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Microbial study of organisms isolated from nutritional fruit juices surrounded by local fruit market in Nanded, Maharashtra, India

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Abstract. *Deshmukh AS, Siddiqui MM, Pathan UK, Dhuldhaj UP. 2020. Microbial study of organisms isolated from nutritional fruit juices surrounded by local fruit market in Nanded, Maharashtra, India. Biodiversitas 21: 4240-4246.* Growing populations depend on the various food products for their nutritional values, but the hidden hunger for nutrients and vitamins are fulfilled by the fruits and their juices. Fruit juices are one of the most dependable food products, available in the Indian markets. But, standard and quality of juices always matters, hence most suggested one are freshly prepared juices as processed and packed one are mostly contains artificial flavors and food additives in the form of preservatives. Even these freshly prepared juices by local staler are also not free from contaminations. Hence, in this study we focus on the quality of fruit juices sold by the local seller. For this purpose, we collected 5 samples of fruit juices from the premises of Swami Ramanand Teerth Marathwada University, Nanded. The fruit juices are on orange, chiku, banana, apple and grapes. These samples were maintained in the basal media nutrient broth and uniculture of bacteria were isolated and maintained in the slant agar for further experiments. Total microbial load was calculated from collected and it was found that these juice samples contain significant bacterial load (2.5×10^6cfu/mL) that can cause diseases. In further investigations and identifications through the biochemical tests, we found that these juices contaminated with coliforms like *E. coli* and *Klebsiella*, along with this we also detected the presence of *Listeria spp* and *Staphylococcus* in juices samples.

Keywords: Bacterial identifications, biochemical test, fruit juices

INTRODUCTIONS

Fruits has important place in our diet, as an easy source of energy and high nutritional values (Slavin and Lloyd 2012). Fruits are more preferred food for the diseased person in comparison to cooked food, because it contains good quantity of antioxidants, vitamins, minerals and also necessary secondary metabolites helps in preventing diseases like cancer, diabetes and heart diseases, it also helps in detoxifications of human body by improving blood lipid profile (Vafa et al. 2011). Fruits and vegetables contain adequate amount of antioxidant and also has significant effect on chronic non-communicable diseases such as neurodegenerative and cardiovascular diseases (Bhardwaj et al. 2014) and its daily uptake reduces the chances of several diseases (Singh et al. 2015; Karabiyikli et al. 2012). Major group of fruits having sufficient amount of vitamin C acts as the antioxidant, helps to neutralize free radicals of the tissues (Jahan et al. 2011; Islam et al. 2014). Fresh fruits and their ready to made concentrate fruit juices are having balance nutrients, sugars, and organic acids. There are several varieties of fruits that are available in the market and their sweetness and deliciousness depend on its maturity and nutrient contents (Zerdin et al. 2003). Fresh and healthy fruits are always good for the better health of human beings (Ara et al. 2016).

It becomes traditions in India to sold seasonal fruit juices at roadside and peoples are nowadays more attracted to it. In summers, more often sold juices are Sugarcane, Pineapple, Watermelon, Grapes, Mangoes, etc. while in winter vegetable juices are preferred and also Amla juice is of more demand (Zheng et al. 2018). Preparation of these ready to made methods are used and it will create more health issues (Health Canada 2006). Fresh fruit juices are having more nutritional value as they are not processed one (Ara et al. 2016), along with these, issues of foodborne illness and food poisonings are also associated (CDC 2016; Scallan et al. 2011a; 2011b). Demands of fruits and packaged fruit juices in the market are increasing very rapidly it creates great risks of spoilage and adulterations.

The modernization of agriculture focuses on the disease-free plant products and also consumers are more aware of the microbial safe food. Shelf life of fruits is very short after ripening, they are more prone to contamination of harmful pathogens through the handling and unhygienic maintenance. The actual source of contamination is soil, water, air, and other environmental factors (Rawat 2015).

