Microbial Quality of Avocado and Guava Fruits used for Preparation of Freshly Squeezed Juices from Juice Houses of Bahir Dar Town, Northwest Ethiopia.

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ABSTRACT

The objective of this study was to assess the microbial quality of fruits used for preparation of juices from juice houses of Bahir Dar town, Ethiopia. The mean aerobic mesophilic counts from avocado surface, avocado peel, guava surface and guava peel were 5.24, 4.88, 4.98 and 4.35log10cfu/g, respectively. The mean total coliform counts from avocado surface was 272.08, avocado peel 67.31, guava surface 183.80 and guava peel 46.63MPN/g. Similarly, the mean fecal coliform counts were 59.45, 21.87, 48.77 and 19.51MPN/g from avocado surface, avocado peel, guava surface and guava peel, in that order. The mean *S. aureus* counts were also 4.91, 3.94, 2.91 and 1.74log10cfu/g on avocado surface, avocado peel, surface of guava and its peel, correspondingly. Finally, the mean yeast and mold counts were 3.50, 2.95, 2.39 and 1.85log10cfu/g from avocado surface, avocado peel, guava surface and guava peel, respectively. The mean microbial counts of avocado fruits were higher than guava fruits and the fruit surfaces were higher than fruit peels as well. Moreover, 12 (15%) *Salmonella* and 5 (6.25%) *Shigella* spp. were also isolated from both surface and peel of fruits. In almost all juice houses, lack of awareness and poor sanitary conditions promote the probability of fruit contamination. Therefore, keeping the sanitary conditions of juice houses and providing regular training for fruit handlers are some of the practices to improve microbial quality, safety and shelf-life of fruits and their products.

Key words: Avocado, guava, hygienic conditions, juice houses, microbial quality.

1. INTRODUCTION

Fruits are exceptional dietary source of nutrients and fiber for human beings hence vital for good health and fitness [1]. Regular consumption of well balanced foods, rich in fruits are especially valuable for their ability to prevent deficiencies of different vitamins, particularly vitamin A and vitamin C and also reduce the risk of several diseases like breast cancer, congestive heart failure (CHF), stroke, Alzheimer disease, cataracts, urinary tract infection (UTI) and also improve blood lipid profiles in peoples affected by hyper-cholesterolemia [2]. Hence, fruit safety has emerged as an important global issue with international food and public health implication. In response to the increasing of foodborne illness, health and other concerned organizations are intensifying their effort to improve fruit safety [3].

Avocado (*Persea americana*) and guava (*Psidium guajava*) fruits are largely consumed in raw, without further processing in most parts of Ethiopia. They are sold under poor hygienic conditions in the market, juice houses, roadsides and hawkers with excess flies and dusts all over the fruits. These can certainly increase the chance of microbial contaminations. Thus, the presence of these microorganisms on these fruits is dangerous for human consumption [4, 5].

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In Ethiopia, particularly in Bahir Dar town there is no information about the microbial quality of fruits that used for preparation of juices and no continuous assessment of fruit safety has been developed in the juice houses of the town. In addition, sanitation practices of juice houses, hygienic conditions of fruit storages and handling practices of fruits are poor. These factors may affect the microbial quality and safety of fruits and their products which cause a serious health risks to the consumers. Therefore, this study was undertaken to evaluate the microbial quality of avocado and guava fruits used for preparation of freshly squeezed juices from juice houses of the town.

2. MATERIALS AND METHODS

2.1 Sampling Techniques

A cross-sectional study was carried out on a total of 80 (40 avocado and 40 guava) fresh fruit samples, five of each from eight purposively selected juice houses of Bahir Dar town were taken from December 2014 to April 2015 for microbiological analysis. From each selected sample juice houses, one kilogram of each of avocado and guava fruits were purchased on the receipt by considering the effect of time and temperature; each of the fruits were decanted into two separated polythene bags. The collected samples were then transported to Microbiology Laboratory in the icebox and then fruit samples were analyzed within an hour of procurement. From the samples, physically damaged and spoiled fruits were excluded in the study. Additional information on risk factors of sanitary conditions and handling practices of fruits were also taken from selected sample juice houses by checklist at the same time.

