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

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ABSTRACT

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. Thirty 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 boiled water for diluting the juice or to use clean equipments should be informed to people involved in preparing and handling of fruit juices.

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1. Introduction

1.1 Background of the study

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 X 10^5 cm^2 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).

However, as a consequence of inappropriate manipulation and storage conditions, both pathogenic and/or deteriorative microorganisms may

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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).

1.2. Statement of the Problem

Fresh products like fruits and vegetables are the normal part of the human diet and are consumed in large quantities in most civilizations. Traditionally, fruits and vegetables have been regarded as microbiologically safer than other unprocessed food items such as meat, milk, eggs, poultry and sea food. These products are rich in carbohydrates and poor in proteins with pH value from 7.0 to slightly acidic and provide a suitable niche to several bacteria, yeasts and moulds (Wiessinger *et al*., 2000; Trias *et al.,* 2008).

Contaminated fruit juices sold in restaurants, cafes and even road side stalls are sometimes unacceptable for human consumption and create significant health problems (Lewis *et al.,* 2006). However, in most parts of Ethiopia continuous assessment of food safety has not been conducted on fruit juices that has been prepared in restaurants and cafes. Therefore, this study was tried to determine the bacteriological safety and quality of fruit juices prepared in Axum town.

1.3. Objectives of the Study

1.3.1. General Objective

The main purpose of this study was to identify the bacteria isolated from fresh juice prepared in cafeteria and restaurants in Axum town.

1.3.2. Specific Objectives

1. To evaluate bacteriological information from prepared unpasteurized fruit juices.
2. To isolate and identify selected pathogens associated with food borne illnesses.
3. To assess the contamination of processing and handling of prepared unpasteurized juices.

1.4. Scope of the Study

The study was conducted in Aksum University. This study was mainly focused on isolation and detection of bacterial strains from fresh juices prepared in different cafes and restaurants in Axum town because most of the society of Axum town including the tourists widely used the fresh juice prepared on the town and are highly affected by various diseases caused by some contaminations present during preparations.

1.5. Significance of the Study

This study used:

- ✓ To create awareness of the people about the use of fresh juice.
- ✓ To identify which strain of bacteria is found in the fresh juice.
- ✓ To assess the handling and processing of fresh juice during preparation.

1.6. Limitation of the Study

Some deficiencies of the current study show as follows:

The reliability of the finding of any study depends on the sample size of the study subjects. However, this study conducted on small size of study subject due to various reasons. It would have been better to identify *E.*coli isolate up to strain level which would not be done in the present study due to time limits and absence of the required media and materials. Therefore, we cannot state with confidence whether the isolates were *enterotoxogenic* or not.

Our study focus only on bacterial quality, the effects of fungus on unpasteurized fruit juices was not stated. Money and Internet access are another shortage during conducting of the research work.

2. LITERATURE REVIEW

2.1. Fruit Juice

Fruit juices are unfermented but fermentable products obtained from fresh, ripe and healthy fruits (Republic of Turkey Ministry of Agriculture and Rural Affairs General Directorate of Protection and Control, 2006). They are also known as very good sources of vitamins and minerals (Kabasakalis *et al.,* 2000; Bates *et al.,* 2001). It is the aqueous liquid expressed or extracted from one or more fruits or vegetables, purees of the edible portions of one or more fruits or vegetables, or any concentrates of such liquid or puree (FDA, 2002).

2.2. Composition of Fruit Juice

The major component of the fruit juice is water. The other most common constituent is carbohydrates which comprise sucrose, fructose, glucose and sorbitol. Also, limited amount of protein and minerals are found in fruit juices. However juice contains no fat or cholesterol. If it is not added, no fiber content can be observed (American Academy of Pediatrics Committee on Nutrition 2001). Fruit juices are known as considerable sources of ascorbic acid (vitamin C).Their consumption has been increasing during last year's (Kabasakalis *et al*., 2000). Especially citrus fruits and juices are good sources of ascorbic acid, folic acid, vitamin B1, thiamine and potassium. It was noted that a cup of citrus juice (240 ml) provides vitamin C in the quantity of more than daily requirement (Bates *et al.,* 2001).

2.3. Water Supply

Water used in processing establishments must be clean unless it is used solely for fire protection or auxiliary services and there must be no connection between the system for that water and the system for potable water. Potable water, hot and cold under pressure, should be provided (Canada food agency, 2001).

