

Bacteriological Safety of Freshly Squeezed Mango and Pineapple Juices Served in Juice Houses of Bahir Dar Town, Northwest Ethiopia

Asmamaw Leul^{a*}, Mulugeta Kibret^b

^aSOS Children's Villages Ethiopia, SOS Hermann Gmeiner School, Bahir Dar, P.O Box 1429, Ethiopia

^bBahir Dar University, College of Science, Department of Biology, Bahir Dar, P.O Box 79, Ethiopia

^aEmail: asmamaw.leul@gmail.com

^bEmail: mulugetanig@gmail.com

Abstract

The objective of this study was to assess bacteriological quality and safety of freshly squeezed mango and pineapple juices in Bahir Dar town, Ethiopia. The mean aerobic mesophilic count of mango juice (4.76 log CFU/ml) was relatively higher than pineapple juice (4.21 log CFU/ml) across each juice house. The mean *Staphylococcus aureus* counts were 3.84 log CFU/ml in mango and 3.74 log CFU/ml in pineapple juices. Total coliform counts were in the range of 9.2 to > 1100 MPN/ml in mango and from < 3 to > 1100 MPN/ml in pineapple juices. Total coliform counts in water samples were in the range of < 0.018 to > 16 MPN/ml. Pineapple juice was more acidic (pH= 4.26±0.44) than mango juice (pH= 4.61±0.42). The %TA of pineapple juice (TA = 0.182±0.164) was slightly higher than mango juice (TA = 0.168±0.046). The dominant bacterial groups isolated from sample juices were *Citrobacter* spp. 16 (45.7%) followed by *Salmonella* spp. 7 (20%), *E. coli* spp. 5 (14.3%), *Enterobacter* spp. 4 (11.4%), *Klebsiella* spp. 2 (5.7%), and *Pseudomonas* sp. 1 (2.9%) species. In almost all juice houses, way of juice preparation, handling practice, and hygiene of juice houses were poor. The bacteriological and overall sanitary condition of juice houses in the present study suggests the risk from the consumption of freshly squeezed fruit juices is high. Therefore, juice vendors that produce freshly squeezed mango and pineapple juices should be aware that preventative measures through food safety control strategies is important.

Key words: freshly squeezed juice; quality; safety; bacteria

1. Introduction

Freshly squeezed and unpasteurized fruit juices are common in restaurants, cafeteria, hotels, and juice houses in Ethiopia. They are widely consumed by millions of people around all over the country, especially in large cities and towns [1]. They are recognized for their mineral and vitamin contents and high nutritive values which offer great taste and health benefit [2]. They also improve blood lipid profiles in people affected by hyper-cholesterolemia and enhance consumers' health through inhibition of breast cancer, congestive heart failure (CHF), and urinary tract infection [3].

* Corresponding author. Tel.: +251 913 015585
E-mail address: asmamaw.leul@gmail.com

However, in the absence of good fruit preparation and juice making practice, the nutritional richness of freshly squeezed fruit juices makes the product good medium for bacterial growth, vehicle of food borne pathogens and associated complications [4]. Source of fruits, quick methods of cleaning fruits and utensils, handling practices of fruits, mechanical methods of squeezing juices, and unhygienic conditions of juice houses also contribute a lot to bacterial contaminations of juices [4]. For example, the outside surface of fruits may not be washed properly before it is placed into juice machine for extracting the juice. Even after extraction, they may not be pasteurized and this may create favorable conditions for growth of aciduric bacterial contaminants [5]. High pH and storage temperature of fruits and juices may also favor growth of pathogenic bacteria. Unhygienic water used for juice preparation can be a major source of total coliforms, faecal coliforms, and faecal streptococci [6]. Environmental formites may also make the juices unsafe and these may have a role in the spread of pathogens like *Salmonella*, *Staphylococcus aureus* and *Escherichia coli* [7]. Contaminated juices are therefore unacceptable for human consumption and create significant health problems for young children, the elderly and people with weakened immune system [8].

Bearing in mind the absence scientific information on different risk factors responsible for bacterial contaminations in the study area, an inevitable question may arise over quality and safety of freshly squeezed fruit juices. Therefore, the objective of this study was to assess bacteriological quality and safety of freshly squeezed mango and pineapple juices.

2. Materials and methods

2.1 Sampling Techniques

Ninety samples (30 each of mango, pineapple, and water) were collected from six purposively selected juice houses in Bahir Dar town from April to June 2012. In each juice house, 250 ml of each of mango and pineapple juices were purchased without addition of flavoring agent and, on receipt; each of the juice was decanted into two sterile Erlenmeyer flasks, 250 ml each. At the same time, 100 ml of water sample was collected in a sterile bottle. The samples were then transported immediately to Microbiology Laboratory of Bahir Dar University in ice box held at approximately 4°C with sealed polythene bottles of frozen water. Samples were analyzed within an hour of procurement. Observation, interview and questionnaire were also used to obtain preliminary information on the demographic characteristics of the fruit juice makers, servers and cares being taken during processing of the fruit juices. All the personnel involved in processing and/or serving of fruit juices in sample juice houses were included.

2.2 Sample Processing

In the laboratory, 10 ml of each juice sample was taken aseptically and blended with 90 ml of sterile buffered peptone water and a serial dilution up to 10^{-3} was prepared. Pour plate technique was used on appropriate culture media to grow, enumerate, isolate, and characterize bacteria from samples.

