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RESEARCH ARTICLE :

Microbial and physico-chemical characteristics of street vended fruit juices

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SUMMARY : Street vended fruit juices are on high demand because of their nutritive value and mineral and vitamin content but the concern on food safety is very high. This study is aimed to detect different food borne micro-organisms along with Physico-chemical characteristics of freshly prepared street vended fruit juices (sapota, pineapple, grape, mixed fruit juices). A total of 12 juice samples from three different stalls of pulivendula were collected and examined for their physico-chemical properties and for their microbiological quality. Physico-chemical properties of fruit juices include pH, Titrable acidity and TSS. The pH of sapota juice (7.26) is high when compared to other fruit juices. The titrable acidity was high for grape juice (0.556) and the TSS of sapota juice (20.3) is high. Microbiological analysis includes the isolation and identification of bacteria. The total colony forming units was found high in mixed fruit juices on an average of 8.5×10^6cfu/ml whereas *E.coli* count was found high in grape with 1.5×10^4cfu/ml and pineapple juices with 1.0×10^6cfu/ml and *Staphylococcus* was found high in sapota juice with 1.9×10^4cfu/ml. Isolated bacteria were identified by using biochemical tests and gram straining.

KEY WORDS :
Fruit juices, pH, Titrable acidity, TSS, Colony forming units

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BACKGROUND AND OBJECTIVES

Fresh fruits and vegetable juices are essential component of human diet and there is considerable evidence of health and nutritional benefits associated with the consumption of fresh fruit juices. There is a significant rise in the consumption of fresh produce for health benefits, there have also been significant changes in the lifestyle and major shifts in consumption trends. In many tropical countries they are common men beverages and are sold at all public places and roadside shops. There are reports of food borne illness associated with the consumption of fruit juices at several places in India and elsewhere. In view of their ready consumption, quick methods of cleaning utensils handling and extraction, they could often prove to be a public health threat. However sources of contamination vary. One potential source of entry of micro-organism into fruits and fruit juices is by environmental exposure. Fruit juices generally attract houseflies because of their sweet odour. So, these swarming house flies can act as source of contamination. This

may be the reason for high contamination though they are acidic in pH (Mudgil *et al.*, 2001). Food borne diseases are harmful, illness mainly affecting the gastrointestinal tract and are transmitted through consumption of contaminated food or drink. Improper washing of fruits add bacteria to extracts leading to contamination. In addition, use of unhygienic water for dilution, dressing with ice, prolonged preservation without refrigeration unhygienic surroundings often with swarming houseflies and fruit flies and airborne dust can act as sources of contamination. Such juices have shown to be potential sources of bacterial pathogens notably *E.coli* 0157:H7, species of *Salmonella*, *Shigella* and *Staphylococcus aureus* (Barro *et al.*, 2006 and Buchaman *et al.*, 1999).

In countries where street vending food is prevalent there is commonly a lack of information on the incidence of food born diseases related to the street vended foods. However microbial studies on such foods in America, Asia and African countries have revealed increased bacterial pathogens in food. There have been documented outbreaks of illness in human associated with the consumption of unpasteurized fruit and vegetables juice and fresh produce (Durgesh *et al.*, 2008). The normal habitat of fecal coliforms is the intestinal tracts of man and animals and they are not known to be found in nature in the absence of fecal contamination from the above sources. They are excreted out from animal body in the form of feces. Some of them are pathogenic and cause diseases like typhoid, dysentery and enteric fever etc. Thus, the presence of these organisms in water and fruit juices is dangerous for human consumption. Most fruit juices contain sufficient nutrients that could support microbial growth. Several factors encourage, prevent, or limit the growth of micro-organisms in juices. The most important are water activity (*aw*), low pH, hygienic practice and storage temperature and concentration of preservative.

