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Research Article

Assessment of microbiological safety of street orange fresh fruit juice sold at Chidambaram, India

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Abstract

Millions of people throughout the world consume fresh juices sold by street vendors as they are rich source of nutrients such as vitamins and minerals. They cause a great health problem in unhygienic conditions. The fresh juices sold by street vendors are preferred by consumers because of their taste, flavor, low price and availability at the right time. Fruit juices sold by the street vendors (as thirst quenching aid) are consumed regularly by the local population in most of the tropical countries. The present study was undertaken to evaluate the quality of orange fruit juices sold by street vendors alongside of roads in Chidambaram, Tamil Nadu, India. The three bacteria were identified after isolation in a specific culture medium and performing the biochemical tests, those bacteria are *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus*. Our results demonstrate the non-hygienic quality of street vended orange fruit juices and ice used for cooling of juices suggesting the urgent need for Government participation in developing suitable intervention measures to improve microbial quality of orange juices.

Keywords: Orange juice, quality, contamination, *Escherichia coli*, TVC, *Salmonella typhi*, *Staphylococcus aureus*.

Introduction

Fruit juices are well recognized for their nutritive value, mineral and vitamin content. In many tropical countries they have become common man's beverages and are sold at all public places along roadside shops (Joy Lewis *et al.,* 2006). In India, especially in the metropolitan and other cities a huge section of the population of all income and age groups consume fresh pressed and squeezed juices, most of these juices are sold by street vendors (Sandeep Mudgil *et al.,* 2004). Most fruits contain bacterial counts up to 1.0 X $10^5/cm^2$ on their surfaces. Improper washing of fruits add these bacteria to extracts leading to contamination. In addition, use of unhygienic water preservation without refrigeration, unhygienic surroundings often with swarming houseflies and fruit flies and airborne dust can also act as sources of contamination. Such juices have shown to be potential sources of bacterial pathogens notable *Escherichia coli* 0157:H7, species of *Salmonella, Shigella* and *Staphylococcus aureus* (Joy Lewis *et al.,* 2006).

Pathogenic organisms can enter fruits and vegetables through damaged surfaces, such as punctures, wounds, cuts and splits that occur during growing or harvesting. Contamination from raw materials and equipments, additional processing conditions, improper handling, prevalence of unhygienic conditions contribute substantially to the entry of bacterial pathogens in juices prepared from these fruits or vegetables (Victorian Government Department of Human Services 2005; Oliveira et al., 2006; Nicolas et al., 2007).

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. A report published by Victorian Government Department of Human services, Australia (2005) reported survival of *E.coli* in apple juice for up to 24 days at 4°C. A total of 48 cases of *E.coli* were reported after drinking unpasteurized apple juice in Washington DC in 1996. *Listeria monocytogenes* has also been identified as a pathogen that is of concern in relation to these products (Victorian Government Department of Human Services 2005).

In 1995, unpasteurized fresh orange juice contaminated with *Salmonella* was linked to an outbreak in a Florida Theme Park, USA. More than 60 visitors were affected (Schmidt *et al.*, 1997). In Australia, 427 confirmed cases of salmonellosis were reported in 1999 after drinking unpasteurized orange juice (Victorian Government Department of Human Services). A cholera epidemic in Pune city, India, was related to street vended sugarcane juice containing ice that was contaminated with *Vibrio cholerae* (Mosupye *et al.*, 1999).

In view of the threat posed by the bacterial pathogens in orange fruit juices and the flourishing demands for such street vended juices, the present work was undertaken to assess the microbiological quality of freshly pressed or squeezed orange juices from street vendors in Chidambaram town.

Materials and Methods

Collection of samples

In the present study, the different parameters were undertaken to evaluate the safety level in street vended fruit juices sold along the road sides at four different public places of Chidambaram. Microscopic examination, colony characteristics and biochemical tests were used here for detection and identification of the presence of different types of microorganisms in freshly squeezed fruit juices. The fresh squeezed orange juices as well as water samples (used in preparation of juices) were collected aseptically from four shops located in different public hot spot areas of Chidambaram, India. The samples were placed in sterile containers, then transferred to the laboratory for sample cultivation and analysis.

