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Research Article

MICROBIOLOGICAL QUALITY OF SOME FRESH FRUIT JUICES SOLD BY THE LOCAL VENDORS IN KANPUR CITY, UTTAR PRADESH, INDIA

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ABSTRACT

The quality of fruit juices is an important area of investigation in India because of community is dependent on local street vendors due to its ease of availability and low price. However, the microbiological quality of most vended juices is ignored. In the present study, microbiological quality of some street vended juices like Beet, Carrot, Pomegranate, Sugarcane and Sweet lime sold at Kanpur city (India) was evaluated during peak season of summer. This study revealed high microbial load in most of the juice samples, moreover, sugarcane juice was found extremely contaminated. The various factors were observed that may contributed the higher number of microbial load in fruit juices and among them poor quality of ice was one of the most important source. The most prevalent bacterial species isolated from fruit juices were *E. coli* followed by *Bacillus* spp. The results suggest necessity of proper hygienic practices and quality attainment to the local vendors to avoid such contamination.

Keyword: Microbial load, fruit juices, microbiological quality.

INTRODUCTION

The increase of resistance in microorganism is not a problem in India but become a global issue. Peoples now are in continuous search of alternate medicine which can treat or modulate the immune system of human beings so that the infection due to pathogens may prevent. The alternate system of medicine include plant based herbal drugs and use of nutraceuticals (i.e., dietary supplement in the form of fruits and vitamins). These days due to change in our life style diseases like diabetes, obesity, acidity etc. become common and affect out health. Thus, we need to focus on healthy food so that disease incidence can be

reduced. The easiest thing which one could get as healthy food is fruits and fresh juices, but, as far as Indian subcontinent is concerned, most of the people prefer juice from road side vendors since they either belong to low or middle income group.

Fruits and fresh juices represent healthier part of daily diet and are rich in minerals, natural antioxidants such as anthocyanins, polyphenolic flavonoids, vitamins, natural sugars like glucose, fructose and sucrose and many other health promoting compounds (Abbo *et al*., 2006; Shakir *et al.*, 2009; Abalaka *et al.*, 2013) and have low cholesterol, fat and proteins. Availability of fruit juices in market depend on cropping season, geographical region and expected choice by consumers. Moreover, different types of fruits juices are readily marketed as raw, processed, fermented, unfermented, tetra packed or canned and in other commercial forms. Indigenous flavor, aroma, color, nutritional and medicinal potentials of fruit juices are so distinctive that decide their economic and ethical values. However, acceptability of any juice is mainly decided by its quality assertion which is affected by source, age, physical appearance, storage, local climate and whether and the most important i.e., contamination by microorganisms (Durgesh *et al.*, 2008). Bottled or canned juices in India are commercialized after approval by one or more quality control systems under the laws and regulations, however, peoples sometimes not preferred due to variations in price, shelf life and use of chemical preservatives, artificial coloring and flavorings agents (Melbourne, 2005; Addo *et al.*, 2008). Freshly squeezed fruit juices are easily available on low price and free from any hesitant chemical and thus liked more. These attract consumers when available in a shop full of raw fruits and served with decorating elements by local vendors. However, their quality is not so persuasive and reported as potential source of microbial contamination by fecal bacteria like *Escherichia coli*, *Salmonella* sp., *Shigella* and *Staphylococcus aureus*, *Vibrio cholera*, etc. (Sandeep *et al.*, 2004; Sharma, 2013; Reddi *et al.*, 2015; Reddy *et al.*, 2016) and may pose a social threat for illness. Many factors have been reported that may responsible for poor quality of fruit juices like inappropriate cleaning of fruits before use, extending preservation without refrigeration, improper handling of tools and squeezing machines, absence any management for insects and flies and poor sanitation (Reddy *et al.*, 2016; Verma and Gaur, 2017). In addition, microbial contaminants may invade fruits through natural openings or route made after physical damaged during harvesting, transporting or handling and colonise easily within cuts, crack, prick, pressed or lesions and persist in unpasteurised juices (Mahale *et al.*, 2008). Many times bacterial counts of some fruit surface may exceed 10^5cfu/cm^2 (Singh *et al.*, 2015; Srinath, 2017). The upsetting load and diversity of bacteria in raw juices may proceed with antibiotic resistance (Chandra *et al.*, 2016). In Kanpur city, Uttar Pradesh (India), there is high demand of fruits juices like sweet lime, pomegranate, sugarcane, mixed fruit, etc. especially in summer season. Most of reputed restaurants serve fruit juices in well hygienic conditions, however, road side

