INTERNATIONAL JOURNAL OF ADVANCE RESEARCH, IDEAS AND INNOVATIONS IN TECHNOLOGY

ISSN: 2454-132X
Impact Factor: 6.078
(Volume 7, Issue 3 - V7I3-1658)
Available online at: https://www.ijariit.com

Bacteriological analysis of different types of fresh fruit juices from market

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ABSTRACT

Bacteriological analysis of samples of fresh orange and pineapple juices collected from different shops confirmed the presence of bacteria. The isolated bacteria were identified as Escherichia coli and Staphylococcus aureus by employing techniques like Microscopy, Culture and Biochemical Reactions. The presence of Staphylococcus aureus and Escherichia coli may be interpreted respectively as contamination of fruit juices by the bacteria present on the skin surface of the juice maker or by the bacteria present on the water used for preparing those fresh juices. It has been proved that Staphylococcus aureus can exist as commensals on skin. Similarly, E.coli being a common water contaminant can easily contaminate fresh juice. Hence it is advised to take necessary precautions to avoid bacterial contamination of juices during the preparation of fresh juices without preservatives. This can be achieved by proper hand washing techniques before juice preparation and also by using filtered cold water for juice preparation. In order to suggest a suitable therapeutic regimen in case of infections occurred during the consumption of such contaminated juices, antibiotic susceptibility testing of Escherichia coli and Staphylococcus aureus was done using antibiotics like Amoxicillin, Chloramphenicol, Tetracycline and Streptomycin. From the results obtained it has been assumed that Amoxicillin was more effective against both Staphylococcus aureus and Escherichia coli. The antibacterial effects of Amoxicillin, Chloramphenicol, Tetracycline and Streptomycin against Staphylococcus aureus and Escherichia coli showed the biocide effects of these antibiotics against the Gram positive and Gram-negative bacteria.

Keywords— *Bacteriological, Juices, Contamination, Preservatives, Antibiotics*

1. INTRODUCTION

Fruit juices are becoming an important part of the modern life in our communities. They are nutritious beverages and can play a significant part in healthy diet. The processing of juices involves washing, extraction, clarification and preservation. Consumption of fruit juices increased dramatically due to their freshness, high vitamin content and low caloric consumption. Juices extracted from fruits contain most substances which are found in the original ripe and sound fruits. The high potassium and low sodium characteristic of most juices help in maintaining a healthy blood pressure. It also helps in the absorption of iron.

Fruit juices contain many microorganisms which is normally present on the surface of the fruits during harvest and post-harvest processing which include transport, storage and processing. Most fruit juices contain nutrients that could support the microorganisms present in the fruit juices often originate from the natural flora of the raw materials used for the preparation and those introduced during the course of processing.

The critical factors affecting the spoilage of include juice pH, oxidation reduction potential, water activity, availability of nutrients, presence of antimicrobial compounds and competing micro flora. Fruit juices have pH in acidic range (< 4.5) serving as important barrier for microbial growth. Fruit juices have pH in acidic range (< 4.5) serving as important barrier for microbial growth. However, food borne pathogens such as *E.coli* and *Salmonella* survive in acidic environment of fruit juices due to acid stress response. Therefore, in the last two decades a number of foods borne outbreaks associated with unpasteurized fruit juices have been documented in many countries.

Contamination and adulteration in edibles can lead to a number of diseases such as paralysis, cancer, mental retardation and hypertension etc. Several diseases associated with the consumption of fruit juices have been reported at several places around the globe. Sources of contamination could be unhygienic water, contaminated ice, preservation without refrigeration, unhygienic surroundings and air borne dust.

