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MICROBIOLOGICAL SAFETY OF STREET VENDED FRESH FRUIT JUICES, DRINKS AND CONVENTIONAL BLENDS IN MULTAN-PAKISTAN

Saeed Akhtar[1], Muhammad Riaz[1,*], Tariq Ismail[1] and Umar Farooq[2]
[1]Department of Food Science and Technology, Bahauddin Zakariya University, Multan-Pakistan;
[2]Institute of Food and Nutrition, University of Sargodha, Sargodha

[*]Corresponding author's e.mail: riaz@bzu.edu.pk

The study aimed at exploring the safety of various fresh fruit juices, blends and drinks sold in the streets of Multan, Pakistan. The city was divided into 4 zones for the purpose of sample collection. Bacteriological analysis of 72 samples of fresh fruit juices, blends and drinks indicated the presence of total viable counts (2.48 ± 0.16 to 7.91 ± 0.62 log CFU/mL), total coliforms (0.70 ± 0.04 to 4.86 ± 0.29 log CFU/mL) and *Escherichia coli* (0.6 ± 0.03 to 3.83 ± 0.32 log CFU/mL). Qualitative data depicted apple juice to be highly contaminated with fecal coliforms and *Salmonella spp*. Coliforms prevalence was highest in Zone IV and Zone II while that of *Salmonella spp.*, in Zone IV and Zone III. The pragmatic levels of contaminants elucidate poor sanitary status of major entities deployed in juice manufacturing process adopted by the street vendors.
Keywords: Fruits, juices, sanitation, hygiene, safety

INTRODUCTION

Developing economies are undergoing a transitional phase as their survival is solely dependent upon their capacity to challenge the emerging issues of food safety. Invisibly massive economic and health loss is being tolerated by these nations due to ineffectual role of regulatory agencies. Apart from numerous other pathogens, microbiological carriers of devastating cholera and epidemic diarrhea are food borne pathogens, typically prevalent among children and aggravate disease burden in the underdeveloped economies. These microorganisms are excessively present in several foods in Pakistan including fresh fruit juices loosely sold in the region. However, the presence of these microbes and the incurred loss owing to food borne infections are hard to estimate in Pakistan due to the absence of any monitoring, surveillance and infection control system (Laurian and Nancy, 2000; Akhtar *et al*., 2010; Akhtar, 2012).

Fresh fruit juices are the potential source of minerals, vitamins and fiber. Moreover, their consumption is considered as a potential thirst-quenching strategy in warm and scorching heat of summer. Conventionally, the juices and drinks extracted on the carts of street vendors are consumed un-pasteurized (Suaad and Eman, 2008; Bagde and Tumane, 2011; Suneetha *et al*., 2011; Akhtar *et al*., 2012). These are preferred by the consumers owing to their natural pleasant taste, fresh flavor, nutritious profile, easy access and cheaper prices however their microbial safety has been remained a potential health risk in the communities overwhelmed by poor food safety management systems. Food borne illnesses are generally associated with the consumption of foods already exposed to pathogenic microorganisms, such as bacteria, viruses and parasites as well as their toxic metabolites, resulting in acute as well as chronic health disorders. Pakistan resides among those countries which are the least developed in terms of food borne disease surveillance and food safety infrastructure. It further lacks reporting systems for food borne illnesses that can effectively communicate with national food control authorities hence, the incidence of food borne diseases is often not used to help define national food safety strategies and policies, thus chemical and microbiological contaminants have never been given the priority they deserve to be addressed (FAO/WHO, 2005).

Street vended foods are generally affordable but often do not meet proper hygiene standards owing to weak regulatory systems, inadequate food safety rules, lack of financial resources to invest in safer equipments and, un-skilled and un-educated food handlers (De-Waal and Robert, 2005). The juices, most commonly sold on stalls in Pakistan, are extracted from oranges, mangoes, apples, carrots, sugarcane and are served with ice in poorly washed tumblers. The method of extraction, inadequate washing of the fruits before extraction and poor cleaning practices of the machines and utensils are some of the significant indicators that render the juices unfit for drinking. Washing with contaminated water

further increases the threat of pathogenic microflora (Splittstoesser, 1979; Harrigan, 1998). Primarily, numerous micro-organisms present in such drinks might be harmless e.g. yeasts and saprophytic bacteria, but the existence of pathogens like *Salmonella*, *Listeria* or *E. coli* may not be disregarded or ignored (Bryan, 1977).

