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MICROBIOLOGICAL ANALYSIS OF PACKED FRUIT JUICES LOCALLY AVAILABLE IN JAIPUR, INDIA

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ABSTRACT

Present investigation attempted to resolve the microbiological and biochemical attributes of the fruit juices collected from outlets of Jaipur city, India. In the study two samples of different brands of packed fruit juice (Brand A and Brand B) were screened for total aerobic mesophilic bacterial counts, fungal count and the coliform counts. Samples were of acidic pH ranging from 4 to 6**.** Samples were found to harbor viable bacteria within the range between 10^1 -10^3 cfu/ml. However the total microbial counts were within acceptable standards for human consumption. These counts are suggestive of bacterial contamination of the packaged fruit juice during handling since they are liquid, which could have contributed to the development as well as multiplication of these contaminants. Drug resistance among the isolates was found against ampicillin and chloramphenicol.

KEYWORDS: **Bacteria, brands, drug resistance, packed fruit juice,**

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INTRODUCTION

Fruit juice is unfermented but fermentable liquid or juice intended for direct consumption, obtained from the edible portion of sound, appropriately mature and fresh fruit by mechanical extraction process and preserved, exclusively by chemical and physical means.[1,2] Juice may be marketed in concentrate form, sometime frozen, requiring the user to add water to constitute the liquid back to its "original state".[3] The addition of sugar or acids can be permitted but must be endorsed in the individual standard. [2] Water is the predominant component of fruit juice along with carbohydrates including sucrose, fructose, glucose and sorbitol.[4] It contains small amount of protein, no fat, cholesterol and unless the pulp is included, contains no fiber.[5] The anti-oxidant components of fruit juice have beneficial long term health effects, such as decreasing the risk of cancer and heart disease.[6] Increased iron absorption from food by ascorbic acid is essential for children who consume diet with low iron bioavailability.[7] Barring chemical and enzymatic changes, serious safety problem associated with juice consumption has risen. 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 fruits or vegetables.[8,9] Fruit juice has increasingly been the source of serious food poisoning outbreak and fatalities. Unpasteurized juice have been implicated in outbreaks due to *spp* of *Salmonella; Escherichia coli, Clostridium* and *Cryptosporidium.* [10, 11, 12] In a processed fruit juice, bacteria are the most diversified micro-organisms causing its spoilage. Lactic acid bacteria *Acetobacter* and *Acetomonas* found on fruit surfaces comprise the most frequent spoilers of fruit juice because they exist on the surface of plant and fruits growing at the expense of secreted plant materials.[13] *Enterobacter spp* is commonly found on visually all types of unsound fruits in wash water. The presence of coliform mostly of the *E. aerogenes* type in both fresh and frozen fruit juices has been attributed to their being natural flora of fruits which may be introduced into the fruit juice if improperly processed.[13] Most fruit juices are acidic enough and have sufficient sugar to favour the growth of yeast. [14] Mould are generally considered to be the least important group of micro-organisms causing spoilage in fruit juice because of their limitation, inability to grow in the absence of air [15], with the exception of few mould such as *Penicillium* and *Aspergillus* spore forming[11]. Lapses in food safety do not only adversely impact the health of consumers; safety errors have and will ruin the reputation and financial health of the offending food company. 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 packed fruit juices from outlets of Jaipur, India. This would provide a background microbiological data for development methods that would effectively reduce the microbial load of fruit juice including those considered to constitution spoilage threat and potentials health hazards to subsequent consumer.

MATERIALS AND METHODS

Collection of samples

Sample selection was based on popularity (most demanded) and only samples within the expiry date as stipulated on the labels by manufacturers was analyzed. Samples of two different packed pineapple fruit juices marketed in retail shops in Jaipur, Rajasthan, India were taken for the study and were designated as sample A and B respectively. Information on the labels was recorded to include manufacturer's address, brand name, manufacture and expiry dates, batch number, type of preservative(s) and compositions. Samples were kept in the refrigerator at 4^0C before commencement of analysis.

Determination of pH

The pH of the samples was determined using pH meter (pH 211 microprocessor, Hanna instruments) immediately after collection.

Isolation and estimation of microorganisms from juice samples

Sample processing

10 ml of the sample was diluted with 90 ml of sterile buffered peptone water and mixed well (10^{-1} dilution). Serial dilutions were prepared and spread plate technique was used on appropriate selective media.

