



# MICROBIOLOGICAL ANALYSIS OF STREET VENDED FRUIT JUICE IN KALIKIRI

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## Abstract

Street vended fruit juices are on high demand because of their nutritive value and mineral and vitamin content but the concern on food safety is very high. The microbiological analysis of street vended fruit juices is an important aspect of food safety, as these beverages are often sold in open-air markets and are susceptible to contamination. This study aimed to investigate the microbial load and diversity in street vended fruit juices in Kalikiri town. Samples were collected aseptically from the local street vendor and were analyzed for total viable count, and total coliform count. Then these samples were further screened to produce the extracellular enzymes like amylase and protease. The results showed extent microbial contamination in street vended fruit juices. These findings highlight the need for improved hygiene and sanitation practices among street food vendors and increased awareness among consumers about the potential risks associated with consuming street vended fruit juices.

**KEY WORDS:** Pineapple juice, most probable number, microbial analysis.

## 1.Introduction

India produces about 9 million tons of fruits every year growing at a rate of 12% per annum. The total market potential for fruit juices is 230 million Liters including both packed and freshly made fruit juices. In many communities, fruit juices are becoming an important part of modern human diet because they are highly nutritious and offer a good taste and variety of nutrients (Jimma et al., 2022). Fruit juices play a role in detoxification of human body and also have a great role in improvement of blood lipid profile in patients of

hypercholesterolemia (Mahapatra *et al* 2015). The demand of fresh juices has increased day by day and is preferred throughout the world (Pem D *et al* 2015). Also, fruit juices are an instant source of thirst quenchers for travellers. The concern on food safety is on street vended fruit juices due to improper handling practices of raw materials, unhygienic water, unclean utensils and hands, preparation process (Hez *et al.*, 2018). Fruit juices sold on roadside were found to be contaminated with faecal coliforms like *Escherichia coli*, *Staphylococcus aureus*, *Shigella sp* (Sharma *et al.*, 2020). This higher incidence of faecal coliforms in fruit juices shows that water and ice used for juice preparation may be of poor quality (Tasnim *et al* 2010). Thus, there have few been instances of food poisoning in fruit juices but microbial spoilage is very common. Bacteria isolated from fruit juice samples included *Escherichia coli*, *Micrococcus sp.*, *Staphylococcus aureus*, *Salmonella sp.*, *Shigella sp.* and *Bacillus sp.* Fungi isolated included *Aspergillus sp.*, *Rhizopus* (malik *et al.*, 2020) In view of the high demand of these fruit juices, a microbiological analysis was carried out to assess the quality and acceptability of fruit juices for human consumption and the aliquots of all the dilutions were plated on nutrient agar plates by pour plate method .

## 2. Materials and Methods

Petri dishes, conical flasks, test tubes, measuring cylinder, pipette, autoclavable, Weighing balance, glass rod, pH meter, Incubator, Fridge, test tube stand, aluminum foil, autoclavable covers, rubber bands, tray, paraffin, marker, cotton.

### 2.1 Chemicals

Nutrient Agar, plate count agar, Starch agar, skimmed milk agar and lactose broth, ethanol, distilled water, sodium chloride were purchased from Hi media.

## 3. Methodology

### 3.1 Collection of the sample

Sample (Pineapple juice) were collected from the street vendors in sterilised cups. Then samples were analyzed within one hour after collected from street vendors.

### 3.2 Isolation of microorganisms from street vended fruit juices.

1 ml of sample was diluted as a 1:10 dilution with 9 ml of 0.9% NaCl. From this diluted sample 0.5 ml was inoculated in nutrient Agar, plate count agar for the total viable count, and incubated at 37 C for 24 h. The mean number of colonies counted was expressed as colony forming units (CFU)/ml.

### 3.3 Coli form count by MPN method:

The coliform count in the fruit juices were performed by MPN (Most Probable Number) by using three tests, namely 1. presumptive Test, 2. Confirmative Test, 3. Completed Test.

In presumptive test Inoculate lactose broth or lauryl tryptose broth, fermentation tubes & incubate at  $24 \pm 2$  hrs at  $35 \pm 0.5^\circ\text{C}$ . one loopful of sample from each positive tubes obtained from presumptive test is inoculated in respective tubes containing Brilliant green lactose bile broth and incubated for 24 hours at  $35.5^\circ\text{C}$ . It is a final test in which a loopful of sample from positive confirmatory tubes is streaked on Eosin methylene blue agar or M-endo agar and incubated for 24 h. Finally, the most probable number (MPN) of coliforms per mL was calculated from the number of tubes showed gas and determined according to the MPN table (ISO 4831:2006).

### 3.4 Screening of extracellular enzymes producing microbes.

Microbes isolated from the pineapple juices were then used to screen the production of extracellular enzymes.

**3.4.1 Amylase:** In the starch hydrolysis, the starch agar media was prepared, inoculate the culture and incubate the media at  $37^\circ\text{C}$  for 3-5 days. Amylase producing microbe was identified with zone of clearance with dark blue background upon the addition of iodine (Shaw et al. 1995).

**3.4.2 Protease:** Milk agar is composed of nutrient agar with milk that contains casein a substrate for proteolytic enzymes. Organism with protease activity will form clear zone around their growth in the plate (APHA 1998).

## 4. Results and Discussion

### 4.1 Total viable count

The total viable count from the freshly prepared pineapple juice was found to be  $4 \times 10^7$  CFU/ml. The number of total viable count was high in the street vended fruit juices. Similar count was also found in other juices that was reported (Suguna, M et al., 2011). this might be due to the contamination from raw materials and equipment, improper handling, prevalence of unhygienic conditions. As far as we are aware, there is no standard for the maximum number of microorganisms that can be present in fruit juices provided in India. The maximum count allowed for total coliform, yeast, and mould colonies is  $1 \times 10^4$ , 100, and  $1.0 \times 10^3$  CFU/ml according to the approved standards for fruit juices sold in the Gulf region (Gulf Standards, 2002).

### 4.2 Coliform count by MPN method

Bacteriological examination of water for total coliform count was done by MPN method for the pineapple juices. From the MPN index table the number of positive tubes is 4, 2, and 1. So the MPN index is 26. Hence the coliform result of the sample was found to be 260 coliforms per 100 mL.

### 4.3 Screening of extracellular enzymes producing microbes.

**Amylases:** Out of the four isolates only one isolates showed the zones of clearance with around the bacterial colonies with dark blue background.

**Proteases:** Similarly, one isolate showed the zone of clearance on the milk agar media.

## 5. Conclusion

In this study the microbial load of the fruit juices examined in this study exceeded the limits established in various regions of the world for fruit juices. It is obvious that our fruit juices had colony counts of the microbial groups that were significantly higher than the norm. However, these high counts could be harmful to consumers' health, particularly if pathogenic species are present in the fruit juices that will be consuming.

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