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Assessment on Bacteriological loads of Street sold Fruits: *Musa Acuminata, Mangifera Indika and Persea Americana* in Arba Minch town, South Ethiopia

Diriba Leta Weleni*

Arba Minch University College of Natural Sciences, Department of Biology, Ethiopia Pobox. 21.

E-mail- dirleta2009@gmail.com

Abstract: **Traditionally, fruit products have been regarded as microbiologically safer than other unprocessed foods. However, many outbreaks of human infections have been associated with the consumption of contaminated fruits. The objective of this study was to evaluate the microbiological safety and quality of fruits being served in various cities .The study was performed to evaluate locally prepared fruit juices such as Avocado, Mango and Banana fruits from some selected areas of shecha and sekela found in Arba Minch Town. Four sample for each Avocado, Mango and Banana of Total 12 fruit samples from secha and sekela were collected and their microbial load was analyzed in Microbiology laboratory on appropriate media by using four fold serial dilution techniques followed by spread plate techniques .The highest colony (over growth) were observed from Avocado fruit samples from Sekela and lowest colony (0 colony) were observed from mango fruit samples taken from Shecha site.**

Keywords: **Fruits, Contaminants, Colony load, serial dilution.**

1. INTRODUCTION

1.1 Background and justification

Fruit Juice being defined in the most general sense as the extractable fluid contents of cells or tissues (Bates and Crandall, 2001). Fruit juices are well recognized for their nutritive value, mineral, and vitamin content. In many tropical countries they are common man's beverages and are sold at all public places and roadside shops. (Chumber *et al*., 2007)

Fruit juices are well recognized for their nutritive value, mineral, and vitamin content. In many tropical countries they are common man's beverages and are sold at all public places and roadside shops. There are reports of food borne illness associated with the consumption of fruit juices at several places in India and elsewhere (*Chamber et al., 2007; Muinde* and Kuria, 2005; Lewis *et al.*, 2006, Ghosh *et al*., 2007; Mosupye and Holy, 2000).

In view of their ready consumption, quick methods of cleaning utensils, handling and extraction; they could often prove to be a public health threat. However, sources of contamination vary. One potential source of entry of microorganisms into fruits and fruit-juices is by environmental exposure. Food borne diseases are harmful illness mainly affecting the gastrointestinal tract and are transmitted through consumption of contaminated food or drink. Improper washing of fruits add bacteria to extracts leading to contamination. In addition, use of unhygienic water for dilution, dressing with ice, prolonged preservation without refrigeration, unhygienic surroundings often with swarming houseflies and fruit flies and airborne dust can act as sources of contamination. Such juices have shown to be potential sources of bacterial pathogens notably E. coli 0157:H7, species of *Salmonella*, *Shegelle*, and *S.*aureus (Buchmann *et al*., 1999; Sandeep *et al*., 2001; Barro *et al*., 2006).

Improper washing of fruits add bacteria to extracts leading to contamination. In addition, use of unhygienic water for dilution, dressing with ice, prolonged preservation without refrigeration, unhygienic surroundings often with swarming houseflies and fruit flies and airborne dust can act as sources of contamination. Water used for juice preparation can be a major source of microbial contaminants including coli forms, fecal coli forms, fecal streptococci, etc. (Tasnim *et al*., 2010). Changes in pH may also promote the growth of pathogens (FDA, 2001). While the quality of fruit juices is strictly being maintained in the developed countries under several laws and regulations, unfortunately, in many developing countries including Bangladesh, the manufacturers are not much concerned about the microbiological safety and hygiene of fruit juices because of lack of enforcement of the law. Thus the transmission of certain human diseases through juice and other drinks becomes a serious problem (Melbourne, 2005).

The most likely cause of the contamination is fruit coming in contact with animal feces, or water, workers, containers or processing equipment contaminated with animal feces. 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).

Pathogenic organisms can enter fruits and vegetables through damaged surfaces, such as punctures, wounds, cuts, and splits. This damage can occur during maturation or during harvesting, handling and processing (Melbourne, 2005). A pathogen that has become internalized within a fruit or vegetable must be able to survive in the product until it reaches the consumer in order to become a public health hazard. Most fruit juice is sufficiently acidic to inhibit the growth of pathogenic organisms. Studies conducted on the survival or growths of microorganisms in juices have showed a number of pathogenic organisms can be present and survive in a wide range of fruit and vegetables (FDA, 2001).