2.3 Culturing Method

Plate Count Agar (PCA) and Manitol Salt Agar (MSA) were used as a culture media for aerobic mesophilic count (AMC) and *Staphylococcus aureus*, respectively. One millimeter from each of three serial dilutions (10^{-1} , 10^{-2} , and 10^{-3}) was taken aseptically and pour plated into three triplicate plates of each agar. The plates were then incubated at 37°C for 24-48 hours. Plates with colonies ranging from 30-300 for AMC and 20-200 for *Staphylococcus aureus* were counted using colony counter (Ahmed *et al.*, 2009). Catalase test was done on presumptive golden yellow colonies of *Staphylococcus aureus* by adding few drops of 3% H₂O₂ on plates of an over-night culture of the pure isolates [8].

MPN of total coliforms in juice and water samples employed the use of Lauryl sulphate Tryptose Broth (LTB) for presumptive test and Brilliant Green Lactose Bile Broth (BGLBB) for confirmed and complete tests. One milliliter of (10^{-1} , 10^{-2} and 10^{-3}) each dilution of juice was inoculated into three test tubes containing 9 ml LTB and Durham's tube. But, a modified procedure was used to calculate the MPN of water. Serial dilution of the water sample was not made. Instead, 10ml, 1ml and 0.1ml of water samples were inoculated into a series of five tubes of triple sets containing 10 ml LTB and Durham's tube. All the test tubes were then incubated at 37 °C for 18-24 hours. From positive test tubes of presumptive tests, loop-full of inoculums from each was taken aseptically and inoculated into

BGLBB and incubated in the same fashion. Then, the MPN ratios of positive tubes were analyzed as per Mc Cardy's table for MPN [9].

Xylose Lysine Deoxycholate (XLD) agar was used to culture *Salmonella* spp. Twenty five milliliter of original juice sample was diluted with 225 ml of buffered peptone water. From pre-enriched culture, 1 ml of inoculum was transferred to 10 ml of selenite cysteine broth and thoroughly mixed for 2 minutes. Following mixing up, it was incubated at 37°C for 18-24 hours (WHO, 2003). A loop-full of inoculum from selenite cysteine broth was then streaked onto Xylose Lysine Deoxycholate Agar (XLD), which was then incubated at 37°C for 18-24 hours. Morphologically, typical red colonies with or without black centers were assumed to be presumptive *Salmonella* spp. [5].

2.4 Biochemical Characterization

Biochemical characterization of other Enterobacteriaceae including presumptive *Salmonella* spp., grown on XLD agar, were also done using Sulfur-Indole- Motility (SIM) agar, Triple Sugar Iron (TSI) agar, Lysine Iron (LI) agar, Simmons Citrate agar, and Urea agar. Before biochemical confirmation was done, the presumptive colonies from XLD agar were streaked to nutrient agar aseptically for purification purpose and incubated at 37°C for 24 hours. The pure cultures were then subjected to biochemical tests as described by [10]. Pure colonies were also transferred aseptically from Nutrient Agar (NA) to Tryptic Soya Agar (TSA) slants as stock cultures and stored in refrigerator at 4°C.

2.5 pH and Titratable acidity Determination

The pH of each juice sample was measured immediately using digital pH meter (Nig 333, Naina Solaris LTD, India). Five milliliters of the collected juice sample was aseptically taken and measured without dilution [11]. Standard method was used to measure titratable acidity [11]. The fruit juice sample (5ml) was homogenized in distilled water (20ml) and filtered through Whatman No.1 filter paper. Two- three drops of phenolphthalein was added to 20 ml of the filtrate as indicator and titrated against 0.1M NaOH to the end point of phenolphthalein. Titratable acidity was expressed as gram citric acid/100 ml of juice and calculated using the formula:

$$\text{Titratable acidity (TA)} = \frac{M \times V_1 \times \text{Eq. wt}}{V_2 \times 1000} \times 100$$

Where, M = molarity of NaOH, V1 = volume of NaOH (ml), Eq. wt. = Equivalent weight of citric acid (64), V2 = volume of juice sample (ml), 1000 = factor relating mg to grams (mg/g), and (1/10 = 100/1000)

2.6 Data Analysis

All the data were analyzed with SPSS version 16.0 for Windows software. The significance between the values was evaluated at 95% confidence level. Statistical significance was set at $p < 0.05$. The significance of any observed differences was determined using X^2 and Student's *t*-test. One-way ANOVA was used to determine bacterial mean differences of samples at each juice site. The results obtained for CFU/ml of juices were transformed into log values.

3. Results and Discussion

Failure to apply good hygienic practices during juice making leads to high microbial loads, thus, reducing the quality of freshly squeezed fruit juices. As shown in Figure 1, the quality of juice samples analyzed in the present study was poor as 58 (96.7%) were deemed above maximum permitted level of Gulf Standard for fruit juices (4 log CFU/ml). Only 2 (3.3%) of the samples were deemed below the standard. Since the time elapsed between preparing the juice and serving it to the consumer was not likely long enough to allow microbial growth, such high counts may be due to cross-contamination from improperly washed utensils or contaminated fruits [8, 12, 13]. Mango and pineapple juices contain sufficient nutrients for microbial growth, thus this may also support such a high bacterial load [14]. In addition, lack of potable water for washing and juice making may also contribute to high AMCs [8]. Unhygienic surroundings often with swarming houseflies, fruit flies and airborne dust in juice houses may contribute to microbial contaminations leading to high AMCs of fruit juices [6]. Thus proper inspection of juice houses should be the regular practice of concerned authorities.