The other serious problem associated with food borne illness is unhygienic water supply that may be used for dilution of fruit juices. According to research conducted in Visakhapatnam City, India, over all the results of the study indicate that all street vended fresh fruit juices in many parts of the city showed contamination with *faecalcoliforms* and *faecal* streptococci. It is contended that contamination is mainly due to poor quality of water used for dilution as well as prevailing unhygienic conditions related to washing of utensils and maintenance of the premises. The location by the side of a busy road with heavy vehicular traffic or by the side of the waste disposal system and overcrowding seem to add to the contamination. Such locations should be avoided for establishing a street vender juice shop. Lack of sanitary conditions in street vended juice shops and the occurrence of pathogenic *E. coli* O157:H7, *Shigella* and *S. typhimurium* is alarming enough for an immediate action by the suitable agency. Regular monitoring of the quality of fruit juices for human consumption must be introduced to avoid any future pathogen outbreaks (Lewis *et al.,* 2006).

A survey on the bacteriological quality of both drinking water and flavored drinks from coin-operated vending machines explains that 44% of 25 drinking water samples examined contained coli forms and 84% had viable counts of greater than 1000 organisms ml at 30^0C. Thirty-one flavored drinks were examined; 6% contained coli forms and 39% had total counts greater than 1000 organisms per mL. It is suggested that the D.H.S.S. code of practice on coin-operated vending machines is not being followed. It is also suggested that drinking water alone should not be dispensed from such machines (Hunter *et al.,* 1986).

2.4. Unpasteurized Juice

Unpasteurized juice/cider does not undergo treatment. Often it can be purchased as freshly pressed from local orchards, roadside stands, farmers markets, country fairs and juice bars. Unpasteurized juice/cider

may also be found on ice or in refrigerated display cases and in produce sections at grocery stores (Health Canada, 2006).

However, consumption of unpasteurized fruit juices causes approximately 16,000 to 48,000 cases of illnesses in a year (Foley *et al.,* 2002). Previously it was believed that fruit juices are safe due to their low pH values. However, recent outbreaks of *Escherichia coli* O157:H7 and *Salmonella* associated with the consumption of unpasteurized juices show the potential of acidic juices to carry pathogenic microorganisms (Cook *et al.,* 1998).

2.5. Pasteurized Juice

Pasteurization describes a mild heat treatment which is applied at temperatures below 100°C (Silva and Gibbs, 2004). The thermal pasteurization criteria for white grape juice are 90- 95 °C for 15-30 seconds (Cemeroglu, 2004). For orange juice temperature and time requirements are 90°C for 1 minute (*Graumlich et al.,* 1986).

2.6. Handling and Processing

Poor handling and processing of fresh fruit juices are some of the main cause of food associated illness to the community who live in developing countries. In most case a number of pathogenic organisms are isolated and identified from locally prepared fruit juices. According to study conducted in Dhaka, Bangladesh, the total viable count of samples ranged from 3.00×10^2 to 9.60×10^8. Out of 114 freshly prepared fruit juices samples collected, 113 samples (99%) showed the presence of coli form and *E. coli*. The other bacteria like *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella*, *Streptococcus* were found in 64.91%, 6.14%, 7.89% and 5.26% of the tested samples, respectively. The number and type of microorganisms recovered from the freshly squeezed fruit juices made them unsafe for drinking. It was concluded that due to unhygienic fruit handling in the unsanitary environmental conditions under which the vendors operate the juices become contaminated with harmful bacteria. The results of this study demonstrate the unhygienic quality of popular types of market vended freshly squeezed fruit juices and their risk to the consumers (Shakir *et al.,* 2009).

2.7. Contamination

The most likely cause of the contamination is fruit coming in contact with animal faeces, or water, workers, containers or processing equipment contaminated with animal faeces. Cattle, deer and sheep, are the most common reservoirs for the pathogen, but usually do not show symptoms themselves. Birds, rodents, insects and poor hygiene may also contribute to the contamination. One contaminated piece of fruit could affect an entire batch of juice or cider (FDA, 1999; Canada food agency, 2001).

Unpasteurized juice products can be contaminated with harmful bacteria such as *Salmonella* and *E. coli*, viruses, and parasites like Cryptosporidium. Although fruits that are used to make juice do not naturally contain harmful bacteria, viruses or parasites, they can become contaminated in the farm environment, through handling, processing or transportation. Contaminated unpasteurized juice and cider can potentially pose a health risk to consumers (Health Canada, 2006).