It is not good towards safety of mankind, food material contains microbes or its contaminations, as the basic food for human beings, come from the plants or their products (Mihiretie and Desta 2015). Contaminations are occurred mainly through the damage surfaces of fruit containers, utensils and untidy instruments (Mukhopadhyay et al. 2011). Pasteurization had done with quick-heat treatments increase the shelf of fruit juices up to one year. Use of rotten fruits and contaminated juices sold by the local staler are harmful to human consumptions, results in severe

disease symptoms (Mahmood and Lahmood 2019). Unhygienic maintenance of fruits and fruit juices are prone to infection through the fruit flies and airborne dust (Singh and Singh 2019). Off-season fruits come in market from the storage plant and such fruits act as the bacterial pool for the spoilage and contamination, unless processed and preserved properly (Grantina-Ievina 2015). Bacteria causing food poisoning are unable to survive in fruit or fruit products containing more acid content, hence such bacteria are more prone to infect vegetables and vegetable products. Some of the bacteria produce toxins and secrete it into the fruits without showing any sign of fruit spoilage. Such fruits are very harmful to the consumers as it contains toxins, hence special care should be taken by the fruit processors with proper processing methods to avoid such contaminations.

Two sorts of fruit juices are available, first freshly prepared or prepared as the concentrate of water extract; second needs stringent processing such as heating, fermentation, etc. Because negligence the processing methods are more often linked to the contamination of pathogens and it directly depends on the variety of crops, processing methods, and practices. Foodborne diseases are caused sometimes because of contaminated water also, if processing of fruit or juices are done by the contaminated water. All bacteria or microbes have taken up through the mouth as food are not pathogenic some of them are beneficial; if the toxin-producing bacteria are swallowed along with food it will create disease conditions. The causal organisms for the foodborne illness are bacteria (66%), parasites (4%), and viruses (4%) are rest of it because of chemical substances (Hezam et al. 2019). Probable presence of pathogens is *E. coli*, *Salmonella*, *Shigella*, and *Staphylococcus* (Buchmann et al. 1999; Barro et al. 2006).

MATERIALS AND METHODS

Fruit samples and fruit juices are collected from local fruit juices sellers in the premises of Swami Ramanand Teerth Marathwada University, Nanded campus, Nanded, Maharashtra, India. Fruit samples were collected during the period of October 2017 to June 2018 and total of 5 juice samples were collected. The collected fruit and their juices were labeled with particular code and isolation of bacteria were done. The bacteria isolated were maintained by repeated sub-culturing on agar medium and glycerol stock of each isolate was prepared. Media were prepared according to standard protocol and recipe for the selective growth, enrichment culture, and for the indication of specific properties. The collected samples are serially diluted in sterile saline water and spread on nutrient agar, Muller-Hinton agar, and Luria-Bertani agar plates and allow it to grow for 24-48 h in standard laboratory conditions and isolation and purification of microbes were done. Further, the colony morphology of grown colonies was recorded in terms of size, margin, elevation, texture, and pigmentation, also for the preliminary identifications were done by the microscopic observations (Cappuccino and Sherman 2005). Before further biochemical analysis of these bacterial isolates, Gram staining was done.

Biochemical analysis

Triple sugar iron agar test

Microbes able to reduce sulfur and ferment carbohydrates are identified by this test. Sterile triple sugar irons slants were prepared in screw cap tube and unknown isolate of bacteria streaked on it, caps kept loosely tightened, and allow it to incubate for 24 hrs at 37 ^{0}C (Cappuccino and Sherman 2005).

IMViC test

Causal organism for the food spoiling generally observed to be Gram-negative enteric bacilli. To identify coliform group of bacteria usually, four tests are used, collectively called IMViC test. It includes Indole production test, methyl red test, Voges- Proskauer test and Citrate utilization test. Indole production test are performed to check the ability of microbes to produce tryptophanase enzymes to degrade tryptophan amino acid for Indole production. Sterile tryptophan broths were prepared and pre-grown (24 h) unknown isolates of microbes inoculated into it with the help of inoculating loop and allow it to incubate for 48 h at 37 ^{0}C. Further, Indole productions can be detected by adding 5 drops of Kovac's reagent in test tube (MacWilliams 2009). Methyl Red tests are performed to check the ability of microbes to oxidize glucose and produce stabilize acid end products. Collective 7 mL of sterile broth of MR-VP were prepared for the Methyl Red test and Voges-Proskauer test. Pre-grown (24 h) unknown isolate of microbes were inoculated in it and allow it to incubate for 24 h at 37 ^{0}C. Half of the MR-VP broth culture was saved for VP test and remaining half of the MR-VP broth culture incubated further 24 h in same laboratory conditions, after 48 h of incubation, 5 drops of the Methyl Red indicator directly added for the formation of red color (Cappuccino and Sherman 2005). Voges-Proskauer test are perform to further differentiate nonacidic or neutral end product (acetylmethylcarbinol) producing enteric microbes such as *E.coli*, *E. aerogenes*, and *K. pneumonia*. To the MR-VP broth save for the Voges-Proskauer test in former procedure, 0.6 mL of 5% α-naphthol and 0.2 mL of 40% KOH added, gently shook and kept exposed to atmospheric oxygen for 30 s-1 min. Further, this medium kept undisturbed for 10-15 min and reading was taken before 1 h after adding above reagents (McDevitt 2009).

