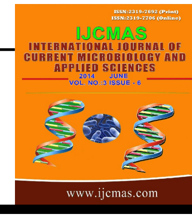

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Original Research Article

Use of Antimicrobial Dips for the reduction of surface microbial load of fresh fruits sold in South Delhi Markets, India

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Keywords

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A B S T R A C T

Fresh fruit and vegetables are important components of a healthy and balanced diet; their consumption is encouraged in many countries by government health agencies to protect against a range of illnesses such as cancers and cardiovascular diseases. Fruits and vegetables are widely exposed to microbial contamination through contact with soil, dust and water and by handling at harvest or during postharvest processing. They therefore harbour a diverse range of microorganisms including plant and human pathogens. In the present study, 50 samples of fresh fruits were collected from South Delhi, both local and retail markets. Then, the total microbial load, yeasts and molds on their surface were estimated. Presence of coliforms and Escherichia coli was also detected. Majority of samples were found to be contaminated and the highest microbial load was found in papaya (4.76 log counts per gram) and lowest in pears (4.18 log count per gram). The bacterial counts were found to be higher than the Yeast and Molds counts. Then, to eliminate the surface microbial load, various types of antimicrobial dips in varying concentration were used. Among, all the three types of antimicrobial dips, the most effective was found to be citric acid at 1% concentration.

Introduction

Fruits and vegetables are exceptional dietary source of nutrients, micronutrients, vitamins and fibre for human beings hence vital for health and fitness. Well balanced foods, rich in fruits and vegetables, are especially valuable for their ability to prevent deficiencies of vitamin C and vitamin A and also reduce the risk of several diseases (Kalia and Gupta, 2006).

These fruits and vegetables promote good health; they harbor a wide range of microbial contaminants, thus undermining their nutritional and health benefits, thereby increasing outbreaks of human infections associated with the consumption of fresh (or minimally processed) fruits and vegetables (Hedberg et al; 1994; Altekruse and Swerdlow, 1996; Beuchat, 1996, Beuchat, 1998, Beuchat, 2002).

Bacteria, viruses and parasites on fruits and vegetables have been linked with illness. Several cases of an outbreak of typhoid fever has been associated with eating contaminated vegetables grown in/or fertilized with contaminated soil or sewage (Beuchat, 1998). These outbreaks differ from a few persons to many thousands affected (SCF, 2002). Olsen et al., (2000) reported that diseases associated with the consumption of fruits and vegetables doubled between 1973 – 1987 and 1988 – 1992. In Canada, 18 outbreaks were documented from 1981 to 2000, with approximately 2000 people affected and 18 deaths (Sewell and Farber, 2001). In developing countries such as Nigeria, continued use of untreated waste water and manure as fertilizers for the production of fruits and vegetables is a major contributing factor for the outbreak of diseases (Olayemi, 1997; Amoah et al., 2009).

Fruits and vegetables vendors generally do not follow good hygienic practices. There is no adequate storage conditions as fruits and vegetables are sold in trays, wheel barrows or on tables by vendors. Vendors who do not maintain personal hygiene can carry microorganism on their skin, hair, hands or cloths and may unintentionally contaminate fresh fruits or vegetables and create the opportunity to transmit food-borne illness. It therefore implies that to maximize all health benefits from adequate consumption of fruits and vegetables, handlers (vendors) should be free from microbial contamination. To this end, Microbiological risk assessment is an emerging tool for the evaluation of the safety of food and water supplies. Different organizations have suggested that microbiological risk assessment should be carried out so that appropriate remedial measures can be adopted to curtail the episodes of food-borne illness as a result of consumption of contaminated foods. Microbes, mainly the coliforms group has been used extensively as an indicator of the main indicators of microbiological quality of water and food.

Their presence indicates improper treatment or post-disinfection contamination. There may be significant differences in the microbiological quality of food products at the market level sold in different socioeconomic areas. Hence, a qualitative survey of two common market foods based on microbiological criteria was envisaged.

