Bacteriological Quality of Locally Prepared Fresh Fruit Juice Sold in Juice Houses of Eastern Ethiopia

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ABSTRACT

BACKGROUND: Currently, fresh fruit juices are widely consumed as a drink worldwide due to their nutritional values and health benefits. Fresh fruit juices are an important source of nutrients, vitamins, and fibers that are important for human health. Fruit juices are nutritious and perishable food that can serve as an ideal medium for the growth and multiplication of pathogenic microorganisms. Therefore, this study aimed to determine the bacteriological quality of locally prepared fresh fruit juices sold in juice houses of eastern Ethiopia from 4 April to 12 June 2020.

METHODS: A cross-sectional study was used that included administrative questionnaires and laboratory-based investigations. A total of 78 fruit juice samples that include mango, avocado, papaya, and mixed juices were collected aseptically from the juice houses. The most probable number method was used to determine the total coliform, fecal coliform and *Escherichia coli*. The pour plate count method was used to determine the total viable bacteria count. Finally, data were analyzed using descriptive statistical tests that included analysis of variance, Chi-square and Fisher's exact tests. A *P*-value of .05 was considered as a cut-off point for statistical significance.

RESULTS: Among the 78 juice samples analyzed, 85.9% of the samples had total viable bacterial count, 64.1% had total coliform count, 60.3% had fecal coliform, and 33.3% of the samples had *Escherichia coli* higher than the maximum permitted level of Gulf standard 2000. The study found a significant association between bacterial contamination and educational status ($\chi^2 = 31.663$), training in food hygiene and safety ($\chi^2 = 23.04$), method of fruit preservation ($\chi^2 = 17.98$), place to keep the juice ($\chi^2 = 13.7$), action done with the juice gone bad ($\chi^2 = 12.78$), frequency of cleaning materials used to keep the juice ($\chi^2 = 12.78$), type of dish washing ($\chi^2 = 19.75$), availability of hand washing equipment ($\chi^2 = 12.78$), and types of waste receptacles ($\chi^2 = 26.25$) (*P*-value $<.05$) (Table 5).

CONCLUSION: In general, majority of fruit juice samples were contaminated with one or more different bacteria species higher than the maximum permitted level. Furthermore, the study found the association between bacterial contamination and other variables such as hygienic and safety conditions. Therefore, the implementation of adequate hygiene and safety practices is very important to prevent the consumption of contaminated fruit juices, which leads to foodborne illness.

KEYWORDS: Bacteria, contamination, fruit juices, Eastern Ethiopia

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Introduction

Currently, locally prepared fresh fruit juices are widely consumed as important sources of nutrients and phytochemicals.[1,2] Fruit juices are rich sources of biologically active components that decrease the risk of chronic diseases such as cardiovascular disease,[3] diabetes,[4,5] and heart disease.[4] Juices are rich sources of bioactive compounds and have antioxidant and anti-inflammatory properties. They are an important source of flavan-3-ols, flavonols, anthocyanins, polyphenols, and vitamin C that are important in the promotion of health.[6-9]

However, fruit juices are highly perishable and can serve as an ideal medium for the growth and multiplication of various pathogenic microorganisms.[4] Fruit juices can be contaminated with *Escherichia coli* and *Salmonella*, *Staphylococcus aureus*, *Enterobacter* spp., *Klebsiella*, and *Serratia* species.[10-16] These pathogens cause typhoid fever, food poisoning, gastroenteritis, enteric fever, and diarrheal disease.[17-19]

Globally, contamination of fruit juices with pathogenic microorganisms has been reported to be associated with various outbreaks of infectious diseases that resulted in high morbidity and mortality.[17,18,20,21] According to a 2017 World Health Organization (WHO)[22] report, the annual global burden of food borne diseases was about 600 million of which 420 000 people die, including 125 000 children under the age of 5 years.

