

Pathogenic Bacterial Species in Locally Prepared Fresh Fruit Juices Sold in Juice Houses of Eastern Ethiopia

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ABSTRACT: Fruit juices are important components of a healthy diet and a dietary source of nutrients, vitamins, and fiber and vital for human health. However, unless it is handled with safety and hygienic conditions, food can be a vehicle for the transmission of various agents of diseases resulting in food borne outbreaks. Thus, this study aimed to determine common pathogenic bacterial species in locally prepared fresh fruit juices sold in juice houses of Eastern Ethiopia. A cross-sectional study design was conducted from 1 January to 27 March 2020, in eastern Ethiopia. Seventy-eight juice samples were collected aseptically using a sterilized collecting jar from each juice house. Pour plate count method was used to determine *Staphylococcus*, *Salmonella*, and *Shigella* species. Finally, the data were analyzed using descriptive statistical tests such as Chi-square and Fisher's exact tests. *P*-value of .05 was considered as a cut point for statistical significance. The study found *Staphylococcus* count ranged from 1.68 log CFU/mL with the mean value of 4.204 log CFU/mL. Overall, 58 (74.4%) of the fruit juice samples had *Staphylococcus* count, 19 (24.4%) had *Salmonella* and 12 (15.4%) had *Shigella* higher than the maximum permitted limit of Gulf standard, 2000 set for any type of fruit juice. In general, the study found more than two-thirds of fruit juice samples had at least 1 pathogenic bacteria species higher than the maximum permitted limit and potentially hazardous to consumer health. Thus, regular supervision and application of food hygiene and safety are essential to improve the quality of fruit juice and to prevent the consumption of contaminated fruit juices, which leads to food borne illness.

KEYWORDS: Bacteriological quality, food quality, food safety, food science, fruit juices

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Background

Fruit juices are defined as unfermented but fermentable liquids obtained from the edible parts of appropriately mature fresh fruits maintained in sound condition by suitable means.^{1,2} Fruit juices are among the food products of great nutritional value, rich in vitamins (vitamins C and E), mineral salts, simple sugars, organic acids, and antioxidants, which are easily assimilated by human and important for human health.^{3–5}

Furthermore, fruit juice contains several important therapeutic properties that may reduce the risk of various diseases such as stroke, diabetes, heart disease, loss of bone, and neural tube defects like spina bifida, anencephaly during fetal development.⁶ Currently, fruit juices constitute a suitable and convenient meal for today's lifestyle because they need no further preparation, they are low in calorie, are rich in fiber, and provide a great variety of vitamins, minerals, and other natural phytochemicals.⁷

However, unless it is handled with safety and good hygienic conditions, food can be a vehicle for the transmission of various agents of diseases resulting in an outbreak of foodborne disease across the globe.⁸ Because, inadequate hygienic condition makes the product a good medium for the growth of microorganisms.⁹ Various studies showed that fruit juice may be a potential source of pathogenic bacteria like *E. coli* (*E. coli* O157:H7), *Salmonella* species, *Shigella* species, and *Staphylococcus aureus*,^{4,10}

Enterobacter species, *Klebsiella* and *Serratia* species,¹¹ and *Listeria monocytogenes*.¹² The most common pathogenic bacterial species that contribute to foodborne outbreaks in un-pasteurized juice include *Escherichia coli* O157:H7, *Salmonella* species and *Cryptosporidium*.¹³

Nowadays, foodborne disease is an international public health problem resulting in potential health and economic impacts. In response to these problems, health, and other concerned organizations are increasing their efforts to improve the quality and safety of fruit and their products.¹¹

However, in developing countries including in Ethiopia, there is no continuous assessment of food quality and safety where fresh fruit juices are prepared and sold.¹⁴ Most of the fruit juices being served to consumers had higher microbial loads than the specification set for fruit juices in some parts of the world, and these products were thought to be the cause of health problems.¹⁵

In Eastern Ethiopia, there was no adequate information existing on the prevalence of pathogenic bacterial species in fresh fruit juices. Availability of adequate and current evidence on pathogenic bacterial species is crucial for evaluating the quality of fruit juices and to protect consumer health. Thus, this study aimed to determine selected common pathogenic bacterial species in locally prepared fresh fruit juices sold in juice houses of Eastern Ethiopia.



