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Microbial Analysis of Fresh Fruit Juice Available in the Market

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ABSTRACT

Juices made from fresh fruits that were improperly prepared are one of the main causes of food borne illnesses. In this study, the microbial qualities of some freshly prepared fruit juices were assessed. A total of 200 fresh fruit juice samples of mango, pineapple, orange, pomegranates and sugarcane were collected. The pour plate method was used to determine the total viable count of the sample. The pathogenic bacteria investigated were *Bacillus E. coli, S. aureus, Lactobacillus, Acetobactor, Salmonella* and *Shigella* using appropriate growth media. *Aspergillus niger, Aspergillus flavus, Penicillium* sp., *Fusarium, Colleotrichum* and *Alternaria* are the predominant moulds found in fruit juices. Yeast isolates such as *Saccharomyces, Candida* and *Rhodotorula* were also observed in freshly prepared fruit juices. It was found that sugarcane and orange juices were highly contaminated with microbes. Fungal contamination was dominant over bacterial contamination. It has been determined that the microbial load in fresh fruit juices is significantly greater than the normal permissible limits, suggesting that it may play an important role in food spoilage and food-borne illnesses. There is a need for some regulations that can improve the quality of fruit juices because some of the microorganisms found in these juice samples can cause disease in human and also produce mycotoxins which cause cancer.

Keywords Fresh fruit juice, Bacterial, Fungal, Contamination, Food-borne illnesses.

INTRODUCTION

There is clear evidence that eating fresh fruits or drinking their juices has positive health and nutritional effects. Fresh fruits are vital parts of the human diet. The most common component of the fruit juice is water, carbohydrates (comprise sucrose, fructose and glucose), protein, vitamins and minerals. Fruit beverages have gained popularity worldwide as they offer nutritional benefits and contain flavonoids that have been shown to inhibit the development of cancer cells (Pinto *et al.* 2022, Faizi 2022). However, improper preparation of these beverages can pose health risks due to the growth of microbes. To ensure the protection of public health, manufacturing processes must

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adhere to significantly stricter guidelines (Ahmed *et al.* 2018). Asghar *et al.* (2018) further demonstrated that apple, carrot, orange, and sugar cane juices had a substantial presence of total and fecal coliforms. Fresh fruit and vegetable juices that have been improperly prepared are known to be one of the principal reasons for food-borne diseases. Each year, approximately one out of every ten individuals experiences the impact of food borne illnesses, resulting in 420,000 fatalities (Petruzzi *et al.*2017). In addition to reducing the risk of numerous diseases, a well-balanced diet full of fruits and vegetables is important for preventing vitamin C and vitamin A deficiency. Juice preparation includes a large involvement of human manipulation, which has been reported to contaminate the juice with microorganisms (Artes and Allende 2012). Pesticides and the use of non-food grade equipment in the processing line can both lead to chemical contamination of the environment, which can affect fruit juice. Fruits and vegetables are generally exposed to microbial contamination through handling at harvest or during postharvest processing as well as through contact with soil, dust, and water. According to study of Bhardwaj in (2013), the presence of bacteria, yeast, and mold in untreated fruit juices and pulp was found to be significantly high. Fresh fruit and vegetable juices that have been prepared improperly are now known to be an emerging source of food related diseases. If contaminated, freshly squeezed fruit juices go through few or no steps in the process step that would lower pathogen levels. Low pH is not ideal for microbial growth, but it is known that pathogenic microorganisms can survive in fruit juices, adapt to the acidic environment, and spread outbreaks of food-borne illnesses. It appears that acidic nature of some juices does not always assure that pathogens like *E. coli, Salmonella*, viruses and *Cryptosporidium* could not survive in them. The quality of fruit juices is strictly retained in developed nations by a number of laws and regulations, but in many developing and undeveloped nations, the lack of legal enforcement has caused an absence of concern on the part of manufacturers for the microbiological safety and hygiene of fruit juice. As a result, it is a serious issue that some human diseases have recently been spread through juice and other beverages. Therefore, this study was aimed at determining the degree of fresh fruit juice contamination by microbes and asses level of awareness about food safety and pathogenic microorganisms as well as the hygienic conditions of the fruit juice.

MATERIALS AND METHODS

Study area

The study was conducted during summer in Patna district. Patna is located in Bihar, India. For the present study, crowded shops were randomly selected. Eight location viz., PMCH, NIT more, Bhikhna Pahari, Hanuman Nagar, Saidpur, Dak Bunglow, Boring Road and Kurji were chosen for the study.

Sample size

200 sample of five different fruit juices (Sugarcane, Mango, Orange, Pine apple and Pomegranates) from eight sites, i.e. Five samples of each juice type were collected. This was achieved by collecting five samples for each type of fruit juice in five rounds.

Sample collection

Two hundred samples of sugarcane, mango, orange, pine apple and pomegranates of locally prepared unpasteurized fruit juices were collected from local juice shops of Patna in five rounds i.e. five juice samples (one sample of sugarcane, mango, orange, pine apple and pomegranates) from each juice shop in different days. Every sample was collected aseptically from juice shop in sterile beakers with proper labeling and brought instantly to the Plant Pathology and Microbiology Laboratory, Department of Botany, Patna University, Patna where they were processed immediately.

pH analyses of fresh fruit juices

A digital pH meter was used to determine the pH of juice sample.

