

Assessment on Bacterial Load of Ready to Use Fruit Juices Served in Cafes and Juice Bars in Hossana Town, Southern, Ethiopia

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Abstract

Contaminated food and drinks are source of various food borne diseases in human. The study aimed on the assessment of bacterial load of locally prepared fruit juice. A cross sectional study was conducted from January 2015 to May 2015 in Hossana town of Ethiopia. Out of Twenty four fruit juice samples, eight samples from each of avocado, papaya and mango were collected at two different time intervals (Day and Night). The pH of original samples was determined before dilution. Streak plate method was used for the growth of bacteria on appropriate media. Structured questionnaire was prepared to assess storage mechanism of fruit, source of fruit, processing and hygienic condition of working environment. Regarding the PH of the juice, here was slight increase in juices collected at night. This may be due to inappropriate storage of fruit and over dilution of fruit juices. Regarding the bacterial load of fruit juice samples, both avocado 9 (38.38%) and papaya 8 (33.67%) were found to be more contaminated with bacteria than mango 6 (28.53%). This may be the water activity, antimicrobial constituents and/or nutrient content. The venders cause subsequent contamination during preparation, handling, by the side of waste disposal system, using water that is not boiled for dilution. Therefore, regular supervision and training about safe processing and handling of fruit juices and hygiene of venders can improve the quality of fresh fruit juices.

Keywords: Bacteria; Contamination; Fruit juice; Streak plating; Hossana

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Introduction

Background and Justification

Juices are the aqueous liquids expressed or otherwise extracted usually from one or more fruits or vegetables, purees of the edible portion of one or more fruits or vegetables, or any concentrates of such liquids or purees (Fraternale, 2011). Fruit juices contain water, sugars, organic acids, vitamins, and trace elements thus providing an ideal environment for spoilage by microorganisms; on the other hand, they generally have a lower pH (pH < 4.5), thus the common feature of their potential spoilage agents is that they must be acid-loving microorganisms. The most commonly encountered microbial genera are Acetobacter, Alicyclobacillus, Bacillus, Clostridium, Gluconobacter, Lactobacillus, Leuconostoc, Saccharobacter, Zymomonas, and Zymobacter. However, yeasts are predominant because of their

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high acid tolerance and the ability of many of them to grow anaerobically. *Pichia*, *Candida*, *Saccharomyces* and *Rhodotorula* are the genera mainly involved in spoiled juices; the species frequently isolated are *Pichiamebranifaciens*, *Candida maltosa*, *C. sake*, *Saccharomyces bailii*, *S. bisporus*, *S. cerevisiae*, *S. rouxii*, *S. bayanus*, *Brettanomyces intermedius*, *Schizosaccharomyces pombe*, *Torulopsis holmii*, *Hanseniaspora guilliermondii*, *Schwanniomyces occidentalis*, *Dekkerabruxellensis*, *Torulasporadelbruckii*, *Zygosaccharomyces microellipsodes*, and *D. naardenensis* (Bevilacqua, et al. 2011).

Freshly squeezed fruit and vegetable juices have little or no process step that reduce pathogen level, if contaminated, such as no kill step. Freshly squeezed juices are simply prepared by extracting, usually mechanical means, the liquid and pulp of mature fruit or vegetable. The final product is an unfermented, untreated juice, ready for consumption. During the process, contamination from raw materials, equipment or food handlers could be easily transferred to final product. If pathogens such as *Salmonellae* were present in freshly squeezed juice, individuals may be exposed (Melbourne, 2005)

In view of their ready consumption, quick method of cleaning utensils, handling and extraction, they could often prove to be a public health threat, however, source of contamination vary. One potential source of entry of microorganism in to the fruit and the fruit juice is by environment expose. Improper washing of fruit add bacteria to extract leading to contamination. in addition use of unhygienic water for dilution, dressing with ice, prolonged preservation without refrigeration, unhygienic surrounding often with swarming houseflies and fruit flies air born dusts are act as source of contamination. (Barro., et al. 2006 Sandeep., et al. 2001 and Bachmann., et al. 1999).