Many researches had been conducted to check the quality of fruit juices whether it processed or unprocessed. The aim of these researches was to identify various microorganisms like

Bacteria, fungi; which generally contaminate food products. One of the researches was conducted in Bangladesh where twenty-six vendor fruit juices and 15 packed juices were examined for the presence of total bacterial load, coliforms and *Staphylococci* (Rashed*et al.*, 2013). Another study conducted to evaluate the quality of six different brands of mango juices packed in Tetra Pak for nutritional quality evaluation. Three samples were found to contain total soluble solids less than the standard limit prescribed by Pakistan Food Laws (Akhter, 2012). One other study was done in which fifteen samples of packaged fruit juices which include pineapple, orange, and apple juice were analyzed for their microbial content using standard microbiological techniques. The fruit juices were purchased from street hawkers in Port Harcourt Metropolis, Nigeria. The study showed the presence of *Bacillus* sp, *Micrococcus* sp, *Flavobacterium* sp., *Lactobacillus* sp, *Penicillum* sp. and *Saccharomyces* species. Some research has confirmed the presence of *Staphylococcus aureus* (T Jaylakshmi *et al.,* 2011), *Pseudomonas spp.*(DBM Virupakshaiah 2016) and *Bacillus spp., Escherichia coli,* and *Proteus spp.* (CO Onuha *et al.,* 2018).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 (Nma and Ola, 2013).

2. MATERIAL AND METHODS

Place of Research: The study was performed at Microbiology laboratory under the Department of Biotechnology, CMS College, Kottayam.

Collection of samples: Freshly prepared fruit juice samples were collected from various shops in Kottayam. Two varieties of juices namely pineapple and orange were chosen. Samples collected were analyzed within an hour.

Sample Analysis: For analysis, the dilution of fruit juice samples were done in the following manner

Table 1: Dilution of Pineapple Juice

20%	2 mL. of pineapple juice sample and 8 mL. of sterile water
30%	3 mL. of pineapple juice sample and 7 mL. of sterile water
40%	4 mL. of pineapple juice sample and 6 mL. of sterile water
50%	5 mL. of pineapple juice sample and 5 mL. of sterile water
60%	6 mL. of pineapple juice sample and 4 mL. of sterile water
70%	7 mL. of pineapple juice sample and 3 mL. of sterile water
80%	8 mL. of pineapple juice sample and 2 mL. of sterile water
90%	9 mL. of pineapple juice sample and 1 mL. of sterile water

Table 2: Dilution of Orange Juice

20%	2 mL. of orange juice sample and 8 mL. of sterile water
30%	3 mL. of orange juice sample and 7 mL. of sterile water
40%	4 mL. of orange juice sample and 6 mL. of sterile water
50%	5 mL. of orange juice sample and 5 mL. of sterile water
60%	6 mL. of orange juice sample and 4 mL. of sterile water
70%	7 mL. of orange juice sample and 3 mL. of sterile water
80%	8 mL. of orange juice sample and 2 mL. of sterile water
90%	9 mL. of orange juice sample and 1 mL. of sterile water

2.1 Culture media and laboratory chemicals

The following media and chemicals are used: Nutrient Agar, MacConkey Agar, Muller Hinton Agar, Simmons Citrate Agar, Triple Sugar Iron Agar, Urease Agar, Agar-agar bacteriological, Kovac's Reagent, Peptone broth, MR-VP broth, Methyl Red Solution, Barrit's Reagent, Crystal violet, Lugol's iodine, Safranine, Acetone, Oxidase discs (impregnated with …), Sodium chloride (NaCl), Hydrogen peroxide, Mannitol, Phenol red.

2.2 Isolation of bacteria from fruit juices

The bacteria are isolated from both freshly prepared pineapple and orange juice by plating the diluted sample.

2.3 Morphological Characterization and Biochemical Tests

Morphological characteristics of isolates viz. shape, size, elevation, surface form, margins and surface texture, color were observed for their characterization. Gram staining technique was to done. Biochemical tests including Indole utilization test, citrate utilization test, TSI test, Urease test, Motility test, Catalase test, Oxidase test, Methyl Red test, Voges Proskauer test were performed for the detection of the isolated bacteria.

2.4 Testing the Antibiotic Susceptibility of the isolated strain

Testing the antibiotic susceptibility of the isolated strains against the antibiotics Streptomycin, Chloramphenicol, Tetracycline, and Amoxicillin by Disc diffusion method.

