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A study on the Microbial quality of fresh fruit juices sold around schools and colleges in Chennai

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Abstract

Millions of people consume fruit juices sold by street vendors in developing countries. There are many health benefits in consumption of fruit juices as they are rich in nutrients. Contamination of fruit juices by pathogenic microbes has led to outbreaks of food borne diseases. Poor sanitation and unhygienic practices of the street vendors is the major cause of these outbreaks. In this study, fresh fruit juices sold by street vendors outside schools and colleges in Chennai city were analyzed quantitatively and qualitatively. The microbiological quality of all the samples analyzed was found to be poor. *Asperigillus niger* (28%) was the dominant fungi isolated in this study followed by *Mucor spp* (10%), *Penicillium spp* (12%), *Aspergillus fumigatus* (8%) and *Rhizopus spp* (8%). All the samples showed the presence of yeast. *Bacillus spp* (56%) was the leading bacteria isolated from the samples tested followed by *Escherichia coli* (20%), *Klebsiella pneumoniae* (8%) and *Pseudomonas aeruginosa* (4%). Regular monitoring of fruit juices sold by street vendors is essential to avoid food borne disease outbreaks. While fruit juice shop locations which are prone to contamination should be avoided.

Key words : Fresh fruit juices, Contamination of fruit juices, Food borne pathogens, Microbial quality of fruit juices, Safety and hygiene of street vended juices.

Fruit juices are prepared from the fleshy part of the fruits or from the whole fruits itself. The method of preparing fruit juice depends on the fruit type, but mostly the juice is prepared by pressing or crushing the fruit to squeeze out the juice. Sometimes pasteurization followed by addition of preservatives is employed before packaging the juice. Fresh fruit juices sold by street vendors are popular because of their great nutritional content, flavor, and taste. Since fruit juices are high in water, fiber, vitamins, sugar, proteins and phytochemicals,

they are well known for their variety of health benefits to people[9].

The consumption of fruit juices has increased nowadays, influencing directly on the economy in a positive way but the outbreaks of food borne diseases in the negative way[7]. Fruit juices sold by street vendors contribute to food safety concerns. Contamination of juices sold by juice sellers is very common due to their poor hygienic knowledge, use of unhygienic water, unclean hands and utensils, handling of raw materials in an improper manner, process of preparation, swarming of flies and the environment of preparation and selling[5].

Large diverse populations of bacteria are present in fresh fruits. Fruits and fruit juices are spoiled by causative microbial agents like bacteria, yeasts and molds. The low pH of most fruits contributes to its spoilage. For many years, food borne pathogens in fruits have been recognized occasionally. Fruits can become contaminated and spoiled in a variety of ways and at a variety of times during the pre-harvest and post-harvest processes. Many countries have reported outbreaks of food-borne illnesses in recent years, and pathogens like enterohaemorrhagic *Escherichia coli* O157:H7 and *Salmonella* have become frequently transmitted by fruit juices[11].

Fruits and vegetables are also contaminated by fungi as a result of substandard handling procedures across the food supply chain, storage conditions, distribution, marketing techniques and transportation. The fungal pathogens such as *Aspergillus*, *Rhizopus*, *Candida*, *Fusarium*, *Phytophora* and *Mucor* have been detected in most common fruits[1]. Mycotoxins produced by the growth of heat resistant molds in fruit products are a major public health concern. Mycotoxins are the toxic metabolites of filamentous fungi and have been found in a variety of foods, including fruits and fruit products. Ingestion or inhalation of mycotoxins leads to mycotoxicoses in men with varying degrees of toxicity. Growth of mycotoxin producing fungi has not only caused serious health problems, but it has also caused significant economic losses, with significant ramifications for the import and export of fruit products[4]. Some species of *Penicillium* and *Aspergillus* produce patulin, a mycotoxin that is immunotoxic, genotoxic, neurotoxic, carcinogenic, and has gastrointestinal impacts on both human and animal health[10]. Ochratoxin A is a nephrotoxin produced by *Aspergillus ochraceus and Penicillium verrucosum* causes kidney failure and is a human carcinogen[3].