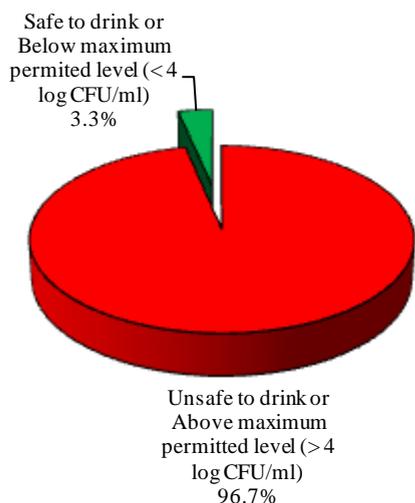


Figure 1 Comparison of aerobic mesophilic microbial counts of juices with Gulf Standard for fruit juices (2000)

As shown in Table 1, the mean aerobic mesophilic count (AMC) of mango juice was slightly higher than that of pineapple juice ($p = 0.02$). A difference may be attributed to relatively high pH and low titratable acidity of sample juices that support survival and growth of bacteria [11]. The mean aerobic mesophilic counts of juices were relatively lower than an earlier works [1] and higher than bacterial loads reported in another study [15]. The probable reason for the discrepancy may be temperature abuse, washing and juicing practices of fruits [15]. There was also statistical significant difference among the mean AMCs of pineapple juice ($p = 0.040$) across juice house. However, there was no statistical difference among the mean AMCs of mango juice against each juice house ($p = 0.875$). This implies that there is a difference in overall hygienic practice of juice houses at different juice houses in handling mango and pineapple juices [6].

In the present study, almost all juice samples were found to be contaminated with *Staphylococcus aureus*. This was in agreement with an earlier work done in Nagpur city, India [16]. Presence of *S. aureus* in fruit juices may be attributed to dirty clothing and contaminated hands of food handlers, which indicates lack of knowledge of hygienic practices and food safety [6, 13, 17]. Therefore, entry of *Staphylococcus aureus* in juices may be attributed to contact with the outer surface of fruits during fruit preparation and juicing process [8]. Training about hygiene during handling of juice is very important. Food handlers should have the necessary knowledge and skills to enable them to handle food hygienically [13].

As shown in Table 2, the mean SACs of mango and pineapple juice samples were within unsatisfactory range (3 log – 4 log CFU/ml) for human consumption [18]. Overall 46 (76.7%) juice samples were potentially hazardous to public health and contain SACs > 4 log CFU/ml. The other 10 (16.7%), 1 (1.6%), and 3 (5%) of the juice samples were unsatisfactory (3 log – 4 log CFU/ml), marginal (2 log – 3 log CFU/ml), and satisfactory (< 2 log CFU/ml), respectively (Figure 3). Such a high counts in majority of juice samples may cause staphylococcal food poisoning due to production of enterotoxins by coagulase positive *S. aureus* [13, 19]. With regard to sample sites, there was no any statistical significant difference among the mean SACs of pineapple juices ($p = 0.517$) and mango juices ($p = 0.374$) across each sample site. In addition, the mean SAC (3.84 log CFU/ml) of mango juice was not statistically significant from the mean SAC (3.74 log CFU/ml) of pineapple juice ($p = 0.670$). This assures that juice handling practices and food safety knowledge across each juice house of the study area was almost similar.

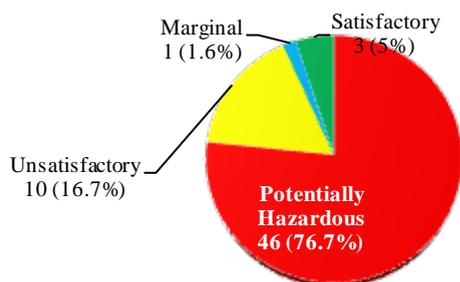
Figure 3 *Staphylococcus aureus* counts in sample juices against Food Standards Australia-New Zealand (2001)

Table 1 Mean aerobic mesophilic counts (AMCs) across each juice house

Juice house	Number of Samples taken	AMCs (log CFU/ml)		
		Mango juice	Pineapple juice	Mean
1	5	4.94	4.25	4.60
2	5	4.63	4.39	4.51
3	5	4.75	3.19	3.97
4	5	4.76	4.40	4.58
5	5	4.83	4.55	4.69
6	5	4.65	4.50	4.58
Mean	5	4.76	4.21	4.49
Stadev.	0.0	1.03	0.08	0.24

Table 2 Mean *Staphylococcus aureus* counts (SAC) counts across each juice house

Juice house	Number of Samples taken	AMCs (log CFU/ml)		
		Mango juice	Pineapple juice	Mean
1	5	2.98	3.54	3.26
2	5	4.28	4.40	4.34
3	5	4.15	3.29	3.72
4	5	3.74	3.54	3.64
5	5	4.16	4.17	4.17
6	5	3.71	3.51	3.61
Mean	5	3.84	3.74	3.79
Stadev.	0.0	0.41	1.04	0.36

