

Microbial status of street vended fresh-cut fruits, salad vegetables and juices in Dhaka city of Bangladesh

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Abstract

An investigation was undertaken for the isolation and identification of food borne microorganisms from different street-vended fresh-cut fruits, salad vegetables and juices. Total bacterial count (TBC), total coliform count (TCC) and total salmonella-shigella counts (TSS) were 3.5×10^3 cfu/g, 4.8×10^2 cfu/g and 3.6×10^2 cfu/g, respectively in fresh cut hog plum (*Spondias mombin* L.). In guava (*Psidium guajava* L.) TBC, TCC and TSS count were 1.5×10^4 cfu/g, 4.9×10^2 cfu/g and 2.3×10^2 cfu/g respectively. Total bacterial count (TBC) and TCC were 4.6×10^4 cfu/g and 3.9×10^2 respectively in plum (*Ziziphus mauritiana* L.). This result suggests that hog plum had the lowest while plum had the highest microbial load among all the fruit samples. In salad vegetables, TBC and TCC varied between 3.9×10^3 to 4.3×10^3 cfu/g and 2.6×10^2 to 6.6×10^2 cfu/g, respectively. Among salad vegetables, tomato (*Lycopersicon esculentum* L.) showed the lowest and carrot (*Daucus carota* L.) showed the highest microbial loads. Total bacterial count (TBC) ranged from 6.3×10^4 to 1.4×10^5 cfu/ml and TCC from 2.3×10^2 to 3.7×10^3 cfu/ml in juice samples. The lowest TBC was found in sugarcane (*Saccharum officinarum* L.) and the highest was found in olot kombol (*Abroma augusta* L.) juices. Total salmonella-shigella counts (TSS) were found to be nil in all salad vegetables and juice samples. Five different organisms were identified from the tested samples including *Escherichia coli* (36%), *Bacillus* (25%), *Staphylococcus* (24%), *Klebsiella* (9%) and *Proteus* (6 %). The isolated pathogens provided a potential health hazard by the consumption of these street vended foods. Therefore, hygienic practices should be ensured for the safety of consumers as well as to prevent food borne illnesses.

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Keywords

Microbial survey
Street vended fruits
Salad vegetables
Juices

Introduction

Fruits and vegetables are important dietary source of vitamins, minerals, fiber and other natural substances. Consumption of fresh fruits and their juices provides potential health benefits to the general population (Allothman *et al.*, 2009; Bhat *et al.*, 2011). A perfect nutrition full of fruits and vegetables reduce risk of some chronic diseases. Well balanced diet rich in fruits and vegetable should be taken to prevent vitamin deficiencies, developing blood lipid profile and detoxification of human body (Kalia and Gupta, 2006; Deanna and Jeffrey, 2007). Eating sufficient amount of vegetables, fruits and fruit juices also control blood pressure, lower the risk of heart diseases, reduces blood cholesterol levels and avoid some kinds of cancer (Hung *et al.*, 2004; Dragsted *et al.*, 2006; Appel *et al.*, 2005; Wiseman, 2008).

Street vended fruits and fruit juice are ready to eat food that can be bought directly from street vendors or

hawkers in streets and other similar public places and eaten immediately without necessarily having to cut, peel or rinse them as they have already been prepared by the vendors (FAO, 1989). In developing countries, street foods provide nutrients to most of the low and middle income people. Recently, there has been a significant increase in the consumption of fresh cut fruits because they are easily accessible, convenient and cheaper than the whole fruits. In Dhaka, the capital city of Bangladesh, fresh cut fruits and vegetables particularly, hog plum, cucumber, carrot, green mango, pineapple are common street vended item. Such fruits and vegetables can be contaminated by bacterial pathogens through wounded surfaces, such as punctures, cuts and splits that occur during growing or harvesting (Durgesh *et al.*, 2008).

Contamination of street vended foods, especially sliced fruits and vegetables are by unhygienic processing, using of dirty utensils and washing with contaminated water. Moreover, the open display

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of these street foods encourages sporadic visits by flies and direct contact with dust. A vast amount of microorganisms including pathogens contain in fruits and salad vegetables (Dunn *et al.*, 1995; Ray and Bhunia, 2007; Ofor *et al.*, 2009). According to World Health Organization (WHO) the exact scenario of food borne disease caused by contaminated food is 300 to 350 times higher than the reported cases (Hetzl *et al.*, 2004).