Materials and Methods

Description of the study area and period

A cross-sectional study was conducted to determine the bacteriological quality and public health risk of locally prepared fresh fruit juices sold in juice houses of Eastern Ethiopia, particularly in Dire Dawa, Harar, and Jigjiga towns from 1 January to 27 March 2020. Dire Dawa, Harar, and Jigjiga towns are found at 520, 525, and 619 km, respectively, from Addis Ababa, the capital city of Ethiopia. Currently, these towns are trade centers for fruits, vegetables, and their products.

Study variables

Dependent variable. Bacteriological quality of locally prepared fruit juices

Independent variables. Socio-demographic characteristics of the food handlers (age, gender, year of service, educational status, training in food hygiene and safety); juice house hygiene conditions (latrine facilities, waste disposal receptacles, dish washing facilities, and hand washing facilities); personal hygiene and safety practices (hand washing habits, clothing/gown/head cover, washing utensils and storage condition of juice); and awareness and knowledge of juice makers.

Sample size and sampling technique

A total of 78 juice samples were collected from 26 juice houses aseptically and analyzed to determine the prevalence and bacterial status of fruit juice samples. From each juice house, the 3 most commonly consumed locally prepared fruit juices (mango, avocado, papaya, and mixed juice) were selected randomly after proportional allocation of the required juice samples to the study sites. At the same time, a total of 78 food handlers were selected randomly and interviewed to collect the required data, particularly on the sociodemographic characteristics and hygiene conditions. Similarly, 78 water samples were collected and analyzed to determine the quality of water used for diluting juice and washing utensils, following the standard procedures used for bacteriological water quality analysis.

Data collection for face-to-face interview

The data related to socio-demographic characteristics and hygiene conditions were collected using a pretested semi-structured questionnaire and an observational checklist. The questionnaire contains 3 sections (socio-demographic; hygienic practice; hygiene; and sanitation facilities). Initially, the questionnaire and checklist were prepared in English version. However, the questionnaire was translated to the local language of the study participants (Afan, Oromo, Amharic, and Somali versions).

Sample collection and processing for bacteriological analysis

Two hundred fifty milliliters of juice sample was collected aseptically from juice storage where the fruit juice was directly given to the consumers. Each fruit juice samples were labeled accordingly to minimize the error. Serial dilutions of 3 folds (10^{-1} , 10^{-2} , and 10^{-3}) were done based on ISO 6887-1:1999 protocols.¹⁶ Then, triplicate diluted samples (3 plates or 3 tubes for each dilution) were used for each set of serial dilution (10^{-1} , 10^{-2} , and 10^{-3}) to determine *Salmonella*, *Shigella*, and *Staphylococcus*.¹⁶ Each procedure was done under ISO 7218.¹⁷

Bacteriological analysis of the samples

Locally prepared juice samples were analyzed for each of the following microorganisms: *Salmonella* species, *Shigella*, and *Staphylococcus aureus*. Each bacteriological analysis of a fruit juice sample was done using appropriate media designed for the identification and/or enumeration of selected bacteria species, following standard procedures. At the same time, quality of water used in the juice house was determined following the standard procedures used for bacteriological water quality analysis.

Detection of Salmonella species. The determination of *Salmonella* species in locally prepared fresh fruit juice samples was done using ISO 6579:2002(E) protocol.¹⁸ Initially, the sample was pre-enriched in Buffered Peptone Water (BPW), then incubated at 34°C to 38°C for 18 hours followed by enrichment in/on selective media using Rappaport-Vassiliadis medium with soya (RVS broth) broth and Muller-Kauffmann tetrathionate broth (MKTT broth). The RVS broth was then incubated at 41.5°C for 24 hours while the MKTT broth was incubated at 37°C for 24 hours. From the cultures obtained, both enriched fruit juice samples were differently plated onto Xylose Lysine Deoxycholate (XLD) agar (selective solid media) and incubated at 37°C and observed after 24 hours. Then, each culture was observed for the presence of typical colonies of *Salmonella* with a black center and a light transparent zone of reddish color. Finally, biochemical tests were done for the confirmation of *Salmonella* using triple sugar iron inoculation on urea agar, and indole test. The formation of a red ring within 10 minutes was considered as a positive reaction for indole tests whereas a yellow/brown color was considered as a negative reaction.¹⁸

