



Estimation of Microbial Contamination in Fresh and Commercial Fruit Juices: A Comparative Study

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Aim and Objective: The objective of the present study is to determine microbial contamination in fresh and packaged commercial fruit juices (including a combination of Aloe vera with fruit juices) available in the Ahmedabad city of Gujarat, India.

Materials and Methods: Seventeen samples were collected from various parts of the city which includes 9 commercial fruit juice samples and 8 street vended fresh fruit juice samples. Samples were examined for Total plate count, Yeast and mould count, coliform count, Escherichia coli, Salmonella, Staphylococcus aureus, Shigella, Enterobacteriaceae, Listeria monocytogens and Vibrio Cholerae.

Results: Commercial fruit juices do not exceed the FSSAI standards for fruit juices and are free of harmful pathogens making them safe for human consumption. Street vended fresh fruit juice samples exceed the FSSAI limit for Total Plate count, Yeast and mould count and Coliform count. Street vended Fresh fruit juices demonstrate the presence of E. coli, Salmonella and Staphylococcus aureus in 75% (6/8) samples. Enterobacteriaceae were identified in street vended fruit juices which exceeds the FSSAI standard limit.

Conclusion: The study demonstrates that commercial fruit juices were safe for human

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consumption but fresh juices showed significant microbial growth and harmful pathogens which must be controlled to ensure consumer's safety and health. However regular monitoring of commercial and fresh fruit juices is recommended to avoid food borne illness resulting from pathogens encountered in the study.

Keywords: Fruit juices; commercial; fresh; microbial contamination; estimation.

1. INTRODUCTION

Fruit juices are considered as one of the important sources of carbohydrates, minerals, vitamins and several proteins and therefore included as the part of diet due to its nutritional values [1]. These juices are either commercial fruit juices or homemade or street vended fresh fruit juices obtained from variety of fruits. The consumption of fruit juices has variety of health benefits which includes detoxification of human body, antioxidant property, lipid lowering agents, etc [2]. The extracted juices can be used in fresh form [as such or concentrated (which is used after dilution)] or in preserved form. Aloe vera juice has nutritional and health benefits such as anti inflammatory, antimicrobial, palliative, antifungal, digestive aid, etc [3]. Aloe vera and fruit juice combination are popular packaged drinks due to functional and health advantages [3]. There is need to evaluate microbiological load in aloe vera and fruit juice samples due to increased consumption of such juices. In the fresh juices, the chances of microbial growth are more due to absence of preservatives. Nutritional value also encourages microbial contamination if the sufficient hygienic conditions are not maintained. To limit the microbial contamination various factors are considered out of those important factors are maintenance of pH, storage temperature, chemical composition, hygienic condition and storage condition [4]. In the developing countries, fresh juices are sold by street vendors which is widely consumed by millions of people every day especially in the summer [5]. Fresh or unpasteurized juice are more preferred because of the fresh flavor and low cost. These fresh juices offer favourable condition for the growth of various types of microbes, some of them may be pathogenic for human. These pathogenic microorganisms may cause borne diseases. Contamination in food may result into many health problems; ranging from mild health problems to serious incident of food poisoning.

Packaged juices include water, small amount of minerals, vitamin A, Vitamin C and carbohydrates. To limit the contamination by

microorganism in the packaged juice preservatives have been added and instructions regarding storage conditions are also given on package. Microbiological analysis of packaged juices has also shown presence of some pathogenic bacteria, moulds and yeasts [6]. However unique properties acidic juices have capability to eliminate the majority of microorganism which are pathogenic but there are still some pathogenic bacteria and yeast which have capability to survive at lower pH and high temperature. These microorganisms may decline the nutrition value by degradation of some important dietary constituents and producing undesirable flavor odour and color changes.

Food Safety and Standards Authority of India (FSSAI) is the regulatory authority of India for food which protects public health by regulation and supervision to ensure compliance to standards. As per the Food Safety and Standards (Food Products Standards and Food Additives) Regulations, 2011 (Version XIII – 26.03.2021) – Table 4: Microbiological Standards for Fruits and Vegetables and their Products – Process Hygiene Criteria were followed for commercial fruit juices and fresh fruit juices [7].

