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Microbiological Risk Assessment of Packed Fruit Juices and Antibacterial Activity of Preservatives Against Bacterial Isolates

Muhammad Naeem Iqbal,[1,3*] Shahzad Ali,[2] Aftab Ahmad Anjum,[3] Khushi Muhammad,[3] Muhammad Asad Ali,[3] Shihua Wang,[1] Waseem Ahmad Khan,[2] Iahtasham Khan,[4] Ali Muhammad,[5] Abid Mahmood,[6] Muhammad Irfan,[6] Aftab Ahmad,[7] Asfa Ashraf[8] and Firasat Hussain[3]

[1]*The School of Life Sciences, Fujian Agriculture and Forestry University, Fuzhou 350002, China*
[2]*Department of Wildlife and Ecology, University of Veterinary and Animal Sciences, Lahore 54000, Pakistan*
[3]*Department of Microbiology, University of Veterinary and Animal Sciences, Lahore 54000, Pakistan*
[4]*Section of Epidemiology and Public Health, University of Veterinary and Animal Sciences, Lahore 54000, Pakistan*
[5]*Department of Zoology, University of Poonch, Rawalakot 12350, Azad Kashmir, Pakistan*
[6]*Department of Biochemistry, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi 46000, Pakistan*
[7]*Department of Zoology, University of the Punjab, Lahore 54000, Pakistan*
[8]*Department of Zoology, Lahore College for Women University, Lahore, Pakistan*

ABSTRACT

The present study was conducted to assess the microbial load of packed fruit juices and determine anti-bacterial activity of the preservatives used in preparation of these fruit juices. Apple, Mango and Orange fruit juice samples (n=90) of five brands collected from retail shops and vendors were subjected to conventional cultural and biochemical methods for microbial enumeration. Anti-bacterial activity was analyzed using agar well diffusion method. Microbiological analysis of fruit juice samples demonstrated the presence of total viable counts (3.23±0.29 to 7.28±0.41 log_{10} CFU/mL), total Staphylococcal counts (0.00±0.00 to 4.89±0.83 log_{10} CFU/mL), total coliform counts (0.00±0.00 to 4.48±1.03 log_{10} CFU/mL) and total fungal counts (1.75±0.58 to 4.40±0.20 log_{10} CFU/mL). Comparative evaluation showed that fruit juices of brands D and E were highly contaminated as these were local brands. The isolated microbes include *Bacillus* spp., *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus* spp., *Saccharomyces cerevisiae*, *Penicillium* sp. and *Rhizopus*. Among the preservatives used, citric acid was found to exhibit highest anti-bacterial activity. Ascorbic acid was active against 2 out of 3 bacteria. No activity was found against sodium benzoate. It is concluded that microbial load in packed fruit juices is significantly higher than standard permissible limits and most preservatives used in manufacture are of low quality which insinuates its possible role in spoilage and food borne illnesses. Periodic monitoring of packed fruit juices should be carried out to make them safe for consumption.

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Authors' Contributions
AAA and KM designed the study. Samples were collected and experiments were performed by MNI, MAA, SA and FH. IK, AM and AM analyzed the data. All others participated in writing manuscript.

Key words
Fruit juices, Microorganism, Microbial load, Preservatives, Anti-bacterial activity.

INTRODUCTION

Fruit juice is the liquid extracted from the edible part of mature and fresh or preserved fruits or any concentrate of such liquid (Codex-Stan, 2005). Various types of packing ensure the availability of fruit juices in the same form (Abshurst, 2005). Preservatives, which are commonly known as natural or synthetic substances, are principally added to fruit juices to enhance their quality and shelf life (Rowe *et al.*, 2012; Anand and Sati, 2013). Aside from their advantages, some of the artificial preservatives may possess life threatening side effects (Anand and Sati, 2013; Mandal and Mandal, 2011; Seetaramaiah *et al.*, 2011). Coliforms and some other microbial contaminants may enter fruit juices from water source (Tasnim *et al.*, 2010). Different methods such as chemical preservatives, freezing, canning and pasteurization are used to process and preserve fruit juices (Fasoyiro *et al.*, 2005).

