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Microbiological Quality Evaluation, Preservation and Shelf Life Studies of Sugar Cane Juices Sold in Peshawar City, Khyber Pakhtunkhwa-Pakistan

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Abstract: The present study was designed to examine the quality, safety, preservation and storage studies of freshly squeezed sugar cane sold by street venders and hawkers in Peshawar City, Khyber Pakhtunkhwa-Pakistan. Forty samples of sugar cane juice were collected from selected areas and analyzed for total plate count, total coliform bacteria, fecal coliform bacteria, *Escherichia coli*, yeast and mould. The results showed that in all the localities the street vended sugar cane juices remained hygienically poor as indicated through high bacterial load i.e. 4×10^2 - 3×10^7 CFU/ml. All samples were contaminated with coliform bacteria ranged from 46 - = 1100 MPN/ml. Seventy five percent of samples were contaminated with confirmed *Escherichia coli*. All the examined samples were contaminated with yeast and mould. Total coliforms was present in all analyzed samples of ice whereas confirmed *Escherichia coli* were present in 37% of samples and total plate count of ice samples ranged from 1×10^2-2×10^4 CFU/ml. Juice was pasteurized just after extraction and its pH was maintained at 4.3 by the addition of citric acid. Pasteurized juice was hot filled in sterilized glass bottles. Bottles were stored at room temperature for one month. Bottled juice samples were merely accepted up to a storage period of thirty days.

Key words: Sugar Cane Juice · Ice · Vendor's Hands · Microbial Quality · Preservation

INTRODUCTION

Sugar cane juice is extensively consumed as a refreshment as well as energetic drink. Sugar cane juice has valuable properties and is regularly consumed along with lemon juice to hepatitis patients. In many tropical countries the sugar cane juice is a common man's drink and is sold at all municipal localities, benches, park and busy market places. Though in view of their ready utilization extraction, quick methods of cleaning and handling, they might often prove to be community fitness fear [1, 2]. The occurrence of native, epiphytic micro flora on sugar cane plant was ascertained [3, 4]. The cane incoming at the sugar cane pulling out may contain a wide micro flora on its surface [5]. Juice of sugarcane is commonly obtained through squeezing sugarcane, therefore improper washing of sugarcane adds bacteria in to extract leading to contamination. Therefore, sugar cane juice has high risk of pathogenic microorganisms [6]. Probable sources of such microbial contamination have been recognized as raw material itself, unclean handling and inadequate cleaning of sugar cane knives, press, clothes, contact surface, ice, vendor's hands and air born contamination. Contaminated raw material used in these drinks has been known to be a source of infectious diseases such as vomiting, nausea, abdominal cramps, typhoid, diarrhea etc [6-8]. To ensure that food is microbiologically safe, both food handlers and food itself must be monitored on permanent bases [9, 10]. These unpasteurized juices have been a potential source of bacterial pathogens notably, *Salmonella* and *E. coli* (O157H7) [11-13]. Epidemiological data indicates that cross contamination during food preparation contribute notably to the occurrence of food born diseases [14].

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In 1991 cholera epidemic in Pune, India was attributed to contaminated sugar cane juice with added ice. In this case the ice was found contaminated with *Vibrio cholerae* [15]. The aim of the current study was to evaluate the quality of sugar cane juice, the microbial flora on vender's hands, ice used for cooling these juices and to preserve a sugar cane.

MATERIALS AND METHODS

Collection of Samples: During the study, eight focal locations in the Peshawar City of Khyber Pakhtunkhwa-Pakistan (Table 1) were investigated. Samples of sugar cane juices and ice were collected from at least five shops in each location. All the samples were collected in sterile Duran bottles of 1L capacity properly sealed.

Microbiological Analysis of Juices and Ice

Total Plate Count (TPC): The samples were opened turn by turn in a laminar airflow chamber under all aseptic measures. Total Viable Count was determined by pour plate method. Serial dilution (10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}) of the juices and ice samples were made and aliquots of 1ml were added to each duplicate Petri dish. Plate count agar (PCA) was added to each Petri dish and incubated at 35°C for 48 hours ±2, after incubation colonies were counted by colony counter and result was expressed as CFU/ml [16, 17].