Nowadays, the demand for freshly squeezed juices in comparison to bottled or canned juices has increased, as unpasteurized juices are preferred by the consumer because of the fresh flavor and no addition of preservatives. Traditionally, juice is consumed more in the morning at breakfast time. Consumption of fresh fruits continues to increase in many countries owing to consumer preferences for fresher, more nutritious foods that also happen to meet the needs of busier lifestyles. Fresh fruit juices have no artificial color, sweetness is natural, and that is why they are preferred over bottled or canned juices (Addo., et al. 2008; Melbourne, 2005).

The rapid increasing number of juice venders has extended consumption of juice products across Ethiopia specifically in Hawassa town. The farmers are attracted toward cultivating different kinds of fruits as well (Abadias., et al. 2008). Therefore, economical contribution of fruit production by the study area is great enormous. Additionally fruit juices processed under hygienic condition could play important role in enhancing consumer's health through inhibition of breast cancer, congestive heart failure (CHF), and urinary tract infection (Ketema., et al. 2008).

The consumption of fruit juices could have both positive and negative effect on the part of consumers. Fruit juices are well recognized for their nutritive value, mineral, and vitamin contents. Fruit juices processed under hygienic condition could play important role in enhancing consumers' health through inhibition of breast cancer, congestive heart failure (CHF), and urinary tract infection (Saenz and Sepulveda, 2001; Hyson, 2011). On the other hand, there are reports of food borne illness associated with the consumption of fruit juices at several places (Chumber., et al. 2007). Food borne diseases are harmful illness mainly affecting the gastrointestinal tract and are transmitted through consumption of contaminated food or drink. Such juices have shown to be potential sources of bacterial pathogens notably *E. coli* 0157:H7, species of *Salmonella*, *Shigella*, and *S. aureus* (; Barro., et al. 2006 Sandeep., et al. 2001; Buchmann., et al. 1999). Food borne or waterborne microbial pathogens are leading causes of illnesses in developing countries, killing an estimated 1.9 million people annually at the global level. Even in developed countries, an estimated one-third of the population is affected by microbiological food borne diseases each year (Andargie., et al. 2008). There are reports of food borne illness associated with the consumption of fruit juices of several places of India and elsewhere (Sandeep., et al. 2001).

Most of the fruit juices being served in Jimma had high microbial load. So that, these products could be the cause of health problems and potential vehicle of food borne outbreaks (Ketema., et al. 2008). Contamination of 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). In response

to the increasing number of food borne illnesses, governments all over the world are intensifying their efforts to improve food safety (Sudershan., *et al.* 2009). However, in Ethiopia no continuous survey/assessment of food safety have been prepared in restaurants and cafes especially on locally prepared fruit juices.

Although fruit juices processed under hygienic condition could play important role in enhancing consumer's health, these products could be the cause of health problems and potential vehicle of food borne outbreaks (Ketema., *et al.* 2008). The study site Hosanna, is one of the town in which juice bars and number of customers have being increased as result food borne illness are common. Since there is no research previously done concerning microbial quality of fruit juice served in cafes and juice bars, this study tried to assess bacterial load of fruit juices that are used in the site.

Significance of the study

Now a day the number of the juice venders is significantly increasing in Hossana in order to not only give services for consumers, but also to create job opportunities to community. On the other hand, unpasteurized fruit juices can pose problems for human health due to poor handling, processing, storage of fruit and fruit juice. The trend of taking fresh fruit juices in the study site is from local venders is relatively higher than before. The study tried to identify which juice types are easily contaminated and point out the route of contamination and generate inputs to remedial action for identified problem. As the study trends to assess the health problem that is facing consumers, it identified hygienic problem that affected quality of locally prepared unpasteurized fresh juices. Generally the study intended to get firsthand information on the safety of fruit juice and promotes other researchers to conduct further research by increasing the sample size since this study was conducted on small sample size. Therefore, this study endeavors to generate relevant data on the bacteria load of ready to use fruit juices that are served in Hosanna town.

