Effectiveness of Various Disinfection Techniques on Vegetables and Fruits: An In-Vitro Study

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ABSTRACT:

Background: Raw and minimally processed vegetables and fruits are an essential part of people's diet all over the world. These food products have also been known to serve as vehicles of human disease for atleast a century.The objectives of this study were to assess the microbial contamination of fruits and vegetables and to evaluate and compare the effectiveness of various disinfection techniques.

Materials and Methods: In this in-vitro study two types of vegetables and two types of fruits were collected for evaluation of their microbiological quality. The disinfection techniques to be evaluated were: washing under running water, soaking in lukewarm water, soaking in salt water and soaking in vinegar solution. ANOVA (Analysis of Variance) was done for statistical analysis of the results.

Results: The contamination of vegetables was significantly more than fruits ($p<0.05$). A microbial reduction in terms of CFU/ml was observed with all the disinfection methods employed in all the fruits and vegetables taken. The reduction observed in the colony forming units per ml among the vegetables, due to the disinfection techniques was statistically significant ($p<0.05$).

Conclusion: Soaking in 5% vinegar solution for 30 seconds may be the most effective for disinfection of raw vegetables and fruits based on its microbial reduction capacity and short time to perform.

Keywords: Disinfection, Fruits, Microbial contamination, Vegetables, Vinegar.

INTRODUCTION

Hippocrates who is considered as the father of modern medicine said, "Let food be your medicine and medicine be your food". But in an age where the food supply chain is often a long and complicated one, where the end consumers live far away from the food producers, the dictum becomes difficult to follow. Especially when food may no longer be retaining all of its nutrition by the time it reaches the table. The importance of fruits and vegetables in nutritious and healthy diets is well recognized, and in recent years consumers have been encouraged to eat more of these products.[1] The consumption of vegetables is highly relevant for a balanced diet as it leads to a healthier life style. In fact, their intake does not only reduce the development of disease, but also their fibers regulate the digestive functions of the human body.[2] Everybody is at risk for foodborne illness, but people who are younger than 5, older than 50, diabetic, take antibiotics or antacids, and whose immunity is compromised are at a higher risk.[3]

Bacteria are the most common food poisoning agents. More than 90% of the cases of food poisoning each year are caused by *Staphylococcus aureus, Salmonella species, Clostridium perfringens, Campylobacter species, Listeria monocytogenes, Vibrio parahaemolyticus, Bacillus cereus,* and *Escherichia coli*.[4-6] Foodborne illness outbreaks linked to fresh produce are becoming more frequent and widespread.[7] Verifying the presence of certain micro-organisms in vegetables and fruits is mandatory within the context of public health.[2]

Today, as our food supply becomes increasingly globalized, the need to strengthen food safety systems is becoming more and more evident. That is why the World Health Organization (WHO) aimed at promoting efforts to improve food safety, from farm to plate (and everywhere in between) on World Health Day, 7 April 2015.[8]

The objectives of the present study were to assess the microbial contamination of vegetables and fruits and compare the effect of disinfection techniques like washing under running water, soaking in lukewarm water, salt water and vinegar solution on the microbiological contamination of the fruits and vegetables.

MATERIALS AND METHODS

In this in-vitro study two types of vegetables (carrots and coriander leaves) and two types of fruits(apples and grapes) were collected from the main vegetable market for evaluation of the microbiological quality. Only those fruits and vegetables that are eaten raw and unpeeled were used.

Methodology:

The sample was divided into 5 groups:

Group 1*:* Microbial contamination evaluated without washing.

Group 2*:* Microbial contamination evaluated after washing and rubbing under running water for 2 minutes.

Group 3*:* Microbial contamination evaluated after soaking in lukewarm water (temperature 45^0C) for 2 minutes.

Group 4*:* Microbial contamination evaluated after soaking in 2.4% salt solution for 2 minutes. To prepare the salt solution 6 grams of salt was dissolved in 250 ml of water.

Group 5*:* Microbial contamination evaluated after soaking in 5% vinegar solution for 30 seconds.

The examiner first washed his/her hands for 20 seconds and only sterile containers were used for the study. The unwashed sample was swabbed and sent separately in a sterile bag to the laboratory for its microbial evaluation. All the disinfected samples were then collected separately after drying with sterile towels. Their outer surface were swabbed and sent to the laboratory in a sterile bag for evaluation of their microbiological contamination which was expressed in terms of colony forming units per ml (cfu/ml).