The contaminated fresh-cut fruits, salad vegetables and juices may cause serious food borne diseases. More than 250 types of microbes and parasites are responsible for different food borne illnesses (Tambekar *et al.*, 2008). The most common pathogens are *Vibrio cholerae*, *Salmonella* Typhi and *Bacillus cereus* which cause several diseases such as food poisoning, diarrhoea, typhoid, dysentery etc. Furthermore, the unhygienic water also carries hepatitis A that causes disease like liver failure. In addition, *E. coli* and *Salmonella* are enteric pathogens and are causal agents for the maximum food related diseases (Buck *et al.*, 2003).

Quality of street foods should be maintained strictly. In developed countries there are some laws to regulate the quality of street foods. Unfortunately, in Bangladesh, street vendors are not concerned about the food security and cleanliness of street foods because of absence of law enforcement against selling of unhygienic foods. This is why some diseases are conveyed to human through infected fresh cut fruits, salad vegetables and juices.

The aim of the study is to assess the microbiological quality and safety of some street vended fruits, salad vegetables and juices for creating awareness of consuming contaminated food. The present study was designed to screen for the possible presence of microbial contaminants (food borne pathogens), isolation and identification of pathogenic microbes associated with street vended foods, such as fresh cut fruits, salad vegetables and juices in Dhaka city and its surrounding area.

Materials and Methods

Collection of samples

Street vended ready to eat fruits, salad vegetables and juices were used as materials for the study. The samples were collected from different streets and markets of Dhaka city. A total of 50 samples comprising 5 each of ten ready-to-eat fruits, salad vegetables and juices were purchased from street vendors. Based on the consumer demand, total 4 types of fresh cut fruits like hog plum (*Ziziphus mauritiana* L.), guava (*Psidium guajava* L.), plum

(*Ziziphus mauritiana* L.) and Pineapple (*Ananas comosus* L.) and 3 types of salad vegetables like Carrot (*Daucus carota* L.), Cucumber (*Cucumis sativus* L.) and Tomato (*Lycopersicon esculentum* L.), and three types of juices such as sugarcane (*Saccharum officinarum* L.), alovera (*Aloe vera* L.) and olot kombol (*Abroma augusta* L.) were selected for microbial analysis. Samples were collected randomly from the vending places. From the vendors all ready to eat fruit and salad vegetable samples (20 gm) were collected in pre-sterilized zip-lock bags (165 mm x 150 mm x 0.05 mm) and freshly extracted juice samples (50 ml each) were collected in sterile bottles. All samples were transported to the laboratory and analyzed immediately. All the microbial analysis was conducted in the laboratory of Food technology, IFRB, AERE, Savar, Dhaka at room temperature.

Sample preparation

For fresh cut fruits and salad vegetables, 10 gm of the sample and for fruites, 10 ml of samples were blended and mixed properly with 90 ml of sterile 0.9% Sodium Chloride (NaCl) solution. One ml of each homogenate sample was added into appropriate dilutions (10^{-1} to 10^{-6}) using 0.9% NaCl solution.

Microbiological analysis

Nutrient agar (DifcoTM, USA, PH 7.0-7.4) was used to determine Total Viable Bacterial Count (TVBC). Media were prepared according to manufacturer's instruction. Briefly, all media except *Salmonella-shigella* were sterilized by autoclave at 121° C for 15 minutes. *Salmonella-shigella* agar was boiled for 15 min before use. From appropriate dilution, 100 µl homogenate of each samples was inoculated in respective culture media by using sterile pipette and was spreaded using sterile glass spreader. Inoculated plates were then kept in an incubator at 37°C for 24 to 28 hours. Following incubation, plates exhibiting colonies were counted. The average number of colonies in a particular dilution was multiplied by the dilution factor to obtained TBC. Microorganisms associated with samples were expressed as colony forming units per gram (cfu/g) for fresh cut fruits and vegetables and colony forming units per ml (cfu/ml) for fruit juices. Total coliform count (TCC) was enumerated in the same way using MacConkey agar (Acumedia, USA) media. *Salmonella-shigella* agar (Scharlau, Spain) was used to enumerate total salmonella-shigella count (TSS) at 37°C for 24 to 28 hours. Cell counts (cfu/g and cfu/ml) were the average of at least 3 independent experiments.

