

Isolation and Identification of Bacteria From Fresh Fruit Juice Prepared in Cafeterias and Restaurants, Axum Town, Ethiopia

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Fruit juices are well recognized for their nutritive value, mineral and vitamin content and are common in many tropical countries. Fresh products like fruits and vegetables are the normal part of the human diet and are consumed in large quantities in most civilizations. The main purpose of this study was to isolate and identify bacteria from fresh juice prepared in cafeterias and restaurants. Six Samples of Avocado and Mango locally prepared fruit juices were collected randomly from different restaurants and cafeterias of Axum town. All data were analyzed through differential statistics and results were expressed by numbers, tables and percentages. Microscopic investigation for Gram reaction and morphological features of suspected colony was determined using standard method of Gram's staining. Most probable the results in number showed that, in Mango and Avocado sample, sample 10^{-1} was most contaminated with a count of 150 and 120 coliforms per 100 ml of the juice sample, respectively. The second highest contamination was seen in juice sample 10^{-2} with a count of 100 and 100 coliforms per 100 ml of Mango and Avocado. The importance of personal hygiene, storage of fruit at cold temperature, using sterilized water for diluting the juice or to use clean equipments should be informed to people involved in preparing and handling of fruit juices.

Keywords: Fruit Juice, Gram staining, Total Coliform Count, Mango and Avocado.

Fruit juices are well recognized for their nutritive value, mineral and vitamin content and are common in many tropical countries. Fresh fruits are essential components of the human diet and there is considerable evidence of the health and nutritional benefits associated with the consumption of fresh fruits or their juices (Shakir *et al.*, 2009).

Most fruits contain bacterial counts up to 1.0×10^5 cm² on their surfaces. Improper washing of fruits add these bacteria to extracts leading to contamination. In addition, use of

unhygienic water preservation without refrigeration, unhygienic surroundings often with swarming houseflies and fruit flies and airborne dust can also act as sources of contamination. Such juices have shown to be potential sources of bacterial pathogens notable *E. coli* 0157:H7, species of *Salmonella*, *Shigella* and *Staphylococcus aureus* (Joy Lewis *et al.*, 2006).

Freshly squeezed juices are simply prepared by extracting the liquid and pulp of mature fruit usually by mechanical means or Blenders. Prior preparation of fruit to avoid bitterness of skin or to remove large stone such as mango, avocado and pineapple followed by separation of juices and pulp by blender. The final product is an unfermented, unqualified, untreated juice, ready for consumption (Melbourne, 2005).

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However, as a consequence of inappropriate manipulation and storage conditions, both pathogenic and/or deteriorative microorganisms may contaminate a product, thus increasing the risk of microbial diseases and spoilage (Beuchat, 1996; Diaz-Cinco *et al.*, 2005). In fact, the number of outbreaks and cases of illness caused by consumption of fresh cut fruits and unpasteurized juices has increased in the last years (Harris *et al.*, 2003).

Quality losses in fresh cut fruits and unpasteurized juices may occur as a consequence of microbiological, enzymatic, chemical, or physical changes. Safety and quality losses by microbiological causes are very important due to two reasons: first, because they constitute a hazard for consumers by the possible presence of microbial toxins or pathogenic microorganisms in the product, and second, by economic losses as a result of microbial spoilage. Many food preservation strategies such as chilling, freezing, water activity reduction, nutrient restriction, acidification, modified atmosphere packaging, fermentation, non thermal physical treatments or the use of antimicrobials have been traditionally applied to control microbial growth (Davidson, 2001).

Objectives of the Study

General Objective

The main purpose of this study was to isolate and characterize bacteria from fresh juice prepared in cafeterias and restaurants in Axum town.