The existence of microorganisms including bacteria, yeasts and molds in fruit juices are responsible for fermentation, food spoilage and food borne illness (Yeh *et al.*, 2004; Essien *et al.*, 2011). According to Association of Food and Drug Officials (AFDO, 1990), contamination may occur due to simple packaging operations in the absence of aseptic conditions (Juhaniakova *et al.*, 2013). Food security is a complex issue. The US Food and Drug Administration (FDA) suggested achieving a 5-log10 reduction of pathogens for fruit juice manufacturing methods (FDA, 2001).

The commercial products should conform microbiological criteria (CAC/GL 21-1997), which require correct processing, storage and constant

* Corresponding author: driqbalnaeem@hotmail.com
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surveillance (Juhaniakova *et al.*, 2013). Pakistan is developing country and lack food borne disease surveillance and food safety infrastructure. The national food safety policies are not defined on the basis of incidence of food borne diseases, hence food contaminants have never been addressed on priority they deserve (FAO/WHO, 2005; Ali *et al*., 2013). Lahore is densely populated city and have hot climate during summer resulting in increased consumption of fruit juices and hence food borne illnesses. The data regarding microbiological quality of fruit juices are important for local authorities to deal violators. The present study was conducted to access microbiological quality of commercial fruit juices and determines antibacterial activity of commonly used preservatives against bacterial isolates.

MATERIALS AND METHODS

Samples collection

Fruit juice samples of three flavors, apple juice, mango juice and orange juice of five various brands (six replicates of each), were procured from retail shops and vendors in Lahore city. The samples of fruit juices were examined in Bacteriology Laboratory, Department of Microbiology, University of Veterinary and Animal Sciences, Lahore.

Isolation and enumeration of bacteria

Packed fruit juice samples collected from retail shops and vendors were analyzed for total viable counts (TVC), total staphylococcal counts (TSC), total coliform counts (TCC) and Salmonella-Shigella detection. Spread plate technique was followed for isolation and enumeration of bacteria. Each of homogenized fruit juice sample (1 mL) was transferred into 9 mL of sterile phosphate buffered saline tubes separately and ten-fold serial dilutions were prepared aseptically. Bacterial isolation and enumeration was done as illustrated in earlier studies (Prescott *et al.*, 2002; Ghengesh *et al.*, 2005) at selective media including nutrient agar for total viable counts (Akhtar *et al.*, 2013), Staph 110 agar for staphylococcal counts and MacConkey agar for coliform counts. Inoculated plates were incubated at standard time-temperature combination (USFDA, 2001). Bacterial colonies were counted and colony forming units per milliliter (CFU/mL) were accessed after overnight incubation at 37ºC.

Pre-enrichment of 25 mL each sample of fruit juices was done in peptone broth followed by overnight incubation of and homogenate at 37ºC. Then 1 mL of primary enrichment was inoculated in 9 mL selenite cysteine broth as selective enrichment. Incubation time for secondary enrichment was 48 h at 37ºC and after that samples were sub cultured on Salmonella-Shigella agar (SS agar). After overnight incubation at 37ºC, the plates were analyzed for identification of *Salmonella* spp. on the basis of biochemical characters and colony morphology.

Isolation and enumeration of fungi

Sabouraud dextrose agar (SDA) was used for isolation and enumeration of yeast and molds from fruit juice samples. Each of homogenized fruit juice sample (1 mL) was transferred into 9 mL of sterile phosphate buffered saline tubes separately and ten-fold serial dilutions were prepared aseptically. Spread plate technique was followed for 0.1 mL of sample dilution on the surface of agar and incubated for 48 h for yeast and 96 h for molds at room temperature and plate yielding counts of 30-300 colonies were chosen (Cheesbrough, 2000). Fungal species were identified according to colony morphology and acid staining (Beuchat and Cousin, 2001).

