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Microbiological Assessment of Fresh Juices Vended in Different Areas of Lahore City

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Research Article

Abstract

This present study was conducted to evaluate the microbiological quality of the fresh juices sold near to the road side and others various location of Lahore city Pakistan. Ten samples of each type of unpasteurized juices like Apple, Carrot, Orange and Sugarcane extract were used for microbiological testing. Most of the samples of fruit juices exhibited heavy bacterial load including other microbial contaminants like coliform, fecal coliform *Escherichia coli*, *Staphylococcus aureus*, yeast and mould count. However, maximum numbers of carrot juice samples were found unsatisfactory. *Salmonella* sp. were also detected in carrot and sugarcane juices. All these findings indicate that the juices were prepared under unhygienic environmental conditions which must be improved for the safety of consumers.

Keywords: Fresh fruit juices; Coliforms; Yeast & mould; *S. Aureus*; *Salmonella* sp.

1. Introduction

Fresh juices are made from raw fruits and are well recognized for high value of natural vitamins, sugars and fibers which are necessary elements for human health. The demand of fresh juices has increased day by day and is preferred throughout the world. Similarly, the fresh juices sold by vendors are easily available in every city of Pakistan. Although fresh juices have a huge amount of nutrients but unhygienic preparation of juices make it potential source of microbial contaminants [1]. These microbial contaminants may cause serious threats to human health on their consumption.

A number of factors like unhygienic water, unclean utensils and hands, preparation process and often swarming of flies on place of preparation promote the contamination of juices [2,3]. Contaminated water has number of pathogenic bacteria which cause serious illness in humans. Similarly, contamination of harmful micro-organisms came out through damaged areas or cuts on the surface of fruits [4]. Utilization of unhygienic ice cubes and prolonged preservation without refrigeration are other potential sources of microbial contamination [5]. The microbial quality assessments and preventions measures are the utmost needs to improve the quality of fresh fruit juices to avoid the contaminations. The present study was therefore undertaken to assess the quality of various types of fresh juices and to find out the microbial load.

2. Materials and Methods

2.1 Sample collection

Samples of different fresh fruit juices (Apple, Carrot, Sugarcane and Orange) were collected in sterilized bottles from different localities of Lahore city, Pakistan and transported to the laboratory in an ice box. All samples were kept at 4˚C before analysis.

2.2 Sample preparations

Initially serial dilutions of each sample were made with sterilized Butterfield's phosphate buffer.

2.3 Microbiological analysis

All the microbiological parameters to assess the quality of fresh fruit juices were conducted according the methods as described in FAO (Food and Agriculture Organization) [6].

2.4 Total plate count

Standard plate count agar (Oxide) was used to estimate total bacterial count/mL of fresh fruit juices. Each sample was diluted serially up to 10^{-3} dilutions in Butterfield's phosphate buffer and 1 mL of each dilution was transferred to a sterilized petri plate. Then 15-20 mL of sterilized molten (40˚C-45˚C) standard plate count agar was poured in the petri plates. Medium was mixed well with sample by thoroughly rotating the plate clockwise and anti-clockwise. After that agar was

allowed to settle down at room temperature and then incubated at 35˚C ± 1 for 48 h.

2.5 Yeast and mold count

Potato dextrose agar (Merck) supplement with chlortetracycline was added for yeast and mould count/mL. One mL of each sample serially diluted up to 10^{-3} dilutions in Butterfield's phosphate buffer was taken and 15 mL of potatoes dextrose agar was added in each petri plates. Each plate was mixed well and allowed to settle and incubate at 25˚C ± 1 for 5 days.

2.6 Total coliform

About 10 mL of Lauryl tryptose broth (Oxoid) was taken in test tubes with inverted Durham tubes and autoclaved at 121°C for 15 min. One mL of 1st three serial dilutions of each sample in Butterfield's phosphate buffer was added into set of three test tubes separately. These tubes were incubated at 37˚C ± 1 for 48 h for presumptive test. Tubes with gas production were used for further confirmatory test.

2.7 Confirmatory test for total coliform

One full loop from positive presumptive tubes were transferred into brilliant green bile broth (Oxoid) having 10 mL volume with Durham tubes. These tubes were incubated at 35˚C ± 1 for 48 h and the tubes with gas production were considered positive for coliforms. Total coliforms were calculated from MPN Tables [6].

