

Demonstration of microbiological status of fresh fruits and determining the efficiency of different decontaminating agents against the isolated bacteria

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Abstract

Consumption of fresh fruits increases along with the promotion of healthier lifestyles. However, many foodborne illnesses have been reported due to the consumption of contaminated fruits. Fresh produces are subjected to various treatments for minimizing these outbreaks. The present study demonstrates the role of various simple household methods including water washing and some other common chemical treatments for the removal of bacterial load. By performing the spread plate technique, total heterotrophic bacteria, *Escherichia coli*, *Staphylococcus* spp., *Salmonella* spp. and *Listeria* spp. were determined in 60 samples of 6 categories including Apple (*Malus domestica*); Grape (*Vitis vinifera*); Guava (*Psidium guajava*); Strawberry (*Fragaria ananassa*); Indian jujube (*Ziziphus mauritiana*); Malabar plum (*Syzygium cumini*) before and after washing with tap water, hot water, 100 mg/L sodium hypochlorite (NaOCl), 50 mg/L calcium lactate, 4% acetic acid and 2 mL/L CleanAva for 20 mins at room temperature. All the tested solutions were found to be effective and reduced bacterial loads in fruits compared to the unwashed fruits samples ($P < 0.01$). Treatment with NaOCl, calcium lactate, acetic acid and CleanAva was the more effective washing technique rather than hot water wash. Two to three log of the bacterial load was reduced when samples were subjected to treatment with chemical decontaminating agents. The efficiency of washing depends on the purity of water, and the types and concentration of the disinfectant solutions. An appropriate washing technique needs to implicate to diminish bacterial load and the risk of foodborne infections caused by fruits.

1. Introduction

In our everyday life consumption of fresh fruits and vegetables are essential as a part of a healthy diet as this fresh produce is a source of phytonutrients, vitamins and minerals (Minocha *et al.*, 2018). Proper washing and hygiene practices are mandatory before consuming raw fruits otherwise contaminated fruits have led to acute foodborne illnesses related to microorganisms and chemical insecticides (Callejón *et al.*, 2015). It has been reported earlier that fresh produce comes into contact with pathogenic microorganisms anywhere from farm to table. Contamination can take place during pre-harvest through contaminated seed, at the time of harvesting by soil, untreated manure, water and pests (Alegbeleye *et al.*, 2018). During postharvest processes, some post-harvesting tools, dirty surfaces can cause contamination. On the other hand, cross-contamination from cutting boards, poor transportation systems, temperature shifting are also responsible for the deterioration of fruits quality

(Machado-Moreira *et al.*, 2019). Usually, the foodborne infection takes place after consuming fruits containing pathogens such as *Salmonella* spp., *Shigella* spp., *Clostridium perfringens*, *Escherichia coli*, *Listeria monocytogenes*, *Bacillus cereus* and *Campylobacter* spp. (Carstens *et al.*, 2019).

Nowadays decontamination of fresh fruits is of concern all over the world. Suitable methods and food-grade disinfecting solutions are developed for the removal of pathogenic microorganisms and undesirable pesticides from fresh produce (Carstens *et al.*, 2019). The most common household processes are not enough for the complete removal of microorganisms. Postharvest heat treatments have been effective to control insects and diseases in fruit and vegetables (Fallik *et al.*, 2000). NaOCl reacts with the cellular enzyme of bacteria and causes plasmolysis leading to the death of the microbes (Bhilwadikar *et al.*, 2019). The acetic acid of vinegar pass into the cell membrane causes acidification of cell

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component which leading bacterial cell death (Chang and Fang, 2007). Calcium based sanitizers are performing an effective role in microbial load reduction (Rico *et al.*, 2007). In recent years some commercial sanitizers are available on the market and they can completely remove microorganisms from fruits sample. Therefore, this study was carried out to isolate the microorganisms from the different categories of raw fruits and to investigate the efficacy of commercial sanitizer (CleanAva), acetic acid, tap water, hot water, NaOCl and calcium lactate as a cleaning agent.