Total coliform counts across juice houses and types of samples are shown in Table 3. Total coliforms were detected in 59 (98.3%) juice and 28 (93.3%) water samples. The highest prevalence of coliforms were recorded in mango juice (100%) followed by pineapple juice (96.7%) and water (93.3%), respectively. Total coliform counts were in the range of 9.2 - > 1100 MPN/ml in mango juice and < 3 - > 1100 MPN/ml in Pineapple juices. Total coliform counts in water samples were in the range of < 0.018 - > 16 MPN/ml. The number of coliforms in juice samples was relatively higher at juice house-2 than the other houses indicating that conditions may be favorable for pathogens to be present [20]. Of course, it doesn't mean that the presence of high number of total coliforms is always associated with presence of pathogens. Total coliforms were absent in one sample of pineapple at sample site 6 (*i.e.*, < 3). This may be attributed to the relative quality of water and fruits. Overall 17 (56.7%) pineapple and 23 (76.7%) mango juices had total coliform counts >100 MPN/ml which is maximum permitted level for any juice sold in the Gulf Region [20]. Mango juice was highly contaminated with total coliforms as compared with pineapple juice. This might be due to over handling and washing practices of mango [6]. In addition, fruits may be harvested using highly contaminated wastewater than pineapples [13]. Only three samples (10%) of water (*i.e.*, < 0.018 MPN/ml) were potable. Thus such high MPN/ml of coliforms of juices may probably come from contaminated water used for washing and juicing purpose [12].

Table 3 Total Coliform counts of samples across each juice house

Juice house	Number of Samples taken	Ranges of total coliforms (MPN/ml)		
		Mango juice	Pineapple juice	Water
1	5	23->1100	36->1100	<0.018-16
2	5	93->1100	43->1100	0.033->16
3	5	15->1100	20->1100	0.45->16
4	5	9.2->1100	11->1100	0.018->16
5	5	15->1100	36->1100	0.078->16
6	5	240->1100	<3->1100	0.34->16

The numbers of bacteria isolated from mango and pineapple juices are shown in Figure 6. Overall 35 samples were analyzed and all were found to be contaminated with different types of bacteria. *Citrobacter* spp. 16 (45.7%) was the dominant contaminant of juices followed by *Salmonella* spp. 7 (20%), *E. coli* spp. 5 (14.3%), *Enterobacter* spp. 4 (11.4%), *Klebsiella* spp. 2 (5.7%), and *Pseudomonas* sp. 1 (2.9%). The high contamination of *Citrobacter* spp. observed in this study is similar to that reported by [21]. The presence of *Salmonella* and *E. coli* in juices indicates possible risks of gastrointestinal infections from their consumption [13]. Thus consumption of freshly squeezed and unpasteurized juice requires special attention.

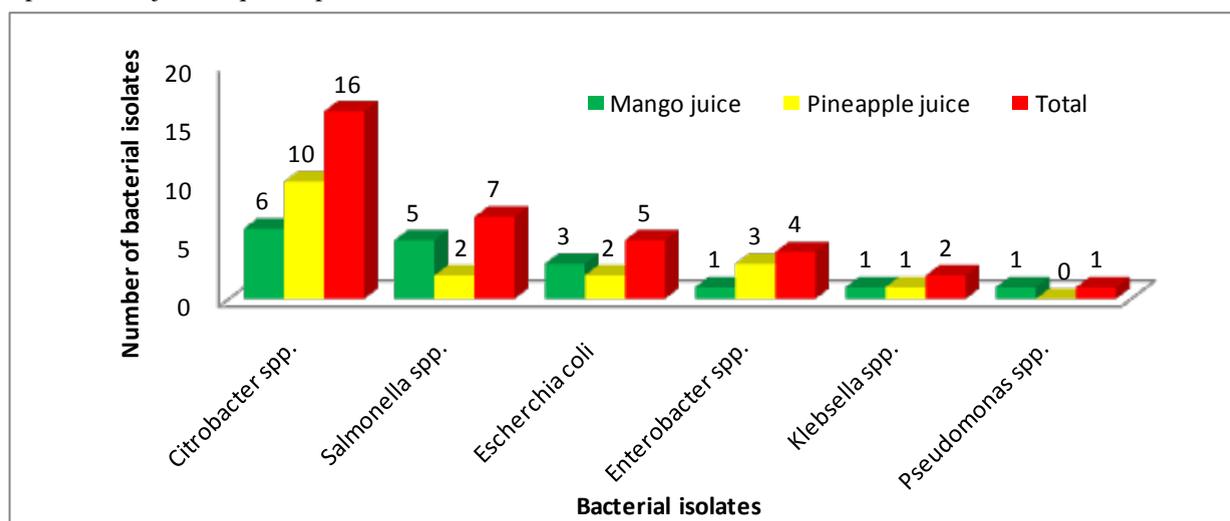


Figure 6 Bacterial isolates in mango and pineapple juices

It is possible that *Salmonella* may have gained entry through contaminated water with animal and human feces because vendors do not use boiled and potable water for washing and juicing process [8]. Sick juice handlers with *Salmonella* may also contribute to contaminations of foods including juices [22]. Presence of *Salmonella* and *E. coli* in low pH juices may be attributed to survival and growth of acid tolerant strains [23]. *Salmonella* serovars and *E. coli* O157:H7 seem to have genetic determinants that enable them to grow at higher acid concentrations (or lower pH) than other strains of the same species. The acid tolerance seems to be related to overproduction of a group of proteins (stress proteins) by these strains [13] indicating their presence in low pH mango and pineapple juices. The main source of *E. coli* contamination of juices might be through contaminated water supplies used to wash utensils or to dilute juices. The presence of *E. coli* and other coliform bacteria could be due to inadequate hand washing by juice handlers, poor processing practices, and unhygienic environment [6]. *Pseudomonas* spp. is environmental fruit contaminant and mainly comes from soil [13] indicating that washing practice of fruits was poor in juice houses.