Specific Objectives

- To evaluate bacteriological information from prepared unpasteurized fruit juices.
- To identify selected pathogens associated with food borne illnesses.
- To investigate specific characteristics of microbes in fresh juice

MATERIALS AND METHODS

Description of the Study Area and Duration of the Study

The study was conducted in Tigray Regional state, Central Zone of Tigray, at Aksum University, Department of Biology and Biotechnology (In Microbiology Laboratory) from March 1 to June 2, 2014. Axum town is located at a distance of 1024 Km from Addis Ababa, which is the capital city of Ethiopia and 241 Km from

Mekelle, which is the capital city of Tigray region. It is located at latitude and longitude of 14°72' N and 33°44' E. The altitude of the town ranges from 2,100 meters above sea level. The total area of this town is 17,128 Km². The annual temperature and rain full of the town is 25°C and 300mm, respectively. According to the 2004 national census report, the total number of the population was 53,884 of this 25,034 (46.47%) males and 28,853 (53.54%) females. In the town there are many Restaurants and Cafeteria that prepare unpasteurized fruit juices that can be consumed by visitors and people of the town.

Materials and Media Used in the Study

Materials	Media
Petri dishes	Nutrient agar
Micro scope	SS agar
Colony counter	Urea agar
Inoculating loop	Triple sugar iron agar
Test tubes	Simmons citrate agar
Bunsen burner	Baird parker agar
Measuring cylinder	Bacillus cereus agar
auto clave	
Flasks	
Water bath	

Data Collection

Data were collected by using primary data collection method. The sample was collected from 4 different cafeterias and restaurants in Axum town.

Laboratory procedure

Laboratory procedures such as sample collection, sample processing, bacterial culture, microscopical examination and biochemical tests were used to determine colony count, isolation and identification of indicator organisms and selected pathogens.

Sources of the Sample

The fresh unpasteurized fruit juices prepared in different Restaurants and Cafeterias were obtained from Axum town.

Collection of the Sample

A total of six (6) Samples of Avocado and Mango locally prepared unpasteurized fruit juices were collected randomly from different restaurants and cafeterias of Axum town in April 16, 2014. All the samples were collected on a voluntary basis from participating restaurants and cafes in a clean beaker (250 ml) container aseptically, labeled and

brought immediately to the laboratory after processed it immediately.

Colony Counting

Serial Dilution

A 1mL of the juice sample was added into 9mL of sterile distilled water to prepare stock solution. Then the test tubes were labeled as (10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} and 10^{-7}). After that, 1mL from the mixture sample was transferred into the first test tube which was 10^{-1} and shaken well in order to get equal distribution of microorganisms. And

then, 1mL from the first test tube was transferred into the next test tube and again shaken. Finally, the procedure was repeated to complete the serial dilution up to 10^{-7} .

Media Preparation

2.8g of nutrient agar powder was weighed using a clean electronic weighing balance; 100 ml of sterile distilled water was poured into a conical flask containing 2.8g of nutrient agar. The mixture was shaken by using hand and covered with a cotton wool, over which an aluminum foil was

Table 1. The number of colonies formed at *Bacillus cereus* agar, SS agar and Baird parker agar media in Avocado sample

Avocado Sample	<i>Bacillus cereus</i> agar		SS agar		Baird parker agar	
	No	%	No	%	No	%
10^{-1}	150	28.7	110	23.9	80	19.7
10^{-2}	100	19.1	80	17.4	78	19.2
10^{-3}	80	15.3	75	16.3	62	15.3
10^{-4}	65	12.4	65	14.1	60	14.8
10^{-5}	50	9.56	55	11.9	44	10.8
10^{-6}	48	9.2	40	8.7	42	10.3
10^{-7}	30	5.7	35	7.6	40	9.9
Total	523	100	460	100	406	100

Table 2. The number of colonies formed at *Bacillus cereus* agar, SS agar and Baird parker agar media in Mango sample

Mango Sample	<i>Bacillus cereus</i> agar		SS agar		Baird parker agar	
	No	%	No	%	No	%
10-1	120	20.7	80	24.2	80	17.9
10-2	100	17.4	64	19.4	75	16.8
10-3	90	15.7	44	13.3	72	16.1
10-4	85	14.8	40	12.1	67	15.0
10-5	80	13.9	36	10.9	60	13.5
10-6	60	10.4	34	10.3	50	11.2
10-7	40	6.9	32	9.7	42	9.4
Total	575	100	330	100	446	100

Table 3. Detection of *Salmonella* and *Shigella* by using different biochemical tests