2. Materials and methods

2.1 Sample collection and processing

For the microbiological analysis, a total of 60 fruits samples of 6 categories including apple (*Malus domestica*); grape (*Vitis vinifera*); guava (*Psidium guajava*); strawberry (*Fragaria ananassa*); Indian jujube (*Ziziphus mauritiana*); Malabar plum (*Syzygium cumini*) were collected from the local market in Dhaka city between December 2019 to February 2020. Samples were collected in sterile bags and transported to the laboratory immediately for microbiological analysis (Das *et al.*, 2018). All tested samples were chopped aseptically and a 10 g sample was added to a sterile conical flask containing 90 mL distilled water. After ten-fold serial dilution with normal saline, the 10^2 dilution factor samples were spread on agar plates for enumeration of the bacterial count. Then the same samples were soaked with desired decontaminating agents such as tap water, hot water (50°C), 100 mg/L sodium hypochlorite (NaOCl), 50 mg/L calcium lactate ($C_6H_{10}CaO_6$), 4% acetic acid and 2 mL/L CleanAva (composed of alkyl polysaccharide) for 20 mins at room temperature (Sun *et al.*, 2012). After soaking, samples were subjected to microbiological analysis and the same procedure was repeated for sample preparation.

2.2 Enumeration of total viable bacteria

For enumerating total viable bacteria (TVB), 0.1 mL of each sample was spread onto the nutrient agar (NA). For TVB, plates were incubated at 37°C (Nur, Mou and Habiba, 2020).

2.3 Isolation of pathogenic bacterial load

For the isolation of coliforms and faecal coliforms, from 10^2 dilutions, 0.1 mL suspension was spread over MacConkey agar and mFC agar. MacConkey agar plates were incubated at 37°C for 18-24 hrs. For faecal coliforms, plates were incubated at 44.5°C for 24 hrs (Shaheduzzaman *et al.*, 2015). On the other hand, for the isolation of *Salmonella* spp., *Shigella* spp., *Vibrio* spp. and *Staphylococcus* spp., 0.1 mL was taken from the

from 10^2 dilution and spread onto Xylose Lysine Deoxycholate (XLD), Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar plates and Mannitol salt agar (MSA) respectively. After incubation at 37°C for 24 hrs, characteristic colonies were counted (Nur *et al.*, 2017; Nur, Ghosh and Acharjee, 2020).

2.4 Statistical analysis

The statistical analysis was performed using the Social Science Statistics [http://www.socscistatistics.com] online software. Using a T-test calculator for two independent means samples were analyzed by a two-tailed hypothesis at a significance level of 0.01 (Das *et al.*, 2018).

3. Results

3.1 Enumeration of bacterial load without washing

Table 1 shows the total bacterial cell count in unwashed fruits samples. The highest and lowest counts of total heterotrophic bacteria in unwashed vegetables were 8.92 ± 0.48 and 7.11 ± 0.31 \log_{10} CFU/g in Strawberry and Olive, respectively. The highest and lowest count of *E. coli* was recorded 7.22 ± 0.41 and 6.11 ± 0.59 \log_{10} CFU/g in Strawberry and Indian jujube, constitutively. *Staphylococcus* spp. and *Salmonella* spp. were also found in all untreated fruits and their range was between 6.57 ± 0.66 to 5.27 ± 0.59 \log_{10} CFU/g. *Klebsiella* spp., *Vibrio* spp., *Shigella* spp. and faecal coliform were absent in all tested samples (Table 1).

3.2 Effect of cleaning agents on viable bacteria

After washing with tap water (Tw), hot water (HW) and NaOCl, the reduced load of total viable bacteria were ranged between 6.52 ± 0.38 - 6.01 ± 0.67 , 5.48 ± 0.14 - 3.98 ± 0.82 and 4.91 ± 0.42 - 3.13 ± 0.83 \log_{10} CFU/g, consecutively ($P < 0.01$). Moreover, 2 logs of the bacterial load were reduced ($P < 0.01$) after washing with HW (50°C) and NaOCl and 1 log reduction after washing with tap water. On the other hand, 2 to 4 log bacterial reduction was observed when the samples were treated with calcium lactate, acetic acid and commercial cleaning agent CleanAva and they ranged between 4.60 ± 0.39 to 2.37 ± 0.44 \log_{10} CFU/g ($P < 0.01$). Acetic acid and CleanAva showed efficacy by reducing a significant bacterial load (Table 2).