Bacteriologically safe fruits and vegetables are essential to maximize the health benefits promised by adequate consumption of these produce. Proper washing of fruits and vegetables is essential for decontamination.

The aim of this study was to get baseline data on microbial load of fruits at market level, and to compare the microbial load of fruits between local and retail market. The present paper deals with the enumeration of the microbial quality of fresh fruits and the use of anti-microbial agents to reduce the microbial population as a preventive measure.

Materials and Methods

Locale of the study

Collection of samples from Local or Retail market

South Delhi Local market (LM)

Retail market (RM)

Sample collection and Preparation

The following fruits were selected for this study: Apple, grapes, sapota, papaya and pears. All samples used for analysis was obtained from local and retail market. The microbiological quality of approximately 50 samples was determined during a 6 months study period. Apart from this, samples were also used for studying the efficacy of various antimicrobial dips. These samples were randomly collected aseptically in a sterilized container maintained in cold conditions, delivered to laboratory immediately and analyzed. All the samples were used for detection of Aerobic Plate Count (APC), Total Coliform, *E.coli* and Yeast and Mold (YMC). The elapsed time between the sample collection and the analysis did not exceed 3 hours.

Sample preparation

25 g of sub sample was randomly taken, aseptically weighed and blended with 225 ml of Peptone water (Himedia, RM001) using Bag mixer (Interscience) for 2 min.

Microbiological analysis

The homogenate from sample preparation were serially diluted using a Gravimetric Dilutor (Interscience) for the following procedures: Total Plate Count, Total Coliform counts, Yeast and Mold counts and *E.coli* detection.

Aerobic Plate Count (APC)

Aerobic plate count of all fruit samples were determined by Plate count as described by APHA using Plate count agar medium (Himedia, M091). Serial dilutions were made. 1ml of the samples from 10^{-2} dilution was inoculated into Plate count agar by pour plate technique in duplicates. The samples were mixed by rotating, and then the pour plates were inverted and incubated at 37^0C for 24 hrs. After incubation, colonies were counted and results were recorded as cfu/gram of the sample

Coliform count

Total Coliform count of all fruits samples were determined by direct plate count as described in standard (APHA) using Violet Red bile Agar (Himedia, M049). 1ml of the samples made in peptone water was inoculated into Violet red bile agar by pour plate technique in duplicates. The samples were mixed by rotating, and then the pour plates were inverted and incubated at 37^0C for 24-48 hrs. After incubation, colonies were counted and results were recorded as cfu/gram of the sample.

Yeast and Mold count (YMC)

The Yeast and Mold count of all fruits samples were determined by Direct Plate Count as in APHA using Potato Dextrose Agar, (Himedia, M096). 0.1 ml of the samples serially diluted in sterile peptone water; was plated on Potato Dextrose Agar medium in duplicates by spread technique. The plates were incubated at 21^0C for 5-7 days. After incubation, colonies were counted and results were recorded as cfu/gram of the sample.

Escherichia coli identification

E. coli were determined by Tube Test method. Aliquots of stock solution (1 ml of the samples made in peptone water) were taken in 9 ml of Brilliant Green Lactose Bile Broth (Himedia M121S). Then broth is incubated at 44.5^0C for 24 hrs; tubes were then observed for acid and gas production. Then for confirmation test, Streak 1 loopful from the positive tubes on plated Eosin Methylene Blue Agar (Himedia M317). The plates were incubated at $37^{o}C$ for 24hrs. After incubation, dark centered colonies with green metallic sheen are subjected to further biochemical test

Treatment with Antimicrobial Dips

Treatment solutions

Samples were treated with three different Antimicrobial dips- 200ppm Chlorine solution (using Suma tab from Diversey (4 tablets in 15 litre water), 1% citric acid solution (RM1023) and 1% Benzoic acid solution (RM1326). All solutions are prepared in sterile distilled water

Treatment of samples

Samples were immersed in respective antimicrobial solutions for 5min and vortexed for 5 minutes. 25g of treated subsamples were aseptically weighed and diluted in 225ml Peptone water and analyzed for their APC, Total Coliform, Yeast and Mold and *E.coli* as per APHA standards. Observations of treated samples were compared with that of APC of their initial counts.

