens in fruits and vegetables in order to determine the efficacy of washing treatment. Data survey/ Questionnaire was also being done to know the knowledge, attitude and practices of people regarding various commercial washing treatments available in the market and importance of food safety and hygiene. Government should also take initiative to educate customer regarding commercial fruit vegetable washes through advertisement in all media sources regarding efficacy of commercial vegetable and fruit washes. People should be advised to thoroughly wash their fruits and vegetables with effective antibacterial washing treatment because they were treated with pesticides and loaded with microorganisms before being put on the shelf in a market.

Acknowledgement

First author wan to thanks Dr. K. K. Singh and Mr. Amit Kumar Shukla for critically reviewing a manuscript.

References

Adams, M. R., Hartley, A. D., and Cox, L. J. 1989. Factors affecting the efficacy of washing procedures used in the production of prepared salads. Food Microbiology. 6: 69-77.

Beuchat, L. R., Nail, B. V., Adler, B. B., and Clavero, M. R. S., 1998. Efficacy of spray application of chlorinated water in killing pathogenic bacteria on raw apples, tomatoes, and lettuce. Journal of Food Protection. 61: 1305–1311.

Brackett, R. E., 1992. Shelf stability and safety of fresh produce as influenced by sanitation disinfection. Journal of Food Protection.55:808–814.

Carter, D. A., D'Souza, F., Simkins, B. J., and Simpson W. G., 2010. The gender and ethnic diversity of US corporate governance: An international review boards and board committees and firm financial performance. 18(5): 396-414.

FAO, 2009. How to feed a world in 2050.

FAO/WHO, 2008. Microbiological Risk Assessment Series 14.

Gonzalez, R. J., Luo, Y., Ruiz-Cruz, S., and Mc Evoy, J. L., 2004. Efficacy of sanitizers to inactivate *Escherichia coli* O157:H7 on fresh-cut carrot shreds under simulated process water conditions. Journal of Food Protection. 67: 2375–2380.

Gupta, S., and Prakash, J., 2008. Studies on Indian green leafy vegetables for their antioxidant activity. Plant Food for Human Nutrition. 64(1): 39-45.

Hung, H. C., Joshipura, K. J., Jiang, R., Hu, F. B., Hunter, D., Smith-Warner, S. A., Colditz, G. A., Rosner, B., Spiegelman, D., and Willett, W. C., 2004. Fruit and vegetable intake and risk of major chronic disease. Journal of National Cancer Institute. 96(21):1577-84.

Ibrahim, M. N. M., Tamminga, S., Zemmelink, G., 1995. Degradation of tropical roughages and concentrate feeds in the rumen. Animal Feed Science and Technology. 54 (1/4): 81-92.

Lynch, I., Sara, L., Vyvyan, C. H., Stepnik, M., Rydzynski, K., Hanrahan, J., Wim de Jong., 2009. Nanointeract: A rational approach to the interaction between nanoscale materials and living matter. Journal of Physics: Conference Series. 170: 1742-6596.

Microbiology, A Laboratory Manual, VII Edition by Cappuccino & Sherman. Pezzuto, A., Belluco, S., Losasso, C., Patuzzi, I., Bordin, P., Piovesana, A., Comin, D., Mioni, R., and Ricci A., 2016. Effectiveness of washing procedures in reducing *Salmonella enterica* and *Listeria monocytogenes* on a raw leafy green vegetable (*Eruca vesicaria*). Frontier in Microbiology. 7:1663.

Miri, S. M., and Roughani A., 2019. The 2nd International Conference on medicinal plants, organic farming, natural and pharmaceutical ingredients. Spinach: An important green leafy vegetable and medicinal herb.

Rao, K.N.V., Tabassum, B., Babu, S.R., Yaja, A., and Banji, D., 2015. Preliminary phytochemical screening of *Spinacia oleracea* L. World Journal of Pharmacy and Pharmaceutical Sciences. 4, 532-551.

Saper, C. B., and Chou, T. C., 2001. The sleep switch: hypothalamic control of sleep and wakefulness. Trends Neuroscience. 24(12):726-31.

Sehgal, V. N., 2009. Plexiform neurofibromas in neurofibromatosis type. 11365-4632.

Sharma, M., Manoharlal, R., Shukla, S., Puri, N., Prasad, T., Ambudkar, S. V., and Prasad, R., 2009. Curcumin modulates efflux mediated by yeast ABC multidrug transporters and is synergistic with antifungals. Antimicrobial Agents Chemotherapy. 53(8): 3256-65.

Smith, S., Dunbar, M., Tucker, D., and Schaffner, D. W., 2003. Efficacy of a commercial produce wash on bacterial contamination of lettuce in a food service setting. Journal of Food Protection. 66(12): 2359-61.

Taormina, R. J., 2009. Organizational Socialization: The missing link between employee needs and organizational culture. Journal of Managerial Psychology. 24:650-676.

Times of India, News Reports. 2002.

Verma, S., 2018. A study on medicinal herb *Spinacia oleraceae* Linn: Amaranthaceae. Journal of Drug Delivery and Therapeutics. 8(4), 59-61.

Vijayakumar, C., and Wolf-Hall, C. E., 2002. Evaluation of household sanitizers for reducing levels of *Escherichia coli* on Iceberg Lettuce. Journal of Food Protection**.** 65(10): 1646-50.

WHO/FAO, 2003. Report on Diet, nutrition and the prevention of chronic diseases. Expert Consultation World Health Organization. WHO Technical Report Series 916.

Xu, C., and Leskovar, D.I., 2015. Effects of *A. nodosum* seaweed extracts on spinach growth, physiology and nutrition value under drought stress. Scientia Horticulturae. 183, 39-47.

Yadav, M., Parle, M., Sharma, N., Jindal, D.K., Bhidhasra, A., Dhingra, M.S., Kumar, A., and Dhingra, S., 2018. Protective effects of *Spinacia oleracea* seeds extract in an experimental model of schizophrenia: Possible behavior, biochemical, neurochemical and cellular alterations. Biomedicine and Pharmacotherapy. 105, 1015-1025.

Zhang, S., and Farber, J. M., 1996. The effects of various disinfectants against *Listeria monocytogenes* on fresh-cut vegetables. Food Microbiology. 13:311-21.