pH and titratable acidity (TA) of mango and pineapple juices are presented in Table 4. The mean pH of mango juice is slightly higher than that of pineapple juice ($p = 0.04$). This may be attributed to differences in strengths of organic acids present in fruit juices or fruit maturity [11, 14]. However, there is no statistical significant difference between the mean TA of both juices ($p = 0.660$). pH can strongly influence the antimicrobial effect of an acid present in fruit juices. An acid is more inhibitory to growth of bacteria in fruit juices at a lower pH than in one at a higher pH [11],

thus maintaining good quality of mango and pineapple juices. The buffering action of the juice components also reduces the antimicrobial effectiveness of low pH [13]. The total acidity (TA) of fruit juices is due to the presence of a mixture of organic acids, whose composition varies depending on fruit nature and maturity conferring individual originality between freshly squeezed fruit juices [14]. Microorganisms differ in their sensitivity to different organic acids. Bacteria are more sensitive to citric acid [13] than any other organic acids present in fruit juices.

Table 4 pH and Titratable acidity (TA) of juice samples

Type of juice	Number of Samples taken	pH	TA
Mango	30	4.61±0.42	0.182±0.164
Pineapple	30	4.26±0.44	0.168±0.046

Where, TA = Titratable acidity (ml citric acid/100 ml of fruit juice)

The socio-demographic profile of respondents is presented in Table 5. Altogether there were 30 respondents and all of them agreed to participate in the present study so the response rate was 100%. The mean age of respondents was 20±4. About 70% of the respondents were aged between 19 - 30 years with median age of 19 years. Majority of workers in juice houses were females (93.3%) and were educated up to secondary school level. Females with at least educational attainments of high school level and less than the age of 40 practice safer food preparation and handling [24]. Only 2(6.7%) respondents had undergone pre-placement training for food handling indicating that professional training takes its own part to reduce food borne illnesses [13].

Table 5 Socio-demographic profile of respondents (n = 30)

Variables	Frequency	Percentage
Sex		
Female	28	93.3
Male	2	6.7
Age		
≤18 years	7	23.3
19-30 years	21	70.0
≥31 years	2	6.7
Educational attainment		
No schooling	2	6.7
Elementary (1-8)	9	30.0
Secondary school (9-10)	13	43.3
Preparatory school (11-12)	2	6.7
Diploma and above	4	13.3
Professional food handling training		
Trained	2	6.7
Not trained	28	93.3

Respondents of a questionnaire were asked how often equipments have been washed and their answers are presented in Figure 9. Majority of respondents stated that all the equipment have been washed after each use. However, observation reveals that juice machines, cutting boards and knives were not frequently washed before each fruit preparation or juicing process. Cross-contamination can be avoided if utensils or equipment is washed with detergents and water in between using it for raw fruits and ready-to-eat juices [25].

Majority of respondents reported that fruits were purchased from open market retailers and temporarily stored in shelf (Table 6). But, observation reveals that majority of juice vending houses store fruits outside in a condition that is exposed to temperature abuse and dust. This may contribute to rapid growth of contaminant microbes in fruits. If the washing practice of these fruits is poor, microbes may get entry during juice making process [6]. The use of good quality raw fruit is also essential to the production of a high quality fruit juice of low microbial count. According to [19], juices produced from soft rot fruit contains many times the total bacteria number found in juice from sound fruit. Thus fruits purchased for juice making should be safe and stored in refrigerators.