Citrate utilization test (Cappuccino and Sherman 2005)

This test is performing to differentiate enteric microbes which are capable for fermenting citrate as the sole carbon source by producing citrate permease enzyme. Sterile Simmons Citrate agar slants of 2 mL media were prepared and unknown isolates of microbes were streaked on it and allow it to incubate for 48 h at 37 ^{0}C (Cappuccino and Sherman 2005).

MIU test (Motility-Indole-Urease test)

This test is performing for collectively for detection of Indole production, motility, and degradation of urea by

urease enzyme. Sterile MIU media were prepared by autoclaving and allow to cool up to 50-55 ^{0}C and 5 mL of 40% urea solution is added to 95 mL of basal media and dispensed 6 mL in sterile loosely cap tubes and allowed to form semi-solid. The pre-grown (24 h) unknown isolates of microbes are inoculated into it by stab inoculation method and allow it to incubate for 24 h at 37 ^{0}C (Acharya 2015).

Nitrate reduction test

This test is performing to check the ability of microbes for the reduction of nitrate into nitrate. Sterile nitrate broth was prepared and dispensed around 6 mL into sterile test tubes. The pre-grown unknown isolate of microbes inoculated onto it and allowed to incubate for 24-48 h at 37 ^{0}C. Further, for the red colorations, 5-5 drops of Reagent A and Reagent B were directly added. Sometimes, traces of zinc also added if there no red colorations with precaution as zinc powder are hazardous (Cappuccino and Sherman 2005). Reagent A: 0.6 g of N, N-Dimethyl-α-naphthylamine added to 100 mL of 5N Acetic acid, mixed properly till solution turns light yellow in color. Reagent B: 0.8 g of sulfanilic acid added to 100 mL of 5N Acetic acid, mixed properly untill solution becomes colorless.

Catalase test

This test is performing to check the catalase producing ability of microbes for the degradation of hydrogen peroxide. Pre-grown unknown isolates of microbes placed on microscopic slide (kept on Petri dishes) with the help of inoculating loop and 1 drop of 3% H_2O_2 were added and observed for the effervescence (Reiner 2010).

Oxidase test

This test is performing for the detection of cytochrome oxidase enzyme in the bacteria. A well soaked small piece of filter paper in Gaby and Hadley oxidase test reagent and allowed it to dry. A pre-grown (24 h) culture of microbes is picked with the help of inoculating needle and rubbed on this filter paper and observed for the change in color (Shields and Cathcart 2010).

Casein hydrolysis test

This test is performing for the detection of caseinase enzyme in microbes by the hydrolysis of casein. Distilled water and agar solutions were sterilized in separate flask by autoclaving. In sterile distilled water skimmed milk powder was added and boiled for 1 min to form complete solution. To this milk solution, agar solution was mixed and poured off on sterile Petri plate in aseptic laboratory conditions and allow solidifying. To these milk plates, pre-grown cultures of unknown isolates were streaked and incubated for 24 h at 37 ^{0}C (Sturm 2013).

Gelatin hydrolysis test

This test is performing for the detection of gelatinase producing bacteria by degrading gelatin. Nutrient gelatin medium was prepared by gentle heating to dissolve all its content and dispense approximately 3 in sterile glass vials. These glass vials were sterilizing by autoclaving and allow cooling in upright position. Adequate biomass of pre-grown cultures of unknown isolates was stab inoculated with the help of inoculating loop and allow to incubate at 37 ^{0}C and observe for 7 days for the liquefaction of the media (Cruz and Torres 2012).

Starch hydrolysis

This test is performing for the detection of amylase producing microbes by the hydrolysis of starch. Sterile starch agar media were prepared and poured off on to Petri plates and allow it to solidity. These starch agar plates were further inoculated with the pre-grown unknown isolates by streaked plate method and allow it to incubate at 37 ^{0}C for 48 h. The starch hydrolysis can be detected by adding Gram's Iodine into it (Cappuccino and Sherman 2005).