Determination of anti-bacterial activity of the preservatives used in fruit juices

The anti-bacterial activity of the preservatives which were used in the analyzed fruit juice samples was performed by using agar well diffusion method as described previously (Ahmed *et al.*, 2013). Lawns of bacterial isolates (*Bacillus* spp., *S. aureus* and *E. coli*) were prepared over nutrient agar plates and holes were made in the nutrient agar using cork borer. Each of the homogenized preservative samples (10µg/mL) was then introduced separately in the specified hole with a positive control (streptomycin, 10µg/mL) and negative control (normal saline). Presence of clear zone around sample suspension indicated the presence of anti-bacterial activity.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of citric acid against bacterial isolates

Macro dilution agar method was used to determine MIC and MBC values of citric acid against bacterial isolates (Ahmed *et al.*, 2013). Two fold serial dilution of citric acid was made in phosphate buffer saline to get 0.031 to 1.0% (v/v) concentrations in eight sterile tubes. Pre-seeded nutrient agar plates with bacteria were used and wells of 8 mm diameter were made using sterile cork borer. 100 µl of each dilution of citric acid were poured in the wells and in control well 100 µl distilled water was added. The plates were observed for growth after overnight incubation at 37ºC. The lowest concentration of a preservative was considered as MIC. MBC was confirmed by incubating bacterial culture plates at 37ºC overnight with the concentrations of preservatives giving MIC.

Statistical analysis

Data retrieved for bacterial load was arranged using Microsoft Excel (MS Excel 2010, Microsoft Corporation). Statistical analyses were done using Statistical Package for the Social Sciences (SPSS version 16.0). Values were revealed as log10 CFU/ml (Bergey, 1984). One way ANOVA employing Duncan Multiple Range (DMR) test statistics were used.

RESULTS

During sampling process it was observed that samples of good quality brands have Pakistan standard PSQCA number while local brands do not have such number. The preservatives were not included in the composition of some of the products.

Total viable count (TVC) in fruit juices

Among the fruit juice samples analyzed, hundred percent were microbiologically contaminated indicating bad food safety situation. Higher contamination in most samples indicated higher threat of pathogenicity except samples of brands A and B. Significantly higher total viable count (TVC) ranging from 3.23±0.29 to 7.28±0.41 $\log_{10}$ CFU/mL was found in fruit juice samples of five various brands (Table I). Mean TVCs among apple, mango and orange flavor juice samples were as 5.62±1.45, 6.06±1.44 and 5.01±1.37 $\log_{10}$ CFU/mL respectively. Comparative evaluation of total microbial contamination among different fruit juice brands depicted mango juice of brand D containing maximum mean TVC, *i.e.* 7.28±0.41 $\log_{10}$ CFU/mL followed by mango juice of brand E while the least mean TVC was noticed in orange juice of brand C, *i.e.* 3.23±0.29 $\log_{10}$ CFU/mL. Fruit juice samples exceeding permissible limits of TVC were 65.55% and all the samples of brand C have TVC within permissible limits (Table II).

Total staphylococcal count (TSC) in fruit juices

Microbial quality of fruit juices is related to TSC. The number of TSC was ranging from 0.00±0.00 to 4.89±0.83 $\log_{10}$ CFU/mL (Table I). Mean TSCs among apple, mango and orange flavor juice samples were 1.93±1.81, 2.28±2.05 and 2.12±2.09 $\log_{10}$ CFU/ml, respectively. Among fruit juice brands, highest staphylococcal contamination was in orange juice of brand D, *i.e.* 4.89±0.83 $\log_{10}$ CFU/mL followed by mango juice of brand D, *i.e.* 4.84±0.46 $\log_{10}$ CFU/mL while all the juice samples of brand A and B do not have staphylococcal contamination. Fruit juice samples exceeding permissible limits of SC were 38.88% and all the samples of brand A and B have SC within permissible limits (Table II).