3.3 Effects of cleaning agent on *Escherichia coli* populations

Initially, the highest and lowest count of total coliform was recorded 7.22 ± 0.41 and 6.11 ± 0.59 in Strawberry and Indian jujube, consecutively ($P < 0.01$). The highest load of *E. coli* was found in apples (5.91 ± 0.28 \log_{10} CFU/g) when fruits were washed with

Table 1. Bacteriological analysis of fresh fruits without wash (WW)

Fruits (n = 60)	Bacterial load (log ₁₀ CFU/g ± SD)				
	TVC	<i>Escherichia coli</i>	<i>Staphylococcus</i> spp.	<i>Salmonella</i> spp.	<i>Listeria</i> spp.
Apple	7.61±0.37	6.17±0.18	6.38±0.45	6.05±0.22	4.61±0.58
Grape	8.75±0.56	7.21±0.73	6.22±0.48	6.11±0.13	4.74±0.53
Guava	7.31±0.39	6.29±0.15	6.38±0.43	6.09±0.07	3.54±1.01
Strawberry	8.92±0.48	7.22±0.41	6.48±0.37	6.19±0.17	4.69±0.75
Indian jujube	8.61±0.24	6.11±0.59	6.31±0.34	5.64±0.65	3.83±1.26
Malabar plum	7.77±0.29	6.25±0.45	6.28±0.96	5.93±0.37	4.48±0.14

Table 2. Counts of bacteria in fruits after washing with water and disinfectant solutions

