

Comparative Microbiological Quality Evaluation of Un-Branded and Branded Juices of Street Vended Sold in Retail Outlet of Peshawar City

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Abstract: This study was performed to evaluate the microbiological quality of Un-Branded street vended and branded juices sold in Peshawar City, Pakistan during June-August 2010. These juices were analyzed microbiologically using standard microbiological methods. The analyzed parameters were Total Plate Count (TPC), Total Coliform Bacteria (TCB), Total Fecal Coliform Bacteria (TFC), *Escherichia coli* O157:H7 and Yeast and Mould. The Un-Branded juices (apple, banana, mango, orange, lemon and sugarcane) were microbiologically analyzed and showed that TPC were in the range of 9×10^9 - 4×10^4 cfu/ml, TCB were in the maximum value 210 (MPN/ml) for sugarcane juice and lowest 9.0 (MPN/ml) were calculated for orange juice. TFCB were absent in orange and lemon Juice, while apple, banana, mango and sugarcane juices were contaminated with TFC (MPN/ml) values 15, 23, 9.0 and 93 respectively. *E. coli* were present in apple, banana, mango and sugarcane juice, while it was absent in orange and lemon juices. The yeast and mould values (cfu/ml) 4×10^5 , 3×10^6 , 3×10^4 , 7×10^5 , 6×10^4 and 45×10^8 were found in Apple, Banana, Mango, Orange, Lemon and Sugarcane juice respectively. The results of Branded juices (P1, P2, P3, P4, P5 and P6) indicated that all juices were free from any microbes, except P6 which have found 10 TPC (cfu/ml) and 50 (cfu/ml) yeast and Mould. The *Escherichia Coli* O157:H7 isolates were evaluated to different antibiotics susceptibility tests. It was observed that these isolates were resistant to Amoxyllin, Ampicillin and Cifloxacin. But the rest of antibiotics (Tetracycline, Gentamycin, Ofloxacin, Augmentin and Ciprofloxacin) showed inhibition zones which range from 22 mm-12 mm.

Key words: Un-Branded Juices • Branded Juices • Microbial Quality • Health hazards • Antibiotics Susceptibility tests

INTRODUCTION

Now a days fruit juices are becoming an important part of the modern diet in many countries. They are nutritious beverages and can play a significant part in a healthy diet because they offer good taste and a variety of nutrients found naturally in fruits. Juices are available in their natural concentrations or in processed forms [1]. Fruit juices and nectars are food products of great nutritional value, rich in vitamins, mineral salts, simple sugars and organic acids which are easily assimilated by the human organism [2]. Water used for juice preparation can be a major source of microbial contaminants such as total coliforms, faecal coliforms, faecal streptococci, etc. Environmental fomites may also make the fruits unsafe

and these may have a role in the spread of Salmonella, Shigella, Vibrio, *Escherichia coli* and other and cause diseases as well fruit spoilage [3]. In countries, where street food vending is prevalent, there is commonly a lack of information on the incidence of food borne diseases related to the street vended foods. However, microbial studies on such foods in American, Asian and African countries have revealed increased bacterial pathogens in the food. There have been documented outbreaks of illnesses in humans associated with the consumption of unpasteurized fruit and vegetable juices and fresh produce [4].

The composition/ ingredients of processed juices are mainly water, sugar, preservatives, color and fruit pulp. The most commonly used preservatives are benzoic acid,

sorbic acid or sulphur dioxide. Natural colors such as anthocynins and betanin are used. Acid is an essential universal constituent of juice and the most commonly used acid is citric acid.

In view of the threat posed by the bacterial pathogens in juices and the flourishing demands for such juices, the present work was undertaken to assess the microbiological quality of branded pasteurized and freshly pressed or squeezed juices from street vendors during June to August 2010 in Peshawar Pakistan and to generate data that can be used to provide awareness to the community and also help to improve the conditions of juice making industry.

MATERIALS AND METHODS

Collection of Sample: During the study, six different branded juices samples (P1, P2, P3, P4, P5 and P6) and six street vended un-pasteurized juices (apple, banana, mango, orange, lemon and sugar cane) were collected from different localities of Peshawar City Pakistan.

Sample Preparation: Each type of sample was analyzed microbiologically. The samples were opened turn by turn in a laminar airflow chamber under all aseptic measures. The chamber provided sterile environment for the microbiological analysis.

Microbiological Analysis of Juices

Total Viable Count: Total Viable Count was determined by pour plate method as described [5, 6]. Place 50 ml of the sample aseptically add 450 ml of sterile Phosphate buffer and blend for 2 min. (this provides 1:10 dilution). Prepare serial dilutions by transferring 1 ml of blend and successive dilutions to 9 ml of sterile phosphate buffer in test tubes. Pipette 1 ml of each dilution into sterile labeled Petri dishes in duplicate. Add 15-20 ml of molten plate count agar at 44-46 °C. Mix the contents by rotating the Petri dishes 5 times clockwise and 5 times anti-clockwise alternately. When agar has set, stack the Petri dishes in inverted position in the incubator at 35 °C for 48 hours \pm 2, after incubation colonies were counted by Colony Counter and result was expressed as cfu/ml.