Types of bacteria	Media	Color observed
<i>Salmonella</i>	Urea agar	Pinkish color
	Triple Sugar Iron agar (Killing agar)	Yellow color
<i>Shigella</i>	Simmon's citrate agar	Block spot
		Blue color

tightly wrapped and then heated for few minutes using hot plate until the foam formed. And the medium together with petri-plates were sterilized in autoclave for 15 minutes at 121°C. Soon after autoclaving, the agar was allowed to cool and placed inside a water bath at about 50°C to maintain the media in a molten stage (to minimize the amount of condensation that forms). Then, the agar medium was allowed to cool to room temperature prior to pouring it into the petri-plate. Plates were dried faster in lower humidity by keeping them at room temperature. The freshly prepared and cooled medium was poured into flat-bottomed petri dishes on a level, horizontal surface to give a uniform depth of approximately 4 mm. This was achieved by pouring 20 ml of the medium for plates with diameters of 100 mm. Then, samples were separately cultured in nutrient agar at 37°C for 24 hrs by streak plate method. Finally, all petri-dishes were incubated in the inverted position at 37°C for 24 hrs.

Gram Stain Procedure

The slide was placed with heat fixed smear on staining tray. Drop of crystal violet was added and allowed for 60 seconds and then washed with distilled water and also one drop of Iodine (mordant) was added and then allowed for 30 seconds after this washed by distilled water. Then 95% ethyl alcohol (decolorization) was added and allowed for 30 seconds then washed by distilled water. After that safranin was added and allowed for 60 seconds and then washed with distilled water. Finally, the slide was put under microscope and then the purple and pink color from a single colony of the slide under microscope was observed. Then, the purple color from a single colony of the slide under microscope indicated that the bacteria were gram positive where as the pink color under microscope indicated that the bacteria were gram negative. Microscopic investigation for Gram reaction and morphological features of suspected colony was determined using standard method of Gram's staining.

Isolation Procedure

0.85 gram of NaCl was added into 100mL of distilled water to prepare normal saline solution then 9mL from the prepared normal saline solution was added with 1mL of sample and then incubated for 24 hours at 37°C. SS agar was prepared and a one loop full from the overnight incubated sample

with normal saline solution was streaked on the prepared SS agar and incubated it for 24 hours at 37°C. Due to this, *salmonella* and *shigella* was isolated.

Biochemical Test

To confirm and characterize the identities of *Enterobacteriaceae*, three standard media for biochemical test were used, namely Urea agar, Simons citrate agar and Triple Sugar Iron agar.

Quality Control

The Quality of the study was kept by training the data collector, preparing, and using standard operational procedures for laboratory investigation and media preparation. Structured Questionnaire was tested using pretest before conducting the study. Sample collection and processing were carried out using aseptic techniques. The samples were labeled properly. Culture and bacterial colony count were determined by experienced laboratory personnel. The performance and sterility test of prepared media were checked by incubating at 24-48 hours and inoculating with control strain organisms, respectively.

Data Analysis

After the data have been collected properly, the researcher have organized, analyzed and summarized the collected data. The data were analyzed through both quantitative and qualitative methods and differential statistics meaning that the result was expressed in numbers, tables and percentage

RESULTS AND DISCUSSION

Total Viable Coliform Count (TVCC)

As clearly observed from the above table, 10⁻¹ avocado dilution was more contaminated than the remaining other dilutions which accounted 150, 110 and 80 number of colonies in Bacillus cereus, SS agar and Baird parker agar, respectively. Whereas 10⁻⁷ avocado dilution was the least contaminated than the others and accounted as 30, 35 and 40 number of colonies in the respective media (in Bacillus cereus, SS agar and Baird parker agar, respectively).

According to the above table 2, in the mango dilution of 10⁻¹, 120, 80 and 80 number of colonies were recorded in the media Bacillus cereus, SS agar and Baird parker agar, respectively. This

indicated that 10^{-1} mango dilution was more contaminated than the others but in the 10^{-7} mango dilution, 40, 32 and 42 number of colonies were counted and this dilution was the least contaminated than the rest all dilutions.

