

## Microbiological Analysis of Street Vended Fruit Juices from Mumbai City, India

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**Abstract:** Fresh squeezed juices of sugarcane, lime and carrot sold by street vendors in Mumbai city were analyzed for their microbial contents during the months of June 2007 to September 2007. The total viable counts of all 30 samples were approximately log 6.5 cfu/100ml with significant load of coliforms, faecal coliforms, *Vibrio* and *Staphylococcal* counts. Qualitative counts showed the presence of coagulase positive *S.aureus* in 5 samples of sugarcane and 2 samples of carrot juice. Almost 70% of the ice samples collected from street vendors showed high microbial load ranging from log 5-8.5. Our results demonstrate the non hygienic quality of three most popular types of street vended fruit juices and ice used for cooling of juices suggesting the urgent need for government participation in developing suitable intervention measures to improve microbial quality of juices.

**Key words:** Sugarcane juice, lime juice, carrot juice, street vendors, faecal coliforms, *Vibrio*

### Introduction

In developing countries, fruit and vegetable juices sold by street vendors are widely consumed by millions of people. These juices provide a source of readily available and affordable source of nutrients to many sectors of the population, including the urban poor. Unpasteurized juices are preferred by the consumers because of the “fresh flavor” attributes and hence, in recent times, their demand has increased. They are simply prepared by extracting, usually by mechanical means, the liquid and pulp of mature fruit and vegetables. The final product is an unfermented, clouded, untreated juice, ready for consumption.

Pathogenic organisms can enter fruits and vegetables through damaged surfaces, such as punctures, wounds, cuts and splits that occur during growing or harvesting. Contamination from raw materials and equipments, additional processing conditions, improper handling, prevalence of unhygienic conditions contribute substantially to the entry of bacterial pathogens in juices prepared from these fruits or vegetables (Victorian Government Department of Human Services 2005; Oliveira *et al.*, 2006; Nicolas *et al.*, 2007).

In countries, where street food vending is prevalent, there is commonly a lack of information on the incidence of food borne diseases related to the street vended foods. However, microbial studies on such foods in American, Asian and African countries have revealed increased bacterial pathogens in the food. There have been documented outbreaks of illnesses in humans associated with the consumption of unpasteurized fruit and vegetable juices and fresh produce. A report published by Victorian Government Department of

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Human services, Australia (2005) reported survival of *E.coli* 0157:H7 in apple juice for upto 24 days at 4°C. A total of 48 cases of *E.coli* 0157:H7 were reported after drinking unpasteurized apple juice in Washington DC in 1996. *Listeria monocytogenes* has also been identified as a pathogen that is of concern in relation to these products (Victorian Government Department of Human Services 2005).

In 1995, unpasteurized fresh orange juice contaminated with *Salmonella* was linked to an outbreak in a Florida Theme Park, USA. More than 60 visitors were affected (Schmidt *et al.*, 1997). In Australia, 427 confirmed cases of salmonellosis were reported in 1999 after drinking unpasteurized orange juice (Victorian Government Department of Human Services). A cholera epidemic in Pune city, India, was related to street vended sugarcane juice containing ice that was contaminated with *Vibrio cholerae* (Mosupye *et al.*, 1999).

In view of the threat posed by the bacterial pathogens in juices and the flourishing demands for such street vended juices, the present work was undertaken to assess the microbiological quality of freshly pressed or squeezed juices from street vendors during the rainy season from June to September 2007 in Mumbai.

## Materials and Methods

### Collection of samples

Five locations from South Mumbai vending fresh fruit and vegetable juices were selected. From every location, two vendors were chosen on the basis of sale of atleast 80 – 100 glasses of juices/day during the rainy season. Based on the consumer demand, three types of juices namely, sugarcane, lime and carrot were selected for microbial analysis and pH measurement. All freshly extracted juice samples (150 ml each) were collected in sterile bottles and transported to the laboratory in an ice box. Juices were analyzed within an hour of procurement. 5 ml portion of juice samples were removed aseptically for pH measurement using pH meter.

### Sample processing

10 ml of the sample was diluted with 90 ml of sterile buffered peptone water and mixed well ( $10^{-1}$  dilution). Serial dilutions were prepared and spread plate technique was used on appropriate selective media.

### Bacteriological analysis of the collected juice samples

Microbiological analysis included enumeration and identification of potential pathogens according to standard procedures for the number of heterotrophic bacteria, *Staphylococcus aureus*, *Vibrio*, *Salmonella*,

*Shigella* and most probable number (MPN) of total coliforms.

Appropriate dilutions were then enumerated for Total aerobic plate counts using Nutrient Agar, Coliforms using Violet Red Bile Agar, *Staphylococcus aureus* using Salt Mannitol Agar, *Vibrios* on Thiosulphate Citrate Bile Sucrose Agar. Xylose Lysine Deoxycholate Agar was used for enumeration of *Salmonella* & *Shigella* (Mosupye *et al.*, 1999; Mudgil *et al.*, 2004). All the selective media were obtained from Himedia Laboratories Ltd, Mumbai, India. All plates were incubated under aerobic conditions at  $36 \pm 1^\circ\text{C}$  for 24 hrs. The mean number of colonies counted was expressed as log colony forming units (cfu)/100 ml. The MPN of total coliforms was determined, following APHA recommendations. Three serial dilutions (0.1ml, 1 ml and 10 ml) were inoculated in MacConkey broth and incubated at 37°C for 24-48 hrs. Positive tubes (gas and acid production) were streaked on Eosin Methylene Blue Agar. Typical *E. coli* colonies were seeded into Tryptone broth and Indol test was done. For confirmation of the pathogens, typical colonies were checked using appropriate biochemical tests as per Collins & Lyne's Microbial Methods 6<sup>th</sup> edition.