vendors near railway station, bus stand, hospitals, shopping mall and recreational areas the rarely manage untainted conditions. Presence of swarming flies on aged fruits, dust particles, unclean utensils, poor quality of ice or water, bad cleaning practices of squeezing machine and improper drainage facilities create microbiological quality issues. In the present study, a prompt assessment of some common fruit juices sold at vendors' shop was undertaken to assess their microbiological quality to increase ample of public awareness and health management.

MATERIALS AND METHODS

Collection of Samples

Different type of fresh juices including sugar cane, pomegranate, sweet lime, carrot, and beet were randomly collected from street vendors in different areas of Kanpur City, Uttar Pradesh (India) during March 2017 to July 2017. Individual sample of juice was collected in a separate sterilized container and analyzed immediately for microbiological qualities in the laboratory.

Enumeration of Total bacterial count

Total bacterial count (TBC) of fresh juice samples was done by using pour plate method as described in IS: 5403 (1999). Ten mL of a juice sample was aseptically mixed with 90 mL of sterilized normal saline solution (0.85%) in an Erlenmeyer flask in laminar air flow. Blended sample was further serially diluted in test tubes. One mL from each tube dilution was aseptically transferred in separate Petri plates (90 mm bottom sized) subsequently and then, sterilized and molten nutrient agar medium (48±2ºC) was mixed thoroughly. After solidification of medium all plates were incubated at 37±1ºC for 18 h in an inverted position in BOD incubator. TBC was calculated by formula given below:

$$\text{TBC (cfu/mL)} = \frac{\Sigma c}{(n_1 + n_2) \times ad}$$

Where,

Σc is the sum of colonies counted in plates has countable colonies (30-300 cfu),

n_1 is the number of plate counted in the first dilution taken,

n_2 is the number of plate counted in the second dilution,

d is the dilution from which the first count was obtained,

a is amount of sample taken in Petri plate.

Identification of isolates

Identification of bacterial isolates was done by microscopy, study of colony characteristics on selective as well as differential media and standard biochemical tests (Holt *et al.,* 1994; Chandra *et al*., 2016).

Determination of total fecal coliforms

The determination of total coliforms in fruit juices was carried out by the Most Probable Number (MPN) method (APHA, 1998), using three series of five tubes with 0.1, 1.0 and 10 ml of juice. MacConkey broth (Hi-Media) was used for the presumptive test. Total coliform was confirmed using Brilliant Green Bile Broth (Hi-Media India); the broth was incubated for 24-48 h at 37±1°C. EC Broth (HiMedia) was used for confirmation of fecal coliform with incubation of 24 h at 44.5±0.2°C in a water bath (Chandra *et al*., 2008). Eosin Methylene Blue agar (HiMedia) medium and standard kit (for identification of Enterobacteriaceae members;

HiMediaKB001) were used to confirm *E. coli* in juice samples.

RESULT AND DISCUSSION

Consuming fruit juice is simplest way to replenish electrolyte and water requirement in our body particularly in hot summer days. The climate of Kanpur city during summer is very hot and consumption of water and juices is increased. Near all public places and official buildings there were several vendors vended fruit juices, though, their frequency varied from zero to three per kilometer or sometimes more. During rush hour vendors peeled and piled up the fruits in an uncovered container(s) in advance of their actual consumption and thus unwillingly encouraged the contamination.

Out of total 30 samples tested for microbiological quality, total bacterial count (TBC) of fruit juices were ranged from 1.2×10^3 to 3.8×10^9 cfu/ml in sugarcane, 3.8 x 10^4 to 7.6×10^4cfu/ml in beet, 5.2×10^4 to 9.0×10^6 in carrot, 2.2×10^4 to 7.1×10^6cfu/ml in pomegranate and 7.6×10^5 to 1.3×10^6cfu/ml in sweet lime (Table 1), respectively. The high value of TBC illustrated very low quality of fruit juices that may cause severe diseases. The sugarcane juice, which was popular among all age and socio economic groups showed highest bacterial count as compared to other juice types. However, no significant relation was established between the juice type and bacterial isolates as revealed by chi-square test of independence, χ^2 (16, N = 69) = 8.1, $p > 0.05$. The contaminating juice is considered fetal for young children and other immune-compromised individuals (Anuranjini and Alex, 2015).