Sample processing

For sample analysis, 1ml of homogenized juice was diluted ten folds using sterile distilled water and then this was further diluted serially to 10^{-2}, 10^{-3}, and 10^{-4}. These dilutions of orange fruit samples are necessary for plating to obtain isolated colonies. Serial dilutions were prepared and spread plate technique was used on appropriate selective media.

Bacteriological analysis of the collected orange juice samples

Microbiological analysis included enumeration and identification of potential pathogens according to standard procedures for the number of heterotrophic bacteria, *Staphylococcus aureus*, *Salmonella* and *Shigella*, *Escherichia coli*. Eosin Methylene Blue [EMB] agar was used for the isolation of *Escherichia coli*, Mannitol salt agar [MSA] was used for the isolation of *Staphylococcus aureus* and Salmonella Shigella agar [SSA] was used for the isolation of *Salmonella* and *Shigella* sp in the orange fresh fruit juice. All the plates were incubated at 37°C for 18-24 hours.

Biochemical tests

To confirm the identity of different members of Enterobacteriaceae, a standard combination of five tests were used, namely Indole test, Methyl Red test, Voges-Proskauer test, Triple Sugar Iron test and Citrate test.

Triple sugar iron medium (TSI medium): This medium is used to differentiate salmonella and shigella from other enteric gram negative rods in culture samples. The medium appear red before reaction (alkaline), yellow after reaction (acidic), black after hydrogen sulphide gas production due to the formation of FeS.

Indole production test: Indole test is used to determine the ability of an organism to spilt amino acid tryptophan to form the compound Indole.

Methyl red test: Methyl Red test determines whether the microbe performs mixed acids fermentation when supplied glucose. MR Positive: When the culture medium turns red after addition of methyl red, because of a pH at or below 4.4 from the fermentation of glucose. MR Negative: When the culture medium remains yellow, which occurs when less acid is produced (pH is higher) from the fermentation of glucose.

Citrate utilisation test: Citrate utilization test is commonly employed as part of a group of tests, citrate utilization test is used to determine the ability of

bacteria to utilize sodium citrate as its only carbon source and inorganic ($NH_4H_2PO_4$) is the sole fixed nitrogen source. Citrate positive, growth will be visible on the slant surface and the medium will be an intense Prussian blue. Citrate negative, trace or no growth will be visible. No color change will occur; the medium will remain the deep forest green color of the inoculated agar.

Voges-Proskauer (VP): This is a test used to detect organisms that ferment but quickly convert their acid products to acetoin. A positive VP test is red on top of the medium. No color change is negative.

Results and Discussion

In developing countries, fruit juice drinks, meals, and snacks sold by street food vendors are widely consumed by millions of people. These street fruit juices provide an affordable source of nutrients to many sectors of population. Street-vended fresh fruit juices are well appreciated by consumers, because of their taste, low price, and availability at right time. However, street foods are frequently associated with diarrheal diseases due to their improper handling and serving practices (Barro et al., 2006; WHO, 2002).

The Total Viable Count (TVC) or colony forming unit (CFU) which is the number of bacteria per ml of dilute sample of freshly prepared orange fruit juice collected from different shops located at Chidambaram town is represented in figures and tables below. It was also observed that the TVC count was highest in all orange juice samples collected in the afternoon than in the morning. In the present study 8 samples were collected in four different shops (A, B, C, and D). Moreover, two samples were collected at each shop among them one was collected during the Morning and the other in the Afternoon. The following table 1 shows the number of colonies counted in each medium used to isolate the specific bacteria, where Mannitol salt agar (MSA) was used to isolate the *Staphylococcus* sp, Salmonella and Shigella agar (SSA) was used to isolate the *Salmonellae* sp and Eosin methylene blue (EMB) used for *Escherichia coli*. C.N (colonies number), **%** (percentage of the counted colonies) and CFU (colonies forming unit). The used dilution was 10^{-4} in all the samples examined in the present research study.