Statistical Analysis

SPSS16 and Variance analysis methods (ANOVA) were used in interpretation of analysis results. T-Test was used in the evaluation of the significance of the difference between the groups. The significance between the values was evaluated at 95% confidence $P < 0.05$.

Results and Discussion

In the present study, the microbiological quality of 50 samples was analyzed from South Delhi local market (LM) and retail market (RM).

Microbiological Analysis

All the samples tested were found to be contaminated although the level of contamination varied. The level of contamination was found to be highest in papaya and lowest in case of pears (refer Table 1).

Apple

In this study, apple samples (n=10) were collected and analyzed for Aerobic Plate Count (APC), Yeast and Molds (YMC), Total Coliform and *E.coli*. The APC ranged from 4.16 to 4.75 log cfu/gram (refer Table 1 and Fig 1). The samples were also analyzed for its Yeast and Mold counts which ranged from Nil to 1.48 log cfu/gram (refer Table 3 and Fig 3). The coliform load range from 1.00 to 2.38 log cfu/gram (refer Table 2 and Fig 2). Out of 10 samples, 7 samples are positive for *E.coli* (refer Fig 6)

Sapota

Sapota fruit deteriorates mildly after harvest due to stress factors such as extreme temperatures or changes in relative humidity. These climatic factors alter both physical and physiological characteristics of the fruits, thus predispose them to spoilage by microbes. In this study, the microbiological analysis of sapota (n=10) was carried out. The samples were contaminated with APC ranged from 4.63 to 4.84 log cfu/gram (refer Table 1 and Fig 1); Yeast and Mold counts which ranged from 1.6 to 2.28 log cfu/gram (refer Table 3 and Fig 3). The coliform load range from 1.30 to 2.41 log cfu/gram (refer Table 2 and Fig 2). All 10 samples are found positive for *E.coli* (refer Fig 6).

Grapes

Microbiological analysis of grapes (n=10) was carried out. The APC ranged from 4.61 to 4.82 log cfu/gram (refer Table 1 and Fig 1). The samples were also analyzed for its Yeast and Mold counts which ranged from 1.00 to 1.48 log cfu/gram (refer Table 3 and Fig 3). The coliform load range from 1.30 to 1.78 log cfu/gram (refer Table 2 and Fig 2). Out of 10 samples, 8 samples are positive for *E.coli* (refer Fig 6).

Papaya

Ten samples were collected from different locations and their microbiological status was determined. The APC ranged from 4.66 to 4.86 log cfu/gram (refer Table I and Fig 1). The Yeast and Mold counts ranged from 1.00 to 1.85 log cfu/gram (refer Table III and Fig 3). The coliform load range from 2.61 to 2.89 log cfu/gram (refer Table II and Fig 2). Out of 10 samples, 9 samples are positive for *E.coli* (refer Fig 6).

Pear

In this study, pear samples (n=10) were collected and analyzed. The APC ranged from 4.00 to 4.4 log cfu/gram (refer Table 1 and Fig 1). The samples were also analyzed for its Yeast and Mold counts which ranged from Nil to 1.48 log cfu/gram (refer Table 3 and Fig 3). The coliform load range from 1.00 to 1.78 log cfu/gram (refer Table 2 and Fig 2). Out of 10 samples, 8 samples are positive for *E.coli* (refer Fig 6)

Effect of Antimicrobial dips

All the anti-microbial agents showed maximum reduction. In this study, the efficacy of three antimicrobial dips – Chlorine, citric acid and benzoic acid at 1% concentration for five minutes; was determined and their suitability and cost effectiveness was analyzed to reduce the microbial load in the fresh produce. Ten samples were taken and assessed for the antimicrobial effect of different sanitizing treatments. All the three antimicrobial dips were found to be equally effective. Citric acid and benzoic acid also reduced the microbial load to considerable levels (refer Table 4, 5 and Fig 4, 5).