Objective of the study

General objective

The overall objective of this study was to investigate bacterial load of freshly squeezed fruit juice served in cafes and juice bars in Hosanna town.

Specific objectives

- To determine the total load of bacteria from the selected sample.
- To study the hygienic condition of preparation.
- To compare bacteriological loads among fruit juices in the study site

Materials and Methods

Study area

This study will be conducted between October 2014 and June 2015 at Hosanna town. This place is the capital city of Hadiya Zone located 232 Km south of Addis Ababa, the capital city of Ethiopia and 212 Km to the capital city of south SNNPR state, Hawassa. It has an average altitude of 1900-2700 meters above the sea level. The core city has an average estimated area of 40.5 Km². Regarding the climate of the town, the area receives an average annual rain fall ranging from about 1001-1200 mm. The mean temperature of the area ranges from 15.1 and 20°C. The climate condition is not favorable for the cultivation of fruit instead it known by its wheat and inset plant.

Study Design

A cross-sectional study design was applied to evaluate the bacterial load of locally prepared fresh fruit juices in Hosanna town. The study sites were grouped as cafes and juice bars by stratified random sampling technique. Four locations in the town were sampled from the total of twenty-six sites.

Sample Size Determination

Total of twenty four fruit juice samples of three types (unpasteurized avocado, unpasteurized papaya and unpasteurized mango) were selected it is by convenience due to limit of time and materials. These juices were chosen by purposive sampling because of the commonly available fruit juices that prepared by the venders from 4 sites (2 cafes and 2 juice bars). Two samples of each type of fruit juices were collected from a single site at different interval (day and night).

Demographic Information and Venders Juice Practices

Interviews were administered to all venders involved in the study. Questionnaire contained variables such as, personal hygiene, environmental hygiene, source of fruit, processing of fruit juices, juice sellers handling and Educational status of juicer and Storage environment of juices was raised up. All the personnel involved in the processing and/or serving of the fruit juices in the selected juice bars and cafés were interviewed. In addition, observation was made to obtain information on the hygienic condition of preparation such as working environment, personal hygiene, water that used for washing and processing.

Methodology

The methodology of Tortora, *et al.* 2013) was used for the determination of bacterial colonies. The samples were collected in sterile containers following aseptic techniques then the p^H of all of undiluted sample was measured by p^H meter immediately after the sample taken to laboratory holding with in ice bag. Serial dilutions of each sample were made using sterile distilled water as diluents. As a guide with clear products dilution to 10^{-3} may be sufficient where as heavily contaminated products required dilution to 10^{-6} . A serial dilution 10^{-1} , 10^{-2} , 10^{-3} and 10^{-4} were made by taking 1 ml from original sample and adding to a series of sterile dilution to be containing 9 ml of distilled water and mixed properly by cleaning and disinfecting by different disinfectants as well as using Bunsen burner flame.

The pH of all undiluted samples was measured by pH meter immediately after collection. It is important to determine the pH of the food sample before undertaking microbiological examination as this can influence the colony count and organisms sought. In general, in foods with a pH below 4.5 pathogens would not be expected to survive; the organisms present would be limited to yeasts, molds and a few acid tolerant bacteria. Foods with a pH above 4.5 required full microbiological examination .

From each sample of previously prepared serial dilution 1ml was transferred into sterile Petridis containing MacConkey agar and nutrient agar. Streak series was made from each of the 10^{-4} dilution. The inoculating loop was sterilized following each streak series then incubated all plates at $37^{\circ}C$ for 24 hour.