In Ethiopian towns and cities, fresh and unpasteurized fruit juice is common and preferred by consumers due to its fresh flavor attributes and nutritious values. Similarly, the number of fruit juice vending houses serving different types of fresh fruit juices are increasing in Ethiopia.[15] However, the lack of an adequate food safety system is a major problem and has become an obstacle to sustainable health, safety, and economic development of the population of Ethiopia. A study conducted in Ethiopia reported the prevalence of food borne pathogen

accounted 8.0% (*Salmonella*, pathogenic *Escherichia coli*, *Shigella*, and *Campylobacter* spp.), of which *Salmonella* and pathogenic *E. coli* contributed to 5.7% and 11.6%, respectively.[23] As a result of poor and under-reporting of outbreaks or foodborne diseases in developing countries, including Ethiopia, this problem is expected to be higher. Today, lifestyle changes in Ethiopian cities and towns have increased the demand for ready-to-eat foods. Despite the increasing use of locally prepared fresh fruit juices, there is no adequate evidence on the bacteriological quality of locally prepared fresh fruit juices.

As a result, there is a need to develop a database on this issue so that the regulatory agencies take action to improve both safety and quality of the fruit juice. Therefore, this study aimed to determine and provide data on the bacteriological quality of locally prepared fresh fruit juices sold in juice houses of eastern Ethiopia.

Materials and Methods

Study area and study design

The cross-sectional study was conducted in selected towns of eastern Ethiopia, where there are high resident populations and juice houses are common, particularly in Dire Dawa, Jigjiga, and Harar towns from 4 April to 12 June 2020. Jigjiga, Harar, and Dire Dawa towns are located at about 619, 525, and 520 km, respectively, from the capital of Ethiopia, Addis Ababa. Dire Dawa, Harar, and Jigjiga towns are located at a latitude and longitude of 9°36′N and 41°52′E, 9°19′N and 42°7′E and 9°21′N and 42°48′E, respectively.

Sample-size and sampling techniques

A total of 78 fruit juice samples were collected from all juice houses (N = 26) found in selected towns in Eastern Ethiopia. From each juice house, 3 most commonly consumed locally prepared mango, avocado, papaya, and mixed juices were collected aseptically (using sterile materials, flaming, refrigeration, and appropriate procedures) and analyzed for their bacteriological status. At the same time, data related to sociodemographic characteristics and hygiene and safety conditions were collected from 78 food handlers (juice makers). There were 26, 24, and 37 juice makers working in juice houses of Harar, Jigjiga, and Dire Dawa, respectively. Based on these number, the sample size (n = 78) was proportionally allocated to the study locations that produced 24, 21, and 33 participants from Harar, Jigjiga, and Dire Dawa, respectively.

Data collection for face-to-face interview

Data related to sociodemographic characteristics (such as age, sex, marital status, educational status, work experience and training in food hygiene and safety), and hygiene and safety conditions (such as method of food preservation, place to store juice and fruit, frequency of cleaning materials, types of dish washing, types of waste collection receptacles, availability of hand washing facilities, and action done with juice gone bad) were collected by face-to-face interview using pretested semi-structured questionnaire and observational checklist. The questionnaire was prepared in the English language and translated into Afan Oromo and Amharic (the local language of the study participants). The questionnaire was pretested before data collection in another town outside the study area, Haramaya Town, eastern Ethiopia, on 5% of the sample size. The accuracy and completeness of the completed questionnaire and checklist were checked to reduce errors by comparing the response from the pretesting with the desired outcome to be obtained using these tools. Finally, completed questionnaires and checklists were checked to determine its completeness before data entry.

Sample collection and processing

About 250 mL of fruit juice sample was collected aseptically from juice storage where the fruit juice was directly given to consumers. An aseptic technique was used throughout sampling and handling procedures by using sterile materials, flaming, refrigeration, and appropriate procedures. The bacteriological analysis of the samples was done at the Environmental Health Laboratory, College of Health and Medical Sciences, Haramaya University. The samples were transported to the laboratory using the ice box and kept below 5°C and immediately analyzed in the range of 10 minutes to 1.5 hours. Serial dilutions of 3 folds (10^{-1}, 10^{-2}, and 10^{-3}) were done based on International Standard Organization (ISO) 6887-1:1999 protocols.[24] Furthermore, each bacteriological test was performed according to the protocol of ISO 7218:2013(E).[25]