The initial APC load on apple sample was found to be 4.38 log cfu/g and it was reduced to 3.97 with chlorine, 4.01 with Citric acid and 4.01 with benzoic acid (refer Table 4 and Fig 4). For sapota, initial APC load was 4.74log /g. It was reduced to 3.96 log /g with Chlorine, 4.03 with citric acid and 4.04 using benzoic acid (refer Table 4 and Fig 4). Initially, the APC load on grapes was found to be 4.71 log /g, Use of chlorine, citric acid and benzoic acid resulted in microbial load reductions to 2.73, 3.01 and 3.01 log /g respectively (refer Table 4 and Fig 4). In case of pears, initially the APC load was 4.18 log /g which was effectively reduced by using Chlorine to 2.99 log /g, citric acid to 3.16 log /g and benzoic acid to 3.2 log /g (refer Table 4 and Fig 4). Initially, the APC load on papaya was found to be 4.76 log cfu /g. Use of chlorine, citric acid and benzoic acid resulted in microbial load reductions to 4.04, 4.09 and 4.09 log /g respectively (refer Table 4 and Fig 4).

The initial Coliform load on apple sample was found to be 1.56 log cfu/g which was effectively reduced to nil log cfu/g by using chlorine; citric acid and benzoic acid (refer Table 5 and Fig 5).

Table.1 Aerobic Plate Counts (log cfu/g) of Fruit samples of local and retail market

Location code	Apple	Grapes	Sapota	Papaya	Pears
LM1	4.75	4.63	4.75	4.81	4.40
LM2	4.29	4.82	4.84	4.74	4.29
LM3	4.45	4.77	4.79	4.73	4.26
LM4	4.62	4.84	4.78	4.84	4.08
LM5	4.30	4.79	4.82	4.86	4.00
RM1	4.39	4.74	4.64	4.66	4.16
RM2	4.16	4.56	4.71	4.71	4.15
RM3	4.19	4.59	4.67	4.72	4.16
RM4	4.29	4.61	4.68	4.73	4.16
RM5	4.61	4.73	4.63	4.71	4.05

**(LM1, LM2, LM3, LM4, LM5, RM1, RM2, RM3, RM4, RM5, RM6 indicates sample numbers from Local and retail market respectively)*

Table.2 Total Coliform Counts (log cfu/g) of Fruit samples of local and retail market

Location code	Apple	Grapes	Sapota	Papaya	Pears
LM1	1.60	1.70	1.30	2.87	1.00
LM2	1.95	1.78	2.11	2.89	1.78
LM3	1.30	1.60	2.18	2.86	1.48
LM4	1.00	1.70	2.41	2.83	1.00
LM5	1.60	1.60	2.28	2.79	1.48
RM1	2.38	1.48	2.18	2.77	1.60
RM2	1.30	1.70	2.04	2.68	1.60
RM3	1.30	1.60	1.85	2.61	1.48
RM4	1.60	1.30	2.00	2.84	1.00
RM5	1.30	1.48	1.70	2.75	1.30

**(LM1, LM2, LM3, LM4, LM5, RM1, RM2, RM3, RM4, RM5, RM6 indicates sample numbers from Local and retail market respectively)*

Table.3 Yeast and Mold Counts (log cfu/g) of Fruit samples of local and retail market

Location code	Apple	Grapes	Sapota	Papaya	Pears
LM1	0	1.30	2.28	1.48	0
LM2	1.48	1.00	2.15	1.70	1.48
LM3	0	1.48	2.23	1.60	0
LM4	1.48	1.00	1.95	1.48	0
LM5	0	1.00	1.95	1.70	0
RM1	0	1.30	2.04	1.00	0
RM2	0	1.48	1.85	1.60	0
RM3	0	1.48	1.78	1.30	0
RM4	0	1.00	1.60	1.85	0
RM5	0	1.30	1.90	1.30	0

**(LM1, LM2, LM3, LM4, LM5, RM1, RM2, RM3, RM4, RM5, RM6 indicates sample numbers from Local and retail market respectively)*