Whitish colonies were formed on streak series. The colonies were counted using digital colony counter. The plate were screened for the presence of discrete colony forming units per ml cfu/ml.

$$CFU/ml = \text{Colony counted} \times \frac{1}{df} \times v(ml)$$

Where df: dilution factor and

V (ml): volume of solution transferred between serial dilution

Result

Survey for Demographic Information and Venders Juice Practices

A total of ten juice maker were interviewed to obtain data on fruit juice processing, source of fruit, storage of fruits educational status of the juicers, hygienic condition of vending sites and garbage sites from stratified randomly selected restaurants and cafes. The study observed that among 10 respondents, 2 (20%) were females and 8, (80%) were males. 6 (60%) of the respondents had completed high school grade and 4 (40%) were attending in elementary school. None of the fruit juice makers had professional training related to safe handling and processing of fruit juices. Among establishment of juice makers, two were cafes and two were juice bars. All of the

venders were using tap water for dilution of fruit juices without any treatment. Except one, all of venders did not boil water for juice dilution. Concerning their garbage site, all of them discard the waste materials in to nearby garbage. Concerning to the source of fruits, all of the venders had bought from open markets. Only one vender was using refrigerator for temporary storage of fruit but the rest were storing on shelves and in baskets (Figure 1).

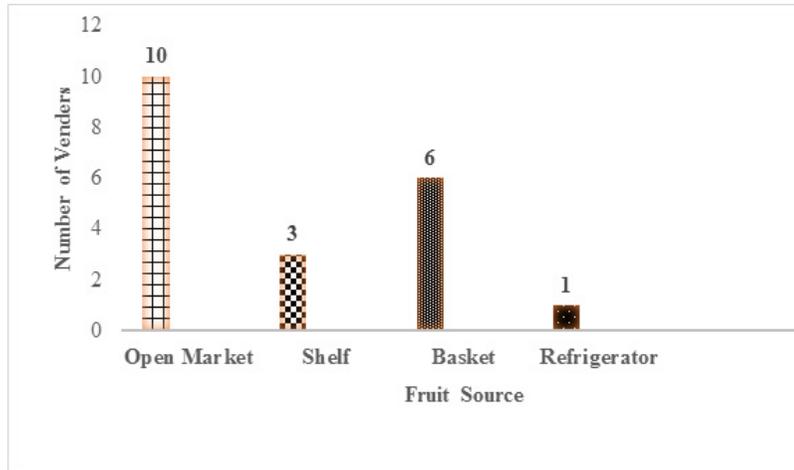


Figure 1: Source of fruits for the juice products.

Source of fruit	No of vender	Percent
Open market	10%	100%
Storage		
Shelf	3	30%
Basket	6	60%
Refrigerator	1	10%

Table 1: Demographic Information of Juice Venders.

Parameter	TVC	coliform	fecal coliform	staphylococci
Maximum bacterial Load anticipated	5.0×10^3	10	0	100
Maximum bacterial Load permitted	1.0×10^4	100	0	1.0×10^3

Table 2: The recommended microbial standard for any fruit juice all numbers are as per ml of juice consumed (GULF standard (2000).

PH measure

A total of twenty four fresh juice were taken for their pH measurement. The pH of both avocado and papaya were (6.86 & 5.94) respectively and more acidic than mango (3.92)