Bacteriological analysis

Total viable bacterial counts (TVBC). The serial dilutions of 10^{-1}, 10^{-2}, and 10^{-3} were done. One mL of the sample was added to 9 mL of sterile peptone water to get the first dilution (10^{-1}). To obtain further dilutions, the same procedure was applied (ie, to obtain the second dilution, 1 mL of the initially diluted sample was added to 9 mL of peptone water). The total colony count of the bacteria was carried out using pour plate count method on plate count agar (PCA) on triplicate plates (3 plates for each dilution) using ISO 4833-1:2013(E) protocol.[26]

One mL of each diluted juice sample and 12 mL of plate count agar were mixed using sterilized Petri dishes and sterile pipette. Triplicate or 3 diluted samples were taken from each set of serial dilution (10^{-1}, 10^{-2}, and 10^{-3}) and analyzed to determine TVBC in fruit juice samples. Then, an inoculum from the molten agar culture medium was mixed accordingly by rotating the Petri dishes. The mixture was allowed to solidify by leaving the Petri dishes on a cool horizontal surface. The same procedure was repeated under the same conditions for each decimal dilution. Then, plates were incubated under an

Table 1. Recommended microbial level for fruit juices (Gulf Standard 2000).

GULF STANDARD 2000	LEVEL	TVBC (LOGCFU/ML)	TCC (LOG CFU/ML)	FCC (CFU/ML)	*E. COLI* (CFU/ML)
	MBLA	3.699	1	Not detected	Not detected
	MBLP	4	2	Not detected	Not detected

Abbreviations: CFU, colony forming Unit; E. coli, *Escherichia coli*; FCC, fecal coliform count; MBLA, maximum bacterial load anticipated; MBLP, maximum bacterial load permitted; TCC, total coliform count; TVBC, total viable bacteria count.

aerobic condition at 30°C for 72 hours in an inverted position. After the incubation period, colonies were counted using a colony counter. The number of microorganisms enumerated per mL of the sample was calculated using the number of colonies obtained from each plate.[26] Finally, the results were presented as the number of total viable bacteria counts per mL and reported as log CFU/mL.

Coliform count (total and fecal coliform). Serial dilutions were performed by adding 1 mL of the sample to 9 mL peptone water to get the initial dilution (10^{-1}), and to get all required dilutions, the same procedure was applied. Detection and enumeration of the coliform were performed according to the ISO 4831:2006(E) protocol.[27] To determine the coliform count, 3 tubes of double-strength Lauryl Sulfate Tryptose (LST) broth (liquid selective enrichment medium) were inoculated with 1 mL of appropriately diluted sample using a sterile pipette and thoroughly mixed.

Furthermore, 3 tubes of single-strength medium, LST medium broth were inoculated with the sample. The same procedure was repeated under the same conditions for each decimal. The tubes contain double and single strength medium were then incubated at 37°C for 24 hours. However, tubes that did not form gas after the incubation period were further incubated for another 24 hours at 37°C, and the presence of gas formation in the Durham tube was considered positive.

For the confirmatory test, Brilliant Green Lactose Broth (BGLBB) was inoculated with cultures from tubes of double and single strength medium and examined for gas formation after incubation time at 37°C for 48 hours. Finally, the most probable number (MPN) of coliforms per mL was calculated from the number of tubes showed gas and determined according to the MPN table.[27]

To determine fecal coliform in the sample, the positive tubes of LST were further incubated at 44.5°C for 48 hours. Then, *Escherichia coli* (EC) broth was used for the confirmatory test and incubated at 45°C for 48 hours. Finally, gas formation in an EC broth was considered as positive for fecal coliform[28] and evaluated according to the MPN table.[27]

Detection of E. coli. The detection of *Escherichia coli* in juice samples was performed using the ISO 7251:2005 (E) protocol.[29] Dilutions were pre-enriched in buffered peptone water and enriched in LSB broth. The tubes were incubated at 37 °C and examined for gas formation after 24 and 48 hours of incubation periods. The positive tubes (tubes produced gas) were further sub-cultured in 3 tubes (MPN) containing a liquid selective medium (EC broth) and examined for gas formation after 24 hours.