Bacteriological analysis of the collected juice samples

Microbiological analysis included enumeration and identification of potential pathogens according to standard procedures for the number of heterotrophic bacteria, *Staphylococcus aureus*, *Salmonella*, *Shigella* and most probable number (MPN) of total coliforms. Appropriate dilutions were then enumerated for Total aerobic plate counts using Nutrient Agar, Coliforms using Violet Red Bile Agar, Mc Conkey Agar. Xylose Lysine Deoxycholate Agar was used for enumeration of *Salmonella* & *Shigella.* [16, 17] Potato Dextrose Agar (PDA) was used for plate counts of yeasts and moulds. [18] All the selective media were obtained from Himedia Laboratories Ltd, Mumbai, India. All plates were incubated under aerobic conditions at 36±1°C for 24 hrs. The mean number of colonies counted was expressed as log colony forming units (cfu)/100 ml. Four serial dilutions of each brand were inoculated on all the media plates. Obtained colonies are isolated. For confirmation of the pathogens, typical colonies were checked using based on the results of the gram reaction and appropriate biochemical tests.[19]

Determination of antimicrobial susceptibility

Isolates were tested against common antibacterial drugs by disc diffusion assay on Mueller-Hinton Agar (Difco, Detroit, MI) with antibiotic discs (Neo-Sensitabs, Rosco, Denmark.[20] Briefly, a single colony of each isolate was introduced into 2 ml of Mueller-Hinton broth, incubated for 4 hours, and the culture turbidity was then adjusted to a 0.5 McFarland standard. Sterile cotton swabs were dipped into the suspensions and were spread evenly over the entire agar surface. Antibiotics impregnated discs (ampicillin 10 µg and chloramphenicol 30 µg,) were then placed onto the surface of the inoculated

plates. After incubation, diameters of the zones of inhibition were measured and interpreted as susceptible, intermediate and resistant.

RESULTS AND DISCUSSION

This study was conducted to evaluate the quality of juices by studying their physio-chemical parameters and microbiology.

Microbiological analysis

The samples collected were examined for their pH. The pH of sample A was come out to be 6 and of sample B was 4; which shows that sample A was more prone towards contamination (Table 1).

Total heterotrophic bacterial count

Total heterotrophic bacteria count of packaged fruit juice for sample A was 30 $x10^1$ cfu/ml and sample B was 1 x 10^1cfu/ml (Table 2 and 3). The bacterial count was low for sample B and higher for sample A (Fig 1). All the results of the bacterial counts from all the packaged fruit juice analyzed were within the acceptable limit. According to the International Commission on Microbiological Specification of Foods, the acceptable limit of mesophilic aerobic bacteria in food products should not exceed a maximum of 10^3cfu/ml.[21] Tasnim *et al*.[22] also found the load of viable bacteria in processed juice samples within the standard limit in the average of 10^3cfu/ml. However, the counts are considerably high since no microorganism should be recovered in any food meant for human consumption.[23, 24]

Total and faecal coliforms, Salmonella, Shigella and Vibrio counts

No coliform bacteria were observed in all packaged fruit juice samples. None of the XLDA plates showed any black colonies of *Salmonella* or pale pink colonies of *Shigella*.

Total fungi count

No traces of fungi and mould were found in either of the samples.