2.2 Sample Preparation for Microbial Analysis

Microbiological analysis was done for both surface and inside of avocado and guava fruits by using one sample for each surface and inside analysis by dividing in to two portions and weighing 25gm for each fruit samples and homogenized in 225ml of sterile peptone water separately for fruit surface (wash) parts for about three minutes. The washed fruit samples were then removed aseptically and crushed properly by sterile mortar and pestles separately. The crushed samples were again homogenized in another flask containing 225ml of peptone water for fruit peel including the inside parts. From each homogenized samples, one ml homogenates were transferred to each test tubes containing 9ml of sterilized saline solutions to prepare each of the ten-fold serial dilutions (10^{-2} to 10^{-5}). The homogenates were used for enumeration of quality indicators (AMC, TCC, FCC, and *S. aureus* counts), microbial spoilage (yeast and mold) counts and isolations of pathogens (*Salmonella* and *Shigella*) spp. by standard procedures on appropriate culture media from both surface and peel parts of each fruit samples [6, 7]. The results of microbial analysis were also compared with microbiological criteria set for ready-to-eat foods [8, 9].

2.2.1 Aerobic Mesophilic Counts (AMC)

The aerobic mesophilic count was determined by pour plate method using pour plate count agar. One ml from each last serially diluted samples (10^{-3},10^{-4} and 10^{-5}) were taken aseptically and pour plated into three sterilized triplicate leveled plates then poured the sterile plate count agar (PCA) (Don Whitley ltd, India) on the samples. Then the plates were incubated in inverted position at 37^{o}C for 24 hours under aerobic atmosphere. After incubation plates with colonies between 30 and 300 were counted using Stuart colony counter (UK) and the result was reported as cfu/g of samples [8, 10].

2.2.2 Enumeration of Coliforms,

Total Coliform Counts (TCC)

This test was employed by using Lauryl SulphateTryptose broth (LSTB) (HiMedia ltd, India) for presumptive test and Brilliant Green Lactose Bile Broth (BGLBB) for confirmatory tests. From each last three ten-fold serial dilutions (10^{-3}, 10^{-4} and 10^{-5}), one

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ml of each sample was transferred in to triplicate tubes containing (LSTB) with inverted Durham's tube. Then the samples were incubated at 37℃ for 24 hours. Tubes with acid and gas formations at the end of incubation periods were selected as presumptively positive for total coliform tests. A loop full of inoculum from each presumptive positive tubes were inoculated in to other tubes containing 10ml of (BGLBB) (HiMedia ltd, India) with inverted Durham's tubes for confirmatory test. Then tubes were incubated again at 37^{o}C for 24 hours. As the result of incubation, those tubes which form gas were considered for total coliform confirmation and evaluated according to the MPN table and reported as MPN/g of sample [8, 11].

Fecal Coliform Count (FCC)

Enumeration of fecal coliform was also determined by using the most probable number (MPN) method and LSTB (HiMedia ltd, India) for presumptive test like that of total coliform and other procedures were also similar to that of total coliform counts. The difference is temperature of incubation that is 44.5^{o}C and MacConkey broth (Blulux ltd, India) was used here for confirmatory test instead of BGLBB. Examined for gas production at 24 hours; if the result was negative, examine again at 48 hours. Results of a test were reported as MPN/g of sample [8].

2.2.3 Enumeration of Staphylococcus aureus

Staphylococcus aureus was enumerated by using pour plate method and Manitol Salt agar (MSA) (Oxoid, England). One ml of samples from each last three ten-fold serially diluted solutions (10^{-3}, 10^{-4} and 10^{-5}) were aseptically taken and poured in to triplicate leveled plates and MSA was poured on the samples. Then the plates were incubated in inverted positions at 37℃ for a maximum of 48 hours for the growth of yellow colonies due to manitol fermentation. The mean number of *S. aureus* per gram of each fruit samples was counted as of aerobic mesophilic bacterial counts but in this case plates with 20 to 200 colonies were selected and numbers of colonies were reported as cfu/g of sample [6, 12].

2.2.4 Enumeration of Yeast and Molds

Enumeration of yeast and mold was determined by using Potato Dextrose agar (Blulux ltd, India) in the presence of 10% tartaric acid. Here surface spread-plate technique was used rather than pour plate techniques. This technique provides maximal exposure of the cells to atmospheric oxygen and avoids heat stress from molten agar [7]. The molten PDA was transferred in to empty sterilized triplicate plates and allowed to solidify with 10% tartaric acid. Each 0.1 ml of the homogenized samples from each three ten-fold serially diluted solutions (10^{-2}, 10^{-3} and 10^{-4}) were aseptically taken and spread on the surface of solidified PDA by sterile bent glass rod. Then the plates were incubated in an upright position at 21-25℃ for about 5-7 days in dark. After incubation colonies between 10 and 150 were counted by using Stuart colony counter (UK). Yeast and mold colonies were identified morphologically by their white creamy and fuzzy colors respectively. The results were reported in the form of cfu/g of sample [6, 7].