Total coliform counts (TCC) in fruit juices

TCC in various fruit juice samples was ranging from 0.00±0.00 to 4.48±1.03 $\log_{10}$ CFU/mL (Table I). Mean coliform counts in apple, mango and orange juice samples were 1.04±1.35, 1.26±1.63 and 1.30±1.86 log10 CFU/ml respectively. Among fruit juice brands, highest coliform contamination was in orange juice of brand D, *i.e.* 4.48±1.03 $\log_{10}$ CFU/mL followed by mango juice of brand D, *i.e.* 3.39±0.86 $\log_{10}$ CFU/mL while all the juice samples of brand A, B and C do not have coliform contamination. Fruit juice samples exceeding permissible limits of TCC were 37.77% and all the samples of brand A, B and C have TCC within permissible limits (Table II). On the analysis of *Salmonella* spp. and *Shigella* spp. none of the fruit juice samples contained these contaminants.

Total fungal count (TFC) in fruit juices

Yeast and molds are common contaminants present in food especially fruit juices. TFC in various fruit juice samples was ranging from 1.75±0.58 to 4.40±0.20 $\log_{10}$ CFU/mL (Table I). Mean fungal counts in apple, mango and orange juice samples were 3.13±0.51, 3.55±0.57 and 1.99±0.22 log10 CFU/ml respectively. Among fruit juice brands, highest fungal contamination was in mango juice of brand E, *i.e.* 4.40±0.20 $\log_{10}$ CFU/mL followed by mango juice of brand C, *i.e.* 3.39±0.86 $\log_{10}$ CFU/mL while the least mean fungal count was noticed in orange juice of brand C, *i.e.* 1.75±0.58 $\log_{10}$ CFU/mL. Fruit juice samples exceeding permissible limits of TFC were 45.55% and all the samples of brand A and B have TFC within permissible limits (Table II).

Prevalence of microorganisms in fruit juice samples

Among the bacteria isolated from fruit juices, *Bacillus* spp. were predominant 66.37%, followed by *S. aureus* 21.68% and *E. coli* 11.94%. (Fig.1). Fungal species isolated from fruit juices were *Aspergillus* spp. 41.37%, followed by *S. cerevisiae* 21.68%, *Penicillium* spp. 20% and *Rhizopus* 11.03% (Fig. 2).

Anti-bacterial activity of the preservatives used in fruit juices

Among the preservatives used, citric acid was found to exhibit highest anti-bacterial activity. Ascorbic acid was active against 2 out of 3 bacteria. No activity was found against sodium benzoate (Table III).

MIC and MBC of citric acid against bacterial isolates

In vitro MIC values of citric acid against bacterial isolates from fruit juices were between 0.5 and 1.0%

Table I.- Mean microbial load of various fruit juices.

Juice Flavor	Juice Brand		TVC (log_{10} CFU/ml) Mean ±S.D	TSC (log_{10} CFU/ml) Mean ±S.D	TCC (log_{10} CFU/ml) Mean ±S.D	TFC (log_{10} CFU/ml) Mean ±S.D
Apple	A		5.10±0.39	0.00±0.00	0.00±0.00	2.85±0.20
	B		5.59±0.49	0.00±0.00	0.00±0.00	2.44±0.47
	C		3.36±0.57	2.24±0.63	0.00±0.00	3.28±0.59
	D		6.97±0.40	2.96±0.45	2.39±0.55	3.26±0.57
	E		7.12±0.28	4.48±0.57	2.82±0.58	3.82±0.67
		Mean±SD	5.62±1.45	1.93±1.81	1.04±1.35	3.13±0.51
Mango	A		5.84±0.52	0.00±0.00	0.00±0.00	3.17±0.43
	B		6.57±0.27	0.00±0.00	0.00±0.00	3.01±0.51
	C		3.50±0.39	2.74±0.66	0.00±0.00	3.86±0.65
	D		7.28±0.41	4.84±0.46	3.39±0.86	3.33±0.58
	E		7.13±0.20	3.86±0.52	2.92±0.57	4.40±0.20
		Mean±SD	6.06±1.44	2.28±2.05	1.26±1.63	3.55±0.57
Orange	A		4.14±0.43	0.00±0.00	0.00±0.00	1.75±0.58
	B		4.68±0.37	0.00±0.00	0.00±0.00	1.83±1.04
	C		3.23±0.29	2.14±0.89	0.00±0.00	1.92±0.50
	D		6.22±0.32	4.89±0.83	4.48±1.03	2.20±0.54
	E		6.77±0.37	3.60±1.07	2.02±0.53	2.25±0.70
		Mean±SD	5.01±1.37	2.12±2.09	1.30±1.86	1.99±0.22