In place like Pulivendula, there is a high demand for both packed and fresh fruit juices especially during summer. The aim of the present study was to isolate and identify the bacteria from street vended fruit juices like sapota, pineapple, grape, mixed fruit juice. The study involves; (a) Collection of street vended fruit juices (b) Microbiological profiling including total bacterial count, coliform count along with the isolation of *Escherichia coli* and *Staphylococcus aureus* from various juice samples (c) Interpretation of the results for the benefit of human welfare by increasing general awareness among the people.

RESOURCES AND METHODS

Collection of samples:

Freshly prepared fruit juices from the three different stalls of pulivendula are collected in sterile 250ml conical flask. Analysis was carried out with in1hr of collection. The stalls from which fruit juices are collected are coded as S1, S2, S3 (S1-Madina, S2-Shivamani, S3-Srinivasa)

Physico-chemical analysis:

Determination of pH:

pH can be determined by using a pH meter. pH meter consists of electrode which is sensitive to hydrogen ions present in the sample. Dilute the sample with equal quantities of water, since dilution does not effect the hydrogen ion concentration. Now dip the electrode into the beaker containing sample. Note the readings of each sample. Electrode should be rinsed with water after each measurement.

Determination of titratable acidity:

The presence of acid in juice can be identified by their effects with acid base indicators such as methyl orange and phenolphthalein. These indicators tell us whether a substance is acidic or basic by change in colour. The acidity of grape juice is expressed in terms of per cent tartaric acid. The acidity of sapota juice and pineapple juice can be expressed in terms of per cent lactic acid and citric acid, respectively. The acidity of mixed fruit juice can be expressed in terms of lactic acid.

$$\% \text{ of acid} = \frac{\text{Titre x normality of alkali x volume make up x eq. wt. of acid x 100}}{\text{Volume of sample for estimation x wt. of sample x1000}}$$

Determination of TSS:

Refractometer measures the refractive index of solution. The Brix scale is based on sucrose and water solution. However, since most samples contain substances other than sugar such as salts, minerals and proteins. Test sample is put on the face of glass prism of known refractive index. A small beam of light is shined at prism surface. The resulting beam of light travels through the prism and out the other side. The angle between light and prism is critical angle. By looking

through an eye piece an observer can measure the critical angle.

Microbial analysis:

Total Plate Count (TPC) :

For total plate count (TPC), the pour plate method was used by following the procedure described in American Public Health Association (APHA, 1992). One milliliter volume of certain three dilutions was inoculated into a Petri dish. This was followed by pouring a volume of 12 ml molten plate count agar (PCA, Oxoid) and mixing it. Petri dish were then placed to allow being set, and incubated at 37°C for 24 ± 2 h. PCA plates showing number of colonies between 30 to 300 were selected and counted using electronic colony counter, and the number of colonies was recorded.

Isolation and identification of micro-organisms :

Bacterial colony of different morphology was streaked on respective media to obtain pure culture. All the plates incubated at 37^0C for 24 hours. Pure bacterial isolates were preserved at 40C. The bacterial isolates were recognized on the basis of morphological and biochemical characteristics such as Indole test (Macfaddin, 2000), Methyl red tests (Macfaddin, 2000), Voges Proskauer (V. P.) tests (Macfaddin, 2000), Citrate test (Claus, 1998). Finally, micro-organisms were provisionally identified according to the Bergey's manual of determinative bacteriology (6^{th} Ed.) and manual for the identification of medical bacteria (Baumann *et al.*, 1998 and Cowan, 1998).

OBSERVATIONS AND ANALYSIS

In pulivendula street vended fruit juices provide an affordable source of nutrients to many sectors of population. Street vended fruit juices are well appreciated by consumers, because of their taste, and their freshness. These freshly squeezed fruit juices have little or no process steps that reduce pathogen levels, if contaminated. In present study freshly squeezed fruit juices like sapota, grape, pineapple, mixed fruit are collected from three different areas of pulivendula. These fruit juices are examined for their physico-chemical properties and for their microbiological quality.