Table.4 Antimicrobial effect of different sanitizing treatments on Aerobic plate count of fruit samples

S.No	Average APC microbial load (log cfu/gram)				
	Sample	**initial count**	**chlorine**	**citric acid**	**benzoic acid**
1	Apple	4.38	3.97	4.01	4.01
2	grapes	4.71	2.73	3.01	3.01
3	Sapota	4.74	3.96	4.03	4.04
4	Papaya	4.76	4.04	4.09	4.09
5	Pear	4.18	2.99	3.16	3.2

Table.5 Antimicrobial effect of different sanitizing treatments on Coliform count of fruit samples

S No	Average Coliform microbial load (log cfu/gram)				
	Sample	**initial count**	**chlorine**	**citric acid**	**benzoic acid**
1	Apple	1.56	Nil	Nil	Nil
2	grapes	1.61	Nil	Nil	Nil
3	Sapota	2.04	1	1.15	1.15
4	Papaya	2.79	1.24	1.24	1.39
5	Pear	1.38	Nil	Nil	Nil

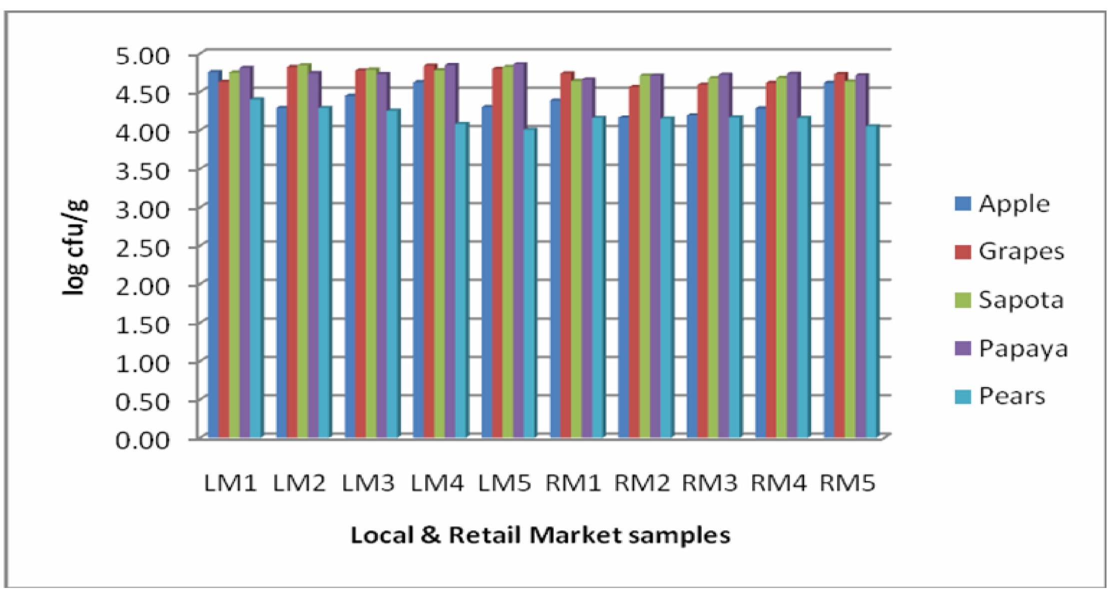

Fig.1 Aerobic plate count of local and retail fruit sample
(LM1, LM2, LM3, LM4, LM5, RM1, RM2, RM3, RM4, RM5, RM6 indicates sample numbers from Local and retail market respectively)