3. RESULT AND DISCUSSION

Two samples each of orange juice and Pineapple juice collected from different juice shops were analyzed for the presence of bacteria by isolation of bacteria from those samples in the Microbiology laboratory. Streak culture method was employed to isolate bacteria from the samples. The bacterial colonies isolated on Nutrient agar and MacConkey agar were identified by Gram Staining and suitable biochemical reactions. (Table 3 to Table 10)

Analysis of Sample 1 of Orange juice confirmed the presence of *Escherichia coli* and Sample 2 of Orange juice confirmed the presence of *Staphylococcus aureus.* (Table 6).

Analysis of Sample 1 and Sample 2 of pineapple juice confirmed the presence *Staphylococcus aureus.* (Table 10).

The isolated *Escherichia coli* and *Staphylococcus aureus* were tested for their susceptibility to antibiotics like Amoxicillin, Chloramphenicol, Tetracycline and Streptomycin. Based on the Kirby Bauer disc diffusion method, we selected appropriate concentration of antibiotics like Amoxicillin, Chloramphenicol, Tetracycline and Streptomycin and applied on MHA plates already inoculated with *Escherichia coli* and *Staphylococcus aureus*. These Petri plates are then incubated at 37^{o}C for 18 – 24 hours. Following incubation, the size of zone of inhibition of bacterial growth formed around the appropriate concentrations of antibiotics were observed and measured in mm using a ruler and the results were tabulated.

From the results obtained it has been assumed that Amoxicillin was more effective against both *Staphylococcus aureus* and *Escherichia coli*.(a zone size of 34mm and 27mm respectively for *Staphylococcus aureus* and *Escherichia coli*). (Table 11 and 12). The results also showed that Streptomycin was least effective against both *Staphylococcus aureus* and *Escherichia coli* in this study. (A zone size of 22mm for both *Staphylococcus aureus* and *Escherichia coli*). (Table11 and 12). The antibacterial effects of Amoxicillin, Chloramphenicol, Tetracycline and Streptomycin against *Staphylococcus aureus* and *Escherichia coli* showed the biocide effects of these antibiotics against the Gram positive and Gram-negative bacteria. So, if any infection occurs by the consumption of these fresh fruit juices, the above antibiotics can be given by the physician as a therapeutic regimen. It has also been suggested to avoid the continuous consumption of fresh juices from one particular shop.

4. RESULTS OBTAINED FROM ORANGE JUICE

Table 3: Morphological Characteristics on Nutrient Agar

SL. NO	BACTERIA	COLOUR	SHAPE	SIZE	SURFACE	ODOR	ELEVATION
1	Sample 1	Greyish white	Rod shaped	Small	Moist and smooth	No putrefactive odor	Low Convex
2	Sample 2	Golden or yellow	Cocci shaped	Small	Smooth and Non-mucoid	No putrefactive odor	Flat

Table 4: Morphological Characteristics on MacConkey Agar

SL. NO	BACTERIA	COLOUR	SHAPE	LACTOSE FERMENTATION	SURFACE	ELEVATION
1	Sample 1	Pink color surrounded by dark pink color	Rod shaped	Lactose fermenting	Dry / Non mucoid	Convex
2	Sample 2	Pink color	Cocci shaped	Lactose fermenting	Smooth	Convex

Table 5: Result of Gram Staining

SL.NO	BACTERIA	GRAM+VE/-VE	COLOR	SHAPE
1	Sample 1	Gram –ve	Pink	Rod
2	Sample 2	Gram +ve	Violet	Cocci in clusters

Table 6: Results of Biochemical tests

SL. NO.	BACTERIA	INDOLE TEST	CITRATE TEST	TSI TEST	UREASE TEST	MOTILITY TEST	CATALA-SE TEST	METHYL RED TEST	VOGES PROSKA-UER TEST	OXIDASE TEST
1	Sample 1	+ve	-ve	A/A/-	-ve	+ve	+ve	+ve	-ve	-ve
2	Sample 2	-ve	+ve	Alk/A/+	+ve	-ve	+ve	+ve	-ve	-ve

