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Bacteriological analysis of some commercially packed and fresh fruit juices available in Jessore city: a comparative look

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Abstract

Fruit juices are considered as the most consumed non-alcoholic beverages throughout the world to all age groups. In general, microbial growth in fruit juices is prohibited by using preservatives and also through other environmental factors during production. Still, there are several reports of illnesses due to the food borne diseases associated with the consumption of fruit juices at several places around the globe. So, from the point of view of public health, it is quite important to assess the microbiological quality of both fresh and commercially packed fruit juices available for the consumers. Present investigation attempted to resolve the microbiological attributes of the fruit juices collected from different areas around Jessore city. Ten fresh fruit juices and ten commercially packed fruit juices were collected. Standard plate count techniques were followed to assess total viable count (TVC), total coliform count (TCC) and total staphylococcal count (TSC) on different culture media. Samples were found to harbor viable bacteria within the range between 10^3-10^8 cfu/ml. 19 samples exhibited the presence of staphylococcai. Total coliforms were detected in 17 samples within the range of 10^3-10^6 cfu/ml which were further detected as *Escherichia coli*, *Klebsiella* spp. and *Enterobacter* spp. From all the assessment, the study demonstrates that the quality of both packed and fresh juices was unsatisfactory and hence the products need to be microbiologically controlled in order to ensure the overall health safety.

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Introduction

Fruit juices are regarded as the most preferred non-alcoholic beverage consumed by millions of people of all age groups throughout the world which may be prepared simply by squeezing the edible part of mature fruit or by extracting with water. These juices can be served as their own concentration without any processing or in processed form. Typically people can get fresh flavor from the unprocessed and unpasteurized fruit juices which can be a great source of minerals like magnesium, calcium, phosphorous, sodium and vitamins especially vitamin C (Basar and Rahman, 2007; Durgesh *et al.*, 2008). However, in the absence of good manufacturing practices, nutritionally rich components of fruit juices makes the product, good medium for microbial growth and vehicle of food borne pathogens (Ketema *et al.*, 2001). One possible source can be damaged surfaces, such as punctures, wounds, cuts and splits that occur during growing or harvesting through which pathogenic organisms can enter fruits (Tambekar *et al.*, 2009). Besides, contamination from raw materials like water used for making juices, ice, fruit pulp itself, equipments, additional processing conditions, improper handling, contribute substantially to the entry of bacterial pathogens in juices (Durgesh *et al.*, 2008).

In case of industrially processed fruit juices, it is well known that the manufacturers commonly use some preservatives to reduce the number of growth of microbes and increase the shelf life of juice products. Sulphur Dioxide (SO_2), sorbic acid, benzoites etc are common preservatives which can significantly damage the vegetative cells. Still, the presence of some indicator organisms in fruit juices is responsible for food borne diseases and even death. (Tasnim *et al.*, 2010). There are several reports of illnesses due to the food borne diseases associated with the consumption of fruit juices at several places around the globe. Such juices have been shown to harbor bacterial pathogens notably *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., *Salmonella* spp., *Shigella* spp., and *Staphylococcus aureus* (Tambekar *et al.*, 2009).

In the developed countries, the quality of fruit juices is strictly being maintained under several laws and regulations, whereas in many developing countries including Bangladesh, the manufacturers are not much concerned about the safety and hygiene of fruit juices because of lack of enforcement of the law. (Tasnim *et al.,* 2010). In Jessore city, there is always a great demand for both fresh and packed fruit juices as the climate remains hot and humid for most part of the year. So, from the point of view of public health, it is quite important to assess the microbiological quality of both fresh and commercially packed fruit juices available for the consumers. Considering the importance, this study has been undertaken to compare the quality of fresh and commercially available fruit juices by assessing their microbial load and the presence of pathogenic bacteria.

Materials and methods

Collection of samples

Twenty samples were collected, of which 10 fresh juice samples were collected from different locations of Jessore city and other 10 juice samples were commercially packed juices available in the market.

Enumeration of total bacteria

1 ml of sample was transferred with a sterile pipette to the tube containing 9 ml of normal saline to make 10^{-1} dilution. In such a way, dilution up to 10^{-7} was made. 0.1ml of each dilution was evenly spread on the nutrient agar medium and incubated at 37^0C for 24 hours. Plates were screened for the presence of discrete colonies after incubation period and the actual numbers of bacteria were estimated as colony forming unit per ml (cfu/ml). Quantitative analysis for the presence or absence of specific microorganisms was done by plating on selective media. Total coliform count (TCC) and total staphylococcal count (TSC) were performed in similar manner as described above using MacConkey agar and Mannitol Salt Agar (MSA) medium, consecutively. Estimation of bacterial load was performed by standard method (ICMSF, 1998). The microbiological condition of safety and hygiene were then assayed by comparing the obtained results with

the limit of Gulf standard (Gulf Standards, 2000) (Table 1), known as the recommended microbiological standard for fruit juices in the Gulf region (Tasnim *et al.*, 2010).