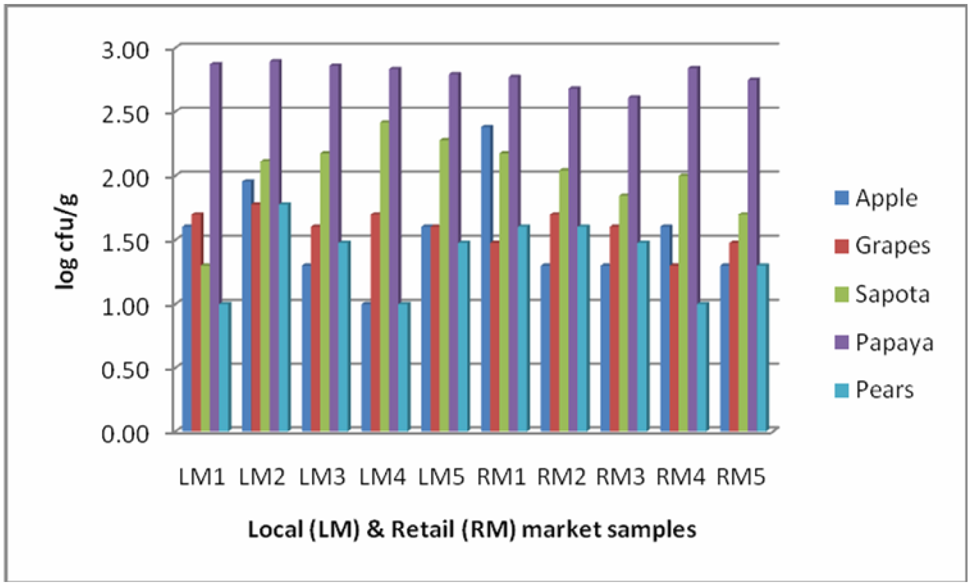

Fig.2 Total coliform count of local and retail fruit sample
(LM1, LM2, LM3, LM4, LM5, RM1, RM2, RM3, RM4, RM5, RM6 indicates sample numbers from Local and retail market respectively)

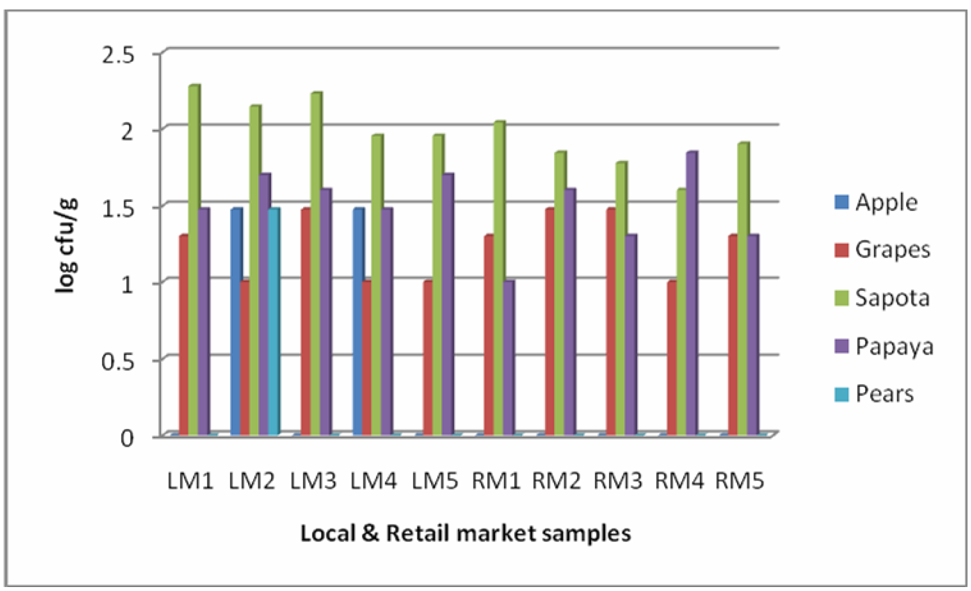

Fig.3 Total Yeast and Mold count of local and retail fruit sample
(LM1, LM2, LM3, LM4, LM5, RM1, RM2, RM3, RM4, RM5, RM6 indicates sample numbers from Local and retail market respectively)