Sample No	Type of juice	Sampling area	Time of collection	pH	Total viable count cfu/ml
P ₁	Avocado	Cafe ₁	Day	6.32	8.1 x 10 ⁻³
P ₂	Avocado	Cafe ₁	Night	6.41	8.4 x 10 ⁻³
P ₃	Avocado	Café ₂	Day	6.48	9.2 x 10 ⁻³
P ₄	Avocado	Café ₂	Night	6.56	9.8 x 10 ⁻³
P ₅	Avocado	Juice bar ₁	Day	6.51	9.1 x 10 ⁻³
P ₆	Avocado	Juice bar ₁	Night	6.67	0.1 x 10 ⁻³
P ₇	Avocado	Juice bar ₂	Day	6.55	7.0 x 10 ⁻³
P ₈	Avocado	Juice bar ₂	Night	6.49	7.2 x 10 ⁻³
P ₉	Mango	Cafe ₁	Day	3.55	5.2 x 10 ⁻³
P ₁₀	Mango	Cafe ₁	Night	3.71	5.8 x 10 ⁻³
P ₁₁	Mango	Cafe ₂	Day	3.81	6.4 x 10 ⁻³
P ₁₂	Mango	Cafe ₂	Night	3.92	6.7 x 10 ⁻³
P ₁₃	Mango	Juice bar ₁	Day	3.66	8.0 x 10 ⁻³
P ₁₄	Mango	Juice bar ₁	Night	3.88	8.6 x 10 ⁻³
P ₁₅	Mango	Juice bar ₂	Day	3.42	5.2 x 10 ⁻³
P ₁₆	Mango	Juice bar ₂	Night	3.48	5.7 x 10 ⁻³
P ₁₇	Papaya	Cafe ₁	Day	5.33	6.8 x 10 ⁻³
P ₁₈	Papaya	Cafe ₁	Night	5.42	7.0 x 10 ⁻³
P ₁₉	Papaya	Cafe ₂	Day	5.44	7.4 x 10 ⁻³
P ₂₀	Papaya	Cafe ₂	Night	5.62	7.8 x 10 ⁻³
P ₂₁	Papaya	Juice bar ₁	Day	5.46	9.2 x 10 ⁻³
P ₂₂	Papaya	Juice bar ₁	Night	5.36	9.6 x 10 ⁻³
P ₂₃	Papaya	Juice bar ₂	Day	5.42	5.8 x 10 ⁻³
P ₂₄	Papaya	Juice bar ₂	Night	5.48	6.2 x 10 ⁻³

Table 3: Bacterial load in vender fruit juice sample (N = 24).

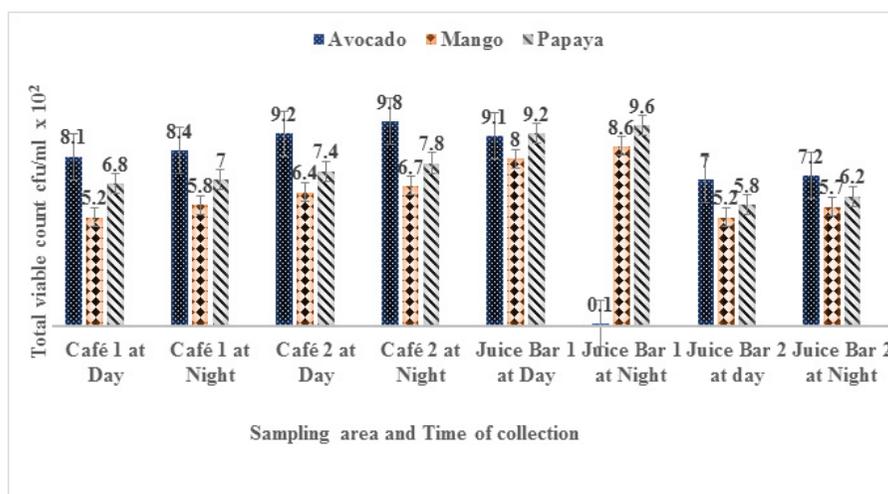


Figure 2: pH Value of the Fruit Juice Types Collected at Different Time and Sampling Area.

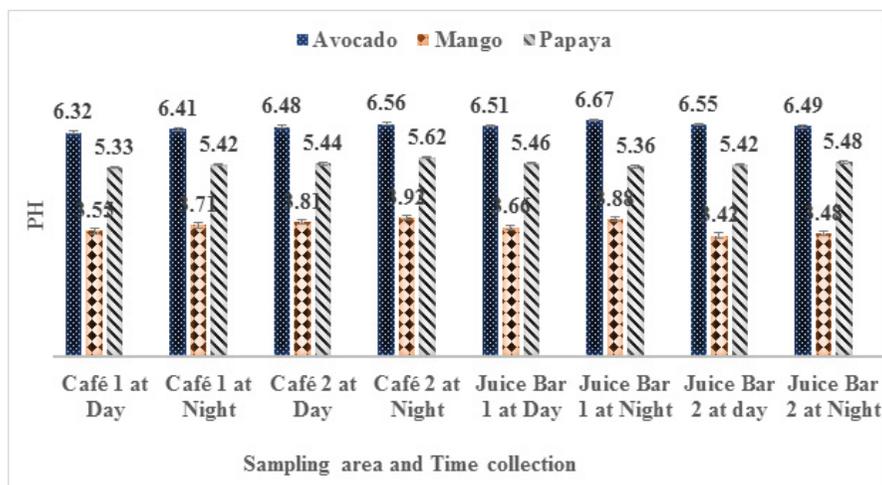


Figure 3: Bacterial load in vender fruit juice sample collected at different time.