Research surveys can play a significant role in curtailing the food safety risks of consuming contaminated foods through identification and evaluation of food safety concerns that may provide better target controls over the problem. Multan is densely populated city situated in the less developed and neglected area of Southern Punjab. The city is regarded as one of the hottest part of the country with a temperature reaching 45-52°C in the months of June-August. Consequently, consumption of the freshly extracted street vended fruit juices increases many times in scorching summer that may result in food borne out breaks frequently. The objectives of the present study were to evaluate the microbiological safety of fresh fruit juices in Multan-Pakistan and to identify the possible risk factors that may significantly contribute in potential health threats resulting from the consumption of most commonly and heavily consumed street vended fruit juices.

MATERIALS AND METHODS

***Zone distribution*:** The entire city of Multan was geographically divided into following four zones with the objective of selection of hot spots of fruit juices consumption for sampling purpose (Table 1).

Table 1. Zone distribution of Multan city for sample collection of street vended fresh fruit juices, blends and drinks

Zone	Types of sampled juices	No. of samples of each juice type	Total no. of samples
Zone I	06	03	18
Zone II	06	03	18
Zone III	06	03	18
Zone IV	06	03	18

Zone I= Pak Arab Fertilizer, Khanewal Road, Bosan Road, Gulgasht, MDA

Zone II =Doulat Gate, Clock Tower, Civil Hospital, Nishtar Hospital, Cantt, Sher Shah

Zone III= Old Shujaabad, Grain Market, Bahawalpur Road, Vehari Road

Zone IV= General Bus Stand, Shah Rukn-e-Alam, Masoom Shah Road, Chowk Kumharan

***Procurement of samples*:** Samples of various kinds of fresh fruit juices, conventional blends and seasonal drinks like sugar cane juice, apple juice, pineapple juice, banana shake, mango shake and *Imli aalo bukhara* (a seasonal drink consumed in the form of water extracts of pre-soaked dried plum and tamarind) in triplicate were procured from the street vended outlets in the months of June and July, 2012. The samples were collected, decanted in sterilized glass bottles, sealed tightly, stored in ice box and transported immediately to the Food Microbiology and Biotechnology Laboratory of the Department of Food Science and Technology, Bahauddin Zakariya University, Multan for bacteriological analysis.

***Isolation and enumeration of bacteria*:** Un-pasteurized juice samples procured from the vendors were analyzed for total viable counts (TVC), total coliforms (TC), *E. coli*, fecal coliforms (FC) and *Salmonella* spp. Pour plate method was used for isolation and enumeration of bacteria from fresh juice samples, drinks and blends. Ten-fold serial dilutions of homogenized suspension were prepared aseptically in laminar air flow by transferring 1 mL of each of the fresh fruit juice sample into 9 mL of sterile phosphate buffer tubes separately. Bacterial isolation and enumeration was made as described by Prescott *et al*. (2002) and Cheesbrough (2002) at selective media including nutrient agar (Merck) for TVC, violet red bile agar (Merck) for TC and eosin methylene blue agar (EMB-Merck) for *E. coli.* Fecal coliforms were detected by sub-culturing juice samples in EC broth (EC Broth-Merck). One mL sample of juice was cultured in 9 mL sterile EC broth containing inverted Durhum tubes at a temperature of 44.5°C for 48 hours. Confirmation of gas positive tubes was done by using EMB agar. Inoculated plates were incubated at standard time-temperature combination (USFDA, 2001).

Pre-enrichment of 25 mL each sample of fresh fruit juices, drinks and blends was made in peptone broth (Merck) and the homogenate was incubated at 37°C for 24 hours followed by inoculation of 1 mL of primary enrichment in 9 mL selenite cysteine broth (Merck) as selective enrichment. Secondary enrichment was incubated at 37°C for 48 hours and sub-culturing of the samples was performed on *Salmonella Shigella* agar (SS agar-Merck). The plates were identified for *Salmonella* spp. on the basis of biochemical and colony characteristics after a period of 24 hours at 37°C.