A research conducted in Ethiopia on microbial spoilage of market bulla and kotcho stated that when stored at room temperature in a loosely wrapped condition, both products resulted in undesirable odor, sticky consistency and dark coloration after 8 days. Drop in pH and a high degree of proliferation of aerobic mesophilic bacteria and molds were observed. Microorganisms active in starch hydrolysis, proteolysis and lipolysis were encountered in both products. The aerobic mesophilic (spoilage) bacterial flora was dominated by Micrococcus and Bacillus spp. About 33% of the products were lost due to such spoilage. Rural producers, vendors and urban consumers of bulla and kotcho use various methods to improve keeping quality. Wrapping the products with fresh enset leaves and burying them in pits are the most frequently used method by rural producers. They can store the products from two to three months using this method. Urban consumers could store the products only for 2-3 weeks (Ashenafi *et al.,* 1996).

2.8. Colony Count

One of the methods for counting of viable bacteria in any fluid is viable colony count by diluting the fluid and culturing for bacteria. Counts of viable bacteria are commonly based on the number of colonies that develop in nutrient agar plates which have been inoculated with known amounts of diluted foods and then incubated under prescribed environmental conditions. Only those bacteria, which will grow under the chosen environmental conditions can be counted. A wide variety of conditions can be obtained by changing the composition of the growth (agar) medium, the gaseous environment of incubation (presence or absence of O_2) and the time and temperature of incubation. The aerobic mesophilic count is most commonly used (Roberts *et al.,* 2003).

3. MATERIALS AND METHODS

3.1. 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 $14^0$72' N and $33^0$44' E. The altitude of the town ranges from 2,100 meters above sea level. The total area of this town is 17,128 Km^2. The annual temperature and rain full of the town is 25^0C 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.

3.2. Study Design

The study design was experimental using the appropriate methods such as isolation and identification of bacteria by the help of microscopic examination and biochemical test.

3.3. Data Collection

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

3.3.1. 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.

3.3.2. Sources of the Sample

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

3.3.3. Collection of the Sample

A total of thirty (30) 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.

At the time of sample collection, swabs were collected from the blender machines in order to get strong evidence for source of contamination. The swabs were collected aseptically using sterile applicator cotton swab and inoculated in sterile bottle containing sterile nutrient broth. Moreover, water samples were also collected from tap and container aseptically using sterile bottle for determination of fecal coli form.

3.3.4. Preparation of the Sample

Steps in preparation of fruit juice:-First the avocado and mango fruits were collected and peeled carefully by using knife .Then the seeds of fruits were withdrawn. After that, the peeled part of the fruit was kept in the machine, which is responsible for preparation of juice that dissolves the fruit and appropriate amount of water and sugar were added for dissolution and increase tastiness of the juice respectively. Then, all those were mixed together and waited for five minutes. But during the preparation of mango juice, the juice was purified by using funnel and the semi-liquid which passed through the funnel was used as juice of mango where as the residue was discarded. The fresh juice prepared was brought to the laboratory immediately and diluted with sterile distilled water to make serial dilution as follows.

3.3.5. Colony Counting

3.3.5.1. 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 shaked by using hand and covered with a cotton wool, over which an aluminum foil was tightly wrapped and then heated for few minutes using hot plate until the foam formed. And the medium together with petri-plates were sterilized by autoclaving for 15 minutes at 121^0 C. Soon after autoclaving, the agar was allowed to cool and placed inside a water bath at about 50^0 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. Finally all petri-dishes were incubated in the inverted position at 37^0C for 24 hrs.

3.3.5.2. 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}and10^{-7}) . After that, 1mL from the mixture sample was transferred into the first test tube which was 10^{-1} and shaked 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 shaked. Finally, the procedure was repeated to complete the serial dilution up to 10^{-7}.

3.3.5.3. Gram Stain Procedure

The slide was placed with heat fixed smear on staining tray. Drop of crystal violate was added and allowed for 60 seconds and then washed with distilled water and also one drop of Iodine (mordent) was added and then allowed for 30 seconds after this washed by distilled water. Then 95% ethyl alcohol (decolarilzation) 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.

3.3.6. 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 37^0C. 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 37^0C. Due to this, *salmonella* and *shigella* was isolated.

3.3.7. 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 Iron Sugar agar.

3.3.8. 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 24-48hurs and inoculating with control strain organisms, respectively.

3.4. 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.