Total Coliform Bacteria/Fecal Coliform Bacteria: The Most Probable Number (MPN) of total coliform bacteria was determined by multiple tube fermentation technique [16,17]. One ml from the previously prepared 10^{-1}, 10^{-2} and 10^{-3} dilutions were inoculated into three replicate tubes containing 10 ml of Lactose Broth with inverted Durham tubes and incubated at 35 °C± 0.5 °C for 24 and 48± 2 hrs after inoculation. Tubes were examined for evidence of gas production/turbidity at the end of 24 hrs incubation. Negative tubes were re-incubated for additional 24 hr and again examined for gas production/turbidity. Positive tubes with gas formation and turbidity were sub-cultured into BGB (Brilliant Green Lactose bile broth) and E.C. Broth having 10ml broth with inverted Durham tubes by means of 3 mm loop. All BGB tubes were incubated at 35 °C for 48 hrs and E.C. Broth tubes at 44.5 in water bath for 24 hrs and examined for gas production. Total coliform and fecal coliform were calculated from MPN index table.

Table 1: Sample collection points

Area code	Location
A	Khyber Teaching Hospital Stop
B	Secondary Board Peshawar Stop
C	Khyber Bazar
D	Bhana Mari Chowk
E	Hashtnagari Stop
F	General Bus Stand
G	Sadder
H	Hayatabad

Escherichia coli (*E. coli*): 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 [16, 17] and also by kits (*E. coli* O157:H7 latex test reagent kit Pro Lab. Canada).

Yeast and Mould: Yeast and mould was calculated following the described method [16]. 25 ml of juice and ice sample (melt at 5 ºC) was obtained and serial dilution (10^{-1} to 10^{-4}) was carried out. One ml portion of each dilution was inoculated on Potato dextrose agar (PDA). Petri dishes were incubated at 22-25 ºC for 5 days and after incubation colonies were counted by colony counter and result were expressed as yeast and mould / ml.

Bacteriological Analysis of Operator's Hands: The swab method was used for coliform determination on hands. Applicator stick 12-15 cm long was used. Sterile swab was moistened in sterile buffer peptone water. Rub the swab head slowly and thoroughly overall surface of hand three times reversing direction between strokes. Rinse the swab in known amount of sterile buffer after the area of hand has been swabbed. Total coliforms, fecal coliforms and *E. coli* were examined according to previously described methods [16, 17].

Preservation and Storage of Sugar Cane Juice: Sugar cane juice was extracted from locally available variety. Juice was filtered by muslin cloth before pasteurization. Pasteurization of juice was carried out at 90°C for five minutes. All the waxy material was removed from the top during pasteurization. pH of the juice was maintained at 4.3 by the addition of citric acid. Juice was hot filled in sterilized glass bottles (250 ml.) and crowned. Filled bottles were then processed in boiling water for 25

minutes. Bottled were stored at room temperature. Stored bottles were analyzed at 15 days interval for up to 30 days. TSS, pH, acidity and TPC, yeast and mold growth was studied. TSS was measured by using Digital Refrectometer Atago RX-1000. Acidity was determined by titration against 0.1 N NaOH solution and results were expressed as percent citric acid. pH was measured by digital pH meter. A panel of six judges following nine-point hedonic scale also evaluated sensory characteristics of the natural sugarcane juice.

RESULTS AND DISCUSSION

Forty (40) samples of sugar cane juice collected from eight selected areas of Peshawar City, Khyber Pakhtunkhwa-Pakistan (Table 1) were analyzed microbiologically for total plate count (TPC), total coliform bacteria, fecal coliform bacteria and yeast/mould (Table 2). These data indicated heavy bacterial load ranging from 4 x 10^2 – 3 x 10^7 CFU/ml. Maximum viable count was found in area B (3 x 10^7) followed by A (2 x 10^6). Minimum TPC was found in area H. The higher TPC of these areas are due to the heavy vehicular traffic, waste water lines and vegetables decomposing waste. The lower TPC might be due to posh area of Peshawar city where hygienic conditions was maintained to some extent. The results of total coliform bacteria in all analyzed samples were >110 MPN/ml except 46 MPN/ml which was found in area H. Fecal coliforms showed variation; the highest observed values 110 and 46 MPN/ml were in the area G and F respectively. The other areas such as A, B, C and D had 21, 24, 12 and 2.3 MPN/ml respectively. All the analyzed samples gave positive result for *E. coli* except samples from area E and H. Yeast and mould count was significantly the highest in area B as compared to other areas.

Table 2: Microbiological analysis of sugar cane juice

Area	Total Plate Count (CFU/ml)	Total Coliform Bacteria (MPN/ml)	Fecal Coliform Bacteria (MPN/ml)	*Escherichia coli*	Yeast and Mould (CFU/ml)
A (N = 5)	2×10^6	=110	21	+ve	2×10^5
B (N = 5)	3×10^7	=110	24	+ve	3×10^7
C (N = 5)	4×10^5	=110	12	+ve	7×10^4
D (N = 5)	8×10^5	=110	2.3	+ve	4×10^4
E (N = 5)	2×10^5	110	9.3	-ve	6×10^3
F (N = 5)	3×10^5	=110	46	+ve	4×10^6
G (N = 5)	8×10^4	=110	110	+ve	5×10^4
H (N = 5)	4×10^2	46	24	-ve	4×10^5

*Note: N= Number of samples collected, CFU = Colony Forming Unit, MPN = Most Probable Number.