Detection of Shigella species. The determination of *Shigella* species in locally prepared fresh fruit juice samples was done using ISO 21567:2004(E) protocol.¹⁹ The sample was inoculated into broth, then incubated at 41.5°C for 16 for 20 hours (enrichment in selective liquid medium). Then, from the enrichment culture, 3 medias such MacConkey agar, XLD agar, and Hektoen enteric agar were inoculated and all media were incubated at 37°C for 20 to 24 hours. Finally, a typical and

suspected colony from each of the 3 agars were selected and purified on nutrient agar and incubated at 37°C for 18 to 24 hours to gain well-isolated colonies. Then, biochemical tests were done using Triple Sugar Iron (TSI) agar at 37°C for 24 hours (for H₂S and gas formation), semisolid nutrient agar (for motility tests) at 37°C for 18 to 24 hours, indole test at 37°C for 24 hours, and Urea agar at 37°C for 24 hours.¹⁹

Determination of *Staphylococcus aureus*. The determination of *Staphylococcus aureus* in locally prepared fresh fruit juice samples was done using ISO 6888-1:1999(E) protocol.²⁰ Surface of a solid selective culture medium was inoculated into triplicate plates with 0.1 mL of the test sample for each decimal dilution. The plates were incubated in an inverted position at 37°C and observed after 24 and 48 hours. The confirmation test (coagulase test) was done using brain-heart infusion broth (BHI) (at 37°C for 24 hours incubation) and rabbit plasma (at 37°C for 4-24 hours). Then, the clot occupying more than half of the original volume of the liquid was considered as a positive coagulase test.²⁰ The result was calculated as:

$$N = \frac{\sum a}{V (n_1 + 0.1 n_2) d}$$

$\sum a$ = the sum of the colonies identified on all plates.

V = the volume of inoculum on each plate in milliliters.

n_1 = the number of plates selected at the first dilution.

n_2 = the number of dishes selected at the second dilution.

d = the dilution rate corresponding to the first dilution.

The total number of microorganisms enumerated per mL of the sample was calculated using the number of colonies obtained from each plate. Finally, the results were presented as the number of positive *Staphylococcus* counts per mL and reported as log CFU/mL.

Physicochemical and water quality analysis

pH and temperature of juice sample were measured using portable digital pH and thermometer meter, respectively. Similarly, the quality of the water used for washing the utensils and preparing the juice were analyzed. For *Salmonella* and *Shigella* identification, 100 mL of water samples was filtered through 0.45 µm cellulose acetate membrane filter and the filter was dipped in sterile 90 mL buffered peptone water and incubated at 37°C for 18 hours. After incubation, enriched broth (BPW) was inoculated to selective enrichment media. Then, 0.1 mL of enrichment broth was added into 10 mL of Rappaport Vassiliadis soya peptone broth and incubated at 41.5°C for 24 hours and then, 1 mL of enrichment broth was inoculated into 10 mL of Selenite cystine broth and incubated at 37°C for 24 hours. After the selective enrichment, colonies appeared on the disk was isolated onto *Salmonella-Shigella* agar (SSA) and Xylose lysine deoxycholate agar (XLD) following incubation at 37°C for 24 hours. Suspected colonies of *Salmonella* spp. and *Shigella* spp. were identified based on colony appearances.^{21,22}

Data quality control

Before data collection, the questionnaire and observational checklist were pretested to check its clarity, sequence, and applicability. To minimize the error, the consistency of the procedure was kept while conducting the bacteriological analysis throughout the study. The bacteriological analysis was done by well-qualified and trained professionals. The samples were analyzed immediately to avoid a significant change. Furthermore, the aseptic technique was used throughout sampling, handling, and analyzing. Additionally, the control was used for each bacterial analysis to determine the quality of laboratory work or analysis. Triplicate samples were analyzed to confirm the contamination levels. Sterilization was done using an autoclave at 121°C for 15 minutes to sterilize the media and some equipments.²³ Alcohol based disinfectant (70% ethanol) was used to disinfect some materials and working environment.