Microbiological analysis

Bacterial isolation and identification

Samples were poured on Mac-Conkey agar and nutrient agar media. They were incubated at 36 ± 2°C for 24 hours. Identification of pure isolates was done on the basis of morphological and cultural characters.

Table 1. Total viable count (TVC) and total coliform count (TCC) in samples of fruit juice.

Sl. No.	Types of juice	Mean TVC (CFU/ml)
1	Orange	209×10^5
2	Mango	164×10^5
3	Pineapple	173×10^5
4	Sugarcane	236×10^5
5	Pomegranates	138×10^5

The isolates were identified by Bergey´s Manual for Determinative Bacteriology (Whitman *et al.* 2012).

Total viable count (TVC)

The pour plate method was used to determine the total viable count of the sample. The sample were appropriately diluted and plated on nutrient agar plates in triplicates and incubated at 36±2°C for 48 h. After the incubation period, the plates were examined for the presence of distinct colonies, and the actual numbers of bacteria were calculated as colony forming units per ml (cfu/ml). The plates with countable colonies were counted and the data was utilized to determine the number of cfu/ml of microbes present in the sample (Table 1).

Fungal isolation and identification

Fruit juice samples were poured on Potato Dextrose Agar (PDA) media at 27 ± 2°C for five days. After pure culture, the fungal colonies were identified on the basis of morphological and microscopic characters with the help of Laboratory Manual of Barnett and Hunter (1972).

RESULT AND DISCUSSION

In the present study, 200 samples of freshly prepared juices (40 samples each of mango, orange, pine apple, sugarcane and pomegranates) were examined for microbiological analysis. Fungi (molds and yeasts) were more frequently found than bacteria in the current study which is due to the low pH levels and high sugar content. Fruit juices contained a total of 458 bacterial, 182 yeast and 510 mould isolates, which were categorized into 7 bacterial species, 3 yeast species and 6 mould species based on phenotypic traits. The frequency of bacteria, yeasts and moulds is described (Table 2). Contamination in sugarcane juice was higher than all juices followed by orange juice, pineapple juice, mango juice and pomegranate juice (Fig. 1). In Tables 3-4, the morphological and biochemical characteristics of bacteria were described. Table 5 summarized the colonial and microscopic characteristics of various moulds. Table 6 provided descriptions of the morphological features of yeasts.

Table 2. Frequency of microbial isolates in different fruit juices sold by local vendors.

	Isolates	Orange	Mango	Pineapple	Sugarcane	Pomegranate	Freq (%)
Bacteria	*Bacillus*	13	07	09	22	16	33.50
	E. coli	12	23	26	29	30	60.00
	S. aureus	21	28	18	31	07	52.50
	Lactobacillus	19	03	08	25	00	27.50
	Acetobactor	03	05	11	14	02	17.50
	Salmonella	00	01	00	02	00	01.50
	Shigella	07	04	37	13	12	36.50
Yeast	*Saccharomyces*	23	17	11	26	03	40.00
	Candida	07	12	00	02	00	10.50
	Rhodotorula	29	16	03	32	01	40.50
Mould	*A. flavus*	36	23	18	31	25	66.50
	A.niger	20	07	12	23	18	40.00
	Alternaria	17	00	26	32	02	38.50
	Fusarium	08	02	06	11	00	13.50
	Colletotrichum	26	32	00	03	14	37.50
	Penicillium	28	35	35	14	06	59.00

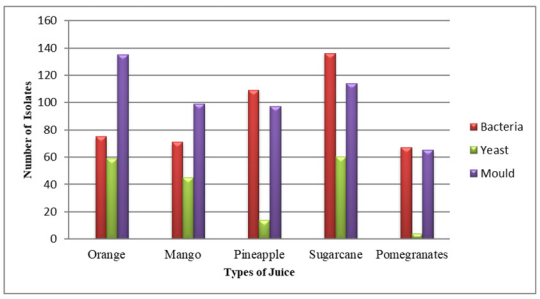

Fig. 1. Showing total number of bacteria, yeast and moulds isolated from fresh juice samples.

materials rather than a closed dustbin. Glasses and cutleries were not frequently washed. The nutrients, temperature, pH, structures, water activity, structures, relative humidity and atmosphere are factors which determine the microbial colonization of juices. Contamination in sugarcane juice was higher than other juices. The study also found that the fungal contamination was dominant over bacterial contamination due to low pH values and high sugar content. The juice underwent microbial analysis specifically focusing on the yeast and mold count (Adedeji and Oluwalana 2013, Michael (2021). *Aspergillus niger, Aspergillus flavus, Penicillium* sp., *Fusarium, Colleotrichum* and *Alternaria* are the predominant moulds found in fruit juices. Mycotoxins, which pose a serious threat to human health, are produced by some of these moulds. In the research conducted in (Ogodo *et al*.2016) found that the presence of *Aspergillus* species, *Rhizopus* species and *Penicillium* species in commercially packaged fruit juices. Notably, *Penicillium* sp. and *Aspergillus* sp. were capable of producing mycotoxins, posing potential health risks for consumers. The highly presence of *E. coli* in fresh fruit juice samples

Table 3. Morphological characteristics of bacteria isolated from fruit juices.