Blood agar

This test is performing for the detection of bacterial hemolytic capability by producing hemolysin and brings about lysis of red blood cells and membranes. Nutrient agar was prepared in conical flask and sterilized it by autoclaving and allowed it to cool. When medium at 45-50 ^{0}C temperature, gently mixed with pre-warmed 5% sterile defibrinated sheep blood and poured off in agar plates and allowed it to solidify. To observe gamma, beta, and alpha hemolysis, solid blood agar plates further streaked with pre-grown culture of unknown microbial isolates and allow it to incubate at 37 ^{0}C for 24 h (Aryal 2015).

Mac Conkey agar

This test is performing for the differentiation of lactose fermenting from non-lactose fermenting Gram-negative enteric bacteria. Gram-negative bacteria having capability of fermenting lactose can be detected by lactose and pH indicator. Sterile MacConkey's agar was prepared by autoclaving and allows it to solidify. To this MacConkey's agar plates, pre-grown unknown bacterial isolates were streaked and incubate at 37 ^{0}C for 24 h. This media allows the growth of Gram-negative bacteria only as the Crystal violet and bile salt present in media inhibits the growth of Gram-positive bacteria.

Mannitol agar (Cappuccino and Sherman 2005)

This test is performing for the detection and isolation of Gram-positive bacteria such as *Staphylococci* from mixed cultures. It is also used for the differentiation of mannitol fermenting *Staphylococci* and acid production can be detected by the phenol red. This technique is commonly used in medical microbiology for the isolation of pathogenic microbes. Sterile plates of Mannitol agar were prepared and pre-grown cultures (24 h) of unknown isolated were streaked on it and allowed to incubate at 37 ^{0}C for 24 h. Mannitol salt agar is both a selective and differential media used for the isolation of presumptive pathogenic *Staphylococcus* species.

RESULTS AND DISCUSSION

Fruit juices are collected from the local seller and tested for their quality in the form of microbial load present in it.

The juices sold by the local seller are freshly prepared the good quality of fruits is also important to know. Sometimes the rotten fruits cannot be easily differentiated; so unhygienic juices possibly can be served to the customers. Hence, we are focus on the quality of fruit juices and probable presence of microbial load in it. Five juice samples were collected from the premises of the University campus and maintained in the laboratory conditions. For convenient regular use and for the experiment purposes the collected juices and bacteria isolated from it labeled with specific code summarized in Tables 1 & 2. Total of 13 bacterial isolates was obtained from the 5 juice samples and maintained in agar slant for further biochemical analysis.

Gram reaction and colony morphology

Collected five samples were maintained in the slant agar and for further identification Gram reaction was evaluated along with the colony morphology (Table 3).

With the broad identification of microbes for the Gram reaction and colony morphology, further continue with the biochemical identification by considering their observations on agar plate in the form of growth pattern, coloration, morphology, shape.

Table 1. Labeling of the fruit juice sample collected from the premises of the SRTM University, Nanded

Fresh Juice	Collection Area	Sample Code
Apple juice	Nearby SGGS Eng. College, Vishnupuri, Nanded	AA
Banana juice	Nearby Petrol pump Latur road, Nanded	AB
Chiku juice	Nearby SGGS Eng. College, Vishnupuri, Nanded	AC
Grape juice	Kaleshwar Gate Vishnupuri, Nanded	AG
Orange juice	Nearby Petrol pump Latur road, Nanded	AO

Table 2. Labeling of the bacterial isolates from respective fruit juices

Isolate No.	Original Name of the isolates	Given name of the isolates
1	Fresh juice Apple- Nutrient Agar- White colony	AA1
2	Fresh juice Apple- Nutrient Agar- Yellow Colony	AA2
3	Fresh juice Apple- Luria Agar- White colony	AA3
4	Fresh juice Banana- Nutrient Agar- Off white colony	AB1
5	Fresh juice Banana- Luria Agar- Off white colony	AB2
6	Fresh juice Chiku- Nutrient Agar- Milky white colony	AC1
7	Fresh juice Chiku- Luria Agar- White colony	AC2
8	Fresh juice Chiku- Nutrient Agar- Yellow colony	AC3
9	Fresh juice Grape- Nutrient Agar- White colony	AG1
10	Fresh juice Grape- Luria Agar- White colony	AG2
11	Fresh juice Orange- Nutrient Agar-Lemon colony	AO1
12	Fresh juice Orange- Nutrient Agar- White colony	AO2
13	Fresh juice Orange-Luria Agar- White colony13	AO3