### Bacteriological analysis of ice samples

10 ice samples (10 pieces each) were collected from the juice vendors in sterile glass bottles and transported in an ice box. They were bacteriologically analyzed using same methods as for juice samples within an hour of collection.

## Results and Discussion

In spite of the potential benefits offered by fruit juices, concerns over their safety and quality have been raised. Freshly squeezed fruit and vegetable juices have little or no process steps that reduce pathogen levels, if contaminated (Victorian Government Department of Human Services 2005).

In the present investigation freshly squeezed juices of sugarcane, lime and carrot showed occurrence of high microbial loads consisting of number of pathogens like coliforms, fecal coliforms, *E.coli*, *S.aureus* and *Vibrio cholerae* as shown in Table 1. Sugarcane juice followed by carrot juice showed high microbial counts consistent with pH values of 5.4 and 6.2 which do not affect the survival of pathogens adversely. In contrast, lime juice with pH 2.3 showed not only much lower total viable count ranging between log 0-8.2, but also showed absence of coagulase positive *Staphylococcus aureus* and *Vibrio cholerae*. A number of factors are responsible for contamination of freshly squeezed fruit juices. Most fruit contains bacterial counts of  $1 \times 10^5$  cfu/cm<sup>2</sup> on their surface (Splittstosser 1979;

Harrigan 1998; Al-Jedah *et al.*, 2002). Improper washing of fruits adds these bacteria to juices leading to contamination. In addition lack of appreciation of basic safety issues by vendors contribute to augmentation of the microbial loads. These include use of crude stands and carts, unavailability of running water for dilution and washing, prolonged preservation without refrigeration, unhygienic surroundings with swarming flies and airborne dust (Lewis *et al.*, 2006).

A number of studies from different countries have shown that microbial quality of ice manufactured for use to cool foods and drinks could be a cause of concern. The microbial safety of commercial ice used in drinks was evaluated by Lateef *et al.* (2006) in Nigeria and it was found that microbial loads of these ice samples ranged from 1.88-3.20 X 10<sup>4</sup> cfu/ml which was largely above the recommended loads of more than 500 and 1000 cfu/ml for ice obtained from manufacturing plants and retail outlets respectively.

In the present investigation, ice samples obtained from vendors also showed high total viable count (log 5-8.5). 70% of the ice samples analyzed showed presence of Total coliforms, Total faecal coliforms and *Vibrio cholerae*. A number of studies from different countries have shown presence of *E.coli*, coliforms and a variety of microorganisms like *Streptococcus pyogenes*, *Streptococcus equi*, *Pseudomonas aeruginosa*, *Staphylococcus* spp, *Micrococcus* spp etc (Lateef *et al.*, 2006; Moyer *et al.*, 1993; Vieira *et al.*, 1997; Nichols *et al.*, 2000). This is an indication of unsanitary conditions, unhygienic practices during or after production and poor quality of source of

water used.

If the source water used is of poor quality, harmful microorganisms may persist in ice since the process of freezing cannot destroy them. When ice is thawed the surviving microorganisms though may be injured, tend to recover their viability so that when the ice melts into the juices, they may be able to survive these too (FEHD 2005).

The presence of coagulase positive *S.aureus* in sugarcane and carrot juice can mainly be attributed to contamination via handlers. Although it is unlikely for the introduced *S.aureus* to survive in juices having low pH, it is possible that they may do so in juices having pH values more than 4 (Mudgil *et al.*, 2004). The results of present studies also showed absence of *S.aureus* in all the lime juice samples studied where the pH was 2.3, whereas 50% of sugarcane juice and 20% of carrot juice showed contamination by *S.aureus*.

All the juice samples tested were devoid of *Salmonella* and *Shigella*. However, presence of *Vibrio cholerae* (in 60% of sugarcane juice and 50% of carrot tested) and *E.coli* (in almost all the samples tested) indicates not only poor hygienic quality of these juices but also places consumers at a high risk of contracting food-borne infections. Lack of sanitary conditions in street vended juice stalls and the occurrence of pathogenic bacteria in juices is alarming enough for an immediate action by the suitable agency. Regular monitoring of the quality of fruit juices for human consumption must be introduced to avoid any future pathogen outbreaks.

**Table 1.** Microbial counts (Log<sub>10</sub>CFU/100ml) of freshly squeezed juices sold in street vended stalls in South Mumbai, India.

Type of Sample	TVC	TEC	TFCC	TSC	TVibC	pH
Sugarcane juice	7.19±1.359	6.06±1.179	5.96±0.872	3.36±3.727	4.34±3.88	5.4
Lime juice	4.03±3.562	1.77±2.924	0.5±0.976	0	0	2.3
Carrot juice	8.29±1.027	3.26±4.222	7.22±1.012	1.49±3.143	4.48±4.733	6.2
Ice	7.27±1.142	4.7±3.277	4.9±3.472	0	4.35±3.048	6.8

Data represent means ±standard deviations of 10 measurements

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