Juice or cider that is pasteurized has been treated to kill harm full bacteria and to extend shelf life (Health Canada, 2006). Not only the locally prepared fruit juice but also juices imported are another important problem in resulting food borne illness. A study conducted in Bahrdar Ethiopia, on microbiological analysis school, that some imported fruit juices indicate significant increase of bacteriological load in the apple and mango juices as they stayed for long period in shelves (Abdullahi and Abdulkareem, 2010). While most people can safely consume unpasteurized juices and ciders, food safety experts do not recommend that children, pregnant women, older adults and people with a weakened immune system consume unpasteurized juices and cider *(*Health Canada, 2006). Vegetables and fruits have been associated with outbreaks of foodborne disease in many countries. Organisms involved include bacteria, viruses and parasites (De Roever, 1998).

These outbreaks vary in size from a few persons affected to many thousands. However, they represent only a small proportion of the total number of cases reported. For example, in the US between 1993 and 1997 fruits and vegetables were associated with only 1.4% to 3% of outbreaks. However, according to the Centers for Disease Control and Prevention, the number of produce-associated outbreaks per year in the US doubled between the periods 1973-1987 and 1988-1992 (Olsen *et al*., 2000). The world's largest reported vegetable borne outbreak to date occurred in Japan in 1996 and of the over 11,000 people affected, about 6,000 were culture confirmed. The outbreak involved the death of three school children and was caused by E. coli O157:H7 (Martin and Moss, 2008).

Edible fruits are those fruits that can be eaten directly from harvest. Most edible fruits have sweet taste, attractive aroma and quality nutritional properties. Fruits are important source of vitamins and to a lesser extent carbohydrate, fats and protein and as such they are indispensable food items needed for healthy living *(Bryan et al., 2006*). In Nigeria, fruits are commonly sold in most public streets and motor parks. In some cases, they are sold using mobile platforms like wheel barrows and trucks loaded with all kinds of fruits such as orange, banana, pawpaw, mango, pineapple, avocado pear, apple, garden egg and coconut. The fruits are sometimes loaded in trays and basin on vendors head. The choice of fruits sold by a particular marketer is influenced by the level of profitability and availability of the fruits as each fruit type is seasonal in nature. The occasional presence of pathogenic bacteria, parasites and viruses capable of causing human infections has been documented in fruits (Adelana, 2004; Hassan *et al*., 2006; Abdullahi and Abdul Kareem, 2010). The safety of these fruits is thus called to question, as regulatory bodies in Nigeria do not enforce sanitary conditions in the handling and presentation of fruits. Also, without much formal education in hygiene and sanitation, the fruit vendors acquire their handling techniques traditionally, considering the above conditions in which these edible fruits are handled and sold, it is expected that the safety of these fruits is not guaranteed, thus not meeting the health standard. Since there is a rapid increase of the already large number of people involved in the consumption of edible fruits in the study area, there

is every need to have first-hand information on the safety of these fruits by investigating the types and volume of potential pathogenic microbes present on the surfaces. Therefore this study was desired to assessment on the bacteriological load of street sold fruit:-*Musa acuminata*, *Mangifera indicia and persea americana* in Arba Minch town southern Ethiopia.

1.2 Statement of the problem

Microbes have significant effect for quality of street sold fruit. The fruit was known in some parts of our country mostly southern parts of Ethiopia especially in Arba Minch town. The fruit and other fruit products can be contaminated by pathogenic microbes especially bacteria and fungi that makes fruit un acceptable for humans consumption .so taking care of fruit preparation is important for temporal storage for quality of locally prepared fruit juice. Regarding bacteriological quality, in this study site no study performed yet, and also shows direction for other researchers for further study.

1.3. Objective of the study

1.3.1 General objectives

- ✓ To assess bacteriological load of street sold fruits:-banana, mango and avocado in Arba Minch Town.

1.3.2 Specific objectives

- ✓ To enumerate bacteriological Load of each fruits samples
- ✓ To compare bacteriological load among the three fruits
- ✓ To determine bacteriological quality of the three fruits

1.4. Significance of the study

Street sold fruits are common in Ethiopia and especially in Arba Minch town since the area is so rich of different types of fruits. Such fruits have high risk of contamination since such fruits are freely exposed to environment. The consumption of such contaminated fruit may leads to serious health effects. So, this study would help to minimize the gap on this area for further study and to create awareness related with bacteriological loads of such street sold fruits of the study area. It also helps to provide information for further study and to know which type of fruit juice can easily contaminated by food born disease.

