JOURNAL OF INDIGENOUS KNOWLEDGE AND DEVELOPMENT STUDIES
JIKDS 03(02)2021
ISSN (online) 2708-2830 ISSN (print) 2707 – 7640
www.bhu.edu.et/jikds

Evaluation of Bacteriological Load and Safety of Fruit Juices Available in Bule Hora Town, Ethiopia

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ABSTRACT

Fruit juices are important components of a healthy diet, an extraordinary dietary source of nutrients, vitamins, and fiber for humans, and thus vital for health and well-being. However, contamination of fruit juices by microorganisms needs great attention in developing countries including Ethiopia. This study evaluated the bacteriological load and safety of avocado and papaya fruit juices prepared and sold in Bule Hora Town, Ethiopia. Ninety-six randomly selected study samples of fruit juices were collected from eight fruit juice houses via a cross-sectional study design. The analysis has shown that the mean pH samples of avocado and papaya ranged from 4.62±0.01 to 5.33±0.14. The mean ± SD of total viable bacteria count in avocado and papaya were 5.05±0.08 log CFU/mL and 5.15± 0.45 log CFU/mL respectively. The mean ± SD of 5.40±0.12 log CFU/mL total coliform count was identified from the avocado juice sample, whereas 5.30±0.22 log CFU/mL was identified from the papaya juice sample. The total *Staphylococcus* count obtained in avocado and papaya was in the range between 5.12±0.73 log to 5.48±0.10 log CFU/mL. The finding of observation and interviews also indicated that there was a lack of training about preparing juice safety and keeping the hygiene of juice for juice makers as well as waiters in juice houses. According to the current study, fruit juice may be contaminated either during processing, harvesting and handling. Therefore, keeping instruments and workplaces hygienic and teaching juice makers how to adhere to hygienic practices are recommended to reduce the bacteriological load and safety of fruit juice.

Keywords: Bacteriological load, Contamination, Fruit juice, safety

INTRODUCTION

Fruit juice is an aqueous liquid usually obtained/extracted from fruits. It is very popular among people of all ages around the world (Tasina H, 2011). It provides health benefits due to its nutritive value, and mineral and vitamin content (Keller S, 2006). The main constituent of juice is water, but also contains carbohydrates, sucrose, fructose, glucose, sorbitol, and a small amount of protein (Vojdani, 2008).

However, fruit juices contain various microorganisms and many of them are harmless bacteria such as saprophytic (Tasina H, 2011) and others are pathogenic, *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., *Staphylococcus* spp. and coliform (Reddy U, 2009). One possible source of entry into the fruit by pathogenic organisms can be damaged surfaces, such as punctures, and wounds, that occur during harvesting (Tambekar DH, 2009). Furthermore, the use of unclean water and types of equipment for juice processing enhance the proliferation of pathogenic microbes

(coliforms bacteria, streptococci, and other members of *Enterobacteriaceae*) that infect the juices (Tasina H, 2011; RM More, 1992). In most cases, hand, instrument and fruit washing is usually done without soap. In addition, there is a possibility of contamination of juice during serving by juice house servers due to personal unhygienic (Tasmina R, 2010). Another important issue influencing juice quality and contributing to a further increase in contamination is juice storage temperature. Due to a lack of proper handling and temperature, the juice can possess pathogens like *Staphylococcus aureus* (Pao S, 2001).

In Bule Hora Town, people consume fruit juice every day which they put in the plastic cup and left on shelves without **usin**g a refrigerator. In Ethiopia, the risk associated with exposure to the outbreak of diseases such as diarrhea, kidney failure, pneumonia, skin infection, respiratory disease, meningitis, and food poisoning, amongst others are common (Danyluk MD, 2012). According to reports from various information sources such as the mass media, outbreaks of food-borne illnesses were observed in Bule Hora town (Bule Hora town, 2019). In addition, there is little information on microbial contamination of fruit juice consumed in Bule Hora town, Ethiopia. Therefore, this study evaluated the bacteriological load and safety of papaya and avocado fruit juices available in fruit juice houses of Bule Hora town, Ethiopia.