Furthermore, positive tubes (gas produced in EC broth) were inoculated and incubated in tryptone water at 44°C for 48 hours. The presence of *Escherichia coli* in the juice sample was then confirmed using indole reagent. Finally, the presence of a red ring in the alcoholic phase was considered as positive test and presented as "detected" or "not detected" and evaluated based on the MPN table index.[29]

Finally, the results of all bacterial species analyzed in this study were determined based on the most probable number (MPN) table and compared with Gulf Standard 2000[30] (Table 1).

Physicochemical and water quality analysis

pH and temperature of juice sample were measured using portable digital pH and thermometer meter, respectively.

Data quality control

Before data collection, the questionnaire and observation checklist were pretested on 5% of the study sample size in Haramaya town, outside the study areas, using face-to-face interviews to ensure clarity and applicability of the questionnaire and observation checklist. It was carried out after permission and consent were obtained from the agencies and individuals included in this study. To minimize errors, the consistency of the procedures was maintained while conducting the experiments throughout the study. The aseptic technique was used for sampling, handling processes and bacteriological testing.[25] Strain specification code, American Type Culture Collection (ATCC), *E. coli* derived from ATCC, 25922 was used as the positive controls for the *E. coli*, total coliform, and fecal coliform. The samples were analyzed immediately to avoid changes. Three diluted samples were analyzed for each serial dilution (10^{-1}, 10^{-2}, and 10^{-3}) to confirm the contamination level and the mean score was taken to determine the level of bacteria in the sampled fruit juice. The equipment and media were sterilized or disinfected depending on the type of equipment. Autoclave was used to sterilize the medium and

Table 2. Sociodemographic characteristics of food handlers working in juice houses of eastern Ethiopia, 2020 (n = 78).

PARAMETER	CATEGORY	FREQUENCY	PERCENTAGE	χ^2 TEST (FISHER EXACT TEST)	*P*-VALUE
Sex of the respondents	Male	24	30.8	19.748	<.001*
	Female	54	69.2		
Age of the respondents	15-24	21	26.9	7.532	.402
	25-34	38	48.7		
	34-44	10	12.8		
	45-54	6	7.7		
	>55	3	3.8		
The educational status of the respondents	No formal	10	12.8	31.663	<.001*
	Primary school	24	30.8		
	Secondary school	31	39.7		
	College/diploma	12	15.4		
	Degree and above	1	1.3		
Service year	<1	23	29.5	3.768	.398
	1-2	41	52.6		
	>2	14	17.9		
Training in food hygiene and safety	Yes	7	9.0	23.040	<.001*
	No	71	91.0		

*Statistically significant (*P*-value <.05). The χ^2 and *P*-value value indicated in the table was calculated based on the total viable bacterial count.

equipment at 121°C for 15 minutes.[25,31] On the other hand, alcohol (ethanol, 70% concentration) was used to disinfect some materials and working surfaces.

Data processing and analysis

Each measurement of the different variables was systematically organized and subjected to statistical analysis using the Statistical Package for Social Sciences (SPSS) version 22.0. One-way analysis of variance (ANOVA) was used to compare the mean bacterial counts among the study locations and types of juices. The association between socio-demographic characteristics and hygiene and safety conditions and bacteriological contamination of fruit juices were assessed using Pearson's Chi-square test and Fisher's Exact Test. A *P*-value of .05 was considered as the cut-off point for statistical significance.

Results

Sociodemographic characteristics of the study participants

The study included a total of 78 study participants (juice makers) of which more than half, 54 (69.2%) were females. Thirty-eight (48.7%) of the study participants were within the ages ranged from 25 to 34 years, the largest proportion, followed by participants with ages ranged from 15 to 24 years, represented 21 (26.7%). Only 10 (12.8%) of the study participants had no formal education, while 68 (87.2%) had formal education. Furthermore, 41(52.6%) of the participants had work experience ranging from 1 to 2 years. Only 7 (9.0%) participants had training in food hygiene and safety. Furthermore, the study found a statistically significant association between bacterial contamination of fruit juice and socio demographic characteristics of the respondents such as educational status and training in food hygiene and safety (*P*-value <.001) (Table 2).

