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Bacteriological analysis of locally vended fresh fruit juices in Khulna city, Bangladesh

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Abstract
Fresh fruit juice contains high levels of natural vitamins, sugars and fibers that are crucial for human health. Fruit juice vending is a widespread business in Bangladesh, where street vendors offer a variety of locally prepared fresh juices in bustling urban areas and markets. Consumption of vended fruit juices worldwide has often been allied with foodborne illnesses, emphasizing the importance of concomitant health risk assessment. The study was focused on the bacteriological assessment of fruit juices that were vended at various locations in Khulna city. Raw mango, ripe mango, apple, sugarcane, pineapple, malta, lemon, papaya and tamarind juice were collected from vendors of popular selling areas in Khulna. The highest bacterial count $2.18x10^4$ CFU/ml was found in lemon juice (Khulna university campus) and tamarind juice (Batiyaghata) and the lowest $0.57x10^4$ CFU/ml was in ripe mango juice (Khulna university campus hall road). Different biochemical tests reveal prevalence of *Proteus* spp. was in raw mango, pineapple and malta juices. *Staphylococcus* spp. was found in ripe mango and lemon juices; *Clostridium* spp., *Bacillus* spp. and *Enterococcus* spp. were found in apple, sugarcane and tamarind juices respectively. 2.12×10^4 CFU/ml coliform bacteria (*Escherichia coli*) was found in papaya juice (from Gollamari) which is higher than the maximum microbiological limit (10^3 CFU/ml) for fresh fruit juices (GSO, 2014). The existence of diverse bacterial species in the vended fruit juices may cause diarrhea, vomiting, stomach ache, skin infection etc., which asserts the importance of adequate monitoring to safeguard consumers safety and improved health.

Keywords: Fruit juice, colony forming unit, gram staining, biochemical characterization, health risk

Introduction
Millions of people enjoy a diverse type of drinks that are vended by street vendors around the world. Fresh fruit juices are prepared from seasonal fresh fruits and are rich in natural vitamins, sugars, and fibers, which are essential for human health [1]. Natural phytonutrients, vital vitamins, and minerals found in fresh fruit juices help prevent a variety of disorders in humans. They are a significant source of flavan-3-ols, flavanols, polyphenols, anthocyanins, and vitamin C, all of which are vital for promoting health [2]. In addition, fruit juice prevents urinary tract infections, congestive heart failure (CHF), and breast cancer [3].
Fresh juices have a huge amount of nutrients but unhygienic preparation of juices makes them potential source of microbial contaminants [4]. However, in the absence of adequate hygiene standards, it provides a perfect opportunity for the growth and survival of bacteria and many types of parasitic and saprophytic fungi. Uddin *et al*. reported that there is a link between the consumption of vended fruit juices and food-borne diseases [5].
As fresh fruit juices are easily perishable, they become the perfect site for a variety of harmful microbes to flourish and multiply [6]. Fruit juices can be contaminated with *Escherichia coli*, *Salmonella*, *Staphylococcus aureus*, *Enterobacter* spp., *Klebsiella*, and *Serratia* species. These pathogens cause typhoid fever, food poisoning, gastroenteritis, enteric fever, and diarrheal disease [7].
The temperature in Khulna, a densely populated city, ranges from 30 to 42 °C from March to October. During the summer months, a significant part of the population (about 94%), involving all age groups and financial classes, including tourists, drinks these freshly squeezed and pressed juices. In order to meet the needs of busier lifestyles and to get freshness, the consumption of fresh fruits is increasing continuously in Khulna.
Unfortunately, the microbiological quality of the offered juices remains uncertain in busy marketplaces, parks, and roadside shops. Hence, it is essential to evaluate the microbiological quality of the fresh fruit juices being sold to customers from the perspective of public health. Therefore, this study investigates the presence of bacteria in vended fresh fruit juice from several sources at Khulna city.

Materials and Methodology

Khulna City Corporation occupies an area of 40.79 square kilometers [8]. People in Khulna city often consume fresh fruit juice vended in different marketplaces, especially during hot humid days. This research emphasizes assessing the bacteriological quality of fresh fruit juices from various sources within Khulna city, Bangladesh.