Data processing and analysis

Each measurement of the different variables was systematically organized into tables and subsequently subjected to statistical analysis. Data analysis was done using SPSS version 22.0 statistical software. The data were analyzed using descriptive statistics such as mean and range. Chi-square and Fisher's exact tests were used to determine the statistically significant association among the variables. The data did not fulfill Chi-square test assumption were analyzed using Fisher's exact test. P -value of .05 was considered as a cut point for statistical significance.

Results

Characteristics of the respondents

Of the 78 participants interviewed, 54 (69.2%) were females, and 38 (48.7%) of the study participants were within the age ranging from 25 to 34 years and constituted the largest proportion. In addition, the study found that 71 (91.0%) of the respondents were not trained in food hygiene and safety. Thirty-one (39.7%) of the participants had attended secondary school while 38 (48.7%) had age ranging from 25 to 34 years old. On the other hand, 41 (52.6%) respondents had work experience ranging from 1 to 2 years. Furthermore, the current study found a significant association between bacterial contamination of juice (*Staphylococcus* and *Salmonella* or *Shigella*) and educational status, and training in food hygiene and safety (Table 1).

Hygienic condition of juice houses

The current study found 41 (52.6%) of food handlers always washed their hands with water and soap, and 65 (83.3%) of food handlers did not wear aprons/uniform. Furthermore, the study found a statistically significant association between contamination of juice with *Staphylococcus* and hygienic and safety conditions of food handlers (Table 2).

Table 1. Overall characteristics of food handlers working in juice houses and its association with bacterial contamination of juice 2020 (N=78).

VARIABLES	PARAMETERS	FREQUENCY (%)	SC	SS
			χ^2 (P-VALUE)	χ^2 (P-VALUE)
Educational status of respondents	Non formal	10 (12.8)	22.287 (.001) ^a	7.323 (.037) ^a
	Primary	24 (30.8)		
	Secondary	31 (39.7)		
	College/diploma	12 (15.4)		
	Degree and above	1 (1.3)		
Training on food safety	Yes	7 (9.0)	15.736 (.000) ^a	4.486 (.034) ^a
	No	71 (91.0)		
Year of service	<1	23 (29.5)	3.452 (.466)	0.157 (1.000)
	1-2	41 (52.6)		
	>2	14 (17.9)		
Wearing aprons while serving juice	Yes	13 (16.7)	17.155 (.000) ^a	0.348 (.555)
	No	65 (83.3)		

Abbreviations: SC, *Staphylococcus*; SS, *Salmonella* and *Shigella*.

^aStatistically significant.

Physicochemical analysis of juice and water quality analysis

pH: The mean value of pH among the analyzed juice samples was 4.515 ± 0.63 (SD), whereas the mean value of temperature among the juice samples was 12.08 ± 2.63 (SD). *Salmonella/Shigella* was detected only in 9 (11.5%) water samples. On the other hand, the study found that there was no any statistically significant association between contamination of fruit juice and pH ($\chi^2=1.422$ [P value=.233]), and temperature of juice samples ($\chi^2=0.24$ [P value=.8]). However, there was statistically significant association between quality of water used and fruit juice contamination, in terms of *Salmonella/Shigella* ($\chi^2=9.63$ and P value=.003) (Table 3).

Bacteriological analysis of samples

Bacteriological status of fruit juice samples was done based on the bacterial load in triplicate of each plate (10^{-1} , 10^{-2} , and 10^{-3} serial dilutions for each juice sample) and later the mean value was taken particularly for *Staphylococcus*. Furthermore, *Shigella* and *Salmonella* species were reported as detected and not detected, and was evaluated toward Gulf standard 2000 (Table 4).