2.2.5 Isolation and Characterization of Salmonella and Shigella spp.

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In the fruit samples, *Salmonella* and *Shigella* spp. may be present in low numbers in addition to other microorganisms, and they may be injured. To diminish the risk of obtaining false negative results, pre-enrichment (peptone water) and selective enrichment (Selenite Cysteine broth) were used. The homogenized samples from surface and peel of each fruits were incubated at 37^{o}C for 24 hours for pre-enrichment. From each pre-enriched samples, one ml was transferred in to tubes containing 10 ml of Selenite Cysteine broth (HiMedia ltd, India) and thoroughly mixed for two minutes. Following mixing up, tubes were incubated at 37^{o}C for 24 hours [6, 8].

A loop-full of inoculum was aseptically taken from each incubated Selenite Cysteine broth and streaked onto Xylose Lysine Deoxycholate agar (XLD) (Oxoid, England) for *Salmonella* and *Shigella* spp. MacConkey agar (Oxoid, England) was also used additionally for *Shigella*, which were then incubated at 37^{o}C for 24 hours. Morphologically, pink colonies with or without black centers were assumed to be presumptive *Salmonella* and red or pink colonies were assumed to be presumptive *Shigella* on XLD agar but on MacConkey agar smooth colorless, opaque or transparent colonies were also assumed to be presumptive *Shigella* [8, 13]. The presumptive colonies from XLD agar were aseptically picked and streaked on to nutrient agar (Don Whitley, India) for purification purpose and incubated at 37^{o}C for 24 hours. Pure colonies were also transferred aseptically in to Triptic Soya agar (TSA) (Don Whitley ltd, India) slants as stock cultures and stored in refrigerator at 4-5^{o}C. The pure cultures were then subjected to biochemical tests like Citrate utilization test, Motility test, Indole test, Lactose fermentation and H_2S production test, Lysine Iron agar test and Urea hydrolysis test [6, 7, 13].

2.3 Data Analysis

All the data were analyzed with SPSS version 20.0 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 Chi-square (X^2) test and one-way ANOVA was also used to determine microbial mean differences of samples at each juice houses. The results obtained for cfu/gm of juices were transformed into log values.

3. RESULTS AND DISCUSSION

3.1 Microbiological Analysis of Fruit Samples

As shown in Table 3, the overall range of aerobic mesophilic bacteria was 3.11 to 5.57log10 cfu/g with the mean value of 4.86log10cfu/g in both avocado and guava fruits including their peel. There were statistically significant differences between the mean AMC of avocado fruit surface and avocado fruit peel ($p= 0.00$) and between guava fruit surface and guava fruit peel as well ($p= 0.00$). The difference might be due to the surface part of fruits are exposed to dusts, human and animal contacts, flies and dusts that can contribute to such bacterial loads. The mean AMC of both fruit types vary due to low pH value in guava fruit (3.5-4.5) than avocado fruit (6.3-6.6) which inhibits the growth of bacteria [14] and surface nature of fruits, avocado fruit has rough surface than guava fruit that harbors high bacterial load. However, there was no statistically significant difference between the mean aerobic mesophilic bacterial count of avocado and guava fruit surfaces ($p= 0.05$).

The present study demonstrated that the overall total coliform counts present in both fruits ranged from 7.30 to 1100MPN/g with mean value of (142.45MPN/g) (Table 3). From these results the higher mean total coliform count was found on avocado fruit surfaces (272.08MPN/g) and the lower mean total coliform count was found in guava fruit peel (46.63MPN/g).

There were statistically significance differences in total coliform counts between avocado fruit surface and avocado fruit peel ($p= 0.00$) and guava fruit surface and guava fruit peel as well ($p= 0.04$). The difference might be due to the exposure of the outer

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surfaces of fruits to various contacts; frequently visited by flies, and airborne dusts which can be major causes of cross-contaminations. The common practice of using poor quality water to wash fruits and the same holding materials could also be responsible for the difference in bacterial loads on both fruit surfaces [15]. However there was no statistically significant variation in total coliform counts between the two fruit types (p=0.31).