Isolation and identification of microorganisms

Bacterial colony of different morphology was streaked on respective media to obtain pure culture. All the plates incubated at 37°C for 24 hours. Pure bacterial isolates were preserved at 4°C. The bacterial isolates were recognized on the basis of morphological and biochemical characteristics such as Oxidase test (Steel, 1961), Indole test (Macfaddin, 2000), Triple Sugar Iron (TSI) test (Macfaddin, 2000), Methyl red (Macfaddin, 2000) tests, Voges Proskauer (V. P.) tests (Macfaddin, 2000), Citrate test (Claus, 1989) and Motility test (Eklund and Lankford, 1967). Finally, microorganisms were provisionally identified according to the Bergey's manual of determinative bacteriology (6th edition) and manual for the identification of medical bacteria (Baumann *et al.*, 1984; Cowan, 1975).

Results and Discussion

A total of ten street vended samples of fresh cut fruits, salad vegetables and juices were examined to investigate the presence of microbial load and level of microbiological contamination. In this present investigation, all the fruits, salad vegetables and juice samples were found to be infected. Microbial load associated with different type of samples are presented in Table 1.

Total bacterial count (TBC)

In the present investigation, microbial quality of four ready-to-eat sliced fruit samples such as pineapple, guava, hog plum and plum were determined. Total bacterial count (TBC) in all tested samples ranged from 3.5×10^3 to 4.6×10^4 cfu/g. In all the fruit samples, hog plum showed the lowest microbial load (3.5×10^3 cfu/g) while plum showed the highest microbial load (4.6×10^4 cfu/g). This result indicated that street fruit mainly plum is contaminated by microbes and contamination might be due to improper washing of fruits, utensils and personal hygiene of vendors (Tambekar *et al.*, 2009). The result of this study also elucidated that hog plum and pineapple was found to be associated with less number of bacteria. This could be due to acidic nature of the hog plum and pineapple. Nwachukwu and Chukwu (2013) reported total bacterial count in pineapple as 3.5×10^5 cfu/g. Eni *et al.*, (2010) reported that total microbial counts in pineapple ranged between 1.3×10^6 to 3.0×10^7 cfu/g. Another study by Oranusi and Olorunfemi (2011) showed total bacterial count as 2.0×10^6 cfu/g in pineapple which is much higher than our experiment.

Three salad vegetables such as carrot, cucumber

and tomato were examined. Total bacterial count in all the salad vegetables varied between 3.9×10^3 to 4.3×10^3 cfu/g. Among salad vegetable samples, minimum bacterial loads were found in tomato (3.9×10^3 cfu/g) and maximum was found in carrot (4.3×10^3 cfu/g). Eni *et al.* (2010) investigated that total microbial count ranged from 3.8×10^6 to 2.9×10^7 cfu/g in carrot and 1.3×10^7 to 4.6×10^6 cfu/g in cucumber. Nwachukwu and Chukwu (2013) reported total bacterial count as 1.8×10^6 cfu/g and 3.2×10^5 cfu/g in carrot and cucumber respectively.

Three juices were sampled such as sugarcane, alovera and olot kombol, where bacterial load ranged from 6.3×10^4 to 1.4×10^5 cfu/ml. This result is almost consistent with the study of Rahman *et al.* (2011). Among juice samples sugarcane showed the lowest (6.3×10^4 cfu/ml) and olot kombol showed the highest (1.4×10^5 cfu/ml) bacterial load. This could be due to high concentration of sugar in sugarcane that might lead to reduce the bacterial growth. Street vended fresh juice samples were found to be highly contaminated with TBC than the commercially packed juice (Rahman *et al.*, 2011).

Total coliform count (TCC)

In fruit samples, the counts of total coliform (TCC) varied from 1.0×10^1 to 4.9×10^2 cfu/g with an average 3.4×10^2 cfu/g. TCC count was minimum in pineapple (1.0×10^1 cfu/g) and maximum in guava (4.9×10^2 cfu/g). In this study, TCC exceeded the acceptable limit and the high level counts are unsatisfactory as suggested by the European Union Commission Regulation (2005). Oranusi and Olorunfemi (2011) found total coliform count (TCC) as 2.0×10^5 cfu/g in pineapple.

Among salad vegetables TCC varied between 2.6×10^2 to 6.6×10^2 cfu/g. Tomato showed the lowest (3.9×10^3 cfu/g) and carrot showed maximum (4.3×10^3 cfu/g) TCC counts. Alam *et al.* (2013) reported that salad vegetables such as tomato and cucumber from vegetable markets were contaminated with total coliforms.