Microbiological examination:

Following the swabbing of the outer surface of the vegetables and fruits, the cotton swabs were incubated in peptone water for 2 hours at 37^0C for enrichment of the micro-organisms. They were then incubated in various culture media for 24 hours at 37^0C for the isolation of the microbes. The confirmatory tests were then done to identify the isolated micro-organisms from the surface of the fruits and vegetables examined.

Microbial cultures used: Nutrient agar, Blood agar, MacConkey agar, Deoxycholate citrate agar, Thiosulphate citrate bile salts and Sabouraud dextrose agar.

Confirmatory biochemical tests: IMVic (Indole, Methyl red, Vogesproskauer, Citrate), Triple Sugar Iron, Urease, Bile esculin hydrolysis, Catalase, Coagulase, Oxidase and Sugar fermentation tests.

Statistical Analysis

The results were analyzed using the Statistical Package for Social Science System (SPSS) Version 15.0. For comparison between the different disinfection methods ANOVA (Analysis of Variance) was employed. The significance level was set at $p<0.05$.

RESULTS

Vegetables were found to be significantly more contaminated than fruits ($p<0.05$). Among the vegetables coriander leaves were found to be more contaminated than the carrots ($p>0.05$) and among the fruits apples were significantly more contaminated than grapes ($p<0.05$) (Table 1). Various pathogens were isolated from the unwashed sample of the vegetables and fruits.*Escherichia coli, Klebsiella, Enterobacter, Salmonella and Shigella* were isolated from the vegetables whereas *Escherichia coli, Klebsiella* and *Enterococcus*, were isolated from fruits (Table 2). The isolated pathogens are commonly associated with diseases like bloody diarrhoea, respiratory and urinary tract infections and typhoid among others (Table 3).

Table 1: Microbial colony count of fruits and vegetables in terms of colony forming units per ml (cfu/ml)

Sample	Type of sample	Contamination (cfu/ml)	p-value	Mean contamination	p-value
Fruits	Apples	$2x10^5$	$p<0.05$	$1.0x10^5$	$p<0.05$ (Statistically significant)
	Grapes	$5x10^3$			
Vegetables	Carrots	$3x10^5$	$p>0.05$	$4.2x10^5$	
	Coriander leaves	$5.5x10^5$			

Table 2: Pathogens isolated from the fruits and vegetables

Sample	Type of sample	Pathogens isolated
Fruits	Apples	*Escherichia coli, Klebsiella* and *Enterococcus*
	Grapes	*Escherichia coli, Klebsiella* and *Enterococcus*
Vegetables	Carrots	*Escherichia coli, Klebsiella, Enterobacter, Salmonella and Shigella*
	Coriander leaves	*Escherichia coli, Klebsiella, Enterobacter, Salmonella and Shigella*

The effect of the disinfection technique were observed by comparing the microbiological contamination of the samples before and after its disinfection, expressed as colony forming units/ml. Maximum difference in colony forming units per ml before and after disinfection was observed when the fruits and vegetables were soaked in 5% vinegar for 30 seconds. Decrease was also noted with other disinfection methods. However, they were not observed uniformly in all samples evaluated. Moreover, the reduction observed in the colony forming units per ml among the vegetables, with different disinfection techniques was statistically significant ($p<0.05$) (Table 4 and Figure 1).

Table 3: The diseases caused by the pathogens isolated

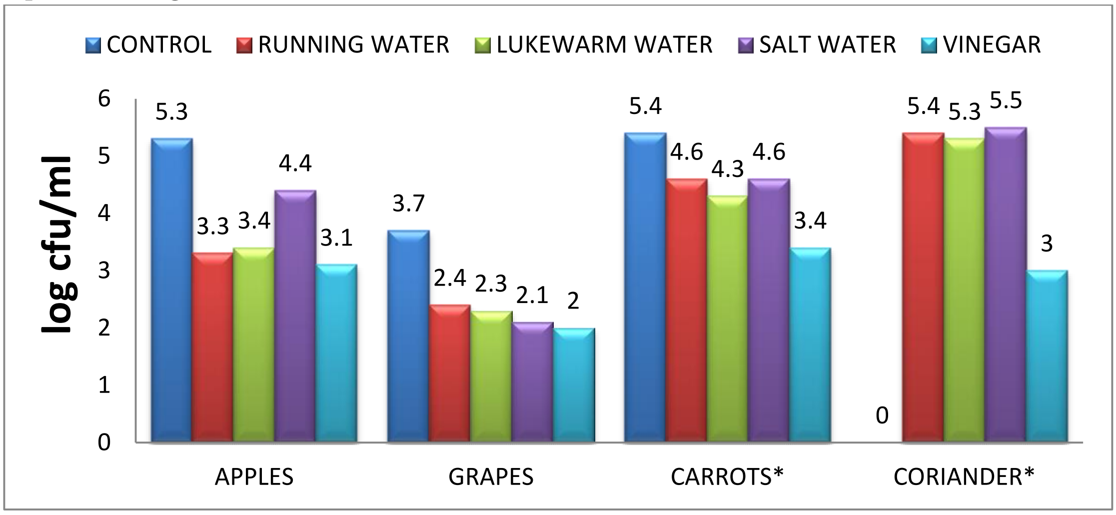
PATHOGEN ISOLATED	DISEASE
Escherichia coli	Bloody diarrhea, Urinary Tract Infections
Klebsiella	Pneumonia, Urinary Tract Infections, Respiratory Tract Infections
Enterococcus	Urinary Tract Infections, Endocarditis, Bacteremia
Enterobacter	Respiratory Tract Infections, Urinary Tract Infections, Skin Infections
Salmonella	Typhoid fever, Paratyphoid fever and Food poisoning
Shigella	Shigellosis