Bacterial identification

Based on the results of the gram reaction and biochemical test performed, (Table 4), *Bordetella pertussis, Brucella species, Eikenella corrodens, Haemophilus species, Moraxella catarrhalis, Neisseria species, Pasteurella multocida*, *Escherichia coli*, *Enterobacter aerogenes* (Table 4) might be present in samples of juices. The bacterial colonies which were isolated from both the samples were sensitive to ampicillin (10 µg) and chloramphenicol (30 µg). Isolate 1 of sample A showed equal zone of inhibition for both the antibiotics and Isolate 2 of sample A showed large zone of inhibition for ampicillin and less for chloramphenicol (Table 5). In comparison to this Isolates 3, 4 of sample B showed same zone of inhibition for ampicillin and chloramphenicol (Fig 2). Hence from results it was concluded that isolates was more sensitive towards ampicillin as compared to chloramphenicol. Biochemical analysis (Fig 3) of the entire isolates (Table 6) showed negative catalase test. Catalase-negative bacteria may be anaerobes, or they may be facultative anaerobes that only ferment and do not respire using oxygen as a terminal electron acceptor (i.e. Streptococci). The results of Positive citrate test was shown by isolate 2 of sample A and rest isolates showed negative citrate test. These results helps in distinguishing between coliforms such as *Enterobacter aerogenes* (positive citrate utilization test) which occur naturally in the soil and in aquatic environments and fecal coliforms such as *Escherichia coli* (negative citrate utilization test) whose presence would be indicative of fecal contamination. Indole test which was done indicated that isolate 1and 4 showed negative test and isolate 2 and 3 showed positive test. Most strain of *E. coli, P. vulgaris; M. morganii* and *Providenica* are indole positive. *Klebsiella pneumonia*, *Citrobacter freundii, Proteus mirabilis* are indole negative. Methyl red test was shown by isolate 1, 3 and 4 as positive. Isolate 2 showed negative methyl red test. *Escherichia coli* show MR Test positive and *Enterobacter aerogenes* shows MR Test Negative. Voges Proskauer test was shown by isolate 2 as positive and rest showed negative test. *Escherichia coli* show VP Test negative and *Enterobacter aerogenes* shows VP Test positive. The sugar fermentation test shown by isolates on dextrose was indicated as isolate 2 and 3 had shown positive test; isolate 1 and 4 had shown negative test. All the isolates showed negative test on lactose sugar and all isolates showed positive test on sucrose sugar. Facultative anaerobic bacteria show positive sugar fermentation test. According to Association of Food and Drug Officials[25], simple packaging or repackaging operations can bring about an opportunity for the contamination or recontamination with pathogens if strict aseptic conditions are not adhered to[26]. Testing for these organisms at specific control points provides the best means of quality control[26]. Constant surveillance and good manufacturing practice are the best methods for prevention of contamination[27, 26].

Table 1
pH analysis of juice samples

Sample	pH
Sample A	6
Sample B	4

Table 2
Microbial Analysis of sample A

S.No.	Dilutions	Total plate count	Coliforms	Yeast	Moulds	Salmonella &Shigella
1	10^{-1}	30	-	-	-	-
2	10^{-2}	14	-	-	-	-
3	10^{-3}	11	-	-	-	-
4	10^{-4}	11	-	-	-	-

cfu/ml= 30x10^{3}

Table 3
Microbial analysis of sample B

S.No.	Dilutions	Total plate count	Coliforms	Yeast	Moulds	Salmonella &Shigella
1	10^{-1}	1	-	-	-	-
2	10^{-2}	1	-	-	-	-
3	10^{-3}	-	-	-	-	-
4	10^{-4}	-	-	-	-	-

cfu/ml=1x10^{1}

Table 4
Gram staining pattern of different bacterial isolates in the fruit juice samples

Isolates		Gram staining		Shape	Bacterial morphology
		Gram positive	Gram negative		
1-	Sample A	Present	Present	Cocci	Streptococci
2-	Sample A	Present	Present	Cocci	Streptococci
1-	Sample B	Present	Present	Cocci	Streptococci
2-	Sample B	Present	Present	Cocci	Streptococci

Table 5
Antibiotic sensitivity (Zone of inhibition) pattern of different bacterial isolates in the fruit juice samples

Isolates	Antibiotics	
	Ampicillin (10µg)	Chloramphenicol (30 µg)
1	30.1mm	30.0mm
2	20.7mm	20.6mm
3	30.0mm	20.7mm
4	30.1mm	20.6mm

Table 6
Biochemical tests for bacterial isolates

Isolates	CAT	CIT	I	MR	VP	Sugar test D	L	S
1-Sample A	N	N	N	P	N	N	N	P
2-Sample A	N	P	P	N	P	P	N	P
3-Sample B	N	N	P	P	N	P	N	P
4-Sample B	N	N	N	P	N	N	N	P

**** N: Negative, P: Positive***
Abbreviations: CAT: Catalase, CIT: Citrate, I: Indole, MR-VP: Methyl-Red and Vogues-Proskauer, D: Dextrose, L: Lactose, S: Sucrose