Identification of coliform bacteria

For presumptive identification of coliform bacteria isolates were randomly selected for major biochemical tests such as Triple Sugar Iron (TSI), Motality Indole Urease (MIU), Methyl-Red (MR), Voges-Proskaure (VP), Citrate Utilization, Catalase test and Oxidase tests. The tests were carried out following the standard methods. Isolates were then identified according to the Bergey's manual of determinative bacteriology (Buchanan and Gibbon, 1984) and manual for the identification of medical bacteria (Cowan, 1975).

Results and discussion

Colony morphology, phenotypic and biochemical traits of the isolates

After incubation for 24 hours, typical pink, circular, convex colonies on MacConkey Agar and yellow colonies on Mannitol Salt Agar were initially considered as coliforms and *Staphylococcus* spp consecutively. Isolates from MacConkey were observed as Gram negative, single, short rods which represent the typical characteristics of coliforms and isolates from MSA were Gram positive in a cluster arrangement which were typical for *Staphylococcus* spp. Based on the biochemical characteristics, isolates were confirmed as *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp and *Staphylococcus* spp.

Table 1. The recommended microbiological standards for any fruit juice; all numbers are as per ml of juice consumed (Gulf Standards, 2000).

Parameter	Total viable count	Coliform	Fecal coliform	Staphylococcal
Maximum bacterial load anticipated	$5.0X10^3$	10	0	100
Maximum bacterial load permitted	$1.0X10^4$	100	0	$1.0x10^3$

Total Viable Count (TVC)

Almost all fruit juice samples showed much higher viable bacterial count than permitted count (Table 1, Table 2 and Table 3) except the sample no p-5, which was a commercially available packed mango juice (sample F-6) taken from Arabpur ($4.5x10^3$ cfu/ml). The highest bacterial load ($3.7x10^8$ cfu/ml) for fresh fruit juice sample was found in a grape juice, collected from Doratona and the lowest load was ($2.14x10^6$ cfu/ml) found in an apple juice (sample F-1), collected from a local café (café 1) (Table 2). On the other hand, the highest total bacterial load for packed fruit juice sample was ($4x10^8$ cfu/ml) was found in a mango juice (sample P-8, Brand H) from Chashra and the lowest load was $4.5x10^3$ cfu/ml, found from same type of juice but from a different brand (Brand E) and different place (Arabpur) (Table 3).

Variations in TVC of the both types of fruit juices may be due to the unhygienic maintenance during preparation of the juices. Rahman *et al.* (2011) reported that total viable bacterial count was found in most of the fresh juice samples was higher than the commercially packed juice, as the highest count was found as $2.4x10^4$ cfu/ml and $3.2x10^3$ cfu/ml respectively which was found to be much lower than our study. Tasnim *et al* (2010) found the load of viable bacteria in processed juice samples within the standard limit in the average of 10^3cfu/ml. In comparison to the study of Tasnim *et al* (2010), we hardly found the load of viable bacteria within the standard limit. As only 1 sample (sample no P-5, Brand E) out of 10 was within the standard limit (Table 3).

Total coliform count

The presence of coliform in fruit juice is not allowed by safe food consumption standard (Andres *et al.*, 2004). All fresh juice samples and 7 out of 10 processed fruit juice samples in our study were found

to be unsafe and unfavorable for consumption because of the exceeding limit of coliforms. The highest and lowest coliform count for fresh fruit juice samples were $8.2x10^6$ cfu/ml (sample F-4, collected from local café no 3, Table 2) and $1.53x10^3$ cfu/ml respectively (sample F-7, collected from road side area of JUST campus, Table 2). In Bangladesh, Ahmed *et al.*, 2009 showed the presence of *E. coli* ranging 43 to >2400/100 ml in different types of vended squeezed fruit juices in Dhaka city. In India the fruit juices were heavily contaminated by *E. coli* (Durgesh *et al.*, 2008) (Tambekar *et al.*, 2009). In comparison with those studies, large no of coliforms were found in our study as well, which we can see from Table 2, all the samples were found to exceed the Gulf standards (Gulf standards, 2000).

Table 2. Bacterial load in fresh fruit juice samples (n= 10).

Sample No.	Type of Juice	Sampling area	Total Viable Count (TVC) cfu/ml	Total Coliform Count (TCC) cfu/ml	Total Staphylococcal Count (TSC) cfu/ml
F-1	Apple	Cafe Mango	2.14×10^6	1.28×10^6	9.3×10^5
F-2	Apple	Cafe delight	$3X10^8$	$2X10^6$	5×10^5
F-3	Orange	Cafe Mango	$2.9X10^8$	8.7×10^5	6.2×10^5
F-4	Orange	Cafe Shuveccha	$3.52X10^8$	8.2×10^6	$1.9X10^3$
F-5	Grape	Cafe Mango	4.3×10^7	$1.9X10^3$	1.2×10^7
F-6	Grape	Road side(Doratana)	$3.7X10^8$	1.6×10^4	$2.6X10^2$
F-7	Watermelon	Road side(JUST campus)	8.5×10^6	$1.53X10^3$	$3.6X10^3$
F-8	Watermelon	Road side(Doratana)	7.9×10^7	5.5×10^6	$4.2X10^3$
F-9	Wood Apple	Road side(JUST campus)	$2.62X10^8$	$1.67X10^6$	$2.3X10^3$
F-10	Wood Apple	Road side(Doratana)	$1.35X10^6$	$8.9X10^5$	$1.25X10^6$