Table 3. Morphological characteristics of bacterial colonies and gram reaction

Bacterial isolates	Colony coloration	Configuration	Margin	Elevation	Gram Reaction	Shape of isolate
AA1	White	Irregular	Undulate	Raised	Positive	Cocci
AA2	Yellow	Circular	Entire	Convex	Positive	Cocci
AA3	White	Irregular	Curled	Raised	Negative	Rod
AB1	Off-white	Circular	Entire	Convex	Positive	Cocci
AB2	Off-white	Circular	Entire	Flat	Positive	Cocci
AC1	Milky white	Circular	Entire	Raised	Negative	Rods
AC2	White	Irregular	Curled	Raised	Positive	Rods
AC3	Yellow	Circular	Entire	Convex	Positive	Staphylococci
AG1	Off White	Irregular	Undulate	Convex	Negative	Rod
AG2	Off white	Irregular	Curled	Flat	Negative	Rods
AO1	White (pale)	Circular	Entire	Raised	Positive	Cocci
AO2	Light yellow	Circular	Entire	Convex	Negative	Coccobacilli
AO3	Mucoid (transparent)	Circular	Entire	Flat	Negative	Rod

Table 4. Biochemical characterization of isolates obtained from different fruit juices

Isolate No.	Isolate	Oxidase	Catalase	Indole	Motility	Indole	Urease	Methyl red	VogesProskaur	Gelatin	Nitrate reduction	Simmon's citrate	Casein hydrolysis	Starch hydrolysis	Blood agar	Mannitol	MacConkey	Slant/butt	Glucose	Lactose	Sucrose	H_2S production	Gas production	Name of species
1.	AA1	-	+	-	-	-	+	+	+	+	+	-	-	-	+	+	-	R/Y	+	+	+	-	-	*Staphylococcus spp*
2.	AA2	-	+	-	-	-	+	-	+	+	+	-	-	-	+	+	-	R/Y	+	+	+	-	-	*Staphylococcus sp*
3.	AG1	+	+	-	+	-	-	-	-	+	+	+	+	-	-	+	+	R/R	-	-	-	-	-	*Pseudomonas spp.*
4.	AB1	-	+	+	-	+	+	+	+	+	+	-	-	-	+	+	-	R/Y	+	+	+	-	-	*Staphylococcus spp*
5.	AB2	-	+	-	-	-	+	+	+	+	+	-	-	-	+	+	-	R/Y	+	+	+	-	-	*Staphylococcus spp*
6.	AC1	-	-	-	-	-	-	-	+	-	+	+	+	-	-	-	-	Y/Y	-	+	+	-	-	*Klebsiella spp.*
7.	AC2	-	+	-	+	-	-	+	+	-	+	-	-	+	-	+	-	Y/Y	+	+	+	+	-	*Listeria spp*
8.	AG2	-	+	-	-	-	+	+	+	+	+	-	-	-	+	+	-	R/Y	+	+	+	-	-	*Staphylococcus spp.*
9.	AA3	+	+	-	+	-	-	-	-	+	+	+	+	-	-	+	+	R/R	-	-	-	-	-	*Pseudomonas spp.*
10.	AC3	-	+	+	+	+	-	+	-	-	+	-	-	-	-	+	-	Y/Y	+	+	+	-	+	*Escherichia coli*
11.	AO1	-	+	-	-	-	+	+	+	+	+	-	-	-	+	+	-	R/Y	+	+	+	-	-	*Staphylococcus spp*
12.	AO2	-	+	-	-	-	-	+	-	-	-	-	-	-	+	+	+	Y/Y	-	+	+	-	-	*Acinetobacter spp*
13.	AO3	+	+	-	+	-	-	-	-	+	+	+	+	-	-	+	+	R/R	-	-	-	-	-	*Pseudomonas spp*