2.8 Confirmatory test for fecal coliform

A loop full was added into the sterilized EC medium (Oxoid) having Durham tubes from Lauryl Tryptose (LT) positive tubes. These tubes were incubated at 45.5 ± 0.2˚C for 48 h and examined for gas production.

2.9 Detection *of E.coli*

Eosin-methylene Blue agar (EMB) (Oxoid) was used to for *E. coli* detection. A loop full from positive EC medium tubes was streaked on EMB agar (oxide) plates and incubated at 35˚C ± 1 for 18-24 h. Positive plates appeared as green colonies with metallic shine.

2.10 Detection of *Staphylococcus aureus*

For detection of *S. aureus* 0.3 mL and 0.4 mL of each sample diluted serially up to 10^{-3} dilutions in Butterfield's phosphate buffer were spread over separate Baired-parker agar plates supplemented with tellurite egg yolk emulsion (Oxoid) and these plates were incubated at 35˚C for 48 h. Black colonies surrounded by clear zone were added into 0.3 mL Brain Heart Infusion broth (BHI) and placed at 35˚C for 18-24 h. After that 0.5 mL reconstitution plasma with EDTA were added in BHI culture and incubated at 35˚C and observed over 6 h for positive coagulates test.

2.11 Detection of *Salmonella* sp.

About 25 mL sample of each fruit juices were added into 225 mL sterilized lactose broth medium (Oxoid) in separate flask and then incubated flask at 35˚C for 24 h for pre-enrichment. One mL of inoculum medium was further transferred into tetrathionate broth for selective enrichment. A loopfull culture from enriched medium was streaked on bismuth sulphite, hecktoen enteric and xylose lysine deoxycholate agars plates and incubated the plates at 35˚C for 24-48 h. The characteristic colonies appeared on each plate were further confirmed by performing biochemical tests according the methods of FAO [6].

3. Results and Discussion

Fresh fruit juices are preferred by consumers because; fresh juices have good source of vitamins and natural mineral and are accessible to everyone. But fresh juices contain heavy load of microbes which can cause serious illnesses [7]. During this study all samples of fresh juices were found contaminated by coliform, fecal coliform, and others harmful pathogens. The recommended microbiological standards for any fruit juice; all numbers are as per ml of juice consumed as shown Table 1. The data

Table 1: Recommended Gulf Standard.

Limits	*Total viable count/l	*Coliform/ml	*Fecal Coliform/ml	**Staphylococcal*/ml	*Yeast and mould count/ml	**Salmonella spp/25 ml
Maximum bacterial load anticipated	$5.0X10^3$	10	0	100	100	ND
Maximum bacterial load permitted	$1.0X10^4$	100	0	$1.0x10^3$	1.0×10^3	

*[8] **[9]

Where:

The number of samples (n) to be examined equals 5.

None of the 5 samples should have counts in excess of Maximum Permitted Limit; any sample with a count above Maximum Count Permitted shall be designated as "defective".

No more than 2 out of the 5 samples should have counts in excess of Maximum Count Anticipated; any sample with a count above Maximum Count Anticipated shall be designated as "marginally acceptable".

presented in Table 2 indicates the microbial quality of apple juice samples sold in different areas of Lahore city. About 80% samples showed higher value and 20% samples showed less total plate count of maximum bacterial load according to gulf standard. In addition, higher value of TPC (Total Plate Count), might indicated the preparation of fresh juice under unhygienic conditions [8,9]. Earlier study reported that coliform is not allowed in fresh fruit juice, coliform was associated with other harmful bacteria such as *E. coli*, *Enterobacter* and *Klebsiella*, that cause severe infection [10,11]. According to standard, 60% samples have higher range of total coliforms and just 40% sample of apple juice showed negative result. The value of fecal coliform should be zero according to standard. However, in present study the 40% samples have positive and 60% samples showed negative result of fecal coliform. Moreover 70% sample of apple showed no detection of *E.coli* and only 30% samples were positive *(E. coli)* that indicates to fecal contamination.

Staphylococcus was found in apple juice, 50% samples have higher value than both of anticipated and permitted, and 50% apple juices were having negative result. The pervious study reported that occurrence of *Staphylococcus* indicates the contamination *via* handling [12,13]. In present study yeast and mould are also existence, 60% sample have lower value and 40% higher than standard value. Moreover, *Salmonella* was not detected in apple juices.