***Statistical analysis*:** All experiments were conducted in triplicate and the data obtained in the form of bacterial counts was converted to logarithmic values for uniformity. Means of the replicates were obtained and standard deviations were calculated.

RESULTS

Owing to the suspected adverse food safety situation in the city, hundred percent of the samples collected from street venders were found microbiologically contaminated. Most of the samples were contaminated with pathogens except *Imli aalo bukhara drink* which was found to be far better having lower threat of pathogenicity. Significantly higher total viable count (TVC) ranging from 2.48 ± 0.16 to 7.91 ± 0.62 (log CFU/mL) was observed from bacteriologically

Table 2. Total viable, coliform and *E. coli* counts (log_{10} CFU/mL) of fresh fruit juices sold in Multan, Pakistan

Type of juice	Zone	TVC	TC (CFU/mL)	*E. coli* (CFU/mL)
Sugarcane juice	Zone I	6.62 ± 0.24 – 6.92 ± 0.20	3.72 ± 0.26 – 3.90 ± 0.22	2.53 ± 0.25 – 2.71 ± 0.17
	Zone II	6.46 ± 0.32 – 6.92 ± 0.31	3.85 ± 0.27 – 4.00 ± 0.26	2.86 ± 3.18 – 3.18 ± 0.14
	Zone III	6.86 ± 0.34 – 7.53 ± 0.34	2.85 ± 0.20 – 3.92 ± 0.31	1.93 ± 0.31 – 3.00 ± 0.19
	Zone IV	5.98 ± 0.27 – 6.81 ± 0.25	2.88 ± 0.32 – 3.67 ± 0.21	1.81 ± 0.08 – 3.04 ± 0.19
Imli aloo bukhara	Zone I	2.48 ± 0.16 – 4.32 ± 0.34	0.70 ± 0.04 – 1.30 ± 0.10	0.60 ± 0.03 – 0.85 ± 0.05
	Zone II	4.43 ± 0.42 – 4.72 ± 0.20	1.60 ± 0.15 – 1.79 ± 0.08	0.70 ± 0.04 – 0.90 ± 0.09
	Zone III	4.15 ± 0.23 – 4.72 ± 0.45	2.83 ± 0.13 – 2.91 ± 0.27	1.08 ± 0.05 – 1.18 ± 0.09
	Zone IV	4.81 ± 0.38 – 4.89 ± 0.21	2.15 ± 0.17 – 2.26 ± 0.14	1.08 ± 0.07 – 1.38 ± 0.06
Apple juice	Zone I	5.30 ± 0.50 – 5.70 ± 0.36	3.68 ± 0.36 – 3.83 ± 0.30	1.08 ± 0.10 – 1.62 ± 0.13
	Zone II	7.71 ± 0.73 – 7.91 ± 0.62	3.70 ± 0.29 – 4.26 ± 0.18	1.94 ± 0.15 – 2.30 ± 0.10
	Zone III	6.83 ± 0.65 – 7.70 ± 0.49	3.73 ± 0.35 – 4.60 ± 0.29	2.28 ± 0.22 – 2.70 ± 0.12
	Zone IV	6.65 ± 0.49 – 7.76 ± 0.29	3.71 ± 0.16 – 4.86 ± 0.29	2.45 ± 0.11 – 3.23 ± 0.25
Pineapple juice	Zone I	5.48 ± 0.30 – 5.80 ± 0.60	3.30 ± 0.26 – 3.45 ± 0.33	2.18 ± 0.14 – 2.30 ± 0.22
	Zone II	5.88 ± 0.46 – 6.81 ± 0.65	2.82 ± 0.22 – 3.49 ± 0.33	1.93 ± 0.10 – 2.62 ± 0.30
	Zone III	5.76 ± 0.55 – 6.80 ± 0.29	2.71 ± 0.25 – 2.89 ± 0.12	1.83 ± 0.18 – 2.11 ± 0.09
	Zone IV	6.18 ± 0.41 – 6.83 ± 0.43	3.92 ± 0.31 – 3.95 ± 0.25	2.08 ± 0.18 – 2.36 ± 0.20
Mango milk shake	Zone I	6.38 ± 0.45 – 6.88 ± 0.86	3.23 ± 0.19 – 3.62 ± 0.25	1.93 ± 0.14 – 2.32 ± 0.22
	Zone II	5.76 ± 0.40 – 6.82 ± 0.60	2.90 ± 0.25 – 3.48 ± 0.36	1.64 ± 0.20 – 2.72 ± 0.26
	Zone III	7.34 ± 0.40 – 7.71 ± 0.69	4.76 ± 0.43 – 4.79 ± 0.30	2.60 ± 0.13 – 2.86 ± 0.27
	Zone IV	7.18 ± 0.45 – 7.74 ± 0.35	3.80 ± 0.30 – 4.15 ± 0.36	2.72 ± 0.16 – 3.83 ± 0.32
Banana milk shake	Zone I	6.15 ± 0.41 – 6.78 ± 0.56	3.80 ± 0.40 – 4.73 ± 0.55	1.84 ± 0.19 – 2.57 ± 0.20
	Zone II	5.62 ± 0.46 – 6.34 ± 0.73	2.36 ± 0.23 – 2.80 ± 0.27	1.91 ± 0.18 – 2.15 ± 0.17
	Zone III	4.70 ± 0.52 – 5.72 ± 0.34	2.73 ± 0.26 – 2.79 ± 0.12	0.70 ± 0.06 – 1.72 ± 0.16
	Zone IV	5.83 ± 0.25 – 6.00 ± 0.40	2.36 ± 0.10 – 2.67 ± 0.20	1.70 ± 0.09 – 1.90 ± 0.15