Fruits	Foodborne Bacteria	Bacterial load (log ₁₀ CFU/g ± SD)					
		TW	HW	NaOCl	Calcium lactate	Acetic acid	CleanAva
Apple	Total viable bacteria	6.27±0.63	5.05±0.07	4.74±0.58	4.60±0.39	3.21±1.10	2.75±0.50
	<i>E. coli</i>	5.91±0.28	4.73±0.57	3.57±1.37	2.43±0.44	NG	NG
	<i>Salmonella</i> spp.	4.89±0.55	3.57±1.37	2.66±0.64	2.34±1.61	1.57±1.51	NG
	<i>Listeria</i> spp.	3.34±0.12	2.21±0.64	NG	NG	NG	NG
	<i>Staphylococcus</i> spp.	5.22±1.04	4.00±0.85	3.53±0.21	2.54±0.54	1.53±1.32	NG
Grape	Total viable bacteria	6.84±0.47	4.00±0.85	4.91±0.42	3.31±0.82	3.11±0.86	2.99±0.81
	<i>E. coli</i>	5.28±0.23	4.39±1.30	2.79±0.55	2.34±0.40	1.25±0.96	NG
	<i>Salmonella</i> spp.	4.79±0.36	3.83±1.26	3.44±0.29	2.23±0.17	NG	NG
	<i>Listeria</i> spp.	3.26±0.13	2.07±0.76	NG	NG	NG	NG
	<i>Staphylococcus</i> spp.	4.79±0.36	3.83±1.26	3.44±0.29	2.23±0.17	NG	NG
Guava	Total viable bacteria	6.85±0.57	4.50±0.35	4.76±0.88	3.83±1.26	3.56±0.46	2.89±1.00
	<i>E. coli</i>	5.18±0.15	4.74±0.58	3.54±1.01	2.81±0.40	NG	NG
	<i>Salmonella</i> spp.	4.83±0.49	3.28±0.10	2.43±0.86	3.83±1.26	NG	NG
	<i>Listeria</i> spp.	2.65±0.57	2.13±0.86	NG	NG	NG	NG
	<i>Staphylococcus</i> spp.	5.07±0.11	4.63±0.95	3.29±0.12	2.53±0.15	1.80±1.61	1.43±1.24
Strawberry	Total viable bacteria	6.92±0.38	4.74±0.58	4.48±0.14	3.84±0.86	3.91±1.04	2.66±0.73
	<i>E. coli</i>	5.80±0.75	5.48±0.14	3.32±1.55	2.54±0.60	1.13±0.94	1.29±0.89
	<i>Salmonella</i> spp.	4.85±0.57	3.28±0.11	2.83±0.53	2.13±1.87	1.92±0.82	NG
	<i>Listeria</i> spp.	3.37±0.76	2.56±0.53	1.23±1.31	NG	NG	NG
	<i>Staphylococcus</i> spp.	5.53±0.46	4.04±0.12	2.73±0.21	2.93±0.15	1.92±0.82	NG
Indian Jujube	Total viable bacteria	6.55±0.61	4.60±0.39	4.69±0.75	4.00±0.85	3.50±0.52	2.37±0.44
	<i>E. coli</i>	5.86±0.45	4.68±0.62	3.31±0.82	2.33±0.29	NG	NG
	<i>Salmonella</i> spp.	4.10±0.40	3.88±0.59	2.81±0.43	2.37±0.44	1.43±1.24	NG
	<i>Listeria</i> spp.	4.10±0.40	3.88±0.59	2.81±0.43	2.37±0.44	1.43±1.24	NG
	<i>Staphylococcus</i> spp.	4.91±0.62	4.60±0.39	3.53±0.21	2.15±0.04	1.90±1.23	NG
Malabar Plum	Total viable bacteria	6.13±0.51	5.48±0.14	4.15±0.44	3.57±1.37	3.56±0.46	3.24±1.12
	<i>E. coli</i>	5.13±0.83	4.63±0.46	3.83±1.26	2.42±0.83	1.63±0.48	NG
	<i>Salmonella</i> spp.	3.97±0.65	2.22±1.37	2.96±0.61	2.66±0.73	NG	NG
	<i>Listeria</i> spp.	3.13±0.83	2.46±0.61	NG	NG	NG	NG
	<i>Staphylococcus</i> spp.	4.73±0.57	3.98±0.82	2.13±0.37	2.19±0.12	NG	NG

Values are presented as mean± SD, n = 3. NG: No growth; TW: Tap water; HW: Hot water.

Reduced bacterial loads in fruits were statistically significantly different when compared to the unwashed fruits samples (P<0.01).

tap water. A remarkable bacterial load reduction was noticed when fruits samples were cleaned with calcium lactate, acetic acid and CleanAva. In the case of calcium lactate treatment, the lowest count was documented in Indian jujube (2.33±0.40 log₁₀ CFU/g) and after treatment with acetic acid, no growth was observed in Apple, Guava, Indian jujube. The effect of CleanAva on the growth of *E. coli* was satisfactory. Except for

strawberry and guava, other samples were free from *E. coli* (Table 2).

3.4 Counts of *Staphylococcus* spp. in fruits after treatment

With tap water wash, the highest load of *Staphylococcus* spp. was noted in strawberries (5.53±0.46 log₁₀ CFU/g) and the lowest count was in

Malabar plum ($4.73 \pm 0.57 \log_{10}$ CFU/g). After treatment with HW (50°C) and NaOCl, the lowest bacterial load was recorded as 3.57 ± 1.37 and $2.13 \pm 0.37 \log_{10}$ CFU/g in olive and Malabar plum, respectively ($P < 0.01$). The acceptable count was obtained following treatment with calcium lactate, acetic acid and CleanAva. Overall, 2 to 3 log reduction of *Staphylococcal* ($P < 0.01$) count took place in all fruits sample and Indian jujube ($2.15 \pm 0.04 \log_{10}$ CFU/g) was remarked as the lowest count. Grape, Malabar plum reported no growth on MSA plates (Table 2).