The overall total *Staphylococcus* count ranged from 1.68 to 4.94 log CFU/mL with a mean value of 4.204 log CFU/mL. The study found *Staphylococcus* count ranged from 1.91 to 4.785 log CFU/mL in mango juice, 2.40 to 4.94 log CFU/mL in avocado juice, 1.99 to 4.92 log CFU/mL in papaya juice and 1.68 to 4.93 log CFU/mL in mixed juice (Table 5).

The current study found 58 (74.4%) of fruit juice samples contaminated with *Staphylococcus*, higher than the maximum permitted level of Gulf standard, 2000 (3log CFU/mL) whereas 5 (6.4%) and 15 (19.2%) were satisfactory and acceptable, respectively. The acceptability distributions of *Staphylococcus* based on the types of juice is presented below (Figure 1).

Salmonella and Shigella. The current study found 19 (24.4%) and 12 (15.4%) of fruit juice samples contaminated with *Salmonella* and *Shigella*, respectively, and unsatisfactory or potentially hazardous to human health. In general, 7 (30.4%) and 5 (21.7%) of mango juice samples were unsatisfactory in terms of *Salmonella and Shigella*, respectively. And also, 4 (18.2%) and 3 (13.64%) of avocado juice samples were unsatisfactory in terms of *Salmonella and Shigella*, respectively (Figure 2).

Discussion

Socio-demographic characteristics of the respondents

Seventy-eight fruit juice samples were collected from juice houses, found in 3 towns of Eastern Ethiopia and analyzed to determine *Staphylococcus*, *Salmonella*, and *Shigella*. Of the 78 participants interviewed, 54 (69.2%) were females. This study agreed with another study conducted in Mozambique reported a higher proportion 116 (79.5%) of female workers.²⁵ This may be due to cultural related issues in developing countries, particularly in Ethiopia, males do not participate in food preparation. In addition, the study found 71 (91.0%) respondents had no training in food hygiene and safety while the study

Table 2. General hygienic and safety conditions of food handlers and juice houses in Eastern Ethiopia, 2020.

VARIABLES	PARAMETERS	FREQUENCY (%)	SC	SS
			χ^2 (P-VALUE)	χ^2 (P-VALUE)
How do you wash your hands (N=78)	With water only	37 (47.4)	9.265 (.044) ^a	1.102 (.294)
	With water and soap	41 (52.6)		
Aware that microorganisms can contaminate food (N=78)	Yes	59 (75.6)	21.640 (.000) ^a	0.711 (.3990)
	No	19 (24.4)		
Aware of symptoms of foodborne illness (N=78)	Yes	42 (53.8)	9.034 (.04) ^a	0.348 (.555)
	No	13 (46.2)		
Wearing aprons while serving juice (N=78)	Yes	13 (16.7)	17.155 (.000) ^a	0.348 (.555)
	No	65 (83.3)		
Waste receiving receptacles (N=26)	Sacks	14 (53.8)	19.634 (.001) ^a	2.989 (.409)
	Bin without cover	6 (23.1)		
	Bin with cover	6 (23.1)		
Hand washing equipment's (N=26)	Available	12 (46.15)	9.930 (.021) ^a	2.292 (.354)
	Not available	14 (53.85)		
Methods of preservation of fruit (N=26)	On Shelf	15 (57.7)	14.809 (.009) ^a	4.468 (.213)
	In a bucket	10 (38.5)		
	On the floor	1 (3.8)		
Place to keep the juice after preparation (N=26)	In Jag	12 (46.15)	13.760 (.014) ^a	2.614 (.443)
	In squeezing machine	2 (7.7)		
	In a refrigerator	12 (46.15)		
Frequency of cleaning material used to keep the juice (N=26)	Every day	14 (53.8)	9.930 (.021) ^a	2.292 (.354)
	After each use	12 (46.15)		
What is done with juice which gone bad (N=26)	Mixing with a fresh juice	14 (53.8)	9.930 (.021) ^a	2.292 (.354)
	I dispose it	12 (46.15)		

Abbreviations: SC, Staphylococcus; SS, Salmonella and Shigella.