2. MATERIALS AND METHODS

2.1 Sample Procurement

Collection of fruit juice samples were done within the city of Ahmedabad, Gujarat, India. Commercial juices of popular brands and fresh fruit juices were procured from various retailers and vendor outlets from different parts of city during month of July to December 2019. For Commercial, Orange, multifruit and aloe vera combination of fruit juices were considered for microbial evaluation. Description of samples are provided in Table 1. For Fresh fruit juices, Orange and multifruit juices were considered for microbial evaluation. Description of sample are provided in Table 2. The samples were collected and transferred in glass sterilized (by autoclave) bottles which were tightly sealed and contained in ice box. Samples were then transported to

laboratory for analysis. For analysis, 1 mL of juice sample was diluted to 10^{-2} in sterile phosphate buffer and the series of the dilutions were plated on the nutrient agar medium on all plates. The plates were incubated at 37°C for 24 hours.

2.2 Commercial Fruit Juices

Table 1. Description of samples of commercial fruit juices

Sample number	Sample description
Sample 1	Orange fruit juice (Brand A)
Sample 2	Orange fruit juice (Brand B)
Sample 3	Aloe Vera juice (Brand X)
Sample 4	Orange fruit juice + Aloe pulp (Brand Y)
Sample 5	Orange fruit juice + Aloe Vera pulp (Brand Z)
Sample 6	Mixed fruit juice [Brand A (Apple, mango, banana, orange, guava, apricot, lime, passion fruit, pineapple)]
Sample 7	Mixed fruit juice [Brand B (Apple, Mango, Guava, Orange, Banana, Apricot, Peach)]
Sample 8	Mixed fruit juice + Aloe Vera pulp [Brand Y (Orange, Pomogranate, Apricot, Apple, Banana, Mango, Peach, Pineapple, Litchi, Guava)]
Sample 9	Mixed fruit juice + Aloe Vera pulp [Brand Z (Apple, Banana, Orange, Peach, Mango, Guava, Litchi, Pineapple)]

2.3 Fresh Fruit Juices

Table 2. Description of samples of fresh fruit juices

Sample number	Sample description
Sample 10	Fresh Fruit Juice (Orange)- Homemade
Sample 11	Fresh Fruit Juice [Mixed Fruit (Apple, Banana, Orange, Peach, Mango, Litchi, Pineapple, Pomogranate, Lime, Grapes, Kiwi, Raspberry)]- Homemade
Sample 12	Fresh orange juice collected from Ahmedabad, Area 1
Sample 13	Fresh orange juice collected from Ahmedabad, Area 2
Sample 14	Fresh orange juice collected from Ahmedabad, Area 3
Sample 15	Fresh mixed fruit juice collected from Ahmedabad, Area 4 (Pineapple, Orange, Grapes, Lime)
Sample 16	Fresh mixed fruit juice collected from Ahmedabad, Area 5 (Pineapple, Orange, Grapes, Kiwi, Lime)
Sample 17	Fresh mixed fruit juice collected from Ahmedabad, Area 6 (Pineapple, Orange, Grapes, Pomegranate)

2.4 Determination of Microbial Load

Commercial and fresh fruit juice samples were analysed for Total Plate Count, Yeast and Mould count, Coliform count, E coli, Salmonella, Staphylococcus Aureus, Shigella, Enterobacteriaceae, Listeria Monocytogens and Vibrio Cholera spp. The technique used for determination of microbial load and presence of pathogenic microorganisms was as per FSSAI guideline which is either Indian standard (IS) or International organization for Standardization (ISO). All the nutrient media were procured from Hi-Media Laboratories. Pour plate method was used for enumeration and isolation of microorganisms for commercial and fresh fruit juice samples. Pour plate method was performed for enumeration of microorganisms from commercial and fresh fruit juices. Fruit juice samples were prepared using decimal dilutions until desired dilution is attained aseptically by transferring 1 mL of each of the fresh fruit juice sample into 9 mL of sterile phosphate buffer (1:10) into test tubes separately [8].