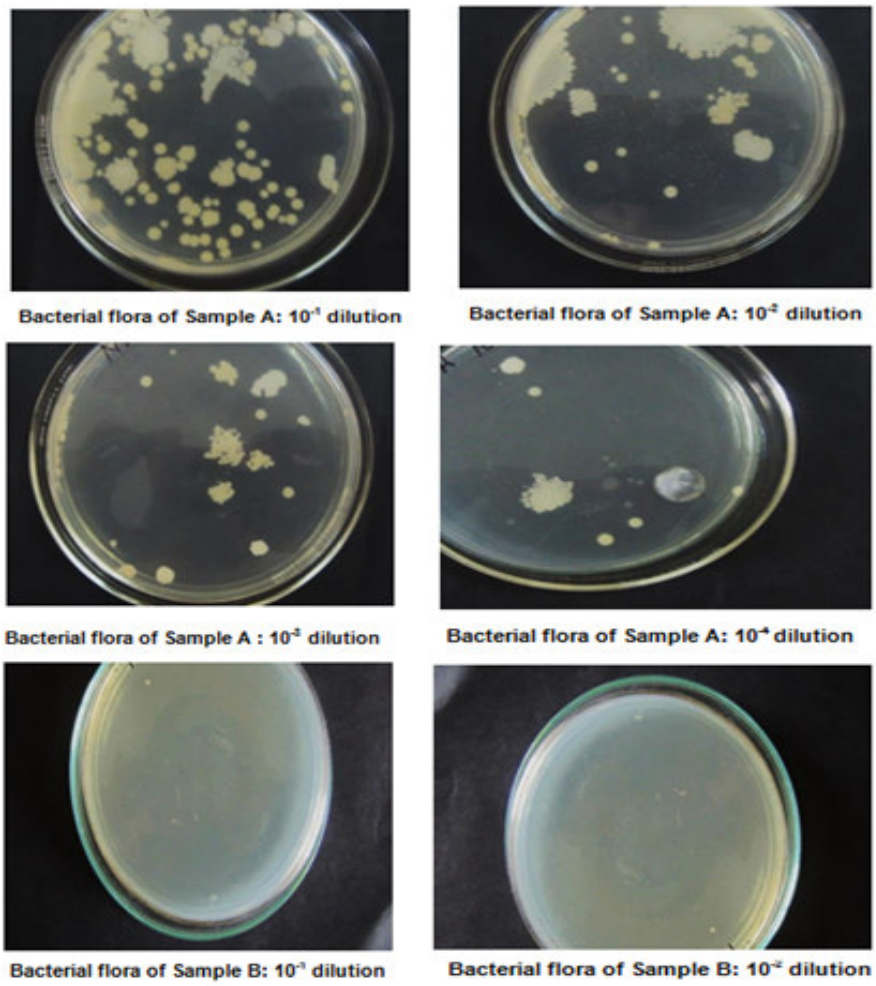

Figure 1
Total heterotrophic bacteria count of packaged fruit juice for sample A and sample B

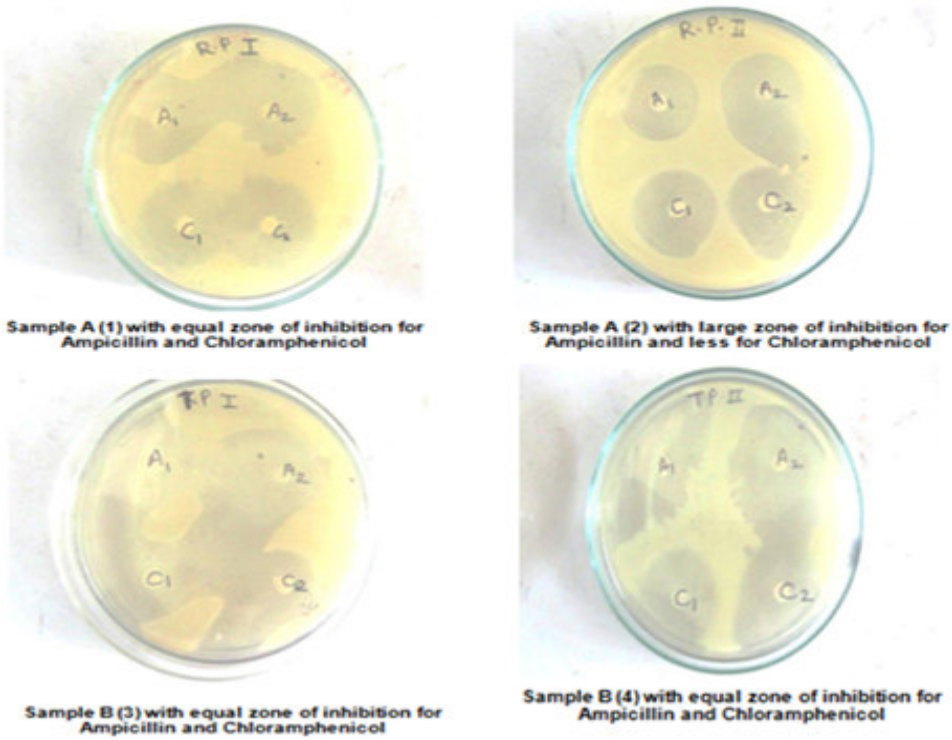

Figure 2
Results of antimicrobial susceptibility of packaged fruit juice for sample A and sample B