TVC = Total viable counts, TC = Total coliforms, *E. coli* = *Escherichia coli;* where Data represents means ± standard deviations of three measurements

analyzed sample population of 72 fresh fruit juices, blends and drinks collected from the four zones of the city (Table 2). Comparative evaluation of total microbial contamination among different juices and drinks samples depicted apple juice as a carrier of maximum TVC, i.e. 7.91 ± 0.62 log CFU/mL followed by mango milk shake while the least TVC was noticed in *Imli aalo bukhara* drink, i.e. 2.48 ± 0.16 log CFU/mL.

Total coliforms (TC) are considered as the reference index for insanitation as well as pathogenesis from fecal contaminants including fecal coliforms and *E. coli*. A similar situation was encountered in TC estimation where the proliferation was ranging 0.70 ± 0.04 to 4.86 ± 0.29 log CFU/mL. Total coliform counts were highest in apple juices ranging from 3.68 ± 0.36 to 4.86 ± 0.29 log CFU/mL. Maximum *E. coli* was observed in mango milk shake (1.93 ± 0.14 - 3.83 ± 0.32 log CFU/mL) while the condition was relatively better in *Imli aalo bukhara* drink (0.6 ± 0.03 - 1.38 ± 0.09 log CFU/mL). Qualitative evaluation of fruits juices and drinks for fecal coliforms (FC) and S*almonella spp.* illustrated apple juices to be heavily contaminated with FC (66.6%) followed by mango milk shake and sugar cane juice i.e. 58.33% (Table 3). Inter-zonal magnitude of FC contamination was highest in Zone IV (66.66%) followed by Zone II (61.11%) (Fig. 1).

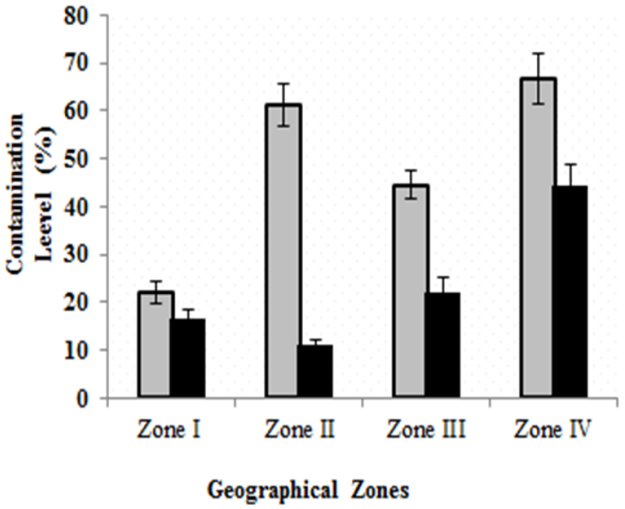

Figure 1. Prevalence of fecal coliform (%) ■ and *Salmonella* spp. (%) ▭ in fresh fruit juices, drinks and blends collected from four zones of Multan-Pakistan. Error bars represent the standard error.