Physico-chemical analysis:

Results of Physico-chemical analysis of fruit juices of the pH, Titrable acidity and TSS are given in the Table 2, 3 and 4. The pH of sapota juice (7.26) is high when compared to other fruit juices. The titrable acidity was high for grapejuice (0.556) and the TSS of sapota juice (20.3°B) is high. Results releaved that pH ranges of orange juice at different temperatures. pH of the orange juice ranged from 3.5-4.6, between the temperatures of 2.9 to 18° C for refrigerated juice and pH ranged from 3.3-4.5 between the temperatures of 23 to 38° C for un refrigerated juice (Agwa1 *et al.,* 2014). Studies revealed that the pH and titrable acidity of grape juice was given

Table 1 : MPN index for coliforms

3 of each 10ml	3 of each 1ml	3 of each 0.1ml	MPN index /100ml
3	1	0	43
3	1	1	75
3	1	2	120
3	2	0	93
3	2	1	150
3	2	2	210
3	3	0	240
3	3	1	460
3	3	2	1100
3	3	3	2400

Ref(standard methods for examination of water and waste water, Roy (1998)

Table 2 : Determination of pH

Juice	pH
Sapota	7.26
Grape	3.84
Pineapple	5.10
Mixed fruit juice	7.0

Table 3 : Determination of titrable acidity

Sample	Titrable acidity
Sapota	0.336
Grape	0.556
Pineapple	0.369
Mixed fruit juice	0.379

Table 4 : Determination of TSS

Sample	TSS
Sapota	20.3
Grape	17.6
Pineapple	16.33
Mixed fruit juice	15.6

as 3.95 and 0.245, respectively (Bello *et al.,* 2014). The pH and Titrable acidity of pineapple was given as 3.50 and 0.223, respectively. Results revealed that the pH of fruit juices like grape, pineapple, sapota and sweet lime are 3.56, 4.0, 6.53 and 3.8, respectively (Reddi *et al.,* 2015).

Total plate counts:

In the present investigation, microbial quality of four street vended fruits juices such as Sapota, Grape, Pineapple and Mixed fruit juice were determined. From Table 5 among the four tested samples, Total bacterial count maximum in mixed fruit juices (of 8.5×10^6cfu/ml) whereas *E.coli* count was found high in grape with 1.5×10^4cfu/ml and pineapple juices with 1.0×10^6cfu/ml and *staphylococcus* was found high in sapota juice with 1.9×10^4cfu/ml. The total coliform count was found high in grape and pineapple juices. Studied about microbological quality of street vended fruit juices in Hyderabad. By this study they stated that about 96.6% of fruit juices were contaminated with *fecal coliforms* (77.3%), *S. Aureus* (73.3%), *Shigella* sp. (48.6%), *and E.coli* (42.6%) (Reddi *et al.,* 2015). The highest bacterial load (3.7×10^8cfu/ml) for fresh fruit juice sample was found in grape juice and lowest load was found in apple (2.14×10^6cfu/ml). The highest value of *staphylococcus* was found in wood apple juice (1.25×10^6cfu/ml) (Kader *et al.,* 2014). They aimed to analyse microbiological safety of fresh fruit juices (apple, avocado, banana, pineapple, mixed fruit juice etc.). The CFU are high in lemon juice (7.5×10^5) and were observed that high coliforms in mixed fruit juice (1.6×10^6) (Al-jedah and Robinson, 2002).

Three juices were sampled such as sugarcane, alovera and olot kombol, where bacterial load ranged from $6.3x10^4$ to $1.4x10^5$ cfu/ml. Street vended fresh juice samples were found to be highly contaminated with TBC than the commercially packed juice (Cowan, 1998). Total Coliform Count (TCC) ranged from $2.3x10^2$ to $3.7x10^3$ cfu/ml in all the juice samples. Among all the juice samples lowest ($6.3x10^4$ cfu/ ml) TCC was found in sugarcane and the highest ($1.4x10^5$ cfu/ml) TCC was found in olot kombol. The washing and processing water contaminated with fecal coliform is one of the major sources for presence of coliforms in street foods. Other studies reported that demonstrated that fruit juices such as sugarcane, lime and carrot juice in Mumbai city, India and apple, orange, pineapple, pomegranate, sweet lemon and mix fruit juice in Amravati city, India, respectively, were infected with *E. coli* in a wide range (Rahman *et al.,* 2011 and Durgesh *et al.,* 2008). All the samples were found contaminated however lower microbial count was recorded in all the tested juices samples compared to the Gulf standard (Tambekar *et al.,* 2011).