Microbial enumeration was made using selective media which includes nutrient agar medium for Total plate count [9], Yeast extract – Dextrose-Chloramphenicol- Agar medium for Yeast and mould count [10], Crystal violet neutral red bile lactose (VRBL) agar medium for Coliform Count [11], Blood agar salt medium and Baird -Parker medium for *Staphylococcus aureus* [12], Violet red bile glucose for Enterobacteriaceae [13]. 25 ml of Sample is inoculated in 3 media Alkaline Peptone Water, Thiosulphate-Citrate-Bile Salts-Sucrose Agar (TCBS) and Bile Salt Agar medium for *Vibrio Cholerae* [14]. For estimation of *Listeria monocytogens*, sample is treated with Half Fraser medium and subculture is treated with secondary enrichment medium and then pour plating is done on OXFORD and PALCAM agar [15]. For estimation of *E. coli*, 25 ml of sample is taken and diluted with 0.1% peptone solution in water and pH adjusted to 6.8 ± 0.1 or 3.4 % KHP in water and inoculation is done using Mckonkey agar medium and Eosin methylene blue lactose agar [16]. For *Salmonella*, non-selective pre-enrichment of 25 mL each sample was made in peptone broth and the homogenate was incubated at 37°C for 24 hours followed by 1 mL in 10 mL Tetrathionate (Muller Kaufman) broth as selective enrichment and then isolation was done on 3 media: Xylose lysine desoxycholate (XLD), Brilliant Green Agar (BGA) and Bismuth Sulphate Agar [17]. For *Shigella*, pre-enrichment with Nutrient broth is done and if enrichment is not required the sample is blended with Selenite F broth per 25g of one portion of sample and 200 ml Tetrathionate broth of 25g of another portion of sample, incubate at 37 °C for 24 hours and pour plating on Deoxycholate citrate agar medium is performed and colonies are identified [18].

2.5 Isolation of Microorganisms from the Fruit Juices

The colonies obtained from the above process were closely examined and distinct colonies subcultured in sterile medium which were counted using Colony Coulter counter. Evaluation of microbial contamination was carried out and the results were compared with the FSSAI standard for fruit juices.

3. RESULTS AND DISCUSSION

In developing countries like India, packaged fruit juices and fresh fruit juices by street food vendor outlets are widely consumed by large population [19]. In local market, people tend to think about

the nutritive benefits but quality aspects are however ignored [20]. Due to improper practices and poor hygiene, there may be chances of diarrhoeal diseases because of microbial contamination. Commercial fruit juices have low chances of microbial contamination as they are properly packaged and contain stabilizers. However, there may be chances due to packaging abnormalities during transport and improper storage conditions. The conditions of fresh fruit juice preparation raise potential risk for consumer's health. Fruit juices are consumed for quenching of thirst. One of the constituent of fresh fruit juice is water which is added for dilution should meet the drinking water standards for bacteriological quality of water as per Indian Standard Drinking Water Specification (IS 10500 : 2012) [21]. Poor quality water usage is applied for washing, cleaning, hand sanitation and even to dilute the juices which are prepared. Fruits and fruit juices are not protected from flies which may carry food borne pathogens. Fruits and fruit juices storage temperatures are not appropriate. There are more chances of microbial contamination from juices prepared from rotten and damaged fruits. In addition, there may be chances of preliminary contamination by rotten fruits which are used for preparation, improper washing of fruits as well as contamination by vendors during preparation, handling and cross contamination. Processing equipments used for the preparation of fresh fruit juice should be cleaned properly to minimize microbial contamination. The present study aims to evaluate commercial fruit juices and fresh fruit juices for microbial contamination and their outcome on consumers health. Seventeen samples were analyzed and the results were shown in Table 3 (Commercial juices) and Table 4 (Fresh fruit juices). The tests included in the analysis were Total plate count, Yeast and Mould count and Coliform count. The pathogens included for analysis were *E. coli*, *Salmonella*, *Staphylococcus Aureus*, *Shigella*, Enterobacteriaceae, *Listeria monocytogens* and *Vibrio Cholera*.

Commercial fruit juices (Sample 1- Sample 9) do not exceed the FSSAI criteria for Microbiological standards for fruits and vegetables and their products- Food safety criteria. Total plate count for all commercial juice samples were below 1 CFU/ml except Sample 2 (5 CFU/ml) and Sample 8 (15 CFU/ml) which are in limit with FSSAI standards. Yeast and mould count and Coliform count for all commercial fruit juices were below 1 CFU/ml and fulfils FSSAI standards. Few

colonies of Enterobacteriaceae were found in commercial fruit juices, however it does not exceed the FSSAI limit. Commercial fruit juices were free from harmful pathogens which are source of various detrimental diseases.