Total Coliform Count (TCC) ranged from 2.3×10^2 to 3.7×10^3 cfu/ml in all the juice samples. Among all the juice samples lowest (6.3×10^4 cfu/ml) TCC was found in sugarcane and the highest (1.4×10^5 cfu/ml) TCC was found in olot kombol. The washing and processing water contaminated with fecal coliform is one of the major sources for presence of coliforms in street foods. Durgesh *et al.* (2008) and Tambekar *et al.* (2009) demonstrated that fruit juices such as sugarcane, lime and carrot juice in Mumbai city, India and apple, orange, pineapple, pomegranate, sweet lemon and mix fruit juice in

Table 1. Quantitative assessment of microbes in ready-to-eat fresh cut fruits, salad vegetables (cfu/g) and fruit juices (cfu/ml)

Types of Sample	Sample	TBC	TCC	TSS
Fruits	Hog plum (<i>Spondias mombin</i> L.)	3.5x10 ³	4.8 x10 ²	3.6 x10 ²
	Guava (<i>Psidium guajava</i> L.)	1.5 x10 ⁴	4.9 x10 ²	2.3 x10 ²
	Plum (<i>Ziziphus mauritiana</i> L.)	4.6 x10 ⁴	3.9 x10 ²	-
	Pineapple (<i>Ananas comosus</i> L.)	3.8 x10 ³	1.0 x10 ¹	7.1 x10 ¹
Salad vegetables	Carrot (<i>Daucus carota</i> L.)	4.3 x10 ³	6.6 x10 ²	-
	Cucumber (<i>Cucumis sativus</i> L.)	4.2 x10 ³	3.2 x10 ²	-
	Tomato (<i>Lycopersicon esculentum</i> L.)	3.9 x10 ³	2.6 x10 ²	-
Juices	Sugarcane (<i>Saccharum officinarum</i> L.)	6.3 x10 ⁴	2.3 x10 ²	-
	Alovera (<i>Aloe vera</i>)	6.7 x10 ⁴	6.4 x10 ²	-
	Ulot-kombol (<i>Abroma augusta</i> L.)	1.4 x10 ⁵	3.7 x10 ³	-

TBC= Total bacteria count; TCC= Total coliform count; TSS= Total salmonella-shigella count
 “-” = Not detectable

Table 2. Bacterial isolates of ready-to-eat fresh cut fruits, salad vegetables and fruit juices collected in Dhaka city

	Name of sample	No. of sample tested	Bacterial Isolates				
			<i>Proteus</i>	<i>Klebsiella</i>	<i>Staphylococcus</i> spp.	<i>Bacillus</i> spp.	<i>E. coli</i>
Fruit samples	Hog plum	5	+	-	+	+	+
	Guava	5	+	+	+	+	+
	Plum	5	-	-	+	+	+
	Pineapple	5	+	+	+	+	+
Vegetable salad samples	Carrot	5	-	+	+	+	+
	Cucumber	5	+	+	+	+	+
	Tomato	5	+	+	+	+	+
Juice samples	Sugarcane	5	+	+	+	+	+
	Alovera	5	+	+	+	+	+
	Ulot-kombol	5	+	+	+	+	+
	Total	50					

Amravati city, India respectively, were infected with *E. coli* in a wide range. All the samples were found contaminated however lower microbial count was recorded in all the tested juices samples compared to the Gulf standard (Gulf Standards, 2000).

Total salmonella-shigella count (TSS)

Total salmonella-shigella counts (TSS) were also observed in fruit samples and the values of TSS were within the range of 7.1x10¹ to 3.6x10² cfu/g. There was no TSS count in plum. Total salmonella-shigella count (TSS) count was the lowest in pineapple (7.1x10¹ cfu/g) and the highest in hog plum (3.6x10² cfu/g). Presence of *Salmonella* and *Shigella* into fresh cut fruits and vegetables were due to contamination of tools and knives (Barro et al., 2006). In addition, sometimes knives used for cutting and chopping of fruits and vegetables are attacked by flies (Mensah

et al., 2002). Through the contact with contaminated water fresh cut fruits can be infected by *Salmonella* spp. and *Shigella* spp. (Beuchat, 1995; Gayler et al., 1995). It has been observed that the ranges of TSS count were below the detectable level in salad vegetables and juices. This finding is in agreement with the study of Eni et al. (2010).