MATERIALS AND METHODS

Sampling Techniques

From January to June 2020, 96 samples of commonly consumed fruit juices (48 each of avocado and papaya juice) were collected twice a day (morning and afternoon) from eight selected juice houses in Bule Hora town, Ethiopia (Figure 1)**.** This was to compare the effect of sampling time on bacterial load. From each juice house, 200 mL of avocado and papaya juice samples were purchased and transported immediately to the Laboratory of Biology Department at Bule Hora University in an icebox (4°C). Samples were analyzed within 30 minutes of procurement. Observations and interviews were also conducted to obtain information about the care being taken during the processing of fruit juices, and a biography of fruit juice makers and servers. Twenty-four respondents who were involved in the preparation of fruit juice and serving of selected fruit juices houses were included (**Figure 1).**

Sample Processing

Serial dilution (10^1,10^2,10^3,10^4,10^5,10^6) was prepared aseptically by taking 1 mL juice from each juice sample and blended with 9 mL of sterile distilled water. The spread plate technique was used to grow, enumerate, isolate and characterize bacteria (Weissmann, 2006).

Culturing Methods

Total viable bacterial count (TVC) and *S. aureus* were cultured using Plate Count Agar (PCA) (Himedia laboratories, Ltd, Mumbai, India) and Mannitol Salt Agar (MSA) (Himedia laboratories, Ltd, Mumbai, India) respectively. Aliquots (1mL) of each dilution was spread plated into triplicate plates of each agar and incubated for 24-48 hour at 37ºC (Hussain, 2017). MacConkey agar (Himedia laboratories, Ltd, Mumbia, India) was used to culture total coliform counts (TCC) (LM, 2012) at 35ºC for 48h. The TVC colonies on plates ranging from 30-300 and *S. aureus* colonies ranging from 20-200 were counted using a colony counter (Sadler, 2010). After enumeration, the

colonies were randomly taken from countable plates of PCA, MSA, and MacConkey and purified through repeated plating on PCA. The resulting bacterial isolates were then identified following the microbiological procedures of Anderson (2014)**)** and Salfinger (2015)**.**

For identification by morphological characteristics, a colony morphology observation is a major identifying criterion for bacteria. The characteristics like shape (circular, irregular, spreading), elevation (flat, slightly raised, or markedly raised), pigmentation (red, white, pink, colorless), size (pinpoint, small, medium, large), and texture were used for morphological identification (Brugger SD, 2012).

For biochemical identification of the general microbiota, a loopful of colonies developed on standard plate count agar was picked at random and purified by repeated plating. Biochemical tests such as catalase test, Triple Sugar Iron Agar test, Voges-Proskauer reaction indole production test, citrate utilization (Adebukunola Mobolaji Omemu, 2018), methyl red test, hydrogen sulfide, and urease test were used to further identify colonies (Cabeen MT, 2005).

pH and Titratable Acidity Determination

For every three types of juice samples, the pH for five milliliters of juice sample was measured using a digital pH meter (Nig 333, Naina Solaris LTD, India) (Brugger SD, 2012; Phoebe, 2017). The method of Bachun et al. (2008); Mehmood et al. (2019), as well as Downes and Ito (Downes F, 2001), were used to measure titratable acidity (TA). According to above mentioned scholars, 5mL of fruit juice sample was mixed with 20mL distilled water and filtered using Whatman No.1 filter paper. Two drops of indicator (phenolphthalein) were added to 20 ml of the filtrate and then titrated against 0.05M NaOH to determine the endpoint. Finally, TA was expressed as follows:

$$\%TA = \frac{MNaOHXmlNaOHX0.09X100}{\text{ml juice sample}}$$

Where, TA = Titratable acidity; MNaOH = Molarity of NaOH used; ml NaOH = amount (in mL) of NaOH used; 0.09 = equivalent weight of lactic acid.

Data Analysis

All collected data were documented and organized in an MS Excel sheet. Also, the TVC, TCC, and *S. aureu*s count values were converted into logarithm, and then data were statistically analyzed by ANOVA, using SPSS software version 20.0, for some variables, mean comparisons were made using the Tukey HSD test at $P = 0.05$ (Amy C. Spriggs, 2009).