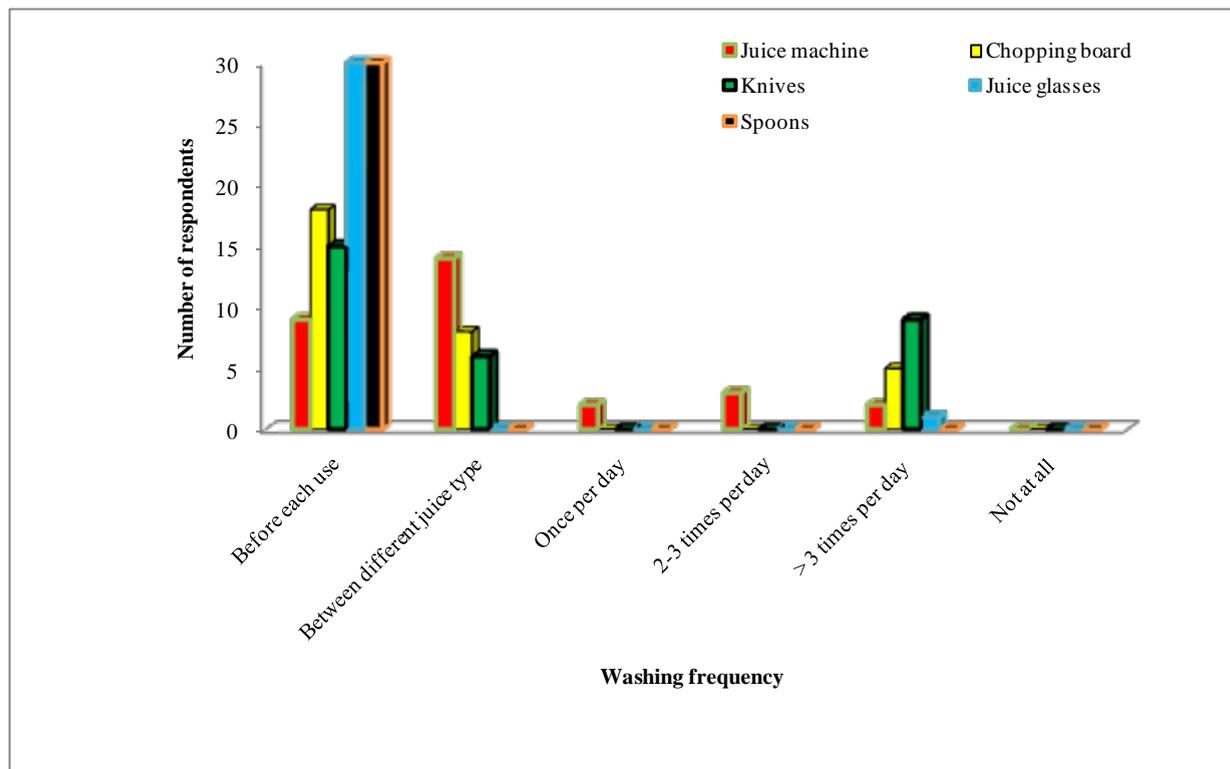


Figure 9 Washing frequency of equipment in juice houses (n =30)

Table 6 Source of fruits and their temporary storage prior to juicing (n = 30)

Variable	Frequency	Percentage
Source of fruits		
Open market retailer	20	66.7
Directly from producers	7	23.3
Whole seller	3	10.0
Temporary storage of fruits		
Shelf	27	90.0
Basket	1	3.3
Refrigerator	2	6.7

When questioned on fruit preparation prior to juicing, the majority of juice houses appeared to prepare the fruits not in a safe manner. Fruit preparation and washing practice prior to juice making is presented in Table 7. Respondents of a questionnaire were asked if they wash fruits at the time of juicing and 18 (60%) reported that they wash fruits early. The remaining 12 (40%) reported that they wash fruits at the time of juicing. The majority of the respondents (60%) reported that fruits have been rinsed with water in container with scrubbed surface. However, fruits products should be washed under cold running tape water before preparation or consumption to reduce or remove microorganisms [26]. Although the practice of not washing the fruits is not desirable, the bacteriological results did not reflect the absence of washing in the present study. The samples that contain coliforms may be attributed to preparation and washing practices of fruits [12]. Preparation of the fruits depended on the fruit type. Juice handlers were asked how they prepare fruits and all of them peeled and cut mango and pineapple fruits with knives before being juiced. Twenty two respondents (73.3%) reported that fruits were peeled and cut at the time of juicing. The remaining 8 respondents (26.7%) reported that fruits used for juice making were peeled early and stored in refrigerator. Mechanical way of fruit peeling and cutting practice in juice houses may contribute access of microbes to fruits and juices [25]. The high contamination of mango and pineapple juices could partly be linked to their high demand, and thus the fruits are peeled and exposed to contaminations well before the juices are prepared.

Table 7 Fruit preparation and washing practice prior to juice making (n = 30)

Variables	Frequency	Percentage
Time of washing fruits		
Washed early	18	60.0
Washed at the time of juicing	12	40.0
Washing practices of fruits		
Rinse with water without scrubbing surface	8	26.7
Scrub surface with hand and cleaned with water	22	73.3
Preparation of fruits prior to juicing		
Peeled and cut with knife	30	100
Time of fruit peeling		
Peeled early	8	26.7
On time	22	73.3

As shown in Table 8, the juicing practice of fruits in juice houses was appeared to be unsafe with regard to source of water and temporary storage of juice. All respondents were involved in stating the practices of juice making process and temporary storage of juices in each juice houses. A total of 23 (76.3%) respondents reported that tap water from containers was used for juice making. The remaining 7 (23.3%) respondents reported that source of water for juicing was running tap water in juice preparation room. But, none of them reported that well water was used for juice making in any of the juice houses. Water quality can greatly influence microbial quality of freshly squeezed fruit juices [5]. Thus the presence of high coliforms in water samples in present study is an indication of contaminations. Water used for juice making, washing fruits, and equipments must be potable [25]. With regard to temporary storage of juices, 24 (80%) respondents reported that juices were prepared for immediate use. But, 6 (20%) respondents reported that juices were prepared in bulk and stored in refrigerator for that same day's use. None of the respondents reported juices were prepared for more than a day of their preparation. Prolonged preservation of juices without refrigeration may create favorable condition for microbial growth [12].