Sl. No.	Isolates	Color of the colony	Gram reaction	Shape of isolate	Configuration	Margin	Elevation
1	*Bacillus*	White	+	Rods in chain	Circular lobate	Irregular	Flat
2	*E. coli*	Mucoid	-	Rod	Circular	Entire	Slightly raised
3	*S. aureus*	Yellow	+	Cocci in bunch	Circular	Entire	Convex
4	*Lactobacillus*	White	+	Rod	Circular	Entire	Raised
5	*Acetobactor*	Pale	-	Rod	Circular	Entire	Flat
6	*Shigella*	Colorless	-	Rod	Circular	Entire	Convex
7	*Salmonella*	Pale yellow	-	Rod	Circular	Entire	Convex

This study was done to determine the microbial contamination of locally prepared fresh fruit juice sold in Patna district of Bihar. Furthermore, factors associated with contamination such as hygiene and safety conditions. The study found that almost all juices were contaminated with microbes. This is because the juice shops put fruits on the shelf and fruits were chopped on table and stored in an open bucket. On the other hand, the juice shops did not have hand washing facility before and after juice extraction. They were using open sacks or plastic bags for collecting waste

Table 4. Biochemical characteristics of bacterial isolates of fruit juices.

Sl. No.	Isolates	Citrate	Oxidase	Catalase	Mortality	Indole	Urease	Starch hydrolysis	Glucose	Sucrose	Lactose	Nitrate reduction	Methyl red	Voges-proskauer
1	*Bacillus subtilis*	+	-	+	-	-	-	+	-	-	-	+	-	+
2	*E. coli*	-	-	+	+	+	+	-	-	+	+	+	+	-
3	*S. aureus*	-	-	+	-	+	+	-	-	+	+	+	+	-
4	*Lactobacillus*	-	-	-	-	-	-	-	+	+	+	-	-	-
5	*Acetobactor*	+	-	-	+	-	-	-	+	+	+	+	+	+
6	*Shigella*	-	-	+	+	-	-	+	-	-	-	+	+	-
7	*Salmonella*	-	+	+	+	+	+	-	+	-	-	+	+	-

'+' = Positive, '-' = Negative.

Table 5. Morphological details of yeast isolates of fruit juices.

Sl. No.	Isolates	Color of the colony	Macroscopic features	Microscopic features
1	*Saccharomyces*	Pale white	Irregular, circular	Spherical, irregular budding
2	*Candida*	White	Regular, circular	Long cylindrical, irregular budding
3	*Rhodotorula*	Pink	Regular, Circular	Ellipsoidal, irregular budding

Table 6. Morphological details of moulds isolates of fruit juices.

Sl. No.	Isolates	Color of the colony	Macroscopic features	Microscopic features
1	*A. flavus*	Yellow green	Irregular, circular	Separate conidiophores. conidia globose to subglobose , phialides uniseriate
2	*A.niger*	Black	Irregular, circular	Smooth walled conidiophores, septate hyphae, globose conidia arranged in basipetal manner
3	*Alternaria*	Brownish black	Regular, circular	Septate conidia arising in chains in acropetal manner
4	*Fusarium*	Pinkish white	Regular, circular	Simple conidiophores, short or branched irregular phialides; macroconidia several celled slightly curved, microconidia 1-celled, ovoid in chains
5	*Colletotrichum*	Grey white	Regular, circular	Simple conidiophores, single celled elongated straight or curved conidia
6	*Penicillium*	Bluish green	Irregular, circular	Conidiophores borne from aerial hyphae with; bearing terminal penicilli; terverticillate but frequently biverticillate or irregular

assumes faecal contamination, which may be related to unawareness as well as poor hygiene practices of vendor and cross-contamination of juice samples. The presence of *S. aureus* in fruit juices may be due to contamination from unsafe handling as they resident on the hair, skin, throats, and nasal passages of people. Fungal contamination is due to storage of fruits in open and keeping the wastes uncovered. This outcome was consistent with a study by Bello *et al.* (2014) that claimed to have isolated *Staphylococcus aureus* from avocado juice. *Staphylococcus aureus* and *E. coli* were observed by Adesetan *et al.*,(2013) in pineapples, pawpaw, watermelons and coconut juice.

CONCLUSION

In the current study, it can be concluded that drinking freshly made fruit juice in the market cause several microbial infections. This is because nearly all types of freshly made juice were collected from different areas of Patna district were found to contain large numbers of bacterial and fungal species. Information about food borne illnesses like hepatitis, cholera, typhoid should be provided to consumers. In the fruit juice market, regular inspections and appropriate training may be the most effective strategy to reduce health risks. Therefore, it is suggested to provide instructions by government for hygiene and sanitation to protect the health of the consumers.

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