RESULTS AND DISCUSSION

pH Measurement and Titratable Acidity Of Juice Samples

The mean pH of fruit juices ranged from 4.62±0.01, as in the case of papaya juices, to 5.33±0.14 in avocado juices (**Table 1**). The highest titratable acidity was recorded in papaya (0.17±0.02) and the lowest in avocado juices (0.08±0.07). The pH values observed in this study for avocado and papaya were consistent with a previous study on fruit juices conducted in Addis Ababa, Ethiopia, by Abalaka et al. (Abalaka RM, 2013), where the pH values observed for avocado were 5.80. This

pH may be favoring the growth of *Staphlococus aurus*. Korting et al. (1992) and Mohamadi et al. (2018) reported that the optimum pH for growth of *S. aurus* is between 5.00 to 8.50. Ghenghesh (2010) and Tasmina (2010), explained that some species of acidophilic microbes could tolerate low pH even though most microbes cannot tolerate low pH. Those that tolerate low pH could **result** in a serious health threat to fruit juice consumers. This indicat**es** that low pH cannot be guaranteed as means of removal of microbial contamination of fruit juices.

The Bacterial Load In Fruit Juices

The bacterial load of avocado and papaya fruit juices ranged from 5.05±0.08 log to 5.48±0.1log CFU/mL (**Tables 2, 3, and 4**). The mean total viable count of avocado and papaya was within the range of (5.05 log-5.15 log CFU/mL). The lowest value for total viable counts in avocado was 3.44 log and the highest one was 5.68 log CFU/mL, whereas in papaya its lowest and highest value was 4.41 log and 5.53 log CFU/mL respectively. The mean total viable counts of all juice house samples were 5.05±0.08 log CFU/mL in avocado, and 5.15±0.45 log CFU/mL in papaya (**Table 2**). This variation of bacterial count depends on types of juice houses, personal hygiene, and storage handling. However, the results of the current study were not similar to that of Fufa and Liben (Fufa BK, 2018) who reported bacterial load in the range of 3.79 logs to 7.49 log CFU/mL for both avocado and papaya juice. Similarly, the total viable bacterial count of fruit juice in this study ranged from 5.05log to 5.15±0.45 log CFU/mL; which was lower compared to that reported by Singh et al. (2015) who reported 5 log to 8 log CFU/mL range of bacterial count for all samples of street vendor fruit juices. The possible reason may be climate change, duration of sample collection, hygienic conditions, and incubation procedure. Moreover, the current results were consistent with that of Ankur et al. (2009), who reported the lowest total viable count 4logCFU/mL, and the highest 6.6logCFU/mL in avocado and papaya juice.

However, the recommended standard for consumption of fruit juices such as avocado and papaya in the Gulf region is less than 4 log CFU/ml for a total viable count and 2 log CFU/ml for coliform count (Standards, 2000). In this study, the colony counts of all bacterial groups (total viable count, *Staphylococci* count, total coliform count) exceeded the Gulf Region standards. The presence of a high microbial load may be an indication of personal unhygienic while serving juice houses, use of contaminated water, and inadequately cleaned equipment or fruits.

The total coliform counts ranged from 5.15log to 5.51 log CFU/mL in avocado, whereas in papaya they were in the range of 4.30log to 5.50logCFU/mL (**Table 3**). The presence of the large total coliform count for investigated avocado (5.11 log CFU/mL) and papaya juice (5.4log CFU/mL) mentioned in **Table 3** is an indication of fecal contamination which may attribute to improper handling, use of contaminated water, use of unsafe processing utensils such as knives and trays during juice preparation. A similar study conducted in Dhaka, Bangladesh by Ketema (2017) also reported that out of 84 freshly prepared fruit juice samples collected, all samples were positive for coliform and he stated that this larger microbial load may negatively affect the health of juice consumers.