Table 3. Qualitative evaluation of fresh fruit juices, drinks and blends collected from four zones of Multan for fecal coliforms (FC) and *Salmonella* spp.

Type of drink	Contaminant	Zone I			Zone II			Zone III			Zone IV			Mean (%)
		1	2	3	1	2	3	1	2	3	1	2	3	
Sugarcane Juice	FC	-	-	+	+	+	-	+	+	+	-	+	-	58.33
	Salmonella	-	-	-	-	-	-	+	+	-	-	+	-	25.00
Imli aloo Bukhara	FC	-	-	-	+	-	-	+	-	-	+	-	+	33.33
	Salmonella	-	-	-	-	-	-	-	-	-	+	-	-	8.33
Apple juice	FC	-	+	-	+	+	+	-	+	-	+	+	+	66.66
	Salmonella	-	-	-	+	-	-	-	+	-	+	+	-	33.33
Pineapple juice	FC	+	-	-	-	+	-	-	-	-	-	+	+	33.33
	Salmonella	-	-	+	-	-	-	-	-	-	+	-	-	16.66
Mango milk shake	FC	-	-	-	-	+	-	+	+	+	+	+	+	58.33
	Salmonella	-	+	-	-	-	-	+	-	-	+	-	+	33.33
Banana milk shake	FC	+	-	-	+	+	+	-	-	-	+	-	-	41.66
	Salmonella	+	-	-	-	-	+	-	-	-	+	-	-	25.00

A similar pattern was reflected in both zones for *E. coli* where the highest log values for Zone II and IV were 3.18 ± 0.14 and 3.83 ± 0.32, respectively. 23.61% of total fresh fruit juice sample examined for bacterial contamination confirmed the presence of *Salmonella* spp. Higher percentile of *Salmonella* was depicted from apple juice and mango milk shake (33.33% each) followed by sugar cane and banana milk shake (25% each). Results indicated Zone IV to be potential *Salmonella* infected region (44.44%) (Fig. 1).

DISCUSSION

Elevated levels of contamination in the juice samples collected from Zone IV (road side vendors) might be associated with the location where environmental micro flora harboring over the fruits and utensils surfaces in the form of dust exploded by the traffic heavily contaminated the fruit juices. Moreover, unhygienic food handling practices in the form of inadequate washing of fruits and tumblers before juice extraction possibly provoked contamination, resulting in higher microbial load in the samples. Similar findings were elaborated in a study on fresh fruit juices in Dhaka Bangladesh by Ahmed *et al.* (2009).

Maximum bacteriological loads in apple juice could be associated with poor fruit handling and juice extraction practices that were followed by almost all vendors of the city. Peel serves as a natural protection of fruit from external micro flora and insects. In contrary majority of the vendors were found to use bruised, poorly stored, already peeled and over ripened apples for juice extraction that could render the fruits as harboring and breeding sites for spoilage and pathogenic micro flora. In addition, the equipments being used for juice extraction were found stuffed with spoiled fruit residues reflecting inadequate cleaning practices of machines even after repeated extraction of juices at different time intervals.

European Commission Regulation (EC 1441/2007) and Spanish microbiological criteria (Real Decreto 3484/2000), suggested that total contaminated samples of fresh fruit must lie below 12% and 43% of the total examined sample for mesophilic aerobic counts and *enterobacteriaceae*, respectively. Our results compared with these permissible limits for bacterial load seem like exceeding to a great extent.

Illiteracy and lack of awareness on hazards and health implication for consuming contaminated juices among the vendors are the major entities that could elucidate magnitude of fresh fruit juices contamination. Poor quality fruits and inappropriate utensils handling practices are the markers for higher microbial load of the fruit juices. The gravity of microbial contamination in fruit juices have been extensively reported in several studies carried out in Indian city of Nagpur (Titarmare *et al*., 2009), Qatar (Al-Jedah and Robinson, 2002), Greece (Vantarakis *et al*., 2011) and Valancia (Sospedra *et al*., 2012) validating the finding of the current study and manifesting higher bacterial contamination in the fruit juice sample despite a substantial socio cultural, economic and geographical diversity.