3.5 Enumeration of *Salmonella* spp. after treatment

Primarily, with tap water wash, the highest load of *Salmonella* spp. was found in Apple ($4.89 \pm 0.55 \log_{10}$ CFU/g). The lowest load was recorded in Malabar plum after washing with HW (50°C) and NaOCl, respectively ($P < 0.01$) and 2 log deduction was exhibited in other samples. Two log population of *Salmonella* spp. was reduced after treatment with calcium lactate and the lowest load was recorded in strawberry ($2.13 \pm 1.87 \log_{10}$ CFU/g). Acetic acid was able to limit the growth of *Salmonella* spp. and no growth was observed in Grape, Guava and Malabar plum. However, no growth of *Salmonella* spp. was found when fruits were washed with CleanAva (Table 2).

4. Discussion

Contamination of fresh produce with *E. coli* strain has been demonstrated previously (Cevallos-Cevallos et al., 2012). *Salmonella* spp. is commonly found in farm effluents and other sources of faeces (Cevallos-cevallos et al., 2012). In our study, we used decontaminating agent on the basis of their pH. Both alkaline pH and acidic pH are vulnerable to bacterial growth (Bhilwadikar et al 2019). If this study was correlated with the previous study, it was observed that tap water wash proved ineffective to reduce the significant load of microorganisms and additionally it could be the source of contaminating microbes (Bernardino et al., 2019). Comparing the different washing treatments, it is clear that treatment with NaOCl, calcium lactate, acetic acid and CleanAva were the more effective washing techniques rather than hot water wash. Sodium hypochlorite (NaOCl) solutions at concentrations of 50 to 200 ppm are generally used as a decontaminating agent in food processing units (Soliva-Fortuny et al., 2003). However, the result of some previous study reflected in our present study where 2 to 3 log reductions of pathogenic bacteria were observed for almost all samples when they are treated with 100 mg/L NaOCl solutions (Sun et al., 2012). Calcium lactate has been reported to be an effective compound (Rico et al., 2007). A group of scientists revealed that calcium lactate

reduced bacterial and fungal populations by about 85% to 99.6% from fresh cut jackfruit (Acedo et al., 2012). Another study demonstrated that up to 3 logs of *E. coli* cell reduction occurred when cabbage was subjected to treatment with 0.1% to 0.4% calcinated calcium for 20 mins (Fukuyama et al., 2009). Different studies showed the efficiency of vinegar (acetic acid) against pathogenic bacteria in fresh produce (Bakir et al., 2017; Budak et al., 2014). Chang and Fang (2007) reported that a 3 log reduction of *E. coli* was observed in lettuce leave when it was treated with 5% acetic acid and in another study, it was documented that a mixture of 4.03% acetic acid and lemon juice significantly reduced the load of *Salmonella* spp. (Sengun and Karapinar, 2004). A general guideline was provided by the European Commission (EC) Regulation 852/2004 on the hygiene of foodstuffs for good agricultural practices (GAPs), which need to be applied from farm to fork (EC, 2004). However, in Bangladesh, most people use tap water for washing fruits. The tradition of using a disinfectant solution is rare in this country and as a result, effective cleaning procedure remains incomplete and microorganisms get chances to reside in the fresh produce. In the present study, some available and inexpensive cleaning agents were used which prove their efficiency against pathogens.

5. Conclusion

Fresh produces are cultivated and consumed worldwide. Conventional washing of fresh produce including tap water and hot water can't reduce the bacterial load to a satisfactory level. Additionally, fruits are taken in raw form. Hence, new washing techniques using more effective sanitizing or disinfecting agents are needed to kill microorganisms that may survive conventional washing systems. For this purpose, sodium hypochlorite (NaOCl (100 mg/L), calcium lactate (50 mg/L), acetic acid (4%) and CleanAva (2 mL/L) can be effective disinfectants to limit the total bacterial load in fruits over the conventional washing procedure. The commercialization of new and effective approaches for disinfection will improve the microbiological quality and safety of fresh and fresh-cut fruits.

Conflict of interest

The authors declare no conflict of interest

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