The finding of the current result also revealed that the mean total *Staphylococci* count was 5.48±0.10 log CFU/mL for avocado and 5.12 ±0.73 log CFU/mL for papaya fruit juice samples (**Table 4**). Fufa and Liben (2018) also reported that the total S*taphylococcus* count of 4.3logCFU/mL and 5.23

log CFU/mL bacteriological loads in papaya and avocado juices respectively. As standard by the Gulf region (Standards, 2000), the presence of 3 log CFU/mL *Staphylococci* in food cause juice spoilage and leads to foodborne illness (Standards, 2000). The existence of *S. aureus* in fruit juice may be due to unhygienic practices during juice processing and serving (Nur et al., 2019).

The mean total viable bacterial count for the avocado juice sample collected during the morning was 5.65±0.02 log CFU/mL, and 5.75±0.03 log CFU/m during the afternoon time, whereas the morning sample of papaya contained 5.31±0.01 log CFU/mL, and afternoon sample contained 5.50±0.04 log CFU/mL of the total viable count. Similarly, coliform count in avocado and papaya juice samples also increased from 5.45±0.01(morning) to 5.56±0.02 (afternoon) and 5.60±0.02log CFU/mL (morning) to 5.66±0.07 (afternoon) respectively (**Table 5**). Analysis of one-way ANOVA showed that there was a significant difference between juice samples collected during morning and afternoon time ($P_{=}.05$), but no significant difference between avocado and papaya juice samples ($P > .05$). The bacterial counts of the fruit juices collected during the afternoon time were higher than the bacterial counts observed for the fruit juices collected in the morning time. A comparative study conducted in Accra, Ghana reported that bacteriological analysis of fruit juices indicates that 20% of the makers had the juices that they sold in the mornings with bacterial loads over 4.7 log CFU/mL and this value increased to 80% in the afternoons (Ketema, 2017). The main reason for this difference might be poor storage habits, a more polluted environment or dust in the afternoon than in the morning, and ambient temperatures that favored the proliferation of bacterial load of respective fruit juices. More specifically, based on the questionnaire results, the absence of a refrigerator in most fruit juice houses (**Table 7**) can lead to the proliferation of microbes during hotter times of the day.

Demographic Characteristics of the Respondents

Among 24 respondents, 91.6% were female and educated up to only elementary school. Only 16.7% of respondents had trained about food handling, but 83.3% of them did not get training on how to prepare fruit juice by keeping bacteriological quality and safety and had no awareness that microorganisms contaminate juices (**Table 6**). Due to this 91.7% of fruits were purchased from the open market and stored on a shelf (62.5%) (**Table 7**). This value was consistent with the work of Mekonen (2016) who reported that the open market was the source of fruits used to make juice and shelf as temporary storage of fruits. This condition might lead to bacterial proliferation and dust particles. Observation also confirmed that fruit juice vendors stored fruits on-site that were exposed to temperature abuse and dust, which may enhance the proliferation of contaminant microbes in fruits. This may infect the juice during preparation if the washing practice of fruits is poor. Respondents also reacted to cleaning habits that 66.7% of them washed their hands only with water but only 33.3% of them washed their hands using both water and soap before juice making. Regarding other personal hygiene, 20.8% reported that they cut their fingernails short, while 20.8%,12.5%, and 8.3% of them covered their hair, wore a clean apron, and wore hand jewelry respectively (**Table 7**). Regarding fruit washing habits, 75% of them reported that they washed fruits at the time of juicing but only 25% washed early (**Table 8**). Respondents also reported that most of them rinse fruits without scrubbing the surface (66.7%) and few of them scrub the surface and clean it with water (33.3). About 29.2% of fruit products, however, are used cold running tap water to prepare fruit juices. Regarding temporary storage, about 41.7% of fruit juice was prepared and used immediately, while 58.3% was stored and used on the same day.

According to most juice houses respondents, profit maximization takes precedence over safety concerns. The current study revealed that **a** major source of microbial load in Bule Hora town was associated with poor washing practices of fruits and equipment, use of contaminated water, and personal unhygienic. Environmental (juice house condition) and personal hygiene during serving consumers possibly increase the extent of the contamination. The environmental conditions such as dust particles and nearby garbage add up to make the situation worse as stated by Getachew and Addisu (2017). In the study area, the concerned body should conduct a fruit juice house inspection. Water supplies for fruit cleaning should be potable and all fruit**s** should be subjected to effective washing and rinsing.