Table 1: Bacteria count x 10^6cfu/ml

Location		Minimum	Maximum	Mean
Shop A	MSA	1.1 x10^6	2.3 x 10^6	1.7 x 10^6
	SSA	1.4 x10^6	1.7 x 10^6	1.55x 10^6
	EMB	0.8 x10^6	2.1 x 10^6	1.45x 10^6
Shop B	MSA	1.4x 10^6	2.6x 10^6	2 x 10^6
	SSA	2.1x 10^6	4.1x 10^6	3.1 x 10^6
	EMB	0.7 x 10^6	2.2x 10^6	1.45x 10^6
Shop C	MSA	0.4x 10^6	3.2x 10^6	1.8 x 10^6
	SSA	1.8x 10^6	12.3x 10^6	7.05x 10^6
	EMB	0.5x 10^6	4.6x 10^6	2.55x 10^6
Shop D	MSA	1.5x 10^6	4.2x 10^6	2.85x 10^6
	SSA	3.1x 10^6	10.7x 10^6	6.9 x 10^6
	EMB	2.2x 10^6	30.9x 10^6	16.55x10^6

On the table 2, Orange fresh fruit juice collected at four different places in the morning has shown a less bacteria colonies growth compared to that of the Afternoon. On MSA, Shop D [15] was the first to have many bacteria colonies than other places followed by Shop B [14] whereas Shop C was the last where only [4] colonies growth was observed. On SSA medium, sample collected at Shop D is the first to have a large number of bacteria colonies [31], Shop B is the second [21] followed by Shop C [23] and lastly was Shop A with [14] bacteria colonies growth. On EMB culture medium, also the colonies growth was abundant in the sample collected from Shop D [22], followed by Shop A [8] and Shop B [7], the last one was Shop C with [5] colonies.

Table 2: Comparative study among the bacterial colonies in the samples collected in the Morning from four different shops

Period of time	Media used	Shop A	Shop B	Shop C	Shop D
Morning	MSA	11	14	4	15
	SSA	14	21	18	31
	EMB	8	7	5	22

On the table 3, The orange fresh fruit juice collected at these four various shops in the Afternoon have shown a lot of number of colonies compared to the sample collected at the same sites in the Morning.

Table 3: Comparative study among the bacterial colonies in the samples collected in the Afternoon from four different places

Period of time	Media used	**Shop A**	**Shop B**	**Shop C**	**Shop D**
Afternoon	MSA	23	26	12	42
	SSA	17	41	123	107
	EMB	21	22	46	309

On MSA, Shop D was remarkably identified to have a large number of bacterial colonies than other sites with [42] colonies followed by Shop B [26], Shop A [38] and last to have less colonies was Shop C [29].

On SSA medium, Shop C was the first to have more number of grown bacterial colonies [123], secondly Shop D [107] followed by Shop B [41] and the least was Shop A with [17] bacterial colonies.

On EMB, Shop D distinguished from others to have a lot of number of bacterial colonies with [309], Shop C come to the second place with [46] colonies followed by Shop B [33] and Shop A [27].

Table 4: Summary of the microscopic and biochemical profile of clinical isolates of orange fruit juice

Gram's reaction	Indole test	MR test	VP test	Citrate test	H_2S	Remarks
-	+	+	-	-	-	*Escherichia coli*
-	-	+	-	-	+	*Salmonella typhi*
$_+$	-	+	+	-	-	*Staphylococcus aureus*

All the above microorganisms were isolated in the orange fresh fruit juice sample collected into street shops in different places at Chidambaram town.

Conclusion

This study indicated that all street vended fruit juices in many parts of the Chidambaram town were contaminated at different stage according to the location of the Shops. It is contended that contamination is mainly due to poor quality of water used for dilution as well as prevailing unhygienic conditions related to washing of utensils, contaminated water and ice, poor personal and domestic hygiene, peeling of fruits before hands, shops in crowed place, dust particles in the evening and maintains of premises. The location by the side of a busy road with heavy vehicular traffic (airborne particles) or by the side of the waste disposal system and overcrowding seems to add to the contamination. Such locations should be avoided for establishing a street vended juice shop. The practice of consuming fresh fruit juice cannot be stopped on unhygienic grounds and not the street vendors prohibited from selling such items, as such activities provide them with a source of livelihood.

References

Ahmed M.S.U., Nasreen T.,Feroza B. and Parveen S. (2009). Microbiological quality of local market vended freshly squeezed fruit juices in Dhaka city, Bangladesh. Bangladesh Journal of Scientific and Industrial Research, 44(4), 421-429.