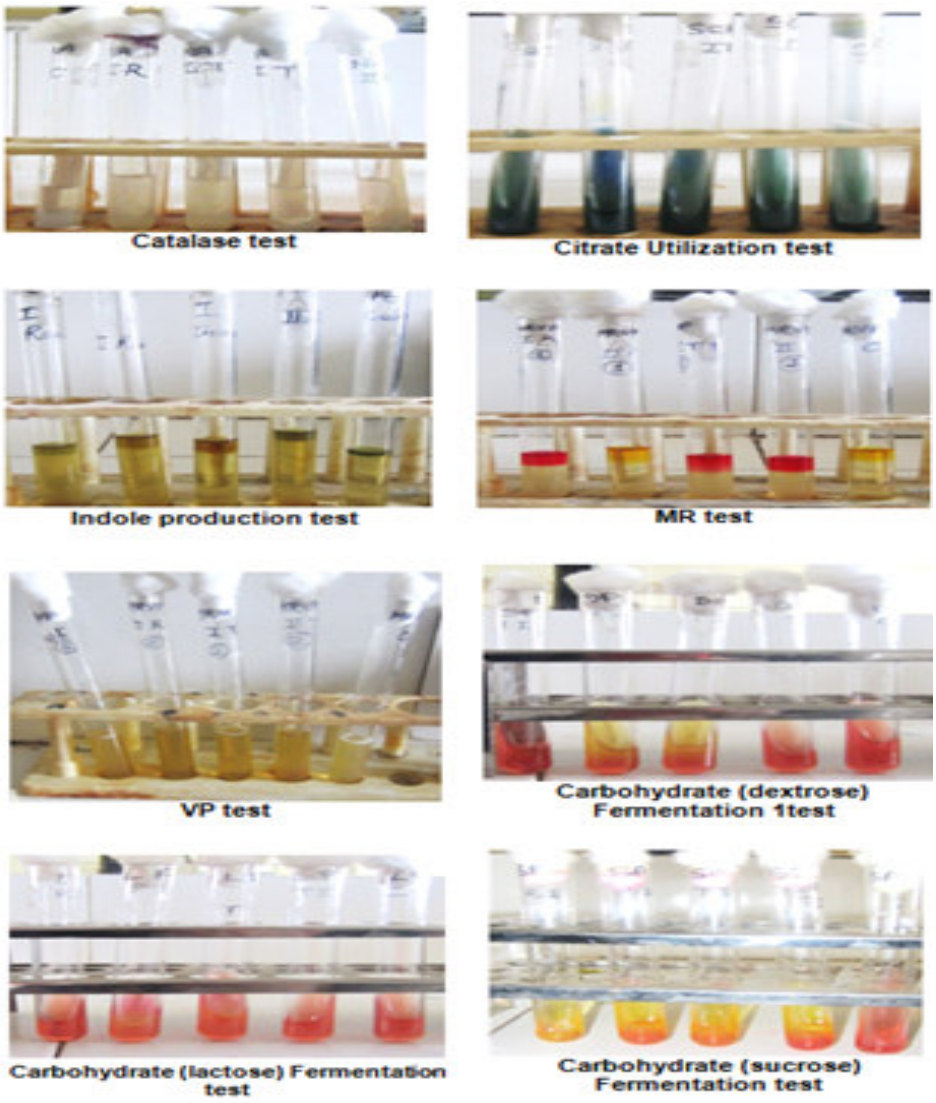

Figure 3
Biochemical analysis of packaged fruit juice for sample A and sample B

CONCLUSION

Present study exhibited the microbiological status of available packed fruit juices to ensure food safety for a precise control over public health risk. Bacterial species isolated from these fruit juice samples was screened for different biochemical tests. No other microorganism was seen during study. The average counts for bacteria of the packaged fruit juice samples examined were below the maximum allowable limit in foods to be marketed for consumption (10^3cfu/ml). Sample A had shown more amount of bacteria as compared to sample B. However, the average ranges obtained for the bacteria indicated a public health concern. These counts are suggestive of bacterial contamination of the packaged fruit juice during handling since they are liquid, which could have contributed to the development as well as multiplication of these contaminants. The study has also shown that these packaged fruit juices are not sterile and thus can favour the growth of microorganisms when conditions become favourable, which could pose a public health risk to their consumers.

CONFLICT OF INTEREST

Conflict of interest declared none.

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