Sample collection and processing :

A total of 50 samples of 5 varieties of fresh fruit juices were collected outside schools and colleges from different locations of Chennai city. Fruit juices included in the study were those that could be consumed readily by the consumers such as Watermelon juice, Sweet lime juice, Pomegranate juice, Lemon juice and Grape juice. Fruit juice samples included were fresh fruit juices collected from fruit vendors around schools and colleges. Fruit juice samples tested were those that were prepared with or without ice. The samples were selected based on consumer demand. All samples were collected in sterile containers, transported in an ice box, and analyzed within an hour of collection.

Enumeration of microorganisms :
Total aerobic count :

About 1ml of fresh fruit juice samples were serially diluted in saline to obtain 10^{-1} to 10^{-8} dilutions. 0.1 ml of each dilution was pipetted out and poured on to the Nutrient agar (NA) and Sabouraud's Dextrose agar (SDA) plates and incubated for 24 hours at 37°C for NA and 2 to 7 days at 25°C for SDA plates respectively. Duplicate plates were maintained. After incubation, the number of colonies on the plates were counted and expressed as Cfu/ gram, using the calculation given below,

$$\text{Total aerobic count} = \frac{\text{Average no. of colonies}}{\text{Volume of the sample}} \times \text{Dilution factor (cfu/g)}$$

Isolation of bacteria,
Direct examination and
Macroscopic appearance :

The macroscopic characteristics of various colonies on Nutrient agar and Sabouraud's dextrose agar were observed and recorded.

Culture :

Different colonies on NA were inoculated onto the culture media such as Nutrient agar (NA), MacConkey agar (MA), Mannitol salt agar (MSA), Eosin methylene blue agar (EMB), Thiosulphate Citrate Bile Salt agar (TCBS) and *Salmonella Shigella* Agar (SS).

Preliminary tests :

Tests such as Gram stain, hanging drop motility technique, Catalase test and Oxidase test were performed.

Identification of bacteria :

The colonies on the selective media were further processed for biochemical identification.

Biochemical tests :

All the biochemical tests were performed with suitable positive and negative controls. The tests performed were Indole test, Methyl red (MR) test, Voges Proskauer (VP) test, Citrate utilization test, Triple Sugar Iron (TSI) test, Urease test, Nitrate reduction test and Oxidation and Fermentation (OF) test.

Sugar fermentation test :

This test was performed to detect the production of acid and gas on fermentation of sugars used such as glucose, fructose, lactose, sucrose and mannitol. Suitable controls were included in the test.

Identification of fungal isolates
Direct examination and
Macroscopic appearance

The macroscopic characteristics of the different colonies on Sabouraud's Dextrose Agar [SDA] plates were observed and recorded.

Culture :

To obtain pure culture and for further identification, each fungus colony was inoculated onto Sabouraud's Dextrose Agar plates. After 5 days of incubation at 25°C, the plates were examined and the colonies were identified.

Lactophenol cotton blue staining :

After macroscopic examination of the fungal culture carefully on Sabouraud's Dextrose Agar plate, the colony was picked up with a sterile loop and placed on the LPCB stain. With the help of a tweasing needle, the specimen was tweased well without disrupting the intact morphology of the fungus. A clean coverslip was placed over the specimen and excess stain was removed with the help of a filter paper and observed under 45X.

A total of 50 samples of 5 varieties of fresh fruit juices were collected around schools and colleges from different locations in Chennai city were examined and the following results were obtained.

Total Aerobic Count :

The average of the total aerobic microbial count of the 5 varieties of fresh fruit juices obtained from Nutrient agar (NA) and Sabouraud's dextrose agar (SDA) were summarized by taking an average count in Tables-1 and 2.