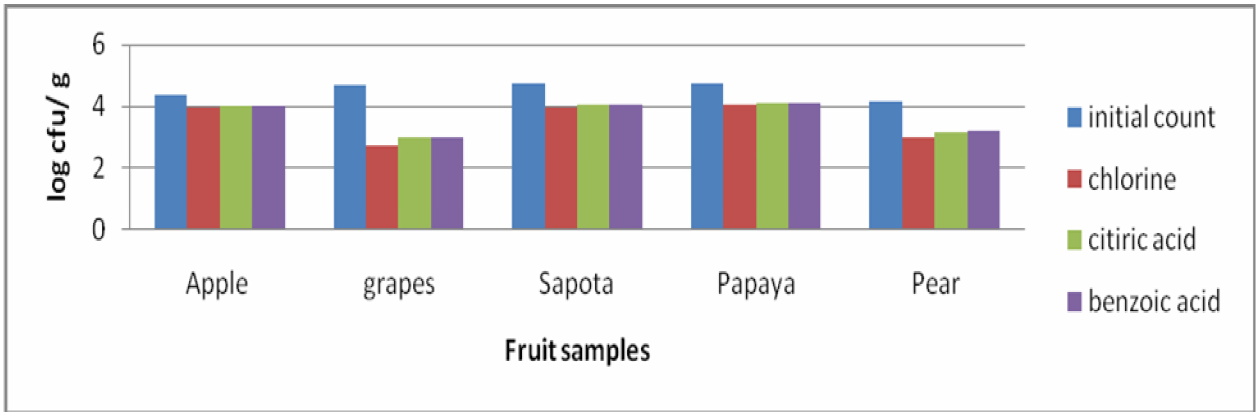

Fig.4 Effect of different antimicrobial dips on the average APC load (mean) of fruit samples

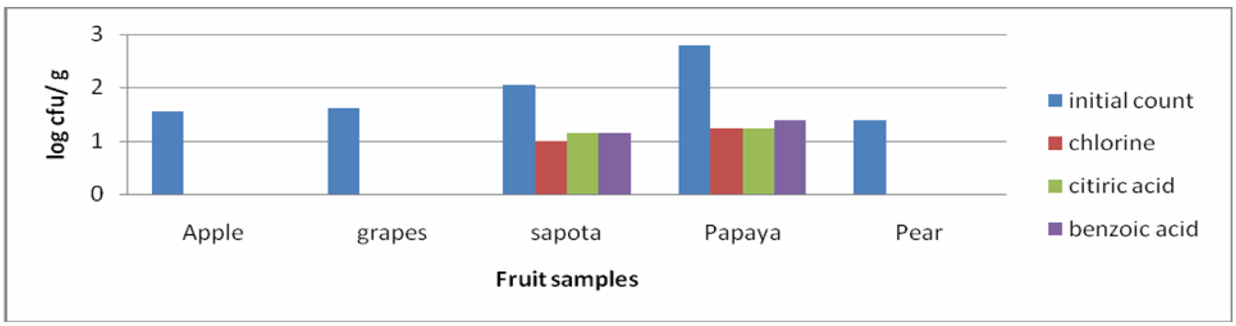

Fig.5 Effect of different antimicrobial dips on the average Coliform load (mean) of fruit samples