Table 8 Juice making practice and its temporary storage (n = 30)

Variables	Frequency	Percentage
Source of water for juice making		
Running tap water	7	23.3
Tap water in container	23	76.7
Temporary storage of juice after preparation		
Prepared for immediate use	24	80.0
In bulk and stored for that same day's use	6	20.0

In the present study, few of the food handlers observed minimal personal hygiene during fruit preparation, juicing and serving it to consumers. As shown in Table 9, 5 (21.7%) respondents reported that they wear apron while juicing or serving juices to customers. However, 18 (78.3%) respondents reported that they would not wear apron. Respondents that wear apron were further questioned on frequency of changing their aprons. Among these, 3 (60%) of them reported that they change their aprons once per day. The other 2 (40%) respondents reported that they change their aprons once per week. Wearing apron during fruit preparation, juicing, and serving may protect bacterial contamination of juices [26]. Majority of the respondents did not wash their hands while juice making or serving to consumers. Among 8 (34.8%) of the total respondents, 5 (62.5%) reported that they wash their hands with water. But, 3 (37.5%) of them reported that they wash their hands with water and soap. Hand washing practice of food handlers is in agreement with a study reported by [27].

During food preparation pathogenic organisms may be transferred to juice by the handler both directly or by cross contamination through hands, hair, hand jewelries and dirt from fingernails that have been inadequately cleaned [27]. Thus it was important to know how the juice makers and waiters acquired their juicing and serving skills to establish their knowledge in handling juice safely. Twenty three juice handlers (15 waiters and 8 juice makers) in six juice sites were observed with regard to their personal hygiene while serving their customers

Table 9 Practice of juice handlers while juicing/serving (n = 23)

Variables	Frequency	Percentage
Wear apron while juice making/serving		
Yes	5	21.7
No	18	78.3
Frequency of changing apron		
Once per day	3	60.0
Once per week	2	40.0
Touch body parts while juicing/serving		
Yes	8	34.8
No	15	65.2
Wash hands with		
Water	5	62.5
Water and soap	3	37.5

As shown in Table 10, majority of juice handlers did not cover their hair, wear clean apron, and wash their hands while juicing and serving. Fourteen (60.9%) juice handlers wore hand jewelries. However, 9 (39.1%) of them did not. Fifteen (65.2%) of juice handlers cut their fingernails short. But, 8 (34.8%) of did not cut their fingers short. There were statistical significant differences in three personal hygiene parameters (hair, apron, and hand washing). However, there were no any associations in two parameters (hand jewelries and fingernails). Food may be contaminated from hair, hand jewelries, unclean apron, hands, and dirty fingernails [26]. Thus proper training on food handling is important to reduce food borne illnesses.

Table 10 Personal hygiene of juice handlers in juice houses (n = 30)

Personal hygiene	Frequency		P value
	Yes	No	
Hair covered	2 (8.7%)	21 (91.3%)	0.00
Wear clean apron	6 (26.1%)	17 (73.9%)	0.02
Wear hand jewelries	9 (39.1%)	14 (60.9%)	0.30
Wash hands while serving customers	3 (1.3%)	20 (98.7%)	0.00
Cut fingernails short	15 (65.2%)	8 (34.8%)	0.30

4. Conclusion

The study revealed that freshly squeezed mango and pineapple juices were unsafe for human consumption. Majority of aerobic mesophilic counts, total coliforms and *Staphylococcus aureus* counts of juice samples were not within the limits of Gulf and Australia-New Zealand Food Standards. The overall sanitary condition of juice houses and knowledge of food handlers with regard to safe juice handling practices were poor across each juice house. Pathogenic bacteria may survive and grow in low acid fruit juices due to adaptation of acidic environments. This indicates the possibility of food borne outbreaks associated with consumption of freshly squeezed mango and pineapple juices. It is clear from both epidemiological and laboratory investigations that pathogenic organisms may be present in fruit juices including those with a low pH. Although food borne illness associated with the consumption of these products is rarely reported in Ethiopia, juice vendors that produce freshly squeezed fruit juices should be aware that preventative measures through food safety control strategies is, in general, important. Further research work is recommended on characterization of coagulase positive *Staphylococcus aureus* and identification of other pathogens in fruit juices.

5. Acknowledgements

My special gratitude and appreciation goes to Dr. Mulugeta Kibret for his expert guidance, enthusiasm and support during preparation of this manuscript. My thanks also goes to microbiology lab assistants, data collectors, respondents of a questionnaire, and owners of juice houses who were unpaid helpers for the present study. Last but not least, I would like to thank my parents Genet Shiferaw and Leul Mengistu for their continuous financial and material support during my investigation.