On the other side, in case of packed fruit juices (Table 3) all but 3 were within the Gulf standard (sample P-1, P-5 and P-9). In a study, Tasnim *et al* (2010) found undetectable coliform count in processed juice samples. In comparison with that, in our study rest 7 samples were found to be unacceptable as they contained large no of coliforms. The highest value of coliform count was $2.7x10^6$ cfu/ml (sample P-2, Brand B, collected from Ambottoal).

Table 3. Bacterial load in commercially packed fruit juice samples (n= 10).

Sample No.	Brand	Type of juice	Sampling area	Total Viable Count (TVC) cfu/ml	Total Coliform Count (TCC) cfu/ml	Total Staphylococcal Count (TSC) cfu/ml
P-1	Brand A	Mango	JUST cafeteria	$8.1X10^6$	0	$1.90X10^3$
P-2	Brand B	Mango	Ambottola	$4.0X10^7$	$2.7X10^6$	$4.0X10^5$
P-3	Brand C	Mango	Ambottola	$3.0X10^8$	$1.8X10^5$	$1.7X10^5$
P-4	Brand D	Mango	Churamonkati	$2.8X10^7$	$1.1X10^6$	$2.0X10^6$
P-5	Brand E	Mango	Arabpur	$4.5X10^3$	0	0
P-6	Brand F	M ango	Pulbari	$5.0x10^6$	$1.2x10^5$	$2.6x10^4$
P-7	Brand G	Mango	Doratana	$5.0x10^5$	$3.4x10^3$	$3.0x10^4$
P-8	Brand H	Mango	Chasra	$4.0X10^8$	$2.0X10^5$	$3.0X10^5$
P-9	Brand I	Apple	Arabpur	$1.4x10^5$	0	$1.0x10^3$
P-10	Brand J	Orange	Arabpur	$1.9x10^6$	$8.0x10^5$	$1.1x10^5$

Total Staphylococcal count

Coagulase positive staphylococcai may cause human diseases through the production of toxins. Effective levels of toxin formation require a large number of microorganisms (approximately 10^5-10^6 cfu/ml of food) (IDF, 1994). A few reports have shown the

prevalence of staphylococcai in fruit juice samples (Ahmed *et al.*, 2009; Tambekar *et al.*, 2009). In our study the highest value of staphylococcai count for fresh juice sample was $1.25x10^6$cfu/ml, found in wood apple juice (sample F-10), collected from Doratona (Table 2). In the contrary, the highest total staphylococcal count for packed fruit juice sample ($2x10^6$ cfu/ml) was found in a mango juice (sample P-4, Brand D) collected from Churamonkathi (Table 3). Interestingly, there was only 1 sample among 20 fresh and packed juice sample (sample P-5, Brand E) was within the Gulf standard as that sample contained no coliform, staphylococcai count and meet the standard limit of total viable bacterial count. Besides, there was another packed juice sample (sample P-9, Brand I) satisfied the Gulf standard for coliform and staphylococcai but unsatisfied for total viable count (Table 3). On the contrary, only 1 sample, grape juice (sample F-6, collected from Doratona) was within the Gulf standard and this sample showed the lowest total staphylococcal count at the value of $2.6x10^2$cfu/ml.

Conclusion

Present study exhibited the microbial status of available local fruit juices (both fresh and commercially packed) to ensure food safety for a precise control over public health risk. From the data presented in the current study, it could be hard to claim that, consumption of commercially packed juice was safe than fresh juices; because almost all types of fresh and commercially packed juice samples collected from different areas of Jessore city were not satisfactory as *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., and *Staphylococcus* spp., were detected in large numbers from samples.

There is a generalized belief among the people that, automated machines and some preservatives are used during processing of commercial fruit juices. Despite of all these issues, large number of coliforms and staphylococcai count were detected from commercially packed fruit juices in this current study, which clearly indicates poor plant management and personnel hygiene. This contamination could also be occurred due to lacking of proper quality control system for juice preparation, lacking of right storage conditions and bad packaging system.

A combination of regular monitoring and proper training could be an appropriate choice to minimize the health risks. In addition to this, not only government authorized institution like BCSIR and BSTI but also some strongly active administrative organization like mobile court should be given more authorization to undertake pre-emptive investigations to check the microbial and chemical quality of fruit juices. Besides, government and non-government institutions should create public awareness about the contamination and adulteration of fruit juices more intensely with the help of electric media.

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