The most prevalent bacteria among all tested sample were *E. coli* (90%) followed by *Bacillus* spp. (73%), *Klebsiella* spp. (50%), *Staphylococcus* spp. (10%) and *Salmonella* spp. (3.3%) (Table 2). Presences of fecal bacteria in fruit juices are neither permissible by any regulatory body nor suitable for consumption. *Bacillus* spp. in juice samples asserted the presence of their endospores on fruit surface or come through dust contamination. This structure can resist chemical disinfectants and remains dormant in hot and dry atmosphere (Chandra *et al*., 2016). Similar findings were also reported from other part of India (Mahale *et al.*, 2008; Sharma, 2013; Singh *et al.*, 2015; Reddy *et al.*, 2016; Srinath, 2017; Verma and Gaur, 2017) and the literatures illustrated the sub-standard quality of fresh juices.

There are several factors had been observed during study that contributing the bacterial contamination. Many vendors stored fruits in inappropriate conditions without proper refrigeration and thus prepared juice from aged and dirtied fruits. They used extremely poor quality of water for dilution of juice and washings. The cleaning practices of utensils and squeezing machine were done improperly on footpath. In addition, other sources of contaminants like, swarming flies on fruits, airborne dusts due to passes of vehicles, overcrowded public gatherings, unsatisfactory personal hygiene, etc. were observed that have also reported in literatures (Khalil *et al*., 1994; Al-Jedah and Robinson, 2002; Lewis *et al*., 2006; Reddi *et al.*, 2015). The poor quality of ice in preset study was specifically established with bacterial load in juice by

purposive samples collected from individual vendor's shop that showed similar TBC range of mesophiles and psychrophiles between 0.2×10^1 and 9.7×10^2 cfu/ml and 0.8×10^1 and 8.4×10^2cfu/ml, respectively (Table 3). Moreover, significantly positive correlation was found between TBC of fruit juices and bacterial count in separate groups i.e., mesophiles ($r=0.52$; $p<0.05$) and psychrophiles ($r=0.38$; $p<0.05$) in ice samples. The lower values of Pearson correlation coefficients (r) confirmed the role of multiple factors on microbiological quality of fruit juices instead of single cause of poor ice quality. Many samples of ice showed considerable high MPN (coliform) values (Table 3) that might be inherited either from pre-contamination by bacteria in water during freezing in the industries or improper storage by vendors as reported by Chandra *et al*. (2008). Mahale *et al.* (2008) had also addressed the accountability of ice as potential source of contamination in street vended juice of sugarcane, lime and carrot.

The MPN values of juice samples showed extensively high level of fecal contamination and varied between 22 coliforms/100ml and 1600 or more coliforms/100ml.

Table 1. Total Bacterial Count and MPN (coliform) value of juice samples

S. No.	Types of juice	Sample	Total Bacterial Count (cfu/mL)	MPN (coliform)/100ml
1	Beet (n=2)	B-1	7.6×10^4	300
		B-2	3.8×10^4	300
2	Carrot (n=4)	C-1	7.2×10^6	1600
		C-2	9.1×10^5	23
		C-3	9.0×10^6	170
		C-4	5.2×10^4	1600
3	Pomegranate (n=2)	P-1	2.2×10^4	350
		P-2	7.1×10^6	1600
4	Sugarcane (n=18)	S-1	3.2×10^5	540
		S-2	2.2×10^4	22
		S-3	5.5×10^6	22
		S-4	4.0×10^4	280
		S-5	8.2×10^4	27
		S-6	8.9×10^5	1600
		S-7	5.5×10^5	1600
		S-8	1.2×10^5	300
		S-9	8.2×10^6	1600
		S-10	1.6×10^3	300
		S-11	3.5×10^4	300
		S-12	9.2×10^4	540
		S-13	2.8×10^5	1600