4. RESULTS AND DISCUSSION

4.1. RESULTS

4.1.1. Total Viable Coliform Count (TVCC)

As table 1 and table 2 shown below, the total viable colony count of Avocado and Mango samples, 10^{-1} sample was more contaminated than the remaining samples whereas 10^{-7} was the least contaminated sample than the others.

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

Avocado	Bacillus cereus agar		SS agar		Baired parker agar	
Sample	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**

As clearly observed from the above table, 10^{-1} avocado sample was more contaminated than the remaining other samples which counted 150, 110 and 80 number of colonies in Bacillus cereus, SS agar and Baired parker agar, respectively. Whereas 10^{-7} avocado sample was the least contaminated than the others and counted as 30, 35 and 40 number of colonies in the respective media (in Bacillus cereus, SS agar and Baired parker agar, respectively).

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

Mango	Bacillus cereus agar		SS agar		Baired parker agar	
Sample	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**

According to the above table 2, in the mango sample of 10^{-1},120, 80 and 80 number of colonies were recorded in the media Bacillus cereus, SS agar and Baired parker agar, respectively. This indicated that 10^{-1} mango sample was more contaminated than the others but in the 10^{-7} mango sample, 40, 32 and 42 number of colonies were counted and this sample was the least contaminated than the rest all samples.

4.1.2. Isolation of bacteria from fresh fruit juice

1 mL of juice sample was mixed with 9 mL of normal saline solution and incubated at 37^0C for about 24 hrs. After overnight incubation, one loopfull from the mixture was aseptically transferred and streaked on the prepared SS agar. Then the SS agar was allowed to cultured overnight until pure colonies were formed. Then from the overnight cultured SS agar, by using a flamed wire loop only a single colony was transferred and mixed with sterile dextrose broth and then incubated at 37^0C for 24 hrs.

4.1.3. 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 37^0C 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.

4.1.3.1. Detection of *Salmonella*

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 CO_2 bubbles were clearly observed in Triple Sugar Iron agar (Killinger agar).

4.1.3.2. Detection of *Shigella*

From the overnight cultured broth, a one loopfull organisms were sub cultured in to the biochemical test of Simmons citrate agar. After overnight incubation, the blue color was successfully observed.

4.1.4. Culture of Staphylococcus aurous and Bacillus cereus

4.1.4.1. Culture of *Staphylococcus aurous*

1mL of juice sample was mixed with 9 mL of normal saline solution and incubated at 37^0C 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 37^0C for 24 hrs. Finally, a successful and pure colony of *Staphylococcus aures* was clearly observed.

4.1.4.2. Culture of *Bacillus cereus*

1mL of juice sample was added into 9 mL of normal saline solution and incubated at 37^0C 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.

4.2. DISCUSSION

From this study, there was more contamination in the juice sample of 10^{-1} with colony count of 150 and 120 coliforms in Bacillus cereus agar of Avocado and Mango samples, respectively. Whereas the juice sample 10^{-7} was the least contaminated than the others with colony count of 30 and 40 coliforms in both samples in the Bacillus cereus agar. In SS agar more contaminated in juice sample of 10^{-1} with colony count of 110 and 80 coliforms in Avocado and Mango samples, respectively was recorded. Whereas the juice sample 10^{-7} was the least contaminated than the others with colony count of 35 and 32 coliforms in both samples and also in Bairde park agar more contaminated in juice sample of 10^{-1} with colony count of 80 and 80 coliforms in Avocado and Mango samples, respectively. Whereas the juice sample of 10^{-7} was the least contaminated than the others with colony count of 40 and 42 coliforms in both samples used SS agar.

The water used in the preparation of fruit juices was highly contaminated with faecal coliforms. 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 clearly indicated that the presence of four different types of fecal coliforms namely; *Shigella, Salmonella, Staphylococcus aureous* 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 are: 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 CO_2 after overnight incubation. Whereas during *Shigella* detection, a successful blue color was simply observed after overnight incubation in Simons citrate agar.

5. CONCLUSION AND RECOMMENDATIONS

5.1. 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, Staphyloccocusaures, Bacillus cereus* .Such foods lead to hazardous effects to the consumers. 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.

5.2. 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 boiled water for diluting the juice/cleaning equipment should be informed to people involved in preparing and handling of fruit juices.
2. There should be regular inspection and routine microbiological analysis in order to assure safe unpasteurized fruit juices for consumers.
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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