3.1 Commercial Fruit Juices

Fresh fruit juices which were either homemade (Sample 1 and 2) or collected from various parts of city (Sample 3- Sample 8) were determined. Total Plate count criteria for all the fresh fruit juices samples exceeds the FSSAI limit. Yeast and mould count and Coliform count also exceeds the FSSAI standards. The results

demonstrate 75% (6/8) of fresh fruit juice samples were contaminated with one or the other foodborne pathogens. Shigella, Listeria monocytogens and Vibrio Cholera were absent in all fresh fruit juice samples. Results demonstrate presence of Ecoli, Salmonella and Staphylococcus aureus in 6 samples. There were also detectable levels of enterobacteriaceae in street-vendor fruit juices exceeding FSSAI standard limit. Pathogens were absent in homemade samples 6 and 11 which are hygienic and safe for consumption. Fruit juice samples from street vendors are contaminated with pathogens that are potentially dangerous to human health.

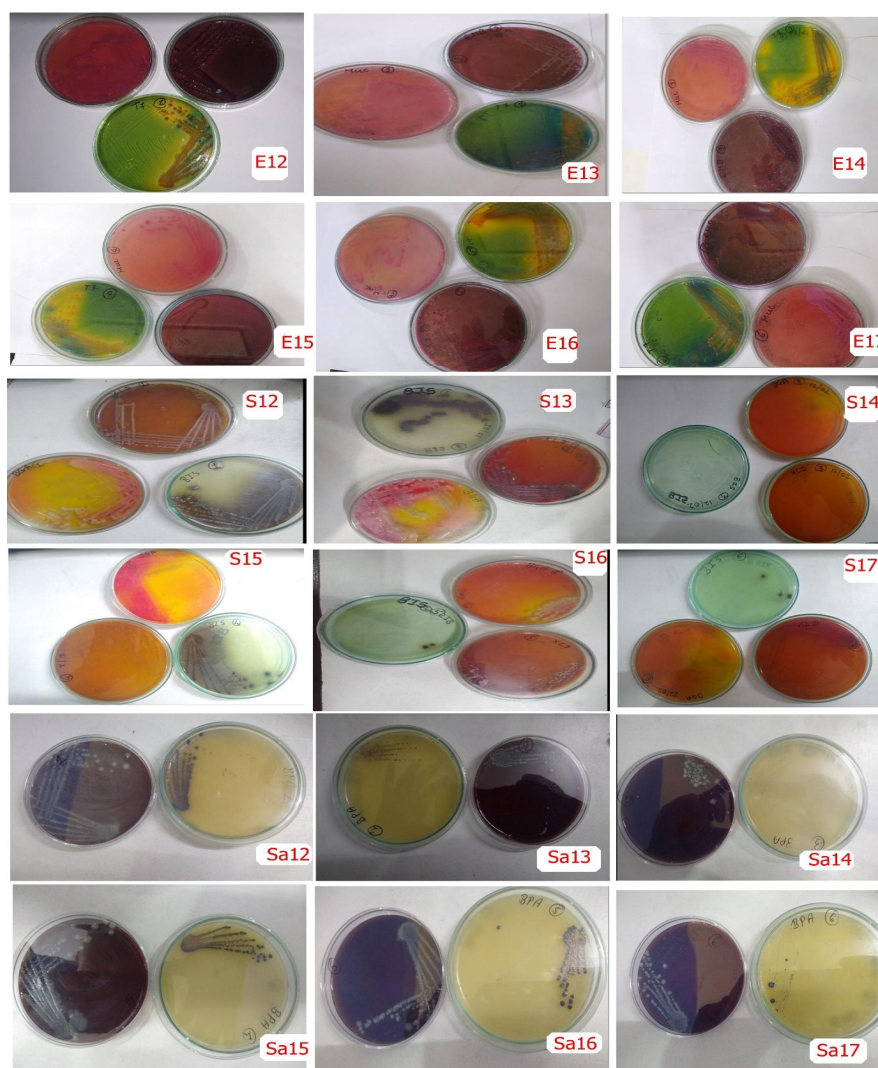


Fig. 1. Presence of microbial pathogens like Escherichia Coli (E12-E17), Salmonella Typhi (S12-S-17) and Staphylococcus aureus (Sa12- Sa17)