Note: + Positive, - Negative, R-Red, Y-Yellow

With the 20 consecutive biochemical tests, the bacterial isolates obtained from 5 fruit juice samples were presumed to be *Escherichia coli*; *Staphylococcus* sp., *Bacillus* sp., *Klebsiella* sp., *Moraxella* sp., *Shigella* sp. and *Aerobacter* sp. The isolates, ferment lactose and produce gas, non-spore-forming, gram-negative, and shows IMViC as ++-- or -+-- are *E. coli and* more often found on fruit surfaces (Singh and Singh 2019). Bacterial isolates were identified by the biochemical tests as *E. coli* in Orange, Apple and Grape juice samples, *Staphylococcus* sp. in Orange, Bacillus in Grape, *Moraxella* sp. in Grape and Orange and also some bacteria such as *Acetobacter* sp., *Acinetobacter*sp., *Francisella* sp., etc. also found. Causal organisms can be varied with the different variety of same fruits and time of harvest (Sever et al. 2012; Weber 2011; Borve et al. 2013).

The observed colony morphology in nutrient agar was white and yellow colorations, circular or entire margin, convex or flat shape of the colony. In case of MacConkey Agar, pink coloration and convex colonies observed which were considered to be coliforms. Yellow colonies in Mannitol considered to be *Staphylococcus* sp., culture in the MSA also indicates the *Staphylococcus* sp. The bacterial species commonly observed in contaminated or rotten fruits are *Salmonella* spp., *Campylobacter jejuni*, *Shigella* spp. and *E. coli*. These fruits and their juices occasionally also showed the presence of *Listeria monocytogenes (L. monocytogenes), Vibrio* and *Clostridium botulinum* (Scallan et al. 2011a).

As we can see freshly prepared juices sold by the local saler contain microbial load of the harmful bacteria. Same situation will be also observed in the packed fruit juices which are available throughout the year, must contain microbial contamination if quality control regulations of the firm are not strict. As the firm of food industry focus on the marketing so these peoples adding preservatives and antimicrobial agents into the fruit juices to reduce the microbial load. These chemicals used as preservatives and antimicrobials are gradually deposited into human body and can cause notorious disease conditions in comparison to harmful disease bacteria.

Variations in the bacterial count in these selected fruits are observed because of the maintenance and storage in unhygienic conditions. The highest bacterial load was observed in chiku (2.5 x 10^6 cfu/mL) and lowest in apple (1.0 x 10^5 cfu/mL). Generally, packed and processed juice sample contains bacterial load within the limit of 10^3 cfu/mL (Tasnim et al. 2010). In similar studies, bacterial load observed in Nagpur which were ranges from 2.0 x10^6 to 1.0 x 10^5cfu/mL (Bagde 2011) and highest load were recorded in one of the study, upto 3.7x10^8 cfu/mL in grape juice. Inner part of the fruits are sterile and presence of coliforms like *E. coli* and *Klebsiella* are more harmful, it seems to be fruits are treated with contaminated water which is not safe (Andres et al. 2004). Most of the authors observed coliforms detected in the fruit juices are non-pathogenic while the main concern regarding the public health is the presence of *Salmonella* spp, *Klebsiella* spp, *Proteus* spp, *Serratia* spp, *S. aureus*, and *Pseudomonas* spp. (Singh and Singh 2019). Some of the studies also reported prevalence of *Staphylococci* which is also observed in this study (Tambekar et al. 2009). Presence of *Staphylococci* is more harmful as it produces toxins responsible human diseases.

Conclusion

In India, manually prepared fresh fruit juices by the local staler are popular as there was minimum loss of taste and nutritional content. Nowadays commercial packed and concentrated ready to made fruit juices are also on-demand, available throughout the year and peoples are more interested because of maintenance fresh taste and nutritional value. Freshly prepared as well as commercial fruit juices are prone to contaminations maybe because of the use of contaminated water for processing or washing, unhygienic storage, and use of low quality or infected fruits. In the market, season, and non-season fruits both are easily available throughout the year as it comes from the cold storage plants. Such mass storage can be a pool for the microbial contaminations and infections. Processed fruit juices contain additives and preservatives reduce microbial load, but often accumulations of these additives in human body create disease conditions. In this study, we found the presence of *Escherichia coli, Klebsiella* spp., *Enterobacter* spp. and *Staphylococcus* spp. in sample of fruit juices which indicates even freshly prepared juices are also not safe for consumptions. It's necessary to increase awareness of common people for quality and storage of fruits and fruit juices and same should be intimated to manufacturing firms.

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