6. References

- [1]Tsigie, K., TsegayE, G., Ketema, B. Microbiological safety of fruit juices served incafes/restaurants. *Ethiopian Journal of Health Science*, 18(3), pp. 96 – 100, (2008).
- [2]Suaads, A., Eman, A.H. Microbial growth and chemical analysis of Bottled fruit juices and drinks in Riyadh, Saudi Arabia. *Research Journal of Microbiology*, 3, pp. 315-325, (2008).
- [3]Kurowska, E.M., Spence, J.D., Jordan, J., Wetmore, S., Freeman, D.J., Piche, L.A., Serratore, P.H.D.L. Cholesterol-raising effect of orange juice in subjects with hypercholesterolemia. *American Journal of Clinical Nutrition*, 72, pp. 1095–1100, (2000).
- [4]Al-Jedah, J.H., Robinson, R.K. Nutritional Value and Microbiological Safety of Fresh Fruit Juices sold through Retail Outlets in Qatar. *Pakistan Journal of Nutrition*, 1, pp. 79 – 81, (2002).
- [5]Mahale, P.D., Khade, R.G ., Vaidya, V.K. Microbiological Analysis of Street Vended Fruit Juices from Mumbai City, India. *Internet Journal of Food Safety*, 10, pp. 31-34, (2008).
- [6]Tambekar, D.H., Murhekar, S.M., Dhanorkar, D.V., Gulhane, P.B., Dudhane, M.N. Quality and safety of street vended fruit juices: a case study of Amravati city, India. *Journal of Applied Bioscience*, 14, pp.782 – 787, (2009).
- [7]Doyle, M.P., Beuchat, L.R., Montville, T.J. (2001). *Food Microbiology*. American Society for Microbiology, ASMPress, WashingtonDC, USA. Available: <http://www.gettextbooks.com/search/?isbn=+food%20+microbiology%20+doyle> [May 11, 2011].
- [8]Ahmed, M.S.U., Nasreen, T., Ferosa, B., Parveen S. Microbiological Quality of Local Market Vended Freshly Squeezed Fruit Juices in Dhaka City, Bangladesh. *Bangladesh Journal of Scientific and Industrial Research*, 44(4), pp. 421-424, (2009).
- [9]Reddy, U.B., Chandrakanth, N., Priya, I.S., Nagalakshmi, V.R., Usha, K.B. Isolation and Characterization of fecal coliforms in street vended fruit juices and its safety evaluation: A case study of Bellary city, India. *Internet Journal of Food Safety*, pp. 11, 35-43, (2009).
- [10]Mudgil, S., Aggarwal, D., Ganguli, A. Microbiological analysis of street vended fresh squeezed carrot and kinnow- mandarin juices in Patiala City, India. *Internet Journal of Food Safety*, pp.3, 1-3, (2004).
- [11]Sadler, G.D., Murphy, P.A. *Food Analysis: pH and Titratable acidity* (4th ed.). Purdue University, West Lafayette, USA, 2010, p. 219.
- [12]Lewis, J.E., Thompson, P., RAO, B., Kalavati, C., Rajanna, B. Human bacteria in street vended fruit juices: a case study of Visakhapatnam city, India. *Internet Journal of Food Safety*, 8, pp. 35-38, (2006).
- [13]Ray, B., Bhunia, A. *Fundamentals of Food Microbiology* (4th ed.) Taylor and Francis Group, LLC, Boca Raton, USA. 2008, pp. 40-41.
- [14]Tasnim, F., Anwar, H.M., Nusrath, S., Kamal, H.M., Lopa, D., Formuzul, H.K.M. 2010. Quality Assessment of Industrially Processed Fruit Juices Available in Dhaka City, Bangladesh. *Malaysian Journal of Nutrition*, 16(3), pp. 431-438, (2010).
- [15]Mukhopadhyay, M., Basu, M.M.M. Microbial Contamination of Street vended Fruit Juices In Kolkata City, India. *Internet Journal of Food Safety*, pp. 13, 1-5, (2011).
- [16]Bagde, N.I., Tumane, P.M. Studies on microbial flora of fruit juices and cold drinks. *Asiatic Journal of Biotechnology Research*, 2 (4), pp.454-460, (2011).
- [17]Titarmare, A., Dabholkar, P., Godbole, S. Bacteriological Analysis of Street Vended Fresh Fruit and Vegetable Juices in Nagpur City, India. *Internet Journal of Food Safety*, 11, pp. 1-3, (2009).
- [18]Food Standards Australia-New Zealand. (2001). *Microbiological guidelines for ready to eat foods*. Australia.
- [19]Jay, J.M. *Modern Food Microbiology* (6th ed.). New York: Aspen Publishers, USA, 2005, p. 388.
- [20]Gulf Standards. (2000). *Microbiological Criteria for Foodstuffs – Part 1*. GCC, Riyadh, Saudi Arabia.

- [21]Mesfin, W. Bacteriological profile of locally prepared fresh juices in Hawassa town. Intenet: [www.http://etd.aau.edu.et/dspace/handle/123456789/2873](http://etd.aau.edu.et/dspace/handle/123456789/2873), June25, 2011[July 7, 2011].
- [22]Bayeh , A., Fantahun, B., Belay, B. Prevalence of *Salmonella typhi* and intestinal parasites among food handlers in Bahir Dar Town, Northwest Ethiopia. *Ethiopian Journal of Health Development*, 24(1), pp. 46-50, (2010).
- [23]Yuk, H.G., Schneider, K.R. Adaptation of *Salmonella* sp. in juice stored under refrigerated and room temperature enhances acid resistance to simulated gastric acid. *Food Microbiology*, 23, pp. 694–700, (2006)..
- [24]Klontz, K.C., Timbo B., Feins, S., Vevy, A. Prevalence of selected food consumption and preparation behaviors associated with increased risks of food borne diseases. *Journal of Food Protection*, 58, pp. 927-930, (1995).
- [25]Victorian Government Department Of Human Services, Food Safety Unit. 2005. Microbiological survey of freshly squeezed juices from retail businesses across Victoria, Melbourne, Australia.
- [26]Mekonnen, H., Habtamu, T., Kelali, A. Source(s) of contamination of ‘raw’ and ‘ready-to-eat’ foods and their public health risks in Mekelle City, Ethiopia. *Journal of Food and Agriculture Science*, 2(2), pp. 20-29, (2012).
- [27]Mulugeta, K., Bayeh, A. The sanitary conditions of food service establishments and food safety knowledge and food handling practices of food handlers in Bahir Dar town. *Ethiopian Journal of Health Science*, 22(1), pp. 27-35, (2012).