Identification of microorganisms

For identification, different bacterial isolates were selected from different media on the basis of agar colony morphology and biochemical characteristics. A total of five different organisms were identified from the samples including *Proteus*, *Klebsiella*, *Staphylococcus* spp., *Bacillus* spp. and *Escherichia coli*.

Staphylococcus spp., *Bacillus* spp. and *Escherichia coli* was the most frequently isolated

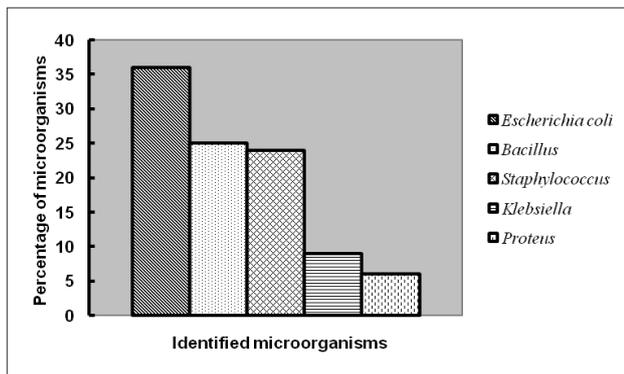


Figure 1. Generic percentage of isolated microorganisms associated with street vended fruits, salad vegetables and juices in Dhaka city

being present in all examined fruit, salad vegetable and fruit juice sample. *Proteus* and *Klebsiella* was the least frequently isolated. *Proteus* was present in all the samples except in plum and carrot. *Klebsiella* isolated from all the samples but not from the hog plum and plum (Table 2). Among various bacterial isolates, 36% were *Escherichia coli*, 25% *Bacillus*, 24% *Staphylococcus* spp., 9% *Klebsiella* and 6% *Proteus* were found (Figure. 1).

Vanderzant and Splittstoesser (1992) demonstrated that *Pseudomonas* spp. and *Bacillus* spp. is the most common vegetable spoilage bacteria. The presence of *Staphylococcus* spp., *E. coli* and *bacillus* spp. are commonly associated with poor sanitary practices (Oranusi, 2006). Furthermore, ambient temperature may increase the bacterial counts of fruit juices (Bryan, 1977). Viswanathan and Kaur (2001) showed the presence of *Salmonella*, *Staphylococcus* sp. and fecal *E. coli* and *P. aeruginosa* in vegetables and fruits. Braide *et al.* (2012) showed high microbial loads in fruit juices such as *Bacillus* sp. and *Staphylococcus* sp.

The present study also showed the presence of *Proteus* and *Klebsiella* in ready-to-eat street vended fresh fruits and vegetable samples. Chukwu *et al.* (2010) found *Salmonella* species, *Proteus* species and *Klebsiella pneumoniae* in pre cut (fruits that have been cut into pieces are stored and displayed for sale in retail outlets) fruit samples. These results corroborate to the present investigation. World Health Organization (WHO) reported that severe illness and deaths especially among children in several countries causes by *E. coli* O157:H7 (WHO, 2002). Lack of proper training, awareness and personal hygiene practice assist the contamination of street foods and can reached to humans through consumption of food (Tambekar *et al.*, 2008; Vanderzant and Splittstoesser, 1992).

Conclusion

The results of the present study clearly indicated that street vended fruits, salad vegetables and fruit juices in Bangladesh are contaminated with different food borne pathogenic bacteria like *Proteus*, *Klebsiella*, *Escherichia coli*, *Staphylococcus* spp. and *Bacillus* spp. This contamination might be due to improper washing of fruits, utensils, poor hygienic conditions, prolonged preservation at ambient environment with swarming flies, locating shop in open air and lack of basic safety issues by vendors etc. All these microorganism causes food borne infection or intoxication and diarrhoeal diseases. Thus street vended fruits and juices represent a substantial level of risk of food borne illness to the consumers. Therefore consumer needs to be aware about the risk of the contaminated foods. Necessary information, education, facilities and training programs for vendors can decrease these risks and provide safe and good quality food. Moreover, regular monitoring of street vended foods for human consumption must be warranted by health ministry and other health sectors for quality improvement of street vended foods as well as to avoid any pathogen outbreaks.

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