Nine varieties of fresh fruit juices were collected from local vendors, which were vended in popular areas of Khulna city. Raw and Ripe Mango juices were collected from Khulna University Hall Road. Apple, Sugarcane, Pineapple, Malta, Lemon, Papaya and Tamarind juices were collected from Dakbanla, Nirala, 7 no. Ghat Khulna, New Market, Khulna University Campus, Gollamari and Batiaghata respectively.

After collection samples were immediately transferred to the laboratory aseptically using the FDA's recommended methodology and subjected to further investigation [9]. Following the serial dilution up to 10^{-7} samples were plated on Nutrient Agar Medium and incubated for 24 hour at 37 °C to count Total Viable Count (TVC) of bacteria [4]. Total Coliform Count (TCC) and Total Staphylococcal Count (TSC) were performed on Membrane Fecal Coliform (m-FC) agar and Mannitol Salt Agar (MSA) respectively [10]. Bacterial isolates were predicted using following biochemical tests: Oxidase test, Catalase test, Citrate utilization test, Urease test, Voges-Proskauer (VP) test, TSI test [11].

Results

Vendors were questioned about the water used for the preparation of fresh juices. Among them the maximum (56%) of vendors used submersible water to prepare fruit juices, 33% used tube well water and the lowest (11%) used filter water.

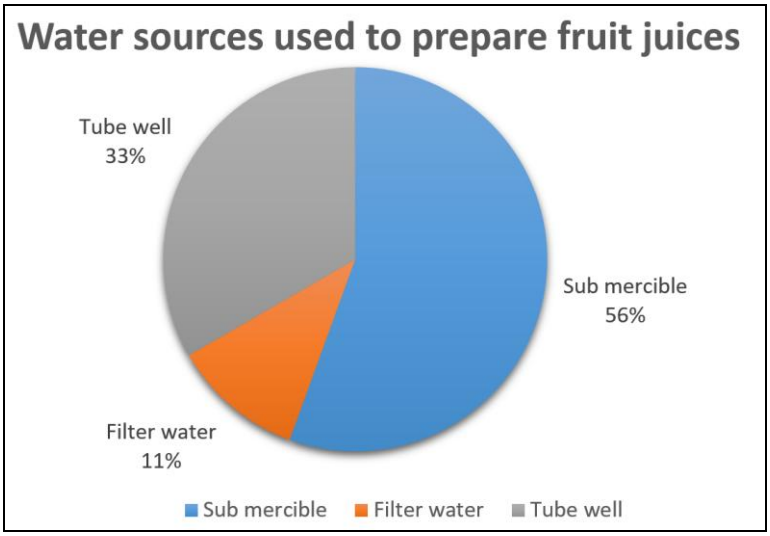

Fig 1: Water sources used to prepare fruit juices

Bacterial count in vended fresh juices on Nutrient Agar (NA), Membrane Fecal Coliform Agar (m-FC) and Mannitol Salt Agar (MSA) are presented in Table 1. All the fruit juices showed Total viable bacterial count (TVC) higher than the permitted amount on Nutrient Agar medium. The highest (2.18×10^4 CFU/ml) Total viable count (TVC) was found in Lemon juice collected from Khulna University Campus, whereas the lowest (0.57×10^4 CFU/ml) was found in Raw Mango juice collected from Khulna University Hall Road.

Table 1: Bacterial load in vended fresh fruit juices in Khulna City

Type of juice	Sampling area	Total viable count (TVC) cfu/ml on nutrient agar	Fecal coliform count (FCC) cfu/ml on m-FC agar	Total staphylococcus count (TSC) cfu/ml on MSA agar
Raw mango juice (n=3)	Hall road, Khulna University	1.89×10^4	0	0
Ripe mango juice (n=3)	Hall road, Khulna University	0.57×10^4	0	1.76×10^4
Apple juice (n=3)	Dak bangla	0.87×10^4	0	0
Sugarcane (n=3)	Nirala	1.36×10^4	0	0
Pineapple (n=3)	7 No. ghat	1.92×10^4	0	0
Malta (n=3)	New market	1.38×10^4	0	0
Lemon (n=3)	Khulna University campus	2.18×10^4	0	2.05×10^4
Papaya n=3)	Gollamari	2.12×10^4	1.96×10^4	0
Tamarind (n=3)	Batiyaghata	2.14×10^4	0	0
Maximum bacterial load permitted (Gulf Standards, 2000) [12]		1.00×10^4	0	1.00×10^3

Bacterial isolates were identified by observing the colony morphology, gram staining and biochemical tests. Around 67% bacterial isolates were found gram positive and 33% were gram negative.