5. RESULTS OBTAINED FROM PINEAPPLE JUICE

Table 7: Morphological Characteristics on Nutrient Agar

SL. NO	BACTERIA	COLOUR	SHAPE	SIZE	SURFACE	ODOR	ELEVATION
1	Sample 1	Golden or yellow	Cocci Shaped	Small	Smooth and Non-mucoid	No putrefactive odor	Flat
2	Sample 2	Golden or yellow	Cocci shaped	Small	Smooth and Non-mucoid	No putrefactive odor	Flat

Table 8: Morphological Characteristics on MacConkey Agar

SL. NO	BACTERIA	COLOUR	SHAPE	LACTOSE FERMENTATION	SURFACE	ELEVATION
1	Sample 1	Pink colour	Cocci shaped	Lactose fermenting	Smooth	Convex
2	Sample 2	Pink colour	Cocci shaped	Lactose fermenting	Smooth	Convex

Table 9: Results of Gram Staining

SL.NO	BACTERIA	GRAM+VE/-VE	COLOUR	SHAPE
1.	Sample 1.	Gram +ve	Violet	Cocci in clusters
2.	Sample 2.	Gram +ve	Violet	Cocci in clusters

Table 10: Results of Biochemical Tests

SL. NO.	BACTERIA	INDOLE TEST	CITRATE TEST	TSI TEST	UREASE TEST	MOTILITY TEST	CATALA-SE TEST	METHYL RED TEST	VOGES PROSKA-UER TEST	OXIDASE TEST
1.	Sample 1	-ve	+ve	Alk/A/+	+ve	-ve	+ve	+ve	-ve	-ve
2.	Sample 2	-ve	+ve	Alk/A/+	+ve	-ve	+ve	+ve	-ve	-ve

6. ANTIBACTERIAL ACTIVITIES OF ANTIBIOTICS

Table 11. Antimicrobial Disc Diffusion Zone Interpretation Guide (Kirby-Bauer Chart

ANTIBIOTIC Disc	Disc Concentration (microgram) (mcg) (µg)	Zone Diameter Interpretive Standards (mm)		
		Resistant (R)	Intermediate or Moderately Sensitive (MS)	Sensitive or Susceptible (S)
Streptomycin (S10)	10	<12	12-14	>14
Chloramphenicol (C30)	30	<13	13-17	>17
Tetracycline (TE30)	30	<15	15-18	>18
Amoxicillin (AMC30)	30	<14	14-17	>17

Table 12. Effect of Antibiotics against *Staphylococcus aureus* and *E.coli*

ANTIBIOTIC	SIZE OF ZONE OF INHIBITION			
	Against *E.coli*	Result	Against *Staph. aureus*	Result
Streptomycin (S10)	22mm	S	22mm	S
Chloramphenicol (C30)	25mm	S	25mm	S
Tetracycline (TE30)	23mm	S	29mm	S
Amoxicillin (AMC30)	27mm	S	34mm	S

7. REFERENCES

[1] Akhter S., Massod S., Jadoon HS., Ahmad I., Ullah S (2012). Quality evaluation of different brands of Tetra Pak mango juices available in market, Pakistan Journal of Food Sciences 23(2): 66-70.

[2] Anshika Tonk., Shubhangi Khare., Anita Rawat (2018). Isolation and Identification of bacteria from fresh juice prepared in local areas, European Journal of Biotechnology & Bioscience, Vol. 6 Iss. 3, pp 12-15.

[3] Batra NG., Sharma A., Agarwal N (2018). Evaluation of Microbiological Criteria and Quality of packed fruit juices, International food Research Journal 25(2): 458-461.

[4] Bikila Wedajo and Awel Kadire (2019). Assessment of bacterial load of some fresh and packed fruit juices in Arba Minch Town, Ethiopia, Journal of Nutrition & Food science, Vol.9 Iss.3 No: 759.

[5] Bulti Kumera Fufu and Melakam Dessalegn Liben (2018). Microbiological quality of fruit juices sold in cafes and restaurants of Shewarobit town, Amhara, Ethiopia, African Journal of Microbiology Research Vol. 12(26), ISSN: 1996-0808, pp. 623-628.