Table II.- Fruit juice samples exceeding permissible limits of microbial load.

log_{10} CFU/ml	Fruit Juice Brands					
	A (n=18) n^a (%)	B (n=18) n^a (%)	C (n=18) n^a (%)	D (n=18) n^a (%)	E (n=18) n^a (%)	Total (n=90) n^a (%)
TVC > 5	10 (55.55)	13 (72.22)	00 (0.00)	18 (100)	18 (100)	59 (65.55)
TSC > 3	00 (0.00)	00 (0.00)	03 (16.66)	16 (88.88)	16 (88.88)	35 (38.83)
TCC > 2	00 (0.00)	00 (0.00)	00 (0.00)	18 (100)	16 (88.88)	34 (37.77)
TFC > 3	00 (0.00)	00 (0.00)	14 (77.77)	15 (83.33)	12 (66.66)	41 (45.55)

n^a , Number of samples with CFU/ml corresponding to the first column of same row

Table III.- Anti-bacterial activity of preservatives used in fruit juices. Data are expressed as mean inhibition zone area (mm^2) ± SD.

Preservative	Zone of inhibition (mm^2 ± SD)		
	***Bacillus* spp.**	***S. aureus***	***E. coli***
Sugar	0	0	225±0.15
Citric acid	225±1.26	144±1.11	289±0.36
Ascorbic acid	144±0.12	0	256±1.28
Malic acid	0	0	100±0.10
Sodium citrate	25±0.05	0	0
Sodium benzoate	0	0	0
Streptomycin (+ve control)	324±1.22	400±2.18	225±0.07
Normal saline (-ve control)	0	0	0

Table IV.- Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of citric acid against bacterial isolates.

Bacterial isolates	Concentration of citric acid (v/v %)							
	0.031	0.062	0.125	0.25	0.5	1.0	MIC	MBC
Bacillus spp.	+	+	+	+	-	-	0.5	0.5
S. aureus	+	+	+	+	+	-	1.0	1.0
E. coli	+	+	+	+	-	-	0.5	0.5

(v/v). *Bacillus* spp. and *E. coli* isolates were most sensitive surviving upto 0.5% concentration and *S. aureus* isolates were most resistant surviving upto 1.0% concentration of citric acid (Table IV). The MBC of citric acid was 0.5 and 1.0% (v/v) equaled MIC (Table IV). It can be concluded that citric is more efficient preservative than other preservatives used in the manufacture of fruit juices.

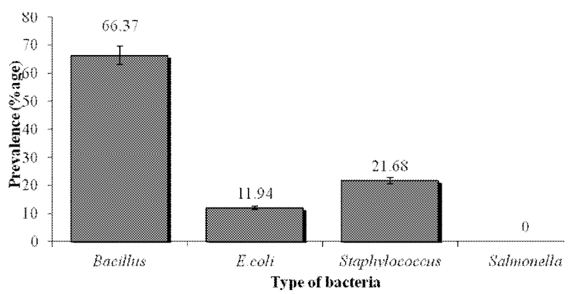

Fig. 1. Prevalence of bacterial isolates among various fruit juices.