Table 2: Bacteria isolated from vended fresh fruit juices

Name of isolates	Biochemical test	Gram staining	Name of juice
Staphylococcus spp	Oxidase (-) Catalase (+) Citrate (+) Urease (+) VP (+) TSI (K/K)	(+)	Ripe mango juice Lemon
Clostridium spp	Oxidase (-) Catalase (-) Citrate (-) Urease (-) VP (-)	(+)	Apple juice
Bacillus spp	Oxidase (-) Catalase (+) Citrate (+) Urease (-) VP (+) TSI (K/A)	(+)	Sugarcane juice
Enterococcus spp	Oxidase (-) Catalase (-) Citrate (-) Urease (-) VP (+) TSI (K/A)	(+)	Tamarind juice
Proteus spp	Oxidase (-) Catalase (+) Citrate (+) Urease (+) VP (-) TSI (A/A)	(-)	Raw mango juice Malta Pineapple
Escherichia coli	Oxidase (-) Catalase (+) Citrate (-) Urease (-) VP (-) TSI (A/A)	(-)	Papaya juice

Bacillus spp, *Clostridium spp*, *and Enterococcus spp* were found in Sugarcane juice, Apple juice and Tamarind juice respectively. While *Proteus spp* was found in maximum types of fruit juices (Raw mango, Malta and Pineapple juice). Presence of fecal coliform (1.96 x 10^4 CFU/ml) was found in papaya juice collected from Gollamari. Presence of staphylococcus were found in Ripe Mango Juice (1.76 x 10^4 CFU/ml) from Khulna University Hall road and Lemon Juice (2.05 x 10^4 CFU/ml) from Khulna University campus.

Discussion

Consumers often prefer fresh fruit juices due to their rich content of vitamins and natural minerals, and because they are widely accessible. However, according to Uddin *et al*., 2017 these juices can harbor high levels of microorganisms, which may lead to serious health risks [5].

Higher load of bacteria may be due to the use of polluted water and unhygienic preparation and maintenance of fruit juices [13]. According to Rahman *et al*. (2011) [14] in vended fresh fruit juices, total viable count of bacteria was higher than the permitted amount, which was similar to our study findings [14]. Similar study of the presence of *E. coli* in vended fresh fruit juices was reported by Subbannayya *et al*. (2007) [15] in India [15]. Warm-blooded organisms frequently have the bacteria *Escherichia coli*, or *E. coli*, in their lower intestines. While the majority of *E. coli* strains are not harmful, some can result in severe food poisoning. However, some strains, including *E. coli* that produces Shiga toxin Escherichia coli (STEC), can result in serious foodborne illnesses [16].

The incidence of staphylococci in vended fresh fruit juices has also been reported in some studies. Presence of *Staphylococcus* spp. in fruit juices might be due to contaminated hands of food handlers and dirty clothing, which ultimately indicates improper hygienic practices while food processing [17]. It may be concluded that the findings of this study may help with the monitoring of bacterial quality of various fruit juices to prevent any future outbreaks of foodborne disease.

Conclusion

This study inferred that all of the fresh fruit juices vended by the street vendors had higher bacterial load than the permitted count. Strains of *Bacillus* spp., *Clostridium* spp., *Proteus* spp. *and Enterococcus* spp. were abundant in maximum types of fruit juices. It can be concluded that the bacteriological quality of the street vended fresh fruit juices collected from different areas in Khulna city were not acceptable as fecal coliform, *E. coli* and *Staphylococcus* spp. were identified from the samples. Therefore, regular monitoring, proper maintenance and creating awareness among the street vendors about hygiene practices by monitoring committee along with the collaboration with health authorities is necessary for vending safe fruit juices in Khulna city.

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