CONCLUSION

Generally, the results in the current study indicate the poor hygienic conditions of these juices(Avocado and Papaya) and the consumers are at risk of foodborne illness. Based on obtained data, both avocado and papaya fruit juices were contaminated with bacteria. Fruit juices studied in this study had **a** large bacterial load than the standard set by Gulf region standards and may affect the health of individual fruit juice consumers and communities. The Lack of training on food hygiene, poor processing, improper storage, and handling may be attributed to the contamination of fruit juices. Generally, the results in the present study indicated the unhygienic processing of fruit juices in Bule Hora Town. Therefore, training on food hygiene, safe processing, and handling is very crucial for juice makers to prevent fruit juice contamination.

Acknowledgment

We thank Bule Hora University Biology Department for providing reagents and equipment for laboratory work.

Ethical clearance

Ethical clearance was issued by the Ethical review and editorial board of Bule Hora University (BHU/RPD/057/13 date 04/03/20). Orientation on how to collect data was given to data collectors and oral consent was also taken.

Conflict of interest

The authors declared there is no any conflict of interest

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Figures and Tables

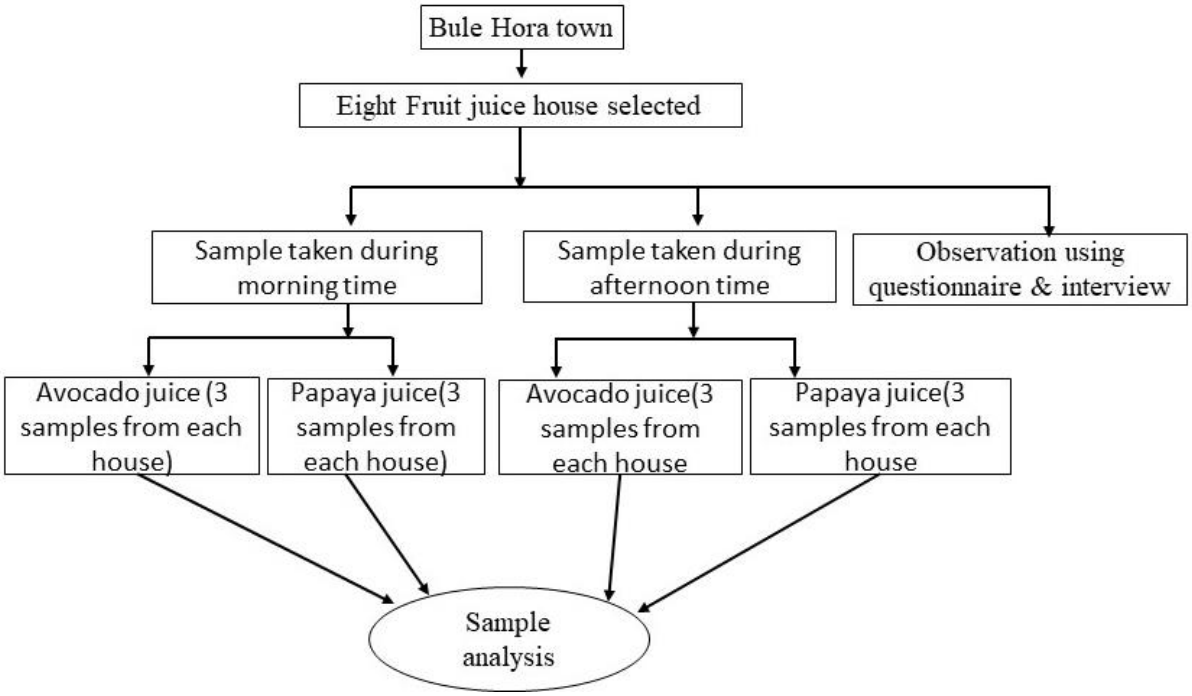

Figure 1. Study design

Table 1: The pH value measurement of fresh fruit juices.