^aStatistically significant.

Table 3. Physicochemical analysis of juice sample, water quality analysis, and its association with fruit contamination.

VARIABLES	PARAMETERS	FREQUENCY (%)	SALMONELLA/SHIGELLA
			χ^2 (P-VALUE)
pH	≤4.6	38 (48.7)	1.422 (.233)
	Above 4.6	40 (51.3)	
Water quality (<i>Salmonella/Shigella</i>)	Detected	9 (11.5)	9.63 (.003) ^a
	Not detected	19 (24.4)	
Temperature of fruit juice sample	Below 10°C	14 (17.9)	0.2 (.88)
	Between 10°C and 60°C (danger zone)	64 (82.1)	

^aStatistically significant.

Table 4. The recommended microbial standards for any fruit juices sold (Gulf standard 2000).²⁴.

STANDARD	LEVEL	STAPHYLOCOCCUS (LOG CFU/ML)	SALMONELLA	SHIGELLA
Gulf standard 2000	MBLA	2	Not detected	Not detected
	MBLP	3	Not detected	Not detected

Abbreviations: MBLA, maximum bacterial load anticipated; MBLP, maximum bacterial load permitted.

Table 5. The range and mean *Staphylococcus* count (log CFU/mL) in locally prepared fresh fruit juice in juice houses in selected towns of Eastern Ethiopia, 2020.

STUDY LOCATIONS	TYPES OF JUICE	MINIMUM (LOG CFU/ML)	MAXIMUM (LOG CFU/ML)	AVERAGE (LOG CFU/ML)
Harar town (n=24)	Mango	1.99	4.20	3.65
	Avocado	3.0	4.94	4.66
	Papaya	1.99	4.91	3.28
	Mixed	1.68	3.88	3.45
Dire Dawa town (n=33)	Mango	1.91	4.79	4.20
	Avocado	2.398	4.53	3.87
	Papaya	2.70	4.30	3.88
	Mixed	2.90	4.15	3.73
Jigjiga town (n=21)	Mango	1.91	4.79	4.43
	Avocado	3.0	4.08	3.63
	Papaya	3.56	4.91	4.58
	Mixed	2.89	4.93	4.32

conducted in Hossana Town, Ethiopia, reported none of the food handlers trained in food hygiene and safety.⁴

Prevalence of selected bacterial species

The overall total *Staphylococcus* count in the fruit juice was ranged from 1.68 to 4.94 log CFU/mL with a mean value of 4.2 log CFU/mL. The study found *Staphylococcus* count ranged from 1.91 to 4.79 log CFU/mL in mango juice, 2.4 to 4.94 log CFU/mL in avocado juice, 1.99 to 4.92 log CFU/mL in papaya juice, and 1.68 to 4.93 log CFU/mL in mixed juice samples. Furthermore, the current study found *Staphylococcus* counts in the juice samples lower than the finding of another study conducted in Ethiopia and found the *Staphylococcus* counts ranged from 4.85 to 5.23 log CFU/mL in avocado juice and 4.3 to 5.2 log CFU/mL in Papaya juice.²⁶

The presence of *Staphylococcus* count in fruit juices above 3.0 log CFU/mL is potentially hazardous to human health.²⁴ However, the current study found 58 (74.4%) of fruit juice samples contaminated with *Staphylococcus* higher than the maximum permitted level of Gulf standard 2000 that was consistent with the finding of another study conducted in India that found 73.3% of fruit juice samples contaminated with *Staphylococcus* higher than the recommended standard.²⁷ However, lower than the finding of another study⁸ found 93.4%

of fruit juice samples contaminated with *Staphylococcus* count higher than the maximum permitted limit of Gulf standard 2000. The difference may be related to poor hygienic and safety practices of food handlers. Because, *Staphylococcus* is commonly presenting in human nasal passage, throat, hair, and skin of food handlers.