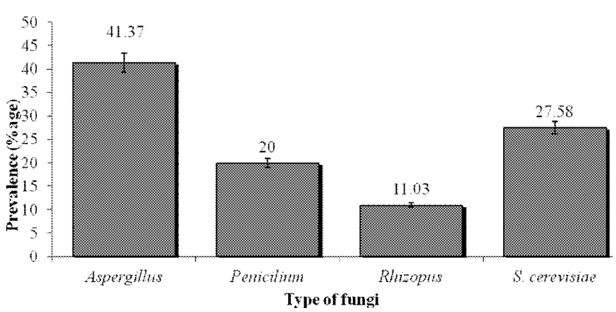

Fig. 2. Prevalence of fungal isolates among various fruit juices.

DISCUSSION

Proper labeling of fruit juice samples manifest that the samples were genuine and prepared according to good manufacturing practice (GMP) and standards. A food is deemed to be adultered if it contains any poisonous or deleterious substance, which renders it injurious to health (WHO, 2003).

The microbial contamination could be due to low quality raw materials, contaminated processing equipment's and environment, packaging materials and untrained workers. The bacterial count was low for some of the packaged fruit juices and comparatively higher for some others. Presence of microbes in high numbers (TVC >5 log10 CFU/ml) is responsible for the spoilage of fruit juices. Maximum permissible range of TVC in fruit juices is 5 log10 (Gulf-Stan, 2000; Codex-Stan, 2005). The results in this study showed that mean total viable counts of the packaged apple and mango fruit juice samples from brands A, B, D and E, and orange fruit juice samples from brands D and E obviously exceeded the maximum recommended standards. TVCs in various brands of apple, mango and orange fruit juice samples vary non-significantly with the standard permissible counts (p>0.05). Significantly higher mean TVCs indicate poor quality and lack of hygienic conditions during manufacturing, processing and packaging of fruit juices. Highest contamination was present in fruit juices of local brand D and E which are mostly served at bus stops, railway stations and open public places. It showed that such juices were prepared by mixing juice flavors in contaminated water at local plants under unhygienic conditions. The other brands were of good quality and have comparatively low contamination and that would be due to good manufacturing practices. In addition, suppressive effect of preservatives coupled with low pH of the juices and limitations of growth in anaerobic environment limited the contaminants (Parish, 1991).

Iqbal *et al*. (2015) reported mean TVCs among non-pasteurized brands of packed fruit juices (6.80±1.91 CFU/ml) were non-significant with standard permissible limits (p>0.05). Higher levels of total viable counts (TVCs) in fruit juices are in accordance with the earlier studies (Durgesh *et al.,* 2008; Bagde and Tumane, 2011; Rahman *et al.,* 2011). Highest total bacterial count of 2.66 x 10^6 CFU/ml in orange juice and lowest bacterial count of 1.59 x 10^2 CFU/ml in mango juice (Rashed *et al.,* 2013) and total heterotrophic bacterial counts of some fruit juices range from 3.0 x 10^2 CFU/ml to 9.0 x 10^4 CFU/ml were reported (Odu and Adeniji, 2013). The relatively higher bacterial counts are due to poor hygienic conditions and are the cause of food spoilage and food borne illnesses. There are some reports about the bacterial counts of fruit juices within the standard limits (Basar and Rahman, 2007; Jackson *et al.,* 2010; Tasnim *et al.,* 2010). The viable bacterial counts of bottled drinks and juice were 3.7 CFU/ml and 4.1 CFU/ml, respectively (Abdalla *et al.,* 2009).