Several food safety reports published to highlight the safety status of street vended fruits, vegetables and their juices associate consumer health threats with unhygienic environment, poor juice extraction and handling practices, extremely low grade raw material and the general health of the vendors (Lewis *et al*., 2006; Tambekar *et al*., 2009; Titarmare *et al.*, 2009). The environmental factors in the form of dust, nearby garbage, sewage spills and insects like flies add up to make the situation worse. Higher levels of TVC in fresh fruits also reflect poor agricultural practices and hygiene codes like post harvest washing with contaminated water (Stannard, 1997).

Use of diluents like ice and water in fresh fruit juices and blends is a common practice among the street vendors of

low income countries hence, people use to adopt unethical means to seek more economical benefits posing a serious threat to the consumer safety. Poorly managed underground sewage systems of different parts of the city especially the old Multan have already been reported to be contaminating the underground potable water supplies with human enteric pathogenic microbial strains including *Streptococci, E. coli,* FC and *Salmonella* ultimately acting as major precursors of several deadly diseases including diarrhea, cholera, acute dehydration and gastrointestinal disorders (Abbas *et al*., 2007; WWF, 2007, Shahzad *et al*., 2012). The water of such red zones is used by the local ice factories for ice manufacturing that is in-turn consumed by the street vendors as coolant, for washing fruits, utensils and equipments. Parallel findings have been argued by Lewis *et al*. (2006) on poor quality potable water used for adulterating fresh fruit juices, drinks and blends.

In addition to water, milk could also serve as a potential source of *Salmonella* in milk based fruit blends (*milk shakes*). Use of un-pasteurized milk is very common among street vendors that could serve as a proliferating source of pathogens including *E. coli* and S*almonella*. In addition, several vendors have also been observed to dilute milk with water to earn more profit that further augments poor hygienic status of finished product.

The overall, microbiological safety situation of fresh fruit juices, drinks and blends procured from the Multan zones not only challenges the safety measures but also poses a great threat to consumers in the form of food borne pathogenesis. Absence of surveillance, monitoring and diagnostic facility seems like paralyzing the health and economic status of the vulnerable population excessively consuming such beverages.

Fresh juice microbial standards in the region are Gulf Standard (2000) that permit maximum total aerobic bacterial counts and TC (CFU/mL) up to 5×10^4 and 100, respectively. Microbiological guidelines from the Center of Food Safety, Hong Kong (Food and Environmental Hygiene Department, 2009) recommend maximum levels of food borne pathogens i.e. *E. coli* less than 10^2 CFU/mL and *Salmonella* spp. to be absent in 25 mL of non-bottled, un-pasteurized fresh fruit juices, drinks and blends samples. Since, the potential microbiological threats are posed by the dirty utensils and the fruits, therefore, simple repeated immersion of machines and utensils in boiling water for 0.5 min, could serve as an effective strategy in reducing bacteriological contaminants level to a significant extent (Bagci *et al*., 2011)

***Conclusions*:** In the light of prevalent microbiological standards for fresh fruit juices, drinks and blends, it is suggested that fresh fruit juices and drinks are the potential sources for common food borne infections in the region and are thus contributing to provoke the disease burden among poor populations. Seemingly, it is hard to ensure consumers' health and safety without seriously addressing this particular issue. The potential natural nutritional significance of fruits juices turns to be a disadvantage if the microbial quality of juices put the consumer at a risk of food borne illness. Finally, the levels of microbial contamination observed throughout the investigation particularly the presence of *E. coli* and *Salmonella* spp., in fresh fruit juices, drinks and blends, raise a serious concern regarding health and economic wellbeing of the population. Microbial resistance against antibiotics is another emerging food safety issue and may provoke the health related issues in Pakistan. A precise and well defined monitoring and surveillance system needs to be in place and implemented on urgent grounds to address the food safety of fresh fruit juices in Pakistan. Implementation of awareness programs on various heath related issues among the vendors, consumers and stakeholder may be another strategy to curtail the enormity of food safety issues.

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