Table 4: Microbial colony count of the fruits and vegetables preceding and following different disinfection techniques

SAMPLE TYPE	DISINFECTION METHOD	CFU/ml	Log CFU/ml	P-value
APPLES	Control	$2x10^5$	5.3	p>0.05
	Running water	$2x10^3$	3.3	
	Lukewarm water	$2.5x10^3$	3.4	
	Salt solution	$2.7x10^4$	4.4	
	Vinegar solution	$1.2x10^3$	3.1	
GRAPES	Control	$5x10^3$	3.7	p>0.05
	Running water	$3x10^2$	2.4	
	Lukewarm water	$2.3x10^2$	2.3	
	Salt solution	$1.5x10^2$	2.1	
	Vinegar solution	$1.0x10^2$	2.0	
CARROTS	Control	$3x10^5$	5.4	p<0.01
	Running water	$4.5x10^4$	4.6	
	Lukewarm water	$2.0x10^4$	4.3	
	Salt solution	$4.2x10^4$	4.6	
	Vinegar solution	$2.5x10^3$	3.4	
CORIANDER LEAVES	Control	$5.5x10^5$	5.7	p<0.01
	Running water	$3x10^5$	5.4	
	Lukewarm water	$3.5x10^5$	5.3	
	Salt solution	$2.0x10^5$	5.5	
	Vinegar solution	$1.0x10^3$	3.0	

When considering the effect of disinfection methods on the various isolated pathogens,running water was found to be effective against *Escherichia coli* only in grapes but not in the other samples. Salt water was effective against *Escherichia coli* only for apples. Both running water and salt water were effective against *Klebsiella* in fruits but not vegetables. 5% vinegar was found to be effective against all the pathogens isolated excepting *Escherichia coli* in the grapes and *Salmonella* and *Shigella* in vegetables. This was followed by lukewarm water which was also effective against all the pathogens isolated except*Enterobacter* in the vegetables and *Salmonella* and *Shigella* in vegetables. None of the methods were effective against *Salmonella* and *Shigella* (Table 5 and Table 6).

Figure 1: Colony count of fruits and vegetables preceding and following different disinfection methods expressed as log cfu/ml



*Statistically highly significant (p<0.01)

Table 5:Effect of disinfection methods on various pathogens isolated from fruits

SAMPLE TYPE	TYPE OF DISINFECTION	Escherichia coli	Klebsiella	Enterococcus
APPLES	Running water	Present	Absent	Absent
	Lukewarm water	Absent	Absent	Absent
	Salt solution	Absent	Absent	Present
	Vinegar	Absent	Absent	Absent
GRAPES	Running water	Absent	Absent	Absent
	Lukewarm water	Absent	Absent	Absent
	Salt solution	Present	Absent	Present
	Vinegar	Present	Absent	Absent

Table 6:Effect of disinfection methods on various pathogens isolated from vegetables (carrots and coriander leaves)

TYPE OF DISINFECTION	Escherichia coli	Klebsiella	Enterobacter	Salmonella	Shigella
Running water	Present	Present	Present	Present	Present
Lukewarm water	Absent	Absent	Present	Present	Present
Salt water	Present	Present	Absent	Present	Present
Vinegar	Absent	Absent	Absent	Present	Present

DISCUSSION

The current study was conducted to ascertain whether raw vegetables and fruits are safe from pathogenic organisms for human consumption. The results obtained in this study testified that the samples were contaminated with pathogenic organisms. It was further observed that vegetables were significantly ($p<0.05$) more contaminated than the fruits.