Total Coliform Bacteria/fecal Coliform Bacteria: The MPN of total coliforms bacteria were determined by multiple tube fermentation technique [5, 6]. Place 50 ml of the sample aseptically in the blender jar, add 450 ml of sterile phosphate buffer and blend for 2 minutes. Prepare two serial dilutions by transferring 1 ml of blend and

successive dilutions to 9 ml of sterile phosphate buffer in test tubes. Pipette 1 ml each of the dilutions 1:10 (blend), 1:100 dilution and 1:1000 dilution into three sets of 10ml sterile Lactose broth tubes (containing Durham tubes) in triplicate and incubated at 35 °C \pm 0.5 °C for 24 and 48 \pm 2 hrs after inoculation. Tubes were examined for evidence of gas production at the end of 24 hrs incubation. Gas production was measured by gas displacement in the inverted vial and also effervescence produced when the tube was gently shaken. Negative tubes were re-incubated for additional 24 hr and again examined for gas production. Positive tubes with gas formation and turbidity were sub-cultured into Brilliant Green Lactose bile broth (BGBB) and E.C. Broth having 10 ml broth with inverted Durham tubes by means of 3mm loop. All BGBB tubes were incubated at 35 °C and E.C. Broth tubes at 44.5°C for 48 hrs and examined for gas production. Total coliform and fecal coliform were calculated from MPN tables [5].

Escherichia coli O157:H7: EMB Agar was used for the enumeration of *E. coli*. All the tubes of E.C. broth showing gas were subculture by streaking on EMB agar plates and incubated at 35 °C for 18-24 hrs. Positive plates contained typical colonies with green metallic sheen were inoculated on PCA slants (plate count agar) and incubated at 35 °C for 18-24 hrs and identified biochemically and also by kits (*E.ColiO157:H7* latex test reagent kit Pro Lab. Canada).

Yeast and Mould: Yeast and mould was calculated following the FAO method described [6]. Place 50 ml of the sample aseptically add 450 ml of sterile Phosphate buffer and blend for 2 min. Prepare serial dilutions by transferring 1 ml of blend and successive dilutions to 9 ml of sterile phosphate buffer in test tubes. One ml portion of each dilution was inoculated on Potato dextrose agar (PDA). Petri dishes were incubated at 22-25 °C for 5 days and after incubation colonies were counted by colony counter and result were expressed as yeast and mould / ml.

Antibiotics Susceptibility Test: A suspension of the isolated organism *Escherichia coli* O157:H7 was transferred into Petri-dishes accordingly and swab over the entire plate, it was then incubated for 1 hrs at 37°C and a forceps was used to transfer each sensitivity disc on the plate and incubated for 24 h at 37°C. The antibiotics used included amoxyllin, ampicillin, tetracycline, gentamycin, ofloxacin, augmentin, ciprofloxacin and cifloxacin as described [7, 8].

RESULTS AND DISCUSSION

Microbiological Analysis of Un-Branded Juices:

The microbiological analysis of un-branded/ un-pauperized street vended juices (apple, banana, mango, orange, lemon and sugar cane) were collected from different premises of Peshawar City, Pakistan and analyzed for their Total Plate Count, Total Coliform bacteria (MPN/ml), Total Fecal Coliform Bacteria (MPN/ml), *Escherichia coil O157:H7* and Yeast and Mould. The results are shown in Table 1. The highest Total Plate Count (TPC) were found in Sugar cane juice 9×10^9 cfu/ml, while the lowest were calculated in Apple juice was 4×10^4 cfu/ml, the other juices banana, mango, orange and lemon calculated values of TPC (cfu/ml) were 6×10^5 , 8×10^4 , 12×10^8 and 45×10^9 respectively.

Generally on the surface of fruit contains microbial load counts were 1×10^5 cfu/cm [9, 10, 11]. Poor washing of fruits have entered these bacteria to juices leading to contamination. In addition lack of appreciation of basic health safety issues by vendors contribute to augmentation of the microbial loads. These include use of crude stands and carts, unavailability of running water for dilution and washing, prolonged preservation without refrigeration, unhygienic environment with swarming flies and airborne dust [12].

A fecal coliform standard is currently used to evaluate the sanitary condition of shellfish growing waters. Its use has been successful and should be continued. However, since the best indicator of fecal contamination. Sugarcane juice were contaminated with TCB (MPN/ml) values 210, its TPC were also higher which indicate that sugarcane juice were mostly contaminated by any possible microbiologically contamination i.e. water contamination, personnel hygiene, air pollution, raw material pollution etc. The second most contaminated juice was mango in term of TCB (MPN/ml) values were 120. The other juices apple, banana, orange and lemon TCB were 43 (MPN/ml), 150 (MPN/ml), 9.0 (MPN/ml) and 23 (MPN/ml) respectively. Total fecal coliform bacteria are an indication of any possible fecal contamination source of animals and human feces. The results indicate that TFCB (MPN/ml) were absent in two samples orange juice and lemon juice. While apple, banana, mango and sugarcane juices TFCB values were 15 (MPN/ml), 23 (MPN/ml), 9.0 (MPN/ml) and 93 (MPN/ml) respectively.