Al-Jedah, J. H., & Robinson, R. K. (2002). Nutritional value and microbiological safety of fresh fruit juices sold through retail outlets in Qatar. Pakistan Journal of Nutrition, 1, 79-81.

Bagci, U. and A. Temiz. 2011. Microbiological quality of fresh-squeezed orange juice and efficacy of fruit surface decontamination methods in microbiological quality. J. Food Protect. 74: 1238-1244.

Bagde N.I. and Tumane P.M., Studies on microbial flora of fruit juices and cold drinks, *Asiatic J. Biotechnology Resources*, 2 (4), 454-460 (2011).

Barro, N., Bello A.R., S. Aly, Ouattara C. A. T., Ilboudo A. Jules and Traoré A. S., 2006. Hygienic status an assessment of dishwashing waters, utensils, hands and pieces of money from street food processing sites in Ouagadougou (Burkina Faso). African Journal of Biotechnology, 5 (11), 1107-1112.

Basar, M. A. and Rahman, S. R. 2007. Assessment of microbiological quality of processed fruit juice. Bangladesh Journal of Microbiology 24 (2): 166-168.

Burt MB, Volel C, Finkel M, 2003. Safety of vendor-prepared foods: Evaluation of 10 processing mobile food vendors in Manhattan. Public Health Rep. 118: 470-476.

Joy E. Lewis, Patrina Thompson, Rao BVVBN, Kalavati C, Rajanna B. 2006. Human bacteria in street
vended fruit juices: A case study of Visakhapatnam city, India. *Internet Journal of Food Safety.* 8:35 - 38.

Mac Faddin J.F. (1980). Biochemical Tests for Identification of Medical Bacteria, 2nd ed. Williams and Wilkins, Baltimore. p.25

Mahale D.P, Khade R.G. and Vaidya V.K., Microbiological analysis of street vended fruit juices from Mumbai city, India, *Internet J. Food Safety*, 10, 31-34 (2008).

Mosupye FM and von Holy A. 1999. Microbiological Quality and Safety of Ready-To-Eat Street Vended Foodsin Johannesburg, South Africa. Journal for Food Protection. 1278-1284.

Nicolas B, Razack BA, Yollande I, Aly S, Tidiane OCA, Philippe NA, De Souza C and Sababénédjo TA. 2007. Street-Vended Foods Improvement: Contamination Mechanisms and Application of Food Safety Objective Strategy: Critical Review. Pakistan Journal of Nutrition. 6(1): 1 -10.

Oliveira ACG, Seixas ASS, Sousa CP, Souza CWO. 2006. Microbiological evaluation of sugarcane
juice sold at street stands and juice handling conditions in São Carlos, São Paulo, Brazil. Cad. SaúdePública, Rio de Janeiro. 22(5):1111 -1114.

Parish, M.E. 1997. Public health and non-pasteurized fruit juices. Critical Reviews in Microbiology 3: 109-119.

Reddy SM., Ram Reddy S. 2000. Microbiology A Laboratory Manual. Revised edition BSC. Publishers and Distributors, Hyderabad.

Salle AJ. 2000. Fundamental principles of Bacteriology. TMH edition, Tata McGraw Hill Publishing Co Ltd., New Delhi. 691 -699.

Sandeep, M., Diwakar. A. and Abhijit. G. 2004. Microbiological Analysis of Street Vended Fresh squeezed Carrot and Kinnow-Manderian Juices in Patiala City, India. Internet Journal of Food safety 3: 1-3.

Sunday P. Ukwo, Nyaudoh U. Ndaeyo. and Etido J. Udoh., Microbiological quality and safety evaluation of fresh juices and edible ice sold in Uyo Metropolis, South-South, Nigeria, *Internet J. Food Safety*, 13,374-378 (2011).

Tambekar DH, Murhekar SM, Dhanorkar DV, Gulhane PB, Dudhane MN. 2009. Quality and safety of street vended fruit juices: a case study of Amravati city, India. *Journal of Applied Biosciences.* 14: 782-787.

Victorian Government Department of Human Services, Food Safety Unit Melbourne, Victoria. 2005.

Microbiological survey of freshly squeezed juices from retail businesses across Victoria. Available at: http://www.health.vic.gov.au/foodsafety. (Accessed September 9 2007).