Isolation and identification of micro-organisms:

For identification, different bacterial isolates were selected from different media on the basis of agar colony

Table 5 : Total bacterial counts

Place	Sample	CFU(colony forming units)/ml	*E.coli* count/ml	*Staphylococcal* count/ml	*Coliform* count/100ml
	Sapota	3×10^3	0.2×10^3	0.5×10^3	120
	Grape	3×10^3	9×10^3	0.3×10^3	2400
S1	Pineapple	2.7×10^3	3.0×10^3	Absent	2400
	Mixed fruit juice	4×10^4	3.5×10^3	1×10^3	2400
	Sapota	1×10^6	2.5×10^3	5.5×10^4	210
	Grape	6×10^4	3×10^4	1×10^4	2400
S2	Pineapple	5.6×10^4	3.0×10^6	2.5×10^6	93
	Mixed fruit juice	2.5×10^6	4.0×10^5	2.5×10^4	120
	Sapota	1×10^3	2×10^3	3×10^3	28
	Grape	2×10^3	6×10^3	Absent	75
S3	Pineapple	4×10^3	2×10^3	Absent	2400
	Mixed fruit juice	2×10^4	5×10^3	3×10^3	1100

Table 6 : Identification of bacteria

Name of organism	Indole test	Methyl red	Voges proskour	Citrate test	Catalase test	Gram staining
E.coli	+	+	-	-	-	-
Staphylococus	-	-	-	-	+	+

morphology and biochemical characteristics. A total of three different organisms were identified from the samples including *coliforms, E.coli* and *Staphylococus.* Staining provides valuable information as to bacterial morphology, gram reaction, and presence of structures such as capsules and endospores. However microscopic observation gives little additional information as to the genus and species of a particular bacterium. To aid in the more definitive identification of bacteria, microbiologists have developed a series of biochemical tests that can be used differentiate even closely related organisms. These various tests were designed to identify various metabolic properties of different bacterial species. For identification of *e. coli* and *staphylococcus* we have used biochemical tests IMVIC test and catalase test. Indole test shows positive result by the formation of cherry red colour ring by addition of kovac'reagent. Methyl red gives positive result by changing color from yellow to red color by addition of methyl red indicator which indicates the production of acid. Voges proskauer shows positive result by changing color from yellow to deep rose color on addition of barriats reagent by the production of neutral end products. Citrate test shows positive by change in color of agar from green to blue color. Catalase test shows positive by the production of bubbles in the H_2O_2. Retaining of crystal violet colour on addition of decoloriser indicates the positive result for gram staining.

From the Table.6 *E. coli* shows positive test for Indole and methyl red tests whereas *staphylococcus* shows positive results for catalase test and gram staining. Under microscope *E. coli* was identified as a rod shaped bacteria and *staphylococcus* as round in the form of grape bunches.

Conclusion:

The fruit juices investigated in this study and conclude that among the four fruit juices (sapota, grape, pineapple, mixed fruit juice) collected from three different stalls of pulivendula mixed fruit juice was highly contaminated. High amount *coli forms* was found in pineapple juice. *E.coli* count was found high in grape juice and pineapple juice whereas *Staphylococcal* count was found high in sapota juice. Isolated *E.coli* was identified by using IMViC tests and gram staining which shows a ppositive result for indole test and methyl red test. Isolated *Staphylococcus* was identified by using catalase test and gram staining.

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