Types of juice sample	Number of samples taken	pH	TA (g of lactic acid/100mL of juice sample)
Avocado	48	5.33±0.14	0.08±0.07
Papaya	48	4.62±0.01	0.17±0.02

Table 2: Total viable count of avocado and papaya fruit juices samples

Juice house	Number of samples	Total viable count (log CFU/mL)		
		avocado	papaya	Mean
01	6	5.68	5.47	5.57
02	6	3.44	5.17	4.30
03	6	5.48	5.36	5.42
04	6	5.57	5.38	5.47
05	6	5.56	4.41	4.98
06	6	4.61	4.47	4.54
07	6	4.48	5.45	4.96
08	6	5.60	5.53	5.56
Mean ± SD		5.05±0.08	5.15±0.45	5.10±0.49

Data represents mean ± SD of 6 samples

Table 3 Total Coliform counts each fruit juice samples

Juice house	Number of samples	Total coliform counts (log CFU/mL)		
		avocado	papaya	Mean
01	6	5.44	4.30	4.87
02	6	5.15	5.00	5.07
03	6	5.40	5.30	5.35
04	6	5.51	5.48	5.49
05	6	5.45	5.32	5.38
06	6	5.49	4.61	5.05
07	6	5.46	5.50	5.48
08	6	5.27	5.39	5.33
Mean ± SD		5.40±0.12	5.11±0.44	5.30±0.22

Data represents mean ± SD of 6 samples

Table 4 Mean *Staphylococcus aureus* counts (SAC) counts of avocado and papaya juice samples

Juice house	Number of samples	***Staphylococci* count (log CFU/mL)**		
		avocado	papaya	Mean
01	6	5.66	5.31	5.48
02	6	5.40	3.32	4.36
03	6	5.54	5.41	5.48
04	6	5.47	5.43	5.45
05	6	5.36	5.41	5.39
06	6	5.56	5.31	5.43
07	6	5.42	5.35	5.38
08	6	5.48	5.48	5.48
Mean ± SD		5.48±0.10	5.12±0.73	5.30±0.39

Data represents mean ± SD of 6 samples

Table 5: The effect of sampling time on the bacterial load of avocado and papaya fruit juices (log CFU/mL).

Sample	No	Morning sample			Afternoon sample		
		Mean of TVC	Coliform count	*Staphylococci* count	Mean of TVC	Coliform count	*Staphlococcus count*
Avocado	24	5.65±0.02[c]	5.45±0.01[a]	5.58±0.10[c]	5.75±0.03[b]	5.56±0.06[c]	5.70±0.05[d]
Papaya	24	5.31±0.01[a]	5.60±0.02[c]	5.45±0.05[a]	5.50±0.04[b]	5.66±0.07[d]	5.53±0.09[b]

Data represents mean ± SD of 24 samples. Different letter along raw indicates significantly different values ($P_{=}.05$).

Table 6 Socio-demographic profile of respondents (n=24)

Variables	Frequency	Percentage (%)
Sex		
Male	2	8.4
Female	22	91.6
Educational background		
No school	3	12.5
Elementary	13	54.2
2ry school (grade 9-12)	6	25
Diploma and above	2	8.3
A profession in Food handling training		
Trained	4	16.7
Not trained	20	83.3

Table 7: Source of fruits, storage site, and cleaning habits (n=24)

Variables	Response	Frequency	Percentage (%)
Source of fruit	Open market	22	91.7
	Direct from producers	2	8.3
Site for temporary storage	Shelf	15	62.5
	Basket	7	29.2
	Refrigerator	2	8.3
Cleaning habit	Wash hands with water	16	66.7
	Wash hands with water & soap	8	33.3
Person hygiene of juice handlers in juice house	Hair coved	5	20.8
	Wear clean apron	3	12.5
	Wear hand jewelry	2	8.3
	Cut fingernails short	14	58.3

Table 8: Washing practice of fruit, and source of water for juice making (n=24).

Variables	Responses	Frequency	Percentage
Time of washing fruits	Washed early	6	25
	Washed at the time of juicing	18	75
Washing practice of fruit	Rinse with water without scrubbing surface	16	66.7
	Scrub surface and cleaned with water	8	33.3
Source of water for juice making	Running tap water	7	29.2
	Tap water in a container	17	70.8
The temporary storage of fruit juice	Prepared for immediate use	10	41.7
	Stored and used on the same days	14	58.3