		S-14	2.2×10^5	1600
		S-15	1.8×10^3	540
		S-16	1.2×10^3	540
		S-17	3.8×10^9	>1600
		S-18	1.6×10^9	>1600
5	Sweet lime	SW-1	1.1×10^6	170
	(n=4)	SW-2	7.6×10^5	60
		SW-3	1.3×10^6	30
		SW-4	8.1×10^5	60

Table 2. Bacterial isolates from different fruit juices

Types of juice	No of samples tested	Bacterial isolates				
		Bacillus spp.	*Klebsiella* spp.	*E. coli*	*Salmonella* spp.	*Staphylococcus aureus*
Beet	02	01	02	02	00	00
Carrot	04	03	02	02	00	00
Pomegranate	02	01	01	02	00	01
Sugarcane	18	15	08	17	01	02
Sweet lime	04	02	02	04	00	00
Sum Total	30	22	15	27	01	03

Table 3. Total Bacterial Count and MPN (coliform) value of purposive ice samples

	Heterotrophic count		
Sample	**Mesophile count (cfu/ml)**	**Psychrophile count (cfu/ml)**	**MPN/Total coliform**
A-1	2.2×10^2	5.3×10^2	1600
A-2	1.7×10^1	7.3×10^1	<2
A-3	3.2×10^2	1.3×10^2	2
A-4	6.7×10^2	4.3×10^2	2
A-5	1.4×10^2	5.4×10^1	900
A-6	4.3×10^2	2.7×10^2	345
A-7	1.7×10^2	1.3×10^2	>1600
A-8	1.2×10^1	9.0×10^1	542
A-9	8.8×10^1	3.4×10^2	24
A-10	2.7×10^2	1.1×10^2	2
A-11	4.9×10^1	6.6×10^2	41
A-12	1.2×10^2	4.0×10^1	175
A-13	1.6×10^2	7.0×10^1	426
A-14	4.1×10^2	3.3×10^2	>1600
A-15	3.2×10^1	7.0×10^1	2

A-16	7.1×10^2	4.4×10^2	540
A-17	5.8×10^2	0.8×10^1	22
A-18	0.3×10^1	8.1×10^2	<2
A-19	0.8×10^1	5.4×10^1	<2
A-20	0.2×10^1	3.9×10^2	<2
A-21	1.1×10^2	4.0×10^1	21
A-22	4.7×10^2	2.4×10^2	17
A-23	1.1×10^2	6.0×10^1	12
A-24	2.1×10^2	6.5×10^2	9
A-26	9.7×10^2	8.4×10^2	7
A-27	1.9×10^2	4.4×10^2	17
A-28	0.4×10^1	8.0×10^1	<2
A-29	7.0×10^1	6.0×10^1	<2
A-30	2.2×10^2	3.6×10^2	17

Present study revealed that fresh juices available at public places have severe contamination by microorganisms. Among the all isolates, *E. coli, Klebsiella* spp. and *Salmonella* spp. were considered potential pathogens responsible for many gastrointestinal diseases. *Bacillus* spp. and *Staphylococcus aureus* on the other hand can produce enterotoxins in fruit juices that cause food poisoning, toxic shock syndrome and other health complications. In India, fresh juices are prescribed by doctors to the patient who is admitted in hospital for fast recovery but the study revealed the poor quality of juices if consumed by hospitalized person could develop secondary infections. The present study showed that the ordinary fruit juices purchased from street vendors would be an inappropriate choice and non-reliable as per the microbiological quality standards.

CONCLUSION

Freshly squeezed fruit juice outside the home in poor hygienic conditions is highly susceptible for microbial contamination and may attribute in serious health problems. The consumption of street vended fruit juices although offer temporary satisfaction for psychological and nutritional improvement but results from present study revealed their different quality. It is not easy to avoid street vended fruit drinks; however, street vendors can be motivated for quality improvement by public awareness programs by the government, NGOs or by individual volunteering. There should be a system which has ability to monitor the quality of street fruit juices so that risk of outbreak of food and water borne illness can be protected. The present study strongly suggests proper hygienic practices for consumable street foods.

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