Table 3 represents the microbiological quality of orange juice obtained from squeezing machines. About 70% samples were found within limits and 30% TPC was out of microbiological limits according to the gulf standards 2002. Among various microbiological parameters, 50% samples showed lower total coliform and 50% were out of range as per standard limits. In 40% samples there was no fecal coliform and 60% samples were out of range. Furthermore, 60% samples exceed limit in presence of *E. coli* and only 40% showed no detection. During this study *S. aureus* was also detected and 60% samples were found within limits while 40% samples were out of range according to standard values. In orange fresh juice, yeast and mould were also counted and observed in 60% and 40% in limits respectively. There was no detection of *Salmonella* in orange samples. Previous studies suggest that juice processing may be major route of contamination [14,15]. Harmful organism introduced into juice through contaminated fruit

Table 2. Microbial quality of fresh juices of apples vended in different areas near roadside of Lahore city.

Apple Juice	TPC (Total Plate Count)/ mL	Total Coliforms (MPN/mL)	Fecal Coliform (MPN/mL)	*E. Coil* (MPN/mL)	*Staphylococcus aureus*/mL	Yeast& mold Count/mL	*Salmonella* Spp./25 mL
1	$1.8x\ 10^4$	93.0	23	23	ND	$8.0x10^1$	ND
2	$2.5x\ 10^3$	1.0	ND	ND	$3.0\ x10^3$	$3.0x10^3$	ND
3	$8.4x10^4$	21	21	21	$2.8x10^4$	$5.0x10^2$	ND
4	$6.5x10^3$	ND	ND	ND	$1.7\ x10^3$	$8.5x10^1$	ND
5	$5.5x10^5$	ND	ND	ND	ND	<10	ND
6	$7.0x10^2$	25	25	ND	$2.5\ x10^4$	$7.0x10^1$	ND
7	$8.2x10^4$	23	ND	ND	$9.8x10^1$	$8.6x10^2$	ND
8	$3.0x10^4$	ND	ND	ND	ND	<10	ND
9	$4.6x10^5$	75	9.1	9.1	$2.0x10^2$	$2.5x10^2$	ND
10	$7.2x10^4$	240	ND	ND	ND	$5.2x10^1$	ND

ND: Not Detected; D: Detected; MPN: Most Probable Number.

Table 3. Microbial quality of fresh juices of orange vended in different areas near roadside of Lahore city.

Orange Juices	TPC(Total Plate Count)/ml	Total Coliforms (MPN/ml)	Fecal Coliform (MPN/ml)	*E.Coil* (MPN/ml)	*Staphylococcus Aureus/ml*	Yeast & mold Count/ ml	*Salmonella* Spp/25 mL
1	$3.2\ x\ 10^4$	20	20	15	$9.8x10^1$	<10	ND
2	$4.0x\ 10^3$	11	7.3	7.3	$5.0x10^3$	$4.5x10^2$	ND
3	$4.1x10^2$	ND	ND	ND	<10	$8.2x10^1$	ND
4	$4.2x10^3$	210	150	23	$2.4x10^2$	$5.2x10^2$	ND
5	$9.0x10^2$	9.1	9.1	9.1	$1.8x10^3$	$6.4x10^3$	ND
6	$7.5x10^3$	21	15	14	$3.5x10^2$	$1.8x10^2$	ND
7	$3.6x10^4$	ND	ND	ND	<10	$3.2x10^2$	ND
8	$3.3x10^4$	ND	ND	ND	$4.5x10^1$	<10	ND
9	$4.0x10^3$	3.0	3	3	<10	$4.5x10^4$	ND
10	$9.8x10^2$	3.6	ND	ND	$7.0x10^1$	<10	ND

ND: Not Detected; D: Detected; MPN: Most Probable Number.

and could exist under improper hygienic standards. Earlier study reported that damaged surface of fruit *via* bruising and cuts to fruit epidermis cause by transport and other necessary processing enhance the growth of micro-organisms (e.g. fungi) [16,17].

Table 4 shows bacterial load in carrot juice samples. During this study, higher microbial load was observed, 99% total plate count was out of limits from standard value. Total coliforms were 2% and fecal coliform 3% within ranged according to Gulf standard 2000. The range of *E. coli* was 60% higher than standard value evaluated from carrot juice. *S. aureus* was also observed in carrot juice which indicates the harsh contamination during juice preparation. Sixty percent samples were out of range than standard value and only 40% samples had negative results. Yeast and mould count was 2% and 70% respectively while *salmonella* contamination was also observed within limits in carrot juice. Carrots are transported from fields and washed with water for removal of soil residues [18]. Entry of microbes might be through soil residues and can also be caused by water which is used for washing of carrots. Similar findings were reported that improper washing and lack of knowledge about basic safety issues of fruits may enhance microbial contamination [19].