Pathogens isolated in this study were *Escherichia coli*, *Klebsiella*, *Enterobacter*, *Enterococcus, Salmonella* and *Shigella* which was similar to a study conducted by Mohammad Khiyami et al (2011)[5] in Riyadh where *Escherichia coli, Salmonella* and *Shigella* were isolated from vegetables. Contamination of vegetables with *Pseudomonas spp., Enterobacteriaceae, yeast* and*moulds*was also seen in a study conducted by S.C. Stringer et al(2007).[9] Similar pathogens from vegetables were isolated from other studies done by Obieze K. O et al(2010)[10] where *Escherichia coli* and *Enterobacter* were isolated and by Hoonmo L. Koo et al (2008)[11] where *Enterobacter* and *Klebsiella* were isolated.

On evaluating the colony forming units per ml, soaking in 5%vinegar for 30 seconds was found to be the most effective method for disinfection of raw fruits and vegetables. Ana Beatriz Almeida de Oliveira et al (2012)[12] conducted a study where washing in potable water followed by dipping in 20% vinegar for 15 minutes was found to be the most effective disinfection method. Whereas, P. Amoah et al (2007)[13] found washing under running water for 2 minutes to be most effective method for disinfection of vegetables. Shigeharu Oie et al(2008)[14] conducted a study where soaking in 0.01% sodium hypochlorite for 10 minutes was proved to be the most effective disinfection method for fruits and vegetables. Other effective methods of disinfection include heat treatment at 52^{O}C and heat shock treatment for 90 seconds as observed in studies by S.C. Stringer et al (2007)[9] and Sara Beirao-Da-Costa1 et al (2014).[15]

Factors that may contribute to real increases in diseases associated with fruits and vegetables include, use of wastewater, increased application of improperly composted manures to soils in which fruits and vegetables are grown, changes in packaging technology such as the use of modified or controlled atmosphere and vacuum packaging, extended time between harvesting and consumption, and changing food consumption patterns (e.g. eating more meals away from home, including greater use of salad bars). Increased global trade in raw fruits and vegetables, as well as increased international travel in general, could also increase the risk of produce-associated diseases. Finally, the susceptibility of the public to foodborne diseases, at least in more developed countries, is changing due to increased numbers of people who are elderly, immune-compromised or have chronic diseases. This change in social demographics is likely to lead to increased risk of illness associated with the consumption of raw produce that otherwise may contain levels of pathogens innocuous to healthy individuals.[16]

The use of properly composted manure and properly treated irrigation and spray waters, as well as pathogen-free water for washing, will minimize the risk of contamination of fruits and vegetables with microbial pathogens. Good hygienic practice during production and transport, including sanitizing of harvesting equipment and transport vehicles, as well as the application of good hygienic practice during processing and preparation are critical. The microorganisms normally present on the surface of raw fruits and vegetables may consist of chance contaminants from the soil or dust, or bacteria or fungi that have grown and colonized by utilizing nutrients exuded from plant tissues. Microorganisms capable of causing human disease can, however, be found on raw produce, and should be viewed as a threat to public health.[16]

The present study had many limitations. Only two types of fruits and vegetables from a single source were considered for microbiological evaluation for feasibility reasons. More variety of vegetables and fruits need to be considered before extrapolating the results of the present study.Though 5% vinegar solution was proved to be an effective method for disinfection, whether the nutritive value of the food is compromised is yet to be considered. Hence, further research needs to be carried out to analyze if the disinfection techniques reduce the nutritive value of the food.Moreover only four disinfection methods were evaluated and none of them were effective against *Salmonella* and *Shigella.* As typhoid and intestinal infections are prevalent among the population, it is necessary to develop other feasible disinfection techniques which will have an effect on *Salmonella* and *Shigella*. Further research in the above mentioned areas will open new vistas in food safety.

CONCLUSION

Surface contamination of both fruits and vegetables was observed and soaking in 5% vinegar for 30 seconds was the most effective method for their disinfection followed by soaking in lukewarm water for 2 minutes. Prevention of contamination is the most efficient way to ensure food safety and prevent foodborne illness. Thus, every effort should be made to protect food from primary sources of contamination.

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How to cite this article: Mahapatra S, Chaly PE, Smiline Girija AS. Effectiveness of Various Disinfection Techniques on Vegetables and Fruits: An In-Vitro Study. Arch of Dent and Med Res 2015;1(3):1-8.