2. METHOD AND MATERIALS

2.1 Description of Study Area

This Study would be carried out in Arba Minch town which is found in southern nation nationalities and peoples of Ethiopia. It is located in the Gamo Gofa zone at about 505 kilometers from south of Addis Ababa, at an elevation of 1285 meters above sea level. It is the largest town in Gamo Gofa zone and the second town in SNNPR next to Hawassa. Arba Minch received its name for the abundant local spring which produces a ground water forest. Located at the base of western side of Great Rift Valley, Arba Minch consists of the uptown administrative center of Shecha and 4 kilo meters away the down town commercial and residential areas of Sekela, which are connected by a paved road . on eastern side of Sekela is the gate to Nechisar national park ,which covers the isthmus between lake Abaya to the north and lake chemo to the south.it is known as a source for fruit , including: mango ,banana, orange, apple ,avocado and as well as known for its fish farms.

2.2. Study Design

Cross sectional study design was used to assess bacteriological load of street sold fruit in Arba Minch Town.

2.3. Source of Sample

Street sold fruit samples were collected from Shecha and Sekele, Arba Minch Town at the same time.

2.4. Sampling Technique and Sample Size Random sampling technique was used to selected street sold from both sites. A total of 12 selected fruit samples (4 samples of each three fruit) in which two fruit samples of each fruit from both sites would be purchased from street.

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2.5 Sample transportation and laboratory procedure

2.5.1 Sample transportation

The selected sample were purchased and transported to Microbiology laboratory of Arba Minch University Abaya Campus by using plastic bag.

2.5.2 Laboratory procedure

Each fruit sample was first pilled and then 30gm would be measured and dissolved in distilled water. 10ml of dissolved fruit sample would have been taken and serial dilution would be performed up to 10^{-4} folds. Following this, by using micro-pipette 1ml of 10^{-4} serial diluted fruit sample will be transferred to Nutrient agar, Mannitol salt agar and MacConkey agar simultaneously and spread by spread plate technique. Finally, each plate would be incubated at 35^{o}C for 24hours.

2.6. Description of Bacteriological Load

Colonies on each overnight incubated plate would be counted by using colony counting machine and described as *cfu/ml (*colony forming unity per gram as of sample) by using the following formula (Sharma and Mazumdar, 2014).

Number bacterial colony in original sample= Number of colony counted x $\frac{1}{Df}$ x v(ml)

Where Df=Dilution factors and V= volume ml(milliliter) transferred after serial dilution

2.7 Data Analysis

The data obtained from laboratory experiment was analyzed using descriptive statistical parameters like tables and graphs

3. RESULTS

After 24 hours of incubation of all fruit samples from both sites of the study on different media such as nutrient agar (for growth of all bacteria), Mannitol salt agar (for staphylococci species) and MacConkey agar (for gram negative bacteria) colonies were counted and then, the loads of bacteria in original fruit samples were estimated by the following formula and indicated in the following table.

Tables 1: Bacteriological load cultivated from each selected fruit samples

Sample areas	Type of fruit	Type of media used	No. of colony counted at Df 10^{-4}	No. of bacterial colony expected in origin samples
Shecha	Banana	Nutrient agar	105 colony	$10.5x10^5$ cfu/ml
		Manitol salt agar	5 colony	$5x10^5$ cfu/ml
		MacConkey	o colony	0
	Mango	Nutrient agar	35 colony	$3.5x10^5$ cfu/ml
		Manitol salt agar	1 colony	$1x10^5$ cfu/ml
		MacConkey	16 colony	$1.6x10^5$ cfu/ml
	Avocado	Nutrient agar	165 colony	$16.5x10^5$cfu/ml
		Mannitol salt agar	1625 colony	$16.3x10^5$ cfu/ml
		MacConkey	0 colny	0
		Nutrient agar	70 colony	$7.0x10^5$ cfu/ml

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Sekela	Banana	Mannitol Salt agar	95 colony	$9.5x10^5$ cfu/ml
		MacConkey	0 colony	0
	Mango	Nutrient agar	31 Colony	$3.1x10^5$ cfu/ml
		Mannitol salt agar	2 colony	$2x10^5$ cfu/ml
		MacConkey	14 colony	$1.4x10^5$ cfu/ml
	Avocado	Nutrient	Overgrowth	Overgrowth
		Mannitol salt agar	Overgrowth	0vergrowth
		MacConkey	0	0

As indicated in above table 1, for Banana samples taken from Sekela and Shecha areas and inoculated on Nutrient agar , Mannitol salt agar and MacConkey and colony counted as $7.0x10^5$ cfu/ml and $10.5x10^5$ cfu/ml, $9.5x10^5$ cfu/ml and $5x10^5$ cfu/ml, 0 and 0, while for over growth and $16.5x10^5$cfu/ml, overgrowth and $1x10^5$ cfu/ml, 0 and 0. On the other hand for, $3.1x10^5$ cfu/ml and $3.5x10^5$ cfu/ml, $2x10^5$ cfu/ml and $1x10^5$ cfu/ml, $1.4x10^5$ cfu/ml and $1x10^5$ cfu/ml colonies were counted from Sekela and Shecha respectively.