Discussion

Most of the samples showing high bacteria counts were Avocado followed by papaya. All of the samples were found to be above Gulf standard (1.0×10^4 cfu/ml) (Table 2). Regarding of the type of juices, Avocado was predominant with 9 (38.38%) and papaya and mango were 8 (33.67%) and 6 (28.53%), respectively. This may be the water activity, antimicrobial constituents and/or nutrient content. In addition to this there was the pH difference during day and night. In the samples that were collected at night time the pH was shown slight increase.

The probable reason may be inappropriate storage of fruit and over dilution of fruit juices. The results of the current study were in agreement with findings which were reported from Hawassa avocado 19 (63.33%) and the rest papaya and mango were 14 (46.67%), and 4 (13.33%) respectively (Mesfin., *et al.* 2011). The probable reason for the similarity may be, seasonal similarity, time of sample collection and hygiene. But, the results of the current study were in contrast to the findings which were reported from Jimma town for Avocado (6.0 log/ml) and Papaya (6.6 log/ml) (Ketema., *et al.* 2008). The probable reason for this difference may be number of factors such as geographical variation, seasonal variation, sanitation habit, collection and transportation of sample, and procedure of inoculation.

Regarding to pH of the fruit juices sample, Avocado in a range of 6.32-6.67, Papaya 5.33-5.62 and mango 3.42-3.92. Both avocado and papaya were shown the pH range that support growth of most bacteria (Figure 1). This may be the possible reason for the high number of Total viable count. The pH result obtained from the current study was also comparable with study conducted in Hawassa, Ethiopia (Mesfin., *et al.* 2008). In addition to this there was the pH difference during day and night. In the samples that were collected at night time the pH was shown slight increase. The probable reason may be inappropriate storage of fruit and over dilution of fruit juices. This study also tried to address source of contamination by assessing storage mechanism of fruit, source of fruit, processing and hygienic condition of working environment.

Fruits should be inspected for clean, dry, and intactness by juice makers who should be trained in inspection and personal hygiene. Only sound whole fruit should be used for fruit preparation. Decayed, wormy, damaged, soiled fruit should be sorted and discarded to prevent contamination of juice product. All fruit should be subjected to effective washing, brushing and rinsing. Water supplies for fruit cleaning must be potable. All workers must be free from communicable diseases. They should be trained not only for their task, but also to keep the venders clean and to practice personal hygiene (Canadian Food Inspection Agency, 2010).

However, none of juice maker that participated to the current study was experienced to training relevant to juice processing and personal hygiene. This finding assures that the possible contamination of juices would be poor processing and handling of fruit juices. Therefore, the conditions of locally prepared fruit juice and vending raise many concerns for consumer's health. In most cases, hands and utensils washing are usually done in one or more buckets, and sometimes without soap. Wastewaters and garbage's were discarded nearby, providing nutrients for insects and rodents. Some of the juices were not efficiently protected against flies, which may carry food borne pathogens.

Conclusion

In this study, high bacterial load was observed in avocado and papaya than mango that could pose health problem. Avocado and papaya were shown high pH range that support growth of most bacterial. According to the result of questionnaire and observations, subsequent contamination by juicer during preparation, handling, storing, waste disposal system (nearby garbage), hygienic status of the juicer and using tap water directly without boiling causes contamination in juices.

The role of different factors such as personal hygiene, the way of storage of fruits, using un boiled water for diluting the juice/cleaning equipment should be clearly recognized by responsible persons in order to understand their effect in the control and prevention of bacterial load on fruit juices in the study area. In order to assure safe unpasteurized fruit juices for consumers, there should be regular inspection health officers.

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