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Antibacterial Agents' Effects on the Bacterial Flora of Fruits Collected from Peshawar City of Khyber Pakhtunkhwa-Pakistan

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Abstract : *The aim of the current study was to investigate the viable plate count, coliform and fecal coliform bacteria on fresh fruits washed with water, and utilization of antimicrobial agents (H_2O_2, ethanol and citric acid). Pour plate count method was employed for the total plate count (TPC), while multiple tube fermentation techniques were used for coliform bacteria determination. Among all the fruit samples washed with water, the highest value of TPC was observed in apples (15000 CFU/g) and the lowest in pears (2000 CFU/g). The presence of Coliform and fecal Coliform bacteria were found <3 in persimmons, grapes and pears washed with water and treated with H_2O_2, ethanol and citric acid. The findings concluded that the bacterial contamination in indigenous wholesale places was incredibly the highest, and hydrogen per oxide could be utilized as a washing source to get the better quality of fresh fruits. The increase in health awareness has led to consuming proper wash –down, or specifically proper treatments of fruits to reduce the bacterial contamination and health problems.*

Keywords: *Fruits, Disease, Coliform bacteria, Fecal Coliform bacteria, Antimicrobial agent*

INTRODUCTION

Food quality is significantly influenced by biochemical, microbiological, chemical and physical processes. Microorganisms case many types of spoilage, which cause quality deterioration leading to limit the shelf life, be undesirable commercially, and raise quality complaints. The growth or presence of foodborne germs is recognized as the most dangerous shape of quality deterioration, because it is seriously related to the costumer health (ICMSF, 1996). Although technological developments and scientific progresses have been achieved in current years, food security issues still exist and may boost in the future (Mead et al., 1999). Gastro-intestinal viruses, Shigella and Salmonella are the main concerning germs related to fresh fruits. Similarly, the human being is the chief source of Streptococcus, Escherichia coli and Shigella, while yeast, Bacillus, Clostridium and Staphylococcus come from the environment (Annon, 1975).

Microbiologically safe vegetables and fruits are necessary, because they accelerate the health benefits when consumed by people. Proper washing of fresh products is essential for the decontamination. The aim of this study was to get baseline data on microbial load of fruits at the market level, and finally apply the antibacterial substances to minimize the germs' load as a preventive measure.

Material and Methods

Sample collection and preparation

One Kg of each fruit samples of apple, guava, pear, grapes and persimmon were collected from the local market. Each fruit sample was kept in sterilized plastic bags and transferred into the laboratory in cold ice boxes.

Microbial counts were determined after imposing treatments. (i) Washing with ordinary tap water and (ii) wash treatments as follow:

Wash treatments

Each sample was washed with 5% concentration treatment solution of H_2O_2, citric acid and ethanol. Three liters of tap water at 30ºC were used for the preparation of each wash. Immediately before that, the treating solution was prepared and tested within 01 hours. Each mesh bag was dipped into one sanitizer solution for ten minutes. In Laminar Air Flow Chamber, the washing treatment processes were experimented. After washing the total plate count, coliform bacteria and fecal coliform bacteria were determined according to the following procedure.

Total Plate count (TPC)

Pour plate method was employed to determine the total plate counts of all the fruit samples (Andrews, 1992). A series of dilutions of the fruit samples was prepared by blending 50 gm of fruit with 450 mL of Better field's phosphate buffer (BPB) for 2 min at 10000 - 12000 rpm which results 10^{-1} dilution, further dilutions (10^{-2} to 10^{-8}) were made through shifting one milliliter of the preceding dilution into nine milliliters of BPB. 01 mL of each dilution (10^{-1} to 10^{-8}) was inoculated into the plate count agar. The inoculated plates were kept for 48±2 hrs at 35 ±2 ºC in incubator. At the end of the incubation, the colonies were counted by a colony counter, and recorded as CFU/gm.

Total Coliform Bacteria

The total Coliform Bacteria was determined by MPN Method using multiple fermentation technique. 50 gm of fruit sample were blended in 450 ml of Better field's phosphate buffer for 2 min at 10000 -12000 rpm resulting 10^{-1} dilution. Other dilutions (10^{-2} and 10^{-3}) were prepared by transferring1mlof the previous dilution to 9 ml blank BPB. 1ml of all the dilutions was inoculated into a set of 3 Lactose Broth tubes, and incubated at 35 ±2 ºC for 48±2 hrs. They were examined for gas presence after 24 hrs, if negative, they were re-incubated for additional 24 hrs. The positive LT tubes were sub cultured on Brilliant Green Bile Broth for 48 hrs at 35 ºC. After that, the incubations were examined for gas production and the No of positive tubes for MPN calculation were recorded (Andrews, 1992).