Presence of staphylococci in high numbers (TSC >3 log10 CFU/ml) is health hazard as they are responsible for the spoilage of fruit juices and food borne diseases. Maximum permissible range of TSC in fruit juices is 3 log10 (Gulf-Stan, 2000; Codex-Stan, 2005). These results showed that mean TSCs of the packaged apple, mango and orange fruit juice samples from local brands D and E exceeded the maximum recommended standards. TSCs in various brands of apple, mango and orange fruit juice samples vary significantly with the standard permissible counts (p>0.05). Significantly higher mean TSCs indicate poor quality and lack of hygienic conditions during manufacturing, processing and packaging of fruit juices. Iqbal *et al*. (2015) reported mean SCs of non-pasteurized brands of packed fruit juices (5.45±1.06 CFU/ml) were non-significant with standard permissible limits (p<0.05). Higher levels of TSCs in fruit juices are in accordance with the previous studies (Ahmed *et al.,* 2009; Pilo *et al.,* 2009; Rashed *et al.,* 2013). In the present study 40% of fruit juice samples had no staphylococcal count which is according to previous findings that reported the absence of staphylococci from fruit juices (Odu and Adeniji, 2013). Some fruit juices have low contamination although they lack preservative and it could be due to low pH. Bacterial counts of fruit juices within the standard limits have been reported (Basar and Rahman, 2007; Jackson *et al.,* 2010).

Coliforms are considered as indicators of quality. Presence of coliforms in high numbers (TCC >2 $\log^{10}$ CFU/ml) is health hazard as they are responsible for the spoilage of fruit juices and food borne diseases. Maximum permissible range of TCC in fruit juices is 2 $\log^{10}$ (Gulf-Stan, 2000; Codex-Stan, 2005). These results showed that mean TCCs of the packaged apple, mango and orange fruit juice samples from local brands D and E exceeded the maximum recommended standards. Safe Food Consumption Standard prohibits the presence of coliforms in fruit juices (Andres *et al.,* 2004), brand D and E are therefore unfavorable for consumption. TCCs in various brands of apple, mango and orange fruit juice samples vary significantly with the standard permissible counts (p>0.05). Significantly higher mean TCCs indicate poor quality and lack of hygienic conditions during manufacturing, processing and packaging of fruit juices. Iqbal *et al*. (2015) reported mean CCs of non-pasteurized brands of packed fruit juices (3.25±1.25 $\log^{10}$ CFU/ml) were non-significant with standard permissible limits (p>0.05). Higher levels of TCCs in fruit juices are in accordance with the previous studies which reported coliform counts of 3.6 x 10^4 CFU/ml and 1.0 x 10^6 CFU/ml, respectively (Al-Jedah and Robinson, 2002; Rashed *et al.,* 2013). The absence of coliforms in 60% of fruit juice samples is according to previous studies (Jackson *et al.,* 2010; Tasnim *et al.,* 2010; Odu and Adeniji, 2013). The presence of coliforms in fruit juice may be due to their being natural flora of fruits which entered into improperly processed fruit juice (Frazier and Westhoff, 1998).

Maximum permissible range of TFC in fruit juices is 3 log10 (Gulf-Stan, 2000; Codex-Stan, 2005). These results showed that mean total fungal counts of the packaged apple fruit juice samples from brands C, D and E and all brands of mango juice samples exceeded the maximum recommended standards. TFCs in various brands of apple, mango and orange fruit juice samples vary significantly with the standard permissible counts (p>0.05). High fungal counts in all the products ranging from 1.4x10^3 to 1.7x10^5 cfu/ml have been reported (Rahman *et al.,* 2011; Oranusi *et al.,* 2012). Fungi are the major causes of spoilage of fruits and vegetables producing aflatoxins and other mycotoxins (ICMSF, 1998; Riby *et al.,* 2001; Kawo and Abdulmumin, 2009).

The generally observed high microbial counts in this study could be attributed to the influence of environmental factors on the microbial populations, which have been shown to play a significant role in affecting the quality of food products. The ways these products are handled in an open air environment are no exception (Kawo and Abdulmumin, 2009).