Similarly, the presence of *Salmonella* or *Shigella* in any food indicates that the food is potentially hazardous to consumers' health.²⁸ However, the current study found 19 (24.4%) of fruit juice samples contaminated with *Salmonella* that was higher than the finding of another study conducted in Ethiopia reported 20.0% of samples contaminated with *Salmonella* species.⁸ However, lower than the finding of another study reported 40 (41.67%) of juice samples contaminated with *Salmonella* species.²⁹ Furthermore, 12 (15.4%) of the samples were contaminated with *Shigella* that was lower than the finding of another study conducted in India reported 48.6% of fruit samples contaminated with *Shigella* species.²⁷ The difference may be due to the presence of *Salmonella* and *Shigella* in the raw materials used for juice preparation, poor quality of water, or poor hygienic practices.

Furthermore, the current study found statistically significant association between contamination of fruit juice by *Staphylococcus* and educational status of participants ($\chi^2 = 22.28$), and training in food hygiene and safety ($\chi^2 = 15.73$). Similarly,

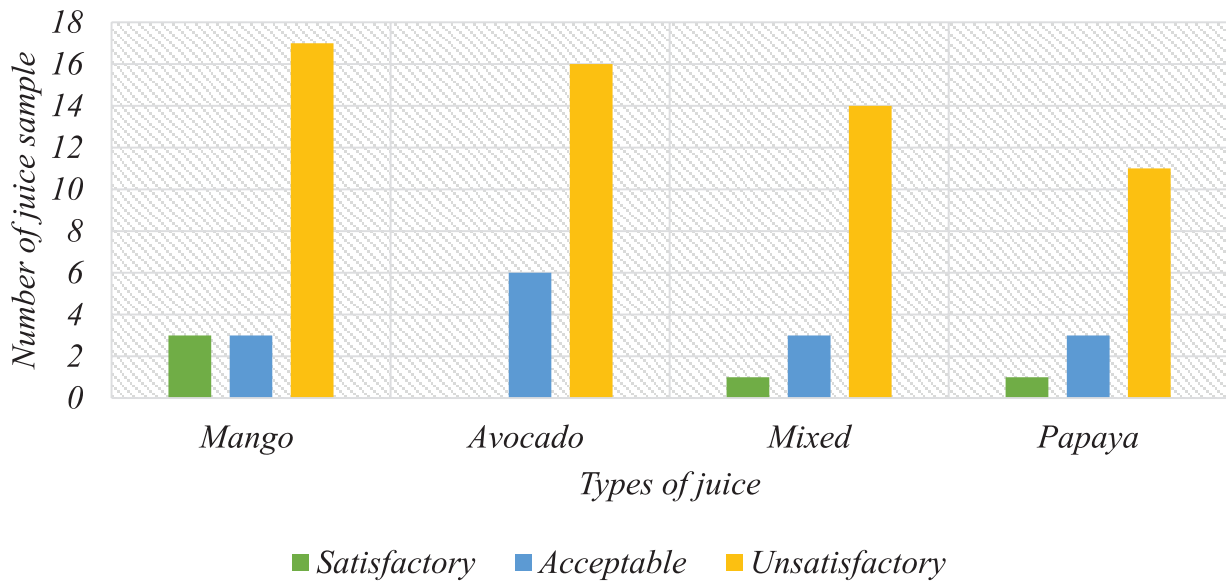


Figure 1. Acceptability distribution of *Staphylococcus* among examined fruit juice samples based on microbiological guideline for any fruit juice (Gulf standard 2000).