Bacteriological load of fresh fruit juices

The total bacterial count of the fruit juice samples was calculated based on the bacterial count on each plate (10^{-1}, 10^{-2}, and 10^{-3} serial dilutions) and later the mean value was taken. Total coliform, fecal coliform, and *E. coli* were determined based on the most probable number (MPN) table and evaluated with Gulf Standard 2000.[30]

The current study found that the overall total viable bacterial count (TVBC) ranged from 2.41 to 5.97 log CFU/mL with a mean value of 5.38 log CFU/mL. The total viable

bacterial count in mango, avocado, papaya, and mixed juices ranged from 2.41 to 5.86 log CFU/mL, 2.95 to 5.91 log CFU/mL, 2.92 to 5.44 log CFU/mL, and 2.68 to 5.46 log CFU/mL, respectively.

The mean total bacterial count among the samples collected from Harar, Dire Dawa, and Jigjiga towns were 5.54, 5.44, and 5.61 log CFU/mL, respectively. Fruit juice samples were contaminated with total coliform, fecal coliform, and *E. coli*, each ranged from not detected to >3.04 log MPN/mL. Based on the study locations, the samples taken from the Harar town were contaminated with total coliform ranged from not detected to >3.04 log MPN/mL, fecal coliform ranged from 0.48 to 2.66 log MPN/mL and *E. coli* ranged from 0.48 to 2.66 log MPN/mL. The samples collected from Dire Dawa town were contaminated with total coliform ranged from not detected to >3.04 log MPN/mL, fecal coliform ranged from 0.48 to 2.46 log. MPN/mL and *E. coli* ranged from 0.48 to 2.46 log MPN/mL (Table 3).

Bacteriological status of locally prepared fresh fruit juices

The current study found that 67 (85.9%) of the samples had TVBC higher than the maximum permitted limit of Gulf Standard 2000 (>4 log CFU/mL) and 50 (64.1%) of the samples had total coliform count higher than the maximum permitted limit (>2 log CFU/mL). Furthermore, the study found that 47 (60.3%) of the samples had fecal coliform and 26 (33.3%) of the sample had *E. coli* greater than the maximum permitted limit.[30]

The study found that 30 (90.9%) of the samples collected from Dire Dawa had TVBC higher than the maximum permitted limit (>4 log CFU/mL). On the other hand, 75.0% of the samples collected from Harar town and 90.5% of the samples collected from Jigjiga town had TVBC higher than the maximum permitted level. Furthermore, a higher percentage of unsatisfactory level of the samples in terms of total coliform (72.2%), fecal coliform (75.7%) and *E. coli* (41.7%) were observed among the samples collected from Jigjiga, Dire Dawa, and Harar towns, respectively (Table 4).

Hygienic and safety conditions of juice houses and food handlers

Among 26 juice houses, 15 (57.2%) of the juice houses were putting fruit on the shelf while 12 (46.2%) of the juice houses reported using the refrigerator to keep the prepared juice. Fourteen (53.8%) of the juice houses were using basins to store the chopped fruits ready for extraction. On the other hand, 14 (53.8%) of the juice houses were using sacks to collect the waste while 14 (53.8%) did not have hand washing facility during observation. Furthermore, the study found a significant association between bacterial contamination of fruit juice and some hygiene and safety conditions, such as the method of fruit preservation, the place used to keep the juice after preparation, the action done with the juice gone bad, the frequency of cleaning materials used to keep the juice such as the refrigerator and materials used to store the juice, the type of dish washing, availability of hand washing facilities and types of waste receptacles (*P*-value <.05) (Table 5).

Physicochemical analysis of juice

pH: The mean value of pH among the analyzed juice samples was 4.59 ± 0.6 (SD). The mean value of temperature among the juice samples was 12.3 ± 2.66 (SD). Additionally, there was no any significant association between pH and contamination of juice and ($\chi^2 = 1.39$ [*P* value = .2]) as well as between temperature of juice samples and bacterial contamination ($\chi^2 = 0.24$ [*P* value = .9]) (Table 6).

Discussion

The aim of this study was to determine the bacterial contamination of locally prepared fresh fruit juice sold in selected towns of eastern Ethiopia. Furthermore, factors associated with bacteriological contamination such as socio-demographic characteristics, and hygiene and safety conditions were assessed to det ermine the variation in bacterial contamination of juice.