Table 3: Bacteriological analysis of ice used in sugar cane juice

Area	Total Plate Count (CFU /ml)	Total Coliform Bacteria (MPN/100ml)	Fecal Coliform Bacteria (MPN/100ml)	*Escherichia coli*
A (N = 5)	2×10^4	46	=0.3	-ve
B (N = 5)	3×10^3	12	2.0	+ve
C (N = 5)	2×10^3	24	=0.3	-ve
D (N = 5)	6×10^3	21	=0.3	-ve
E (N = 5)	8×10^3	110	2.8	+ve
F (N = 5)	3×10^3	7.5	=0.3	-ve
G (N = 5)	8×10^3	9.3	1.5	+ve
H (N = 5)	2×10^2	15	=0.3	-ve

*Note: N= Number of samples collected, CFU = Colony Forming Unit, MPN = Most Probable Number.

Table 4: Bacteriological analysis of operator's hands during juice preparation

Area	Total Coliform Bacteria	Fecal Coliform Bacteria	*Escherichia coli*
A	+ve	-ve	-ve
B	+ve	+ve	-ve
C	+ve	-ve	-ve
D	+ve	-ve	-ve
E	+ve	+ve	-ve
F	+ve	-ve	-ve
G	+ve	+ve	-ve
H	+ve	-ve	-ve

Table 5: Effect of storage time on the sugar cane physicochemical parameters and microbial growth

Storage (Days)	pH	Acidity	Total Soluble Solid	Total Plate Count (*CFU/ml)	Yeast/Mould
0	4.31	0.64	21.3	3	Nil
5	4.30	0.63	21.2	3	Nil
10	4.27	0.65	21.1	3	Nil
15	4.22	0.68	21.1	4	Nil
20	4.18	0.70	21.0	4	Nil
25	4.14	0.73	21.0	5	Nil
30	4.10	0.80	21.0	6	Nil

*CFU = Colony Forming Unit.

Table 6: Effect of storage time on the sensory characteristics of sugar cane juice

	Storage (Days)						
Parameters	0	5	10	15	20	25	30
Appearance	8.0	7.0	8.0	7.0	7.2	7.0	7.0
Taste	8.0	8.0	6	7.0	7.0	7.0	7.0
Overall acceptability	8.0	8.2	8.0	7.0	7.0	7.0	6

*Note: 1=extremely dislike, 2=Strong dislike, 3=Moderate dislike, 4=Slight dislike, 5=Neutral, 6=slight like, 7=Moderate like, 8=Strong Like, 9 = extremely like.

The sugar cane juice was served along with ice to the consumers. Therefore the ice samples were also analyzed for microbial quality. The results are shown in Table 3. TPC of ice samples were low as compared to sugar cane juice. The minimum TPC was 2 x 10^2 CFU /ml (area H) and highest was 2 x 10^4 CFU/ml (area A). The maximum number of total coliforms (110 MPN/100ml) was found in ice sample of area E followed by sample from area A which had 46 MPN/100 ml total coliforms. The presence of coliform bacteria may also be due to the use of contaminated water in ice formation. Out of 40 ice samples collected from different areas, three samples showed positive indication of fecal coliform as well as *E. coli*. Samples collected from the operator hands from the studied areas (Table 1) by swab methods showed that all the operator hands were contaminated with coliform bacteria. *E. coli* was not recovered from any operator hand (Table 4).

Preservation and Storage of Sugar Cane Juice: Results regarding changes in physiochemical and microbial growth are given in Table 5. PH of the prepared juice was maintained at 4.3. There was gradual decrease in pH during storage. After 30 days of storage the pH decreased from 4.31 to 4.10. Similarly acidity in fresh juice after pasteurization was 0.64 % while it was gradually increased due to degradation by microorganisms. The total soluble solids at the time of filling were measured at 21.3%. There was negligible change in the TSS. After storage period of 30 days TSS were decreased to 21.0 %. All the results are in accordance with previous findings, but with short time period preservation [18]. Regarding microbial growth, the results were also encouraging; TPC was 03 CFU/ml at 0 days, while yeast and mould were not detected while after storage of 30 days TPC were 6.0 CFU /ml. Sensory evaluation regarding appearance, taste and overall acceptability were carried out and mentioned in Table 6. Naturally preserved juice remained acceptable in appearance, taste and overall acceptability up to storage period of 30 days.