Table 3. Microbiological evaluation of commercial fruit juice samples

Name of test/ Pathogen	Limits as per FSSAI standards (mg/kg)	Results (mg/kg)								
		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 7	Sample 8	Sample 9	Sample 10
Total Plate Count	1×10^6 /ml to 1×10^7 /ml	<1 CFU/ml	5 CFU/ml	<1 CFU/ml	<1 CFU/ml	<1 CFU/ml	<1 CFU/ml	15 CFU/ml	<1 CFU/ml	<1 CFU/ml
Yeast and mould count	1×10^2 /ml to 1×10^4 /ml	<1 CFU/ml	<1 CFU/ml	<1 CFU/ml	<1 CFU/ml	<1 CFU/ml	<1 CFU/ml	<1 CFU/ml	<1 CFU/ml	<1 CFU/ml
Coliform Count	Absent/100ml	<1 CFU/ml	<1 CFU/ml	<1 CFU/ml	<1 CFU/ml	<1 CFU/ml	<1 CFU/ml	<1 CFU/ml	<1 CFU/ml	<1 CFU/ml
E coli	Absent/ml	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Salmonella	Absent/25 ml	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Staphylococcus Aureus	Absent/25 ml	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Shigella	Absent/25 ml	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Enterobacteriaceae	1×10^2 /g to 1×10^4 /g	63 CFU	10 CFU	130 CFU	120 CFU	10 CFU	20 CFU	350 CFU	50 CFU	180 CFU
Listeria monocytogens	Absent/25 ml	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Vibrio Cholera	Absent/25 ml	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

Table 4. Microbiological evaluation of fresh fruit juice samples

Name of test/ Pathogen	Limits as per FSSAI standards (mg/kg)	Results (mg/kg)							
		Sample 6	Sample 11	Sample 12	Sample 13	Sample 14	Sample 15	Sample 16	Sample 17
Total Plate Count	1×10^6 /ml to 1×10^7 /ml	1.1×10^5 CFU /ml	1.4×10^6 CFU /ml	3.7×10^6 CFU /ml	2.1×10^5 CFU /ml	7.1×10^6 CFU /ml	4.0×10^6 CFU /ml	2.0×10^5 CFU /ml	2.3×10^6 CFU /ml
Yeast and mould count	1×10^2 /ml to 1×10^4 /ml	6.3×10^4 /ml	2.8×10^4 CFU /ml	4.0×10^5 /ml	4.0×10^5 /ml	2.9×10^5 /ml	4.0×10^5 /ml	1.5×10^4 /ml	1.2×10^5 CFU /ml
Coliform Count	Absent/100ml	70 CFU/ ml	7.0×10^5 CFU /ml	2.2×10^6 CFU /ml	4.5×10^4 CFU /ml	6.0×10^4 CFU /ml	3.8×10^6 CFU /ml	1.0×10^4 CFU /ml	2.0×10^5 CFU /ml
E coli	Absent/ml	Absent	Absent	Present	Present	Present	Present	Present	Present
Salmonella	Absent/25 ml	Absent	Absent	Present	Present	Present	Present	Present	Present
Staphylococcus Aureus	Absent/25 ml	Absent	Absent	Present	Present	Present	Present	Present	Present
Shigella	Absent/25 ml	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Enterobacteriaceae	1×10^2 /g to 1×10^4 /g	20 CFU	30 CFU	2.0×10^5 CFU /ml	1.0×10^5 CFU /ml	4.0×10^5 CFU /ml	6.0×10^5 CFU /ml	2.0×10^5 CFU /ml	1.1×10^5 CFU /ml
Listeria monocytogens	Absent/25 ml	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Vibrio Cholera	Absent/25 ml	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

4. CONCLUSION

The study indicated that all the commercial fruit juices were free of microbial growth due to presence of stabilizers, acidity regulators and preservatives. Quality of commercial juices are improved and storage conditions are notably clean and temperatures are maintained at outlets where it is sold. The commercial fruit juices are safe for human consumption. However, freezing conditions should be maintained during storage. Street vended fruit juices had a higher microbial load and were contaminated by various pathogens. It can be caused due to varying factors such as quality of water used in preparation, unhygienic practice of washing of utensils and equipment, contaminated water and ice, personal hygiene, handling of fruits such as cutting and peeling before preparation, crowded area, dust particles in the areas and improper waste disposal system [19]. Food borne outbreaks can be avoided if quality of fresh juices is maintained [22]. To improve quality, awareness amongst street vendors regarding hygiene and safety of different fruit juices should be required [23]. Concerned Government authorities should regularly conduct awareness programmes on handling of raw materials and hygiene practices in preparation of fruit juices. Commercial juices and fresh fruit juices, particularly those sold in street stalls and popular choices among consumers for human consumption, should be regularly monitored for microbial growth and presence of harmful pathogens.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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