4. DISCUSSION

Fruits are well recognized known for their nutritive value, vitamin content, minerals and consumed by consumer for their fresh flavor. Therefore, the numbers of fruits is significantly increasing in order to not only services for the consumers, but it also creates job opportunities to community. On the other hand contaminated fruit can cause human health problems specifically to children, pregnant women and also elderly people and people with weakened immune system due to poor handling, processing and fruit storage product (health Canada, 2006).

From this finding of this study, more colonies were observed from samples taken from Sekela cultivated on nutrient agar and Mannitol salt agar were over growth, while less colonies were observed from samples taken from Shecha that show no growth (zero colony) for all fruit samples except from mango, $1x10^5$ cfu/ml and$1.4x10^5$ cfu/ml **from** Sekela and Shecha respectively on MacConkey agar. Too many numbers of bacterial colonies grown from avocado fruits samples from Sekela on Nutrient agars and Mannitol salt agars medium indicate that, as there were many contaminants at the site. From the fruits sample avocado shows more sensitivity for the contaminants which may due to its richness of nutrients. The others less bacterial colonies from fruit samples on MacConkey medium indicates that, there were less gram negative bacterial contaminants.

The microbial contamination present in street sold fruits samples collected from two sites of Arba Minch town from Shecha and Sekela total around commercial bank of Ethiopia .Regarding to types of fruit sample collected from Shecha Street, avocado account$16.6x10^5$cfu/ml colonies on nutrient agar, the rest banana$10.5x10^5$cfu/ml and mango were $3.1x10^5$cfu/ml. The results of the current study was not in agreement when comparing with an others studies, Avocado($1.4x10^7$cfu/ml),Mango($8.4x10^7$cfu/ml) and Banana($6x10^7$cfu/ml) for Mango and Avocado (Aguoru.,*et al.*, 2015). As well as for Banana (Javid Ali *et al., 2013)*.The differences on result may be due to difference in environmental conditions and sample size.

According to the research conducted in University of Valencia in Spain, the poor handling and processing of uncleaning equipment in the food service setting can stimulate bacterial contamination that can results in food-borne illness outbreak.

According to the study conducted on bacteriological quality of fruits the mean total bacterial colony count was generally causes pathogenic microbes were the highest in avocado fruit, second was banana and the least on mango in all places. This indicates that Avocado fruit can easily contaminated (Ketema *et al*., 2008). All sampled fruits show growth of colonies on Mannitol salt agar and these colonies are considered as staphylococci species which were from the fruit handlers, these bacteria leads consumer to different food-borne disease. Study conducted in USA and UK show that 7.8% (47) of the 600 bacterial food poisoning and 1.9% (54) out of a total of 2815 respectively of food born outbreaks were caused by Staphylococci species (Martin, *et al*, 2008). Unless measurements were taken, the current study reveals the information for the outbreaks will be at the study site.

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5. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The practice of consuming fresh fruit, vegetables and other fruit in unhygienic form is unsafe for human consumed, and fruit juice prepared cannot also be prohibited from selling such items. From this study, fruit samples taken from both Sekkela and Shecha Street shows different bacteriological load which may relate to their nutrient contents.

Generally, from this finding, more bacterial loads were observed on avocado (over growth) followed by banana ($9.5x10^5$ cfu/ml), while minimum/no growth was observed on mango (0cfu/ml) which may be biological structure of the fruit. Certain fruits such as mango that show 1.4 $x10^5$cfu/ml and $1.6x10^5$ cfu/ml bacterial colonies from Shecha and Sekela respectively indicates the presences of gram negative bacteria, this shows that such fruit was contaminated with contaminants from human fecal which causes risk for human health. The numbers of high bacteriological colonies were observed from avocado fruits from sekela. When compared to others bacteriological colonies from Shecha.

5.2. Recommendation

The fresh fruits produced for consumption and to produce non contaminated fruit for consumers the following ideas are recommended .Based on the finding of this study, the following recommendations were made:

- Health agencies must be adopt measures to educate the vendor on food quality and safety of hygienic practices.
- Before manufacturing fruits juices, the manufacturer must be take care from where they have to take fruits.
- The consumer must minimize the habit purchasing and consuming fruit from street.
- Further studies must be conducted for identification of bacterial at species level and to identify sources of contains for fruit sold at street by taking many fruit samples from different sites.

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