The current study found that the total bacterial count ranged from 2.41 to 5.97 log CFU/mL with a mean value of 5.38 log CFU/mL. However, another study conducted in Ethiopia found a microbial count in the juice samples ranged from 4.0 to 5.46 log CFU/mL.[15] Furthermore, the study found the mean TVBC in mango, avocado, papaya, and mixed juices was 5.15, 5.44, 5.44, and 5.46 log CFU/mL, respectively. This indicates that about an equal bacterial load was observed among all types of juice samples. However, this finding was lower than the finding of another study conducted in Bahir Dar Town, Ethiopia, that found the mean TVBC in avocado, papaya, and mango juice accounted for 7.49, 6.59, 2.51, and 5.23 log CFU/mL, respectively.[32] The variation may be related to the difference in the implementation of hygiene and safety measures. At least 3-quarters of the samples from each study location (Dire Dawa, Harar, and Jigjiga town) had TVBC higher than the maximum permitted limit, with a mean bacterial count of 5.44, 5.54, and 5.61 log CFU/mL, respectively. This may be as a result of relatively similar socioeconomic conditions and regulatory agencies of the study locations.

Overall, the current study found 67 (85.9%) of the samples had TVBC higher than the maximum permitted level of the Gulf standard, 2000 (>4 log CFU/mL) that was lower than the finding of another study conducted in Nigeria that reported 90.0% of fruit juice samples contaminated with TVBC higher than the maximum permitted level of Gulf standard 2000 (>4 log CFU/mL).[33] However, the current study found higher proportion of juice samples had TVBC higher than the maximum permitted level than another studies conducted in Ethiopia[34]

Table 3. Bacterial count of locally prepared fresh fruit juice samples based on the locations and type of fruit juices (n=78).

BACTERIAL COUNT	STATUS	BACTERIOLOGICAL LOADS OF JUICE SAMPLES											
		HARAR TOWN (N=24)				DIRE DAWA TOWN (N=33)				JIGJIGA TOWN (N=21)			
		MANGO	AVOCADO	PAPAYA	MIXED	MANGO	AVOCADO	PAPAYA	MIXED	MANGO	AVOCADO	PAPAYA	MIXED
TVBC (log CFU/mL)	*Min*	3.62	3.62	2.92	2.68	4.25	2.95	5.0	5.23	2.64	5.10	4.85	4.54
	Mx	5.34	5.86	5.95	5.0	5.86	6.81	5.90	5.97	5.32	5.91	5.87	5.83
	Av	5.10	5.40	5.38	5.74	5.28	5.32	5.52	5.64	5.93	5.61	5.41	5.48
	SD	1.70	1.39	0.50	1.63	1.34	1.31	1.50	1.48	1.90	1.42	1.50	1.39
TCC (log MPN/mL	*Min*	1.88	1.81	ND	1.97	1.18	0.78	1.38	1.86	ND	1.88	0.48	0.48
	Mx	>3.04	>3.04	>3.04	>3.04	>3.04	>3.04	>3.04	>3.04	>3.04	>3.04	>3.04	>3.04
FCC (log MPN/mL)	*Min*	ND	1.45	ND	1.32	1.11	0.48	0.56	1.10	ND	0.48	ND	ND
	Mx	2.38	2.32	1.98	2.66	2.20	2.10	2.46	2.46	>3.04	3.04	2.46	2.38
E. coli (log MPN/mL)	*Mn*	ND	1.45	ND	1.32	1.11	0.48	0.56	1.1	ND	0.48	ND	ND
	Mx	2.38	2.32	1.98	2.66	2.20	2.10	2.46	2.46	>3.04	3.04	2.46	2.38

Abbreviations: Av, average; CFU, colony forming unit; *E. coli*, *Escherichia coli*; FCC, fecal coliform count; MPN, most probable number; ND, not detected; TCC, total coliform count; TVBC, total viable bacteria count; SD, standard deviation.

Table 4. Acceptability distribution of examined fruit juice samples based on microbiological guideline for any fruit juice (Gulf standard 2000).