Identification of isolates showed the presence of *Bacillus* sp., *Staphylococcus aureus*, *E. coli*, *Aspergillus* spp., *Penicillum* sp., *Rhizopus* and *Saccharomyces* sp., particularly important is the *Bacillus* sp., *Bacillus* spp. is known causative agent of food poisoning and intoxication (FAO, 1979; Kawo and Abdulmumin, 2009). The presence of *Bacillus* sp. (66.37%) in almost all the fruit juices may be attributed to unhygienic environmental conditions, poor handling and its ability to form spores which are heat resistance. The spores of *Bacillus* species could survive high temperatures of processing (Essien *et al.,* 2011). In addition, its immediate source is usually the plant equipment, but it may also have originated from one of the major ingredients of fruit juice *e.g.* sugar (Banwart, 1989). The presence of *Bacillus* sp. was also reported in previous studies (Abdalla *et al.,* 2009; Iqbal *et al.,* 2015).

Staphylococcus aureus are facultative and tolerant and may enter fruit juices due to food handlers and environment (Oranusi *et al.,* 2012). *E. coli* is a gram negative facultative bacterium and its presence in fruit juices is attributed to its acid adaptive and acid tolerant properties. It is an important pathogen that cause hemolytic uremic syndrome in human beings (Batool *et al.,* 2013). The presence of these microbes though in low amount needs to be restrained to prevent spoilage and foodborne illness (Oranusi *et al.*, 2012). In the present study, none of the samples contained *Salmonella* spp. Previous studies have reported the absence of any viable microorganisms in fruit juice samples (Ghengesh *et al.*, 2005; Jackson *et al.,* 2010; Odu and Adeniji, 2013). These findings suggested use of higher amount of preservatives in fruit juice preparations that had bacteriostatic effect on microbes. It can be suggested to use low amount of preservatives (Basar and Rahman, 2007). Some studies have reported the presence of *Salmonella* in fruit juices (Ahmed *et al.,* 2009; Rahman *et al.,* 2011).

Organic acids are popular preservatives with marked anti-bacterial traits (Nwachukwu and Ezeigbo, 2013). In our study, citric acid exhibited anti-bacterial activity against all bacteria. It suggests that citric acid could be used to prevent food spoilage. In another study, citric acid was found satisfactory preservative both in terms of microbiological criteria and anti-bacterial traits (Sultana *et al.,* 2014). Although preservatives are used in almost all the packed fruit juices, high bacterial load may be due to unhygienic conditions and improper use of preservatives.

The presence of fungi in packaged fruit juice samples indicates that the handling of fruits and the extraction of juices leaves a lot to be desired with respect to sanitary practices. *Penicillum* sp. and *Saccharomyces* sp. were also isolated from the samples. This may be due to contamination of the surface of fruit by these organisms which end up in the fruit during processing (FAO, 2002). The fungi isolated in this study are mostly contaminants. The surrounding air, packaging materials and the personnel concerned with the packaging processes could all serve as sources of these contaminants. *Aspergillus* species is specifically known to produce mycotoxins, which cause food intoxication in man and other animals. Various products have been linked with food poisoning because of quality, composition and handling (Kawo and Abdulmumin, 2009).

All the commercial packed fruit juices sold on roadside and at retail shops were found highly contaminated with pathogenic bacteria. Lack of pasteurization is one of the major factors responsible for contamination of fruit juices. Testing for these organisms at specific control points could be the best way of quality control. Constant surveillance and good manufacturing practice are the best procedures to control contamination (Juhaniakova *et al.,* 2013). Therefore, it is suggested that these juices should be monitored periodically in food laboratories for quality and human consumption. The application of Hazard Analysis Critical Control Point (HACCP) system should be introduced in the food industry sector to improve the quality of food products manufactured in Lahore (Pakistan).

CONCLUSIONS

It can be concluded that packed fruit juices of local brands are responsible for common food borne illnesses in the region and are thus playing their part to raise the disease burden among poor populations. A precise and well defined monitoring and surveillance system needs to be implemented to address the food safety of packed fruit juices in Pakistan. Implementation of awareness programs on various heath related issues may be another strategy to deal food safety issues.

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Conflict of interest

The authors declare that they have no competing interests.

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