Biochemical Tests for identification of *Salmonella* and *Shigella*

To identify *Salmonella* and *Shigella* from the SS agar, the suspected colonies performed on this agar were sub cultured into sterile dextrose broth and incubated at 37°C for 24 hrs until the broth being cloudy. Different biochemical tests such as Urea agar, Simmons citrate agar and Triple sugar iron agar were used for identification of *Salmonella* and *Shigella* and results were shown as follows.

Detection of *Salmonella* and *Shigella*

As clearly observed from table 3 above, from the overnight cultured broth, a one loopfull organisms were sub cultured in to the biochemical tests of Urea agar and Triple sugar iron agar. After overnight incubation, the clear pinkish color was observed in the Urea agar and yellow color, black spots as well as CO₂ bubbles were clearly observed in Triple Sugar Iron agar (Killingner agar) for *Salmonella*. Whereas in *Shigella* blue color was successfully observed by using Simmons citrate agar.

Culture of *Staphylococcus aureus* and *Bacillus cereus*

Culture of *Staphylococcus aureus*

1mL of juice sample was mixed with 9 mL of normal saline solution and incubated at 37°C for 24 hrs. Then after overnight incubation, one loopfull of the mixture was aseptically transferred and properly streaked on the prepared Baird parker agar and then incubated at 37°C for 24 hrs. Finally, a successful and pure colony of *Staphylococcus aureus* was clearly observed.

Culture of *Bacillus cereus*

1mL of juice sample was added into 9 mL of normal saline solution and incubated at 37°C for about 24 hrs. After overnight incubation, a loopfull of the mixture was aseptically transferred and streaked on the media of *Bacillus cereus* agar that was prepared. Then after, the media was incubated and pure colonies of *Bacillus cereus* species were observed.

From this study, there was more contamination in the juice dilution of 10^{-1} Avocado and Mango samples, respectively. Whereas the juice dilution 10^{-7} was the least contaminated than the others. By this result clearly indicated that the water used in the preparation of fruit juices was highly contaminated with many enterobacterial spores. In addition to this, the contamination of juices was also due to the use of unhygienic conditions of water storage and use of unclean utensils and unhygienic physical and biological contaminants. The results of the present study obviously indicated that the presence of four different types of enterobacteria namely; *Shigella*, *Salmonella*, *Staphylococcus aureus* and *Bacillus cereus*. These organisms are highly pathogenic and may cause serious diseases in human beings. This result was agreed with the earlier reports (Lewis *et al.*, 2006).

According to this study, *Salmonella* and *Shigella* were identified by using the different biochemical tests such as Urea agar, Simons citrate agar and Triple Sugar Iron agar. During detection of *Salmonella* by using Triple Sugar Iron agar, three basic characteristics has been shown, these were: one yellow color was observed after overnight incubation (this means it was glucose fermented), second H₂S was performed that means a black spot was observed and the third one there was bubbling of CO₂ after overnight incubation. Whereas during *Shigella* detection, a successful blue color was simply observed after overnight incubation in Simons citrate agar.

CONCLUSION

In the present study it can be concluded that, the most probable number analysis showed high levels of contamination in juice samples that were prepared in cafeterias and restaurants. This would be possible because of the poor quality of water was used in juice preparation; moreover, water is one of the major sources of sewage contamination. The results of the present findings clearly demonstrated that the fresh juices did not meet public health standards and many kinds of enteropathogenic bacteria were found namely; *Shigella*, *salmonella*, *Staphylococcus aureus*, *Bacillus cereus*. Such foods lead to hazardous effects to the consumers.

Recommendations

Based on the findings of the present study, the following recommendations were given:

1. The importance of personal hygiene, storage of fruit at cold temperature, using sterilized water for diluting the juice/cleaning equipment should be informed to people involved in preparing and handling of fruit juices.
2. Government agencies must adopt measures to educate the vendors about food safety and hygienic practices and enforce adequate guidelines for juice preparations, especially street vended fruit juices.
3. Further study should be conducted to isolate and characterize different bacterial and fungal species and know the quality of fresh juice by increasing the sample size because the current study was carried out only on small number of bacteria and small sample size

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