PARAMETER	CATEGORY	COUNTS/ ABSENT/ PRESENT	HARAR TOWN (N=24)	DIRE DAWA TOWN (N=33)	JIGJIGA TOWN (N=21)	TOTAL (N=78)
			FREQUENCY (%)	FREQUENCY (%)	FREQUENCY (%)	FREQUENCY (%)
TVBC (log CFU/mL)	Satisfactory	<3.699	5 (20.8)	2 (6.1)	1 (4.8)	8 (10.2)
	Acceptable	3.699-4.0	1 (4.2)	1 (3.0)	1 (4.8)	3 (3.9)
	Unsatisfactory	>4.0	18 (75.0)	30 (90.9)	19 (90.4)	67 (85.9)
TCC (log MPN/mL)	Satisfactory	<1	4 (16.7)	2 (6.1)	2 (9.5)	8 (10.3)
	Acceptable	1-2	9 (37.5)	8 (24.2)	3 (14.3)	20 (25.6)
	Unsatisfactory	>2	11 (45.8)	23 (69.7)	16 (72.2)	50 (64.1)
FCC (log MPN/mL)	Satisfactory	Not detected	13 (54.2)	8 (24.2)	10 (47.6)	31 (39.7)
	Unsatisfactory	Detected	11 (45.8)	25 (75.8)	11 (52.4)	47 (60.3)
E. coli (log MPN/mL)	Satisfactory	Not detected	14 (58.3)	23 (69.7)	15 (71.4)	52 (66.7)
	Unsatisfactory	Detected	10 (41.7)	10 (30.3)	6 (28.6)	26 (33.3)

Abbreviations: CFU, colony forming unit; E. coli, *Escherichia coli*; FCC, fecal coliform count; MPN, most probable number; TCC, total coliform count; TVBC, total viable bacteria count.

Table 5. Hygiene and safety conditions of juice houses and food handlers working in juice houses of eastern Ethiopia, 2020.

PARAMETER	CATEGORY	FREQUENCY (%)	χ^2 TEST (FISHER EXACT TEST)	*P*-VALUE
Methods of preservation used/place to put fruits	On Shelf	15 (57.7)	17.977	<.05*
	In a bucket	10 (38.5)		
	On the floor	1 (3.8)		
Place to put chopped fruit	In saucepan	6 (23.1)	8.591	>.05
	On table	6 (23.1)		
	In a basin	14 (53.8)		
Place to keep the juice after preparation	In Jag	12 (46.2)	13.709	<.05*
	In squeezing machine	2 (7.7)		
	In a refrigerator	12 (46.2)		
Action done with the juice gone bad.	Mixing with a fresh juice	14 (53.8)	12.776	<.05*
	I dispose it	12 (46.2)		
Frequency of cleaning material used to keep the juice (refrigerator and other materials used to store juice)	Every day	14 (53.8)	12.776	<.05*
	After each use	12 (46.2)		
Type of dish washing	One compartment sink	3 (11.5)	19.748	<.05*
	Two compartments sink	19 (73.1)		
	Three compartments sink	4 (15.4)		
Hand washing equipment	Available	12 (46.2)	12.776	<.05*
	Not available	14 (53.8)		

(Continued)

Table 5. (Continued)

PARAMETER	CATEGORY	FREQUENCY (%)	χ^2 TEST (FISHER EXACT TEST)	*P*-VALUE
Types of waste receptacles	Sacks	14 (53.8)	26.247	<.05*
	Bin without cover	6 (23.1)		
	Bin with cover	6 (23.1)		
Latrine	Present	20 (76.9)	6.602	>.05
	Absent	6 (23.1)		
Hand washing facilities attached to the toilet (n=20)	Present	4 (20.0)	3.459	>.05
	Absent	16 (80.0)		

The χ^2 and *P* value indicated in the table was calculated based on the total viable bacterial count;
*Statistically significant (*P* value <.05).

Table 6. Physicochemical analysis of juice sample and its association with fruit contamination.