Fecal Coliform

The positive Lactose Broth tubes were inoculated into EC broth for sub culturing. The EC broth tubes were incubated at 45.5 ºC for 48±2 hrs. The MPN of fecal coliform from the proportion of the confirmed turbidity/gas EC broth tubes was calculated (Andrews, 1992).

Results and Discussion

The effects of antibacterial agents on bacterial flora of fruits are shown in table 1. The results indicated that the fruits washed with water have more TPC as compared to the treatments with H_2O_2, ethanol and citric acid. The overall results showed that H_2O_2 is the most potent antibacterial fruit rinsing agent as compared with the ethanol and citric acid.

A study (Shalini & Swati, 2014) showed the lowest microbial load in pears (4.18 log/g) and the highest microbial load in papayas (4.76 log/g). Salmonella sp. could not be isolated from any of the samples involved in this research, although the numbers of coliforms and E. coli were above the legal limits (Anonymous, 2001). The most effective (1%) antimicrobial agent was recorded for citric acid (Shalini and Swati, 2014). The report of Perchonok and French (2005) indicated that 0.5% of food grade Hydrogen peroxide dip was effective in preventing decay, because it strengthens the walls of fruits and vegetables thereby preventing the invasion of

the cell walls by disease causing spores. This inhibitory activity of Hydrogen peroxide then prevents decay, weight loss, and also maintains firmness.

The existence of microbes in vegetables and fruits was blamed on harvesting, cultivation, water quality, storage, processing and transportation of the products (Beuchat, 1996). Microbes go to the intercellular places with the help of physical openings like broken trichomes, lenticels, stomata and stem scars and might persevere in the tissue of healthy plant (Bartz and Wei, 2003). Generally, due to the poor hygienic conditions of vendors and growers, the Coliform bacteria was found.

The mechanisms involved the action approach of sorbic, benzoic, tartaric, malic, succinic, citric and acetic acids which caused the direct lowering of pH, the distribution of substrate transports through cell membrane's permeability alteration, or changing the interior acidity (pH) of the microorganisms' cells through ionization of the un-dissociated acid compounds (Beuchat, 1998).

Conclusion

Although many fruit samples were recorded unhygienic, so it was essential to find a cost effective and easily available antibacterial agent. In this study, citric acid, ethanol, H_2O_2 and water were used as antibacterial dips. Generally, the first three (citric acid, ethanol and H_2O_2) dips were observed effective; however, Hydrogen peroxide was the primary active. So, it could be utilized as a feasible efficient antibiotic agent in a budding country like Pakistan.

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Table1. Effects of Antibacterial Agents on Bacterial Flora of Fruits

Fruits	Parameters	Treatment			
		W	H	E	C
Apple	TPC (CFU/g)	15000	800	1500	1000
	Coliform Bacteria (MPN/g)	22	<3	21	15
	Faecal Coliform Bacteria (MPN/g)	22	<3	11	9

Guava	Parameters	W	H	E	C
	TPC (CFU/g)	5000	400	750	500
	Coliform Bacteria (MPN/g)	>1100	<3	39	23
	Faecal Coliform Bacteria (MPN/g)	1100	<3	<3	<3
Persimmon	Parameters	W	H	E	C
	TPC (CFU/g)	9000	600	800	700
	Coliform Bacteria (MPN/g)	<3	<3	<3	<3
	Faecal Coliform Bacteria (MPN/g)	<3	<3	<3	<3
Grapes	Parameters	W	H	E	C
	TPC (CFU/g)	9000	450	900	480
	Coliform Bacteria (MPN/g)	<3	<3	<3	<3
	Faecal Coliform Bacteria (MPN/g)	<3	<3	<3	<3
Pear	Parameters	W	H	E	C
	TPC (CFU/g)	2000	800	1000	950
	Coliform Bacteria (MPN/g)	<3	<3	<3	<3
	Faecal Coliform Bacteria (MPN/g)	<3	<3	<3	<3

Legend: W=Water wash, H=H_2O_2, E=Ethanol, C=Citric acid, MPN= most probable number, CFU= colony forming unit