Escherichia coli are the best indicator of fecal contamination presently available. Microbiological criteria involving *E. coli* should be applied to sensitive foods as indicators of fecal contamination. The apple, banana, mango and sugarcane juice were found contaminated with *Escherichia coil O157:H7*, while the remaining two juices orange and lemon were safe from these bacteria.

Table 1: Microbiological Analysis of Un-branded/ un-pasteurized Juices sold in Peshawar City, Pakistan

Type of Juice	TPC (cfu/ml)	TCB (MPN/ml)	TFCB (MPN/ml)	E. coli O157:H7	Yeast and Mould
Apple	4×10^4	43	15	+ve	4×10^5
Banana	6×10^5	150	23	+ve	3×10^6
Mango	8×10^4	120	9.0	+ve	3×10^4
Orange	12×10^8	9.0	-ve	-ve	7×10^5
Lemon	45×10^9	23	-ve	-ve	6×10^4
Sugarcane	9×10^9	210	93	+ve	45×10^8

TPC (cfu/ml) = Total Plate Count (colony forming unite); TCB (MPN/ml) = Total Coliform Bacteria (Most Probable Number); TFCB (MPN/ml) = Total Coliform Bacteria (Most Probable Number) and E. coli O157:H7= *Escherichia coli* O157:H7

Table 2: Microbiological Analysis of Branded Juice sold in Peshawar City, Pakistan

S#	Parameters	Lab. Code #					
		P1	P2	P3	P4	P5	P6
1	TPC	Nil	Nil	Nil	Nil	Nil	10
2	TCB (MPN/ml)	Nil	Nil	Nil	Nil	Nil	Nil
3	TFCB (MPN/ml)	Nil	Nil	Nil	Nil	Nil	Nil
4	<i>E. Coli O157:H7</i>	Nil	Nil	Nil	Nil	Nil	Nil
5	Yeast and Mould	Nil	Nil	Nil	Nil	Nil	50

TPC (cfu/ml) = Total Plate Count (colony forming unite); TCB (MPN/ml) = Total Coliform Bacteria (Most Probable Number); TFC (MPN/ml) = Total Coliform Bacteria (Most Probable Number) and E. coli O157:H7= *Escherichia coli* O157:H7.

Table 3: Antibiotics Susceptibility profile of *E. coli* Isolates

S. No.	Tested Antibiotics	Zone of Inhibition (mm)
1	Amoxyllin	*R
2	Ampicillin	R
3	Tetracycline	12
4	Gentamycin	22
5	Ofloxacin	14
6	Augmentin	17
7	Ciprofloxacin	15
8	Cifloxacin	R

*R= Resistance.

Yeast and mould were also found in the analyzed samples. The results indicate that the highest (45×10^8 cfu/ml) yeast and mould were present in Sugarcane Juice and the lowest were observed in mango juice (3×10^4 cfu/ml). While the other count of yeast and mould viz., 4×10^5 cfu/ml, 3×10^6 cfu/ml, 7×10^5 cfu/ml and 6×10^4 cfu/ml were found in the apple, banana, orange and lemon juices respectively. A wide variety of fungal species were also present in sugarcane juice samples all over Karachi [13].

Microbiological Analysis of Branded Juices: The branded juices were procured from different shops of Peshawar City Pakistan. They examined at the time of purchased for leakage and expiry date. The microbiological analysis results (Table 2) of theses branded juices showed that TPC, TCB, TFC, *E. coli* O157:H7 and Yeast and Mould were not detected in any P1, P2, P3, P4, P5 and P6 samples except that TPC 10cfu/ml and yeast and mould 50cfu/ml were found in P6 branded juice. The study [14] on the pasteurized beverages packed in sealed plastic containers was contaminated with yeast (86%), mold (69%), coliform (59%) or *E. coli* (13%).

Antibiotics Susceptibility Test against Isolated *E. coli*: *Escherichia coil O157:H7*, were isolated from apple, banana, mango and sugarcane juice and they were tested against selected antibiotics. The antibiotics were amoxyllin, ampicillin, tetracycline, gentamycin, ofloxacin, augmentin, ciprofloxacin, cifloxacin. The results of antibiotic susceptibility were observed that isolate of *Escherichia coil O157:H7* resistance to Amoxyllin, Ampicillin and Cifloxacin. While Gentamycin showed the highest inhibition zone (22mm) to isolate, which were followed by Augmentin (17mm) Ciprofloxacin (15mm), Ofloxacin (14mm) and Tetracycline (12mm) showed sensitivity.

CONCLUSION AND RECOMMENDATION

In order to improve the microbiological quality of un-pasteurized and pasteurized juices produced on small-scale premises within a short period of time, the following strategies must be performed:

- Regular monitoring of un-pasteurized and pasteurized juices. (2) Basic GMP at the premises especially on personal hygiene, equipment environment and utensils. (3) Training on GMP and the workers or street vended hawkers. (4) Encouragement and enforcement of food legislation.

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