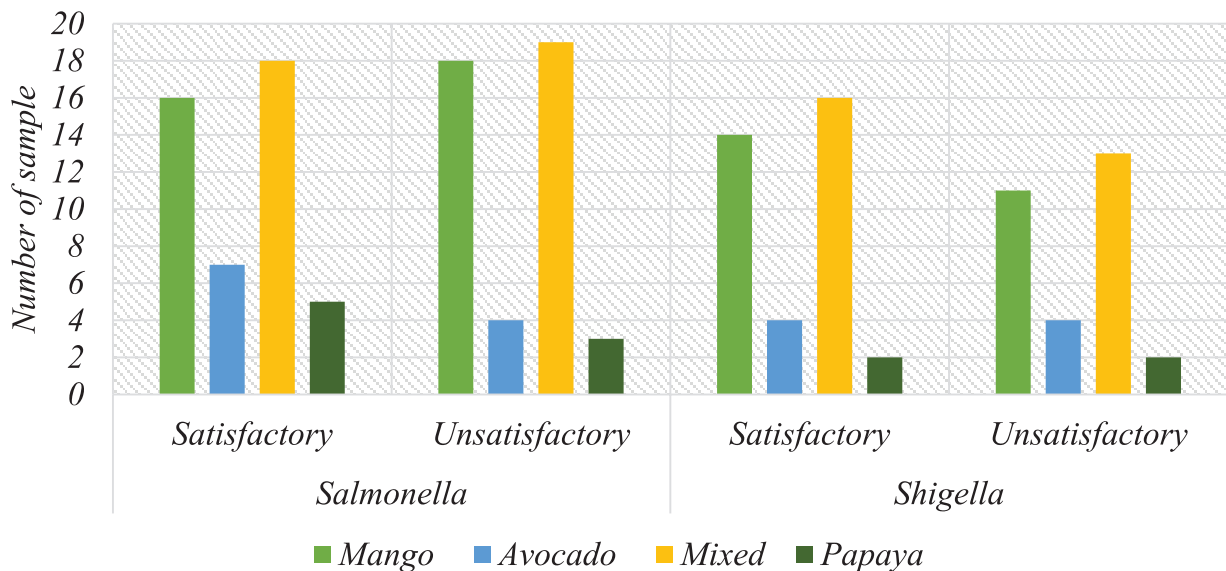


Figure 2. Acceptability distribution of *Salmonella* and *Shigella* among examined fruit juice samples based on microbiological guideline for any fruit juice (Gulf standard 2000).

there was statistically significant association between detection of *Salmonella/Shigella* in fruit juice and educational status of participants ($\chi^2=7.323$), and training in food hygiene and safety ($\chi^2=4.486$) that was in line the finding of another studies.^{30,31}

Overall, about three-fifth (74.4%) of fruit samples were contaminated with at least 1 pathogenic bacterial species higher than maximum permitted limit. Therefore, it is suggested to provide food safety training,³² and adequate hygiene and safety.³³ Similarly, temperature control,³⁴ preventing cross-contamination and sanitation for facility and utensils are important to protect the health of the consumers and the public by improving the quality of fruit juice. Implementing good manufacturing practice is also essential to prevent food borne disease and to protect the consumers health. Precautionary

procedures,³⁵ and periodic quality assessment of food also plays a major role in improving quality food.³⁶

Conclusion

Among fruit juice samples collected for bacteriological analysis, more than two-thirds of fruit juice samples had at least 1 pathogenic bacterial species (*Salmonella, Shigella,* and *S. aureus*) and potentially hazardous or risk to consumer health. Thus, regular supervision and monitoring to improve the quality of fruit juice is essential to prevent the consumption of contaminated fruit juices, which leads to food borne illness.

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Author Contributions

DAM conceived the idea and collected all necessary data for the study. All authors (DAM, TGT, and NB) have contributed in data analysis, writing, and editing the document. DAM, TGT, and NB gave valuable ideas of the manuscript and revised the manuscript. Finally, all authors read and approved the final version to be published and agreed on all aspects of this work.

Availability of Data and Other Materials

In addition to the data available in the study, the data used during this study are available from the corresponding author on reasonable request.

Ethical Approval

Ethical clearance was taken from Institutional Health Research Ethics Review Committee (IHERERC) of Haramaya University College of Health and Medical sciences. Permission letters were obtained from all concerned bodies. Informed, voluntary, written, and signed consent was obtained from each vendor, owner(s), and participants after explaining the purpose and importance of the study before the commencement of interviews and sampling.

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