Table-1. Total bacterial count in fruit juice samples

Sample type	Total bacterial count		Average count Cfu/ml
	1	2	
Watermelon juice	21×10^6	27×10^6	24×10^6
Sweet lime juice	19×10^7	17×10^7	18×10^7
Pomegranate juice	16×10^6	11×10^6	13.5×10^6
Lemon juice	15×10^5	19×10^5	17×10^5
Grape juice	29×10^7	27×10^7	28×10^7

Table-2. Total fungal count in fruit juice samples

Sample type	Total bacterial count		Average count Cfu/ml
	1	2	
Watermelon juice	41×10^2	36×10^2	38.5×10^2
Sweet lime juice	22×10^4	21×10^4	21.5×10^4
Pomegranate juice	36×10^4	44×10^4	10×10^4
Lemon juice	12×10^2	20×10^2	8×10^2
Grape juice	21×10^2	7×10^2	14×10^2

The fungal pathogens isolated from various fruit juice samples with their percentage prevalence are tabulated in Table-3.

Table-3. Percentage of fungal pathogens isolated

S. No	Fungal isolates	Percentage prevalence
1	*Yeast*	100
2	*Mucor species*	16
3	*Aspergillus niger*	28
4	*Penicillium species*	12
5	*Rhizopus species*	8
6	*Aspergillus fumigatus*	8

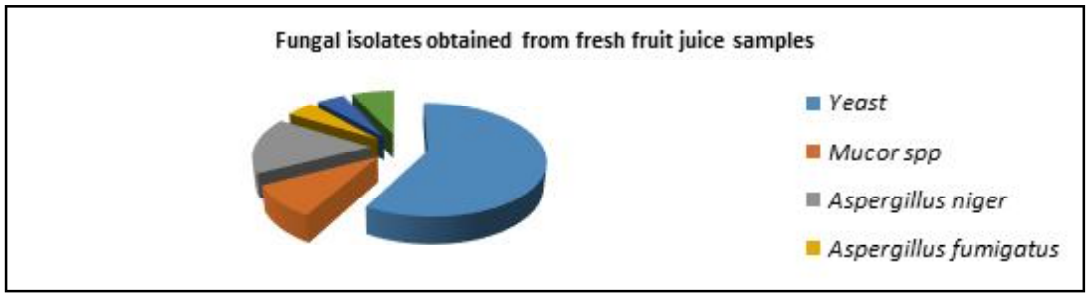

Figure 1. Percentage prevalence of fungal isolates in fresh fruit juice samples

The bacterial pathogens isolated from various fruit juice samples with their percentage prevalence are tabulated in Table-4.

Table-4. Percentage of bacterial pathogens isolated

S.No	Bacterial isolates	Percentage prevalence
1	*Bacillus species*	56
2	*Escherichia coli*	20
3	*Klebsiella pneumoniae*	8
4	*Pseudomonas aeruginosa*	4

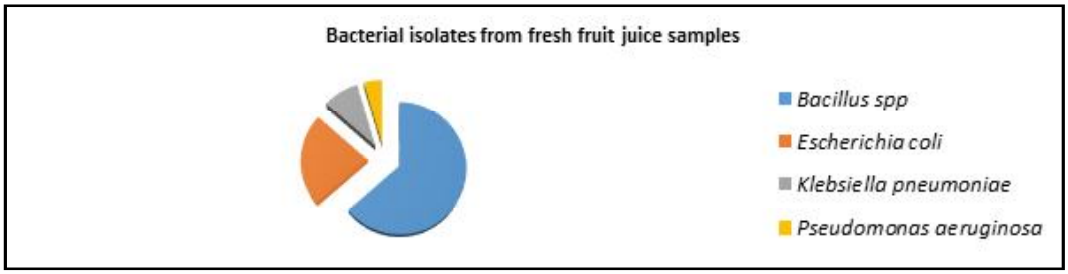

Figure 2. Percentage prevalence of bacterial isolates in fresh fruit juice samples

Table-5. Identification tests for bacterial isolates

Bacterial Isolate	Gram stain	Mot	Spore	Cat	Oxi	Ind	MR	VP	Cit	TSI
Escherichia coli	-	+	-	+	-	+	+	-	-	A/A+G
Klebsiella pneumoniae	-	-	-	+	-	-	-	+	+	A/A+G
Pseudomonas aeruginosa	-	+	-	+	+	-	-	-	+	K/K
Bacillus spp	+	+	+	+	-	-	-	+	+	ND