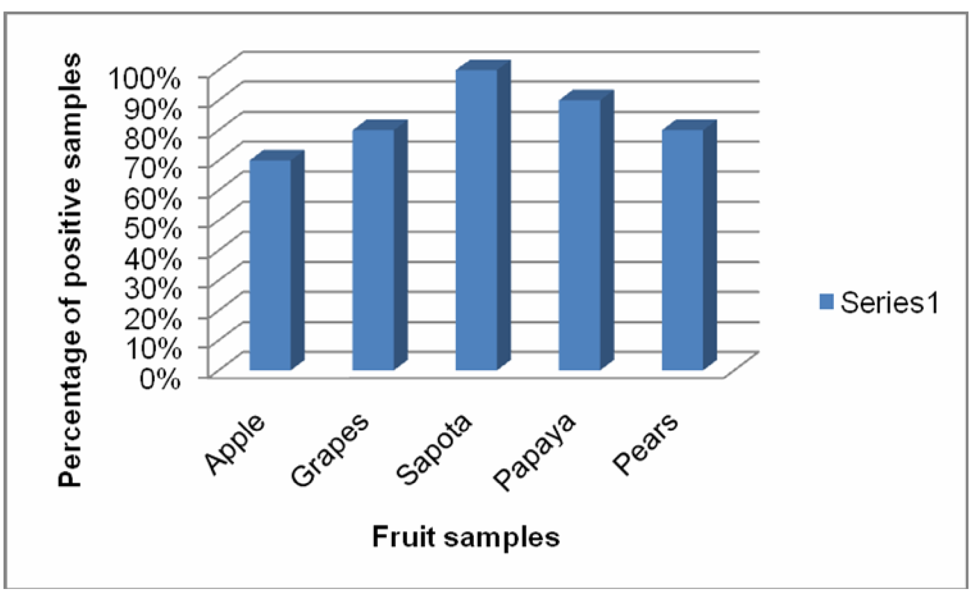

Fig.6 Percentage of positive samples of *E.coli* in fruits

Initially, the Coliform load on sapota was found to be 2.04 log /g (refer Table 5 and Fig 5), Use of chlorine, citric acid and benzoic acid resulted in microbial load reductions to 1.00, 1.15 and 1.15 log /g respectively (refer Table 5 and Fig 5). In case of papaya, initially the Coliform load was 2.79 log /g which was effectively reduced to 1.24 log /g with Chlorine, 1.24 with citric acid and 1.39 using benzoic acid (refer Table 5 and Fig 5). In case of grapes and Pears initial Coliform load were found to be 1.61 and 1.38 log cfu/g and it was reduced to Nil by using all three antimicrobial dips (refer Table 5 and Fig 5).

The initial load of Yeast and Mold was found 1.48, 1.98, 1.23, 1.52 and 1.48 log cfu/g in case of apple, sapota, grapes, papaya and pear respectively. Use of chlorine, citric acid and benzoic acid resulted in microbial load reductions to Nil. All the samples were initially positive for *E.coli* (refer Fig 6) and reduction was seen in *E.coli* load after using all three antimicrobial dips as found negative in all the samples.

Microbiological control is very important in food industry to prevent food poisoning and other health hazards. . In developing countries, continued use of untreated waste water and manure as fertilizers for the production of fruits and vegetables is a major contributing factor to contamination (Sehgal, S. 2013).In the present study, the microbial status of various fruit samples was assessed. All the samples studied were found to be contaminated. The highest microbial load was found in case of Papaya followed by sapota, grapes, apple and then pears, which can be attributed to the various internal and external sources of contamination .The usual trend is that the fruits growing closer or within the soil are more contaminated. Similar trend was observed in this study too. Beside soil microbes, other sources of their contamination are improper handling, unhygienic storage and transportation conditions. Coliforms were present on majority of samples. Yeasts and molds are the natural micro flora of the fruits and vegetables and hence they were detected in majority of the samples. In case of apples, it can be due to the wax coating on their surface, which makes it impermeable to water. Presence of *E.coli* indicates poor water quality, untreated manure for the production of these crops. Fruits may be contaminated with pathogenic microorganisms during growing in the field or during harvesting, post harvesting, handling, processing and distribution. Therefore, vegetables may act as a reservoir for many microorganisms from which they will be colonized inside these vegetables and infect susceptible host.

Our study involved both unorganized and organized sector – local market and retail market. The level of contamination was highest in the local market where the fresh produce comes directly from the farmers / cooperatives or farmer societies. The trend also indicated that the local produce within Delhi was contaminated, only the retail samples were found to be of good quality as the fresh produce is usually dipped in the chlorine solution (200ppm) prior to packaging and distribution as found out by the survey of various retail outlets .

Since the majority of the samples were found to be contaminated, there was a requirement of finding an easily available and cost effective antimicrobial dip. Three solutions -200 ppm of chlorine solution, 1% Citric Acid solution and 1% benzoic Acid solution were studied for their antimicrobial property. All the three antimicrobial dips were found to be effective, but citric acid was the most effective as per statistical analysis (Paired test). Thus, it can be introduced as a cost effective antimicrobial dip.

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