VARIABLES	PARAMETERS	FREQUENCY (%)	TVBC
			χ^2 (*P*-VALUE)
PH	⩽4.6	40 (51.3)	1.39 (.2)
	Above 4.6	38 (48.7)	
Temperature of fruit juice sample	Below 10°C	16 (20.5)	0.2 (.9)
	Between 10°C and 60°C (danger zone)	62 (79.5)	

Abbreviation: TVBC, total viable bacteria count.

and Ghana[35] that reported 80.0% and 52% of the samples had TVBC higher than the maximum permitted level, respectively. The variation may be due to poor hygiene practices or cleanliness of working areas and poor quality of raw materials used or improper storage conditions. The higher microbial load in food makes a food unfit for consumption and potential risk to consumer health.[36]

Furthermore, the current study found the total coliform count ranged from not detected in the sample to higher than 3.04 log MPN/mL. The study found 50 (64.1%) fruit juice samples had a total coliform count higher than the maximum permitted level of Gulf Standard 2000 (2 log MPN/mL), that was lower than the finding of another study conducted in Ethiopia that found 76.7% of the samples had a total coliform count higher than the maximum permitted level.[34] However, it was in line with the finding of another study conducted in Lahore City that found 60% of samples had a total coliform count above the maximum permitted level.[37] The variation may be related to the difference in educational status of the respondents, training on food hygiene and safety and/or hygienic and safety practices such as method of food preservation, quality of raw materials used, and hand washing facilities that contribute to the entry of various microorganisms.

The presence of fecal coliform in any food is not allowed for consumption.[30] However, the current study found 47 (60.3%) of fruit juice samples contaminated with fecal coliform, which was lower than the finding of another study conducted in India that reported 77.3% of fruit juice samples contaminated with fecal coliforms.[38] The difference may be related to the national food safety system, exposure of fruits to feces, poor hygiene practices, or quality of raw materials used for juice preparation and training in food hygiene and safety.

The current study found a statistically significant association between bacterial count and training in food hygiene and safety ($\chi^2 = 23.04$; *P* value of <.001) that was in line with the finding of another study conducted in Ethiopia.[39] Furthermore, the study found that the method of preservation and the frequency of cleaning materials used to preserve the juice (*P* value of <.001) were associated with bacteriological contamination of juice samples that was consistent with the finding of another studies.[39,40] Additionally, educational status of the respondents was associated ($\chi^2 = 31.66$; *P* value of <.001) with bacteriological contamination of fruit juice samples that was consistent with the finding of another study conducted in Ethiopia.[39]

In general, the study found higher percentage of fresh fruit juice contaminated with bacterial species examined in

this study. Therefore, it is suggested to provide food safety training,[41] personal hygiene,[42] temperature control (keeping below 40°F and pasteurization (heat treatment),[43] prevent cross-contamination, and sanitation for facility and utensils to protect the health of the consumers and the public by improving the quality of fruit juice.

Limitations of the study

This study relied on self-reported data, which may lead to an overestimation of positive hygiene practices. However, juice preparation and hygiene practices were directly observed and recorded to minimize self-reported bias. Additionally, only 3 towns were included in this study. We recommend a study with a larger sample size, including collection of samples over different seasons of the year and locations.

Conclusions

In general, the study found that majority of the fruit juice samples were contaminated with one or more different bacteria species higher than the maximum permitted level. The results of this study found that 85.9% of the juice samples had a total viable bacterial count, 64.1% had total coliform, 60.3% had fecal coliform, and 33.3% had *Escherichia coli* higher than the maximum permitted level. Similarly, some sociodemographic characteristics, and hygiene and safety conditions were significantly associated with bacteriological contamination of fruit juices. Therefore, the implementation of adequate hygiene and safety practices is very important to prevent the consumption of contaminated fruit juices, which leads to foodborne illness.

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Author Contributions

DAM conceived the idea and collected the data and played a major role. The authors (DAM, NB, and TG) contributed to data analysis, writing, and editing the document. DAM, DM, YM, NB, and TG gave valuable ideas for the manuscript and revised the manuscript. All authors read and approved the final version of the manuscript. Finally, the authors read and approved the final version to be published and agreed on all aspects of this work.

Data Availability

Almost all data are included in this study. However, additional data will be available from the corresponding author upon reasonable request.

Ethical Approval

Ethical approval for this study was obtained from the Institutional Health Research Ethics Review Committee (IHRERC) of the Faculty of Health and Medical Sciences of Haramaya University (Ref number: IHRERC/069/2020).

Informed Consent

Informed consent has been obtained from all individuals included in this study.

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