CONCLUSIONS

It was concluded from the results of this study that all the sugar cane juices collected from eight focal areas of Peshawar City of Khyber Pakhtunkhwa were highly contaminated with fecal coliform, *E. coli* and yeast and mould. Bottled juice samples were merely accepted up to a storage period of 30 days. Sugar cane juice preservation is very difficult process and requires standard of the art technology (refrigeration and packing) with further research.

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REFERENCES

1. Parish, M.E., 1997. Public health and un-pasteurized fruit juices. Crit. Rev. Microbiol., 23: 109-119.

2. Sandeep, M., A. Diwaker and G. Abhijit, 2001. Microbiological analysis of street vended fresh squeezed carrot and kinnow mandarian juices in Patiala City, India. Internet J. Food Safety, 3: 1-3.
3. Wolzogen, K.C.A.H., 1923. Investigations on the microflora of normal sugar cane and of cane affected by sereh disease. Arch. Suikerind. Ned. Indie I Mededeel. Proefsta. Java Suikerind, 9: 321-484.
4. Mayeux, P.A., 1960. Some studies on the microbial flora of sugar cane, M.Sc. Thesis, Faculty of Life Sciences, Louisaina state University, Boton Rouge.
5. Geerlings, P., 1924. Cane sugar and its manufacture, 2nd ed. Normal Roger, London.
6. Jeffrey, C.P., 2004. Alcamo's Fundamentals of Microbiology, 7th ed., Sudbury, UK., pp: 309.
7. Imran, P., S. Parveen, S. Rehman and H. Nawaz, 2008. Baking quality of wheat flour cookies supplemented with fiber from different sources. Pak J. Food Sci., 18(1-4): 1-8.
8. Qurishi, I.H., I. Manna and I. Fatima, 1987. In the Proceedings of 2nd International conference on Elements in health and Diseases-Karachi, Published. Hamdard University Press karachi, pp: 471.
9. Coste-cruz, J.M., M.L.G. Cardoga and D.E. Marques, 1995. Parasitas intestinias, em manipuladares de alimentos de escalas na cidade de Uberlandia. Minas Gerais, Brasil. Rev. Inst. Med Trap Sao Paulo, 37: 191-196.
10. APHA, 2001. Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association 4th Edition Washington DC.
11. Arias, B., E. Sato, L. Sepulveda and A. Herrera, 1987. Infeceiones intestinales por parasites Y10 comensales en manipuladores de alimentos de hospitals del sector morte de Santiago. Chile Bol. chilen Parasitol., 42: 84-86.
12. Ryu, J.H. and L.R. Beuchat, 1998. Influence of acid tolerance responses on survival, growth and cross protection of Escherichia coli O157:H7 in acidified media and fruit juices. Int. J. Food Microbiol., 45: 185-193.
13. Ujlas, H.E. and S.C. Ingham, 1998. Survival of *Escherichia coli* O157:H7 in sunthetic fluid after cold and acid habitation in apple juice or trypticase soy broth acidified with hydrochloric acid or organic acids. Food Prot., 61: 939-947.
14. Zhuang, R.Y., L.R. Beuchat and F.J. Anglo, 1995. Fate of salmonella Montevideo on and in raw tomatoes as affected by temperature and treatment with chlorine. Appl Environ microbial., 61: 2127-2131.
15. Kusumaningrum, H.D., E.D. Asselt, R.R. Beumer and M.H. Zwiltering, 2004. A quantitative analysis of cross-contamination of salmonella and camphylobacter spp. Via domestic kitchen surface. J. Food Prot, 67: 1892-1903.
16. Javid, A., N. Ullah, F.A. Khan, S. Akhtar, Zia-ur-Rahman and I. Ahmad, 2013. Comparative Microbiological Quality Evaluation of Un-Branded and Branded Juices of Street Vended Sold in Retail Outlet of Peshawar City. American-Eurasian J. Agric. and Environ. Sci., 13(8): 1155-1159.
17. Javid, A., N. Ullah, F.A. Khan, Zia-ur-Rahman, I. Ahmad, S. Hassan and I. Ahmad, 2013. Bacteriological Quality Analysis of Drinking Water of Rural Areas of Peshawar, Pakistan. American-Eurasian J. Agric. and Environ. Sci., 13(9): 1202-1206.
18. Chauhan, O.P., D.T. Singh, S.M. and D.K. Balyan, 2002. Studies on Preservation of Sugarcane Juice. International Journal of Food Properties, 5(1): 217-229.