[6] Durgesh P Mahale., Ranjana G Khade., Varsha K Vaidya (2008). Microbiological Analysis of street vended fruit juices from Mumbai city, India, Internet Journal of Food Safety, Vol.10, pp 31-34.

[7] Getachew Tafere and Adissu Dessalegn (2017). Microbial load and safety of locally prepared fresh fruit juices in cafeteria and restaurants in Mekelle city, Northern Ethiopia, Indian Journal of Traditional Knowledge Vol. 16(4) pp.598-604.

[8] Grace Baby., Simi P., Chinchu V. R., Saritha K.R., Ria Elsa Roy and Dr. Mukundan M.K (2001). Assessment of microbial quality of ready to drink foods and water from a public health perspective. The Food Quality Monitoring Laboratory under the Council for Food Research and Development, Government of Kerala, Perinjottakkal.

[9] Gulzar Ahmad Nayik., Tawheed Amin., Suman vikas Bhat (2013). Microbial Analysis of some fruit juices available in the markets of Kashmir valley, India, Asia Journal of Microbiology, Biotechnology &Environmental Sciences Paper, Vol.15 Iss.4, Page No. 733-737.

[10] Jayalakshmi T., Krishnamoorthy P., Ramesh Kumar G., Sivamani P (2011). The Microbiological Quality of fruit containing soft drinks from Chennai, Journal of Chemical and Pharmaceutical Research 3(6): 626-630

[11] Kamal Rai Aneja., Romika Dhiman., Neeraj Kumar Aggarwal., Vikas Kumar., Manpreet Kaur (2014). Microbes associated with freshly prepared juices of Citrus and Carrots, International Journal of Food Science Volume 2014/Article ID 408085

[12] Mashat B (2011). Microbiological Quality of fresh (unpasteurized) fruit juices in Makah, Saudi Arabia, WIT Transactions on Ecology and Environment, Vol 152, ISSN 1743-3541

[13] Muhammad Naeem Iqbal, Shahzad Ali, Aftab Ahmad Anjum, Khushi Muhammad, Muhammad Asad Ali, Shihua Wang, Waseem Ahmad Khan, Iahtasham Khan, Ali Muhammad, Abid Mahmood, Muhammad Irfan, Aftab Ahmad, Asfa Ashraf and Firasat Hussain (2016). Microbiological risk assessment of packed fruit juices and antibacterial activity of preservatives against bacterial isolates. Pakistan J. Zool., vol. 48(6), pp. 1695-1703, 2016.

[14] Ogodo, Ugbogu, ,Ekeleme, and Nwachukwu (2016). Microbial Quality of Commercially Packed Fruit Juices in

South-East Nigeria. Journal of basics and applied research ISSN2413-7014.

[15] Onuoha CO., Braide W., Orji JO., Uzoh CV., Adeleye SA., Okoronkwo Chimaobi., Oguejiofor Benigna (2018). The Microbiological Quality Assessment of Commercially

[16] Poonam U Sharma (2013). Bacteriological Analysis of street vended fruit juices available in Vidharbha, International Journal of Current Microbiology and Applied Sciences V2/Number5/178-183.

[17] Rashed N., Md. Aftab U., Md Azimul H., Saurab KM., Mrityunjoy AM., Majibur R (2013). Microbiological study of vendor and packed fruit juices locally available in Dhaka city, Bangladesh, International Food Research Journal 20(2): 1011-1015.

[18] Umar Asghar., Muhammad Nadeem., Rubina Nelofer., Sania Mazhar., Quratulain Syed., Muhammad Irfan (2018). Microbiological Assessment of fresh juices vended in different areas of Lahore city, Electronic Journal of Biology Vol. 14(4): 106-110.

[19] Virupakshaiah DBM (2016) Isolation and Identification of *Pseudomonas* species from fruits and vegetables, International Journal of Informative and Futuristic Research /V3/E10/042: 3783-3790.