Mot - Motility test, Cat - Catalse test, Oxi - Oxidase test, Ind - Indole test, MR- Methyl Red test, VP- Voges Prauskeur test, Cit-Citrate test, TSI - Triple Sugar Iron test, K/K - Alkaline slant/Alkaline butt, A/A+G -Acid/slant/Acid butt with Gas production, ND- Not Done

Fresh fruit juices contain microorganisms that may be harmful to public health. The present study has shown that the majority of fruit juices collected around schools and colleges from various locations in Chennai were contaminated and many potential sources of contamination were identified. All the samples analyzed had poor microbiological quality. The results clearly showed that the aerobic colony count (ACC) (Cfu/ml) with respect to bacterial counts for all the samples in all the localities in Chennai were above the acceptable ranges in accordance with the recommendations for the microbiological quality of ready to eat foods *i.e* ACC>10^5 [6].

Low pH of fruit juices attribute to spoilage by high levels of mould and yeast. The yeast was found in all of the fresh fruit juice samples tested in the study. *Candida*, *Pichia*, *Rhodotorula*, *Torulopsis*, *Saccharomyces*, *Zygosaccharomyces* and *Trichophyton* are the yeast genera responsible for fruit juice spoilage[2]. Yeasts such as *Rhodotorula*, *Pichia*, *Saccharomyces*, *Candida parapsilosis*, and *C. krusei* were also found in orange and sweet orange juices[7].

The prevalent types of mold recorded in fresh fruit juice samples belong to *Asperigillus niger* (28%) followed by *Mucor* spp (10%), *Penicillium* spp (12%), *Aspergillus fumigatus* (8%) and *Rhizopus spp* (8%) respectively whereas in another study, the most frequent moulds encountered were *Aspergillus flavus*, *A. tereus* and *Penicillium islandiarm*[7]. Fungal pathogens such as *Aspergillus*, *Rhizopus*, *Candida*, *Fusarium*, *Phytophthora* and *Mucor* have been isolated in most common fruits[1].

The present study showed the presence of pathogenic bacteria such as *Bacillus* spp, *Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa. Bacillus spp* was the leading bacteria isolated from 56% of the tested fruit juice samples followed by *Escherichia coli* (20%), *Klebsiella pneumoniae* (8%) and *Pseudomonas aeruginosa* (4%) in fresh fruit juice samples respectively. This is similar to the study in which *Bacillus cereus* was the leading bacteria isolated from freshly squeezed fruit juice samples[8].

Escherichia coli was isolated from 20% of the tested samples. Contaminated water supplies used to wash utensils or dilute fruit juices could be the main source of *Escherichia coli* contamination. The presence of *E. coli* and other coliform bacteria could be attributed to insufficient hand washing by food workers and poor processing practices[11]. *Pseudomonas aeruginosa* may be present due to poor personal hygiene, unsanitary surroundings, vehicular activity and proximity to sewage.

This work proved the presence of pathogenic bacteria and fungi in fresh fruit juice samples collected around schools and colleges in Chennai with a high bacterial and fungal load. Pathogenic microorganisms found in fresh fruit juices are a clear indicator of food-borne outbreaks. Because consumption of fresh fruit juice cannot be stopped on unsanitary grounds, and selling such items in streets cannot be controlled because it provides a source of income for street vendors, government health agencies must take measures to educate vendors about food safety and hygienic practices, as well as enforce adequate guidelines for street food vending. To avoid infection, the quality of fruit juices intended for human

consumption should be monitored on a regular basis, and shops that contaminate juices should be avoided. Other important factors to consider are the time of preparation of fruit juices, the personal hygiene of street vendors, and the area surrounding vending sites.

Future Scope :

This study can provide information to bring about safety and quality in shops and streets that sell juices with poor quality which can be prone to food borne outbreaks in children and young adults studying in schools and colleges.

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