Table 5 shows microbial quality of fresh juices of sugarcane vended in different areas near roadside of Lahore city. Sugar cane juice was also collected for microbiological testing and it was observed that most of the samples contained higher range of TPC, total coliform and fecal coliform according to standard value, which indicated that juices were highly contaminated by microorganism. The contaminated water is the main source, which is used in juice preparation [6,20]. Other factor is also affected like bare hands used for handling the ice and sieving of juice. The utensils were washed just by dipping in the contaminated water. As shown in Table 4, 70% samples have *S. aureus* and 80% yeast and mold count was out of range from standard value. *Salmonella* spp. were also detected in 2% of samples that expressed the poor hygienic conditions. Another study reported that most of the fruit juices used in markets were heavily loaded with a variety of microbes which could cause food borne illness [21].

4. Conclusion

Present study carried out to evaluate the microbiological quality of fresh fruit juice obtained from different vendors. The result of samples revealed high microbiological status of available local fruit juices. It's indicated that contamination in fresh juices

Table 4. Microbial quality of fresh juices of carrot vended in different areas near roadside of Lahore city.

Carrot Juice	TPC (Total Plate Count)/mL	Total Coliforms (MPN/mL)	Fecal Coliform (MPN/mL)	E.*Coil* (MPN/mL)	*Staphylococcus Aureus*/mL	Yeast & mould Count/ mL	*Salmonella Spp* /25mL
1	9.8 x 10^3	75	23	9.1	6.0x10^3	2.3x10^3	Detected
2	5.5x 10^5	43	23	23	7.2x10^2	8.5x10^3	ND
3	6.1x10^4	93	43	ND	1.4x10^3	9.2x10^2	ND
4	3.7x10^4	20	20	15	4.4x10^3	7.5x10^2	ND
5	6.1x10^5	9.1	ND	ND	2.2x10^1	5.7x10^4	ND
6	4.5x10^3	3.0	ND	ND	1.0x10^2	6.7x10^1	ND
7	5.6x10^4	460	240	93	5.0x10^3	7.8x10^1	Detected
8	5.3x10^5	ND	ND	ND	2.0x10^1	1.9x10^2	ND
9	4.7x10^4	21	15	9.1	<10	1.7X10^2	ND
10	9.4x10^4	29	21	21	4.3x10^3	6.7x10^3	Detected

ND: Not Detected; D: Detected; MPN: Most Probable Number.

Table 5. Microbial quality of fresh juices of sugarcane vended in different areas near roadside of Lahore city.

Sugarcane Juice	TPC (Total Plate Count)/mL	Total Coliforms (MPN/mL)	Fecal Coliform (MPN/mL)	*E.Coil* (MPN/mL)	*Staphylococcus aureus*/mL	Yeast & mold Count/ mL	*Salmonella* Spp/25 mL
1	1.5 x 10^5	43	43	9.1	4.6x10^3	3.2x10^3	ND
2	3.4x 10^5	240	93	43	4.9x10^2	7.2x10^3	D
3	3.2x10^4	21	21	9.1	5,5x10^2	7.8x10^1	ND
4	8.0x10^4	75	75	43	7.0x10^3	4.5x10^2	ND
5	8.2x10^3	2.0	ND	ND	7.1x10^1	<10	ND
6	2.5x10^6	11	11	11	8.1x10^4	3.8x10^4	D
7	8.1x10^3	28	21	15	1.5x10^3	3.2x10^2	ND
8	3.4x10^4	23	23	23	9.8x10^3	7.5x10^4	ND
9	4.8x10^5	3.6	ND	ND	1.0x10^2	3.9x10^3	ND
10	4.5x10^5	23	9.1	ND	1.9x10^3	4.0x10^4	ND

ND: Not Detected; D: Detection; MPN: Most probable Number.

comes from poor quality of water and unhygienic condition which employed during preparation of fresh fruit juices. All these findings pointed out the need of proper implementation of hygienic rules to make sure the quality of fresh fruit juices.

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