In the case of fecal coliform enumeration, the overall counts found in both fruits ranged from 3 to 210MPN/g with mean value of 37.40MPN/g. The higher mean value of fecal coliform count was found on avocado fruit surface (59.45MPN/g) and the lower mean value of fecal coliform was found in guava fruit peel (19.51MPN/g) (Table 3). There were statistical significance differences in fecal coliform counts between avocado fruit surfaces and their peel (p= 0.00) and guava fruit surfaces and their peel as well (p= 0.01).

The difference might be due to the surface parts of fruits exposed to contacts to human with unwashed hands, flies, airborne dusts and physical damages. The presence of coliform bacteria, especially fecal coliform also indicated that fecal contamination of fruits which may be contributed from different sources such as the water used for irrigation during pre-harvest and postharvest activities, improper storage conditions and poor handling practice at any stage could be responsible for the difference in bacterial loads on the fruit surface [15]. However there was no statistically significant difference in fecal coliform counts between the two fruit types (avocado and guava) (p= 0.66).

Fecal coliform contamination of fresh fruits and vegetables may be due to pre-harvest factors such as irrigation water, animal manure and effluent of wastes and/or postharvest factors like washing water, handling equipments, storage environment, contact with unwashed hands and transportation vehicles [16]. The presence of coliforms in foods including edible fruits is an indicative of recent fecal contamination and there is a greater risk that pathogens may also be present. So, more attention must be given to careful handling practices and sanitary conditions from cultivation to marketing or consumption processes to prevent such contaminations [17].

The overall *S. aureus* counts in both fruits including their peel ranged from 0 to 5.26log10cfu/g with the mean value of 3.38log10cfu/g. But higher *S. aureus* mean count was found in avocado fruits and lower *S. aureus* mean count was found in guava fruits (Table 3). There were statistically significant differences between avocado fruit surface and avocado fruit peel (p= 0.00), guava fruit surface and guava fruit peel (p= 0.00) and between avocado and guava fruit surfaces as well (p= 0.00).

The differences might be most surface parts of fruits are exposed to human contact during picking for preparation and/or marketing, sorting to get healthy fruits, contact to dusts, frequently visited by flies and exposed to damages that can contribute to bacterial loads. The presence of lower pH in guava fruits (3.5-4.5) can also inhibit microbial growth on their parts [14]. The rough surface natures of avocado fruit also harbor high microbial loads and have great contribution for these differences. The pH value of avocado is almost neutral (6.3-6.6) which is favorable for microbial growth and increases the *S. aureus* count on both parts of the fruits. This was also supported by other works [18]. The presence of *S. aureus* in this study indicated that the poor personal hygienic practice and related factors such as fruit handlers do not use glove, hair cover and overcoat to avoid contacts between body parts. Fruit buyers also touched by their hands for sorting healthy fruits during marketing. These and other related factors have significant contributions for cross-contamination of fruits [19].

From the total of fruit samples, 58 (72.5%) were contaminated by *S. aureus*. Out of this almost all 39 (97.5%) of avocado fruit surface, 24 (60%) of avocado peel and only 6 (15%) of guava fruit surface were potentially hazardous based on [11]. While the

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remaining of fruit samples fell into satisfactory level in which, only 1 (2.5%) of avocado fruit surface categorized as good; 1 (2.5%), 3 (7.5%) and 12 (30%) of avocado fruit peel categorized as good, acceptable and unsatisfactory, respectively. In case of guava fruit surface, 7 (17.5%), 2 (5%) and 25 (62.5%) categorized as good, acceptable and unsatisfactory, respectively. Similarly 13 (32.5%) and 27 (67.5%) of guava fruit peel categorized as good and acceptable, respectively. (Table 1)

Table 1: Number and percentage of NSWFA standard categories of *S. auerus* count on surface and in peel of fruits in juice houses of Bahir Dar town, 2015.

Types of fruits	NSWFA Standard Categories				Total
	Good	Acceptable	Unsatisfactory	Potentially	
		no (%)	no (%)	no (%) hazardous	
Avocado surface	1 (2.5)	0	0	39 (97.5)	40
Avocado peel	1 (2.5)	3 (7.5)	12 (30)	24 (60)	40
Guava surface	7 (17.5)	2 (5)	25 (62.5)	6 (15)	40
Guava peel	13 (32.5)	27 (67.5)	0	0	40

In this study, the total yeast and mold counts ranged from 0 to 5.04log10cfu/g with mean value of 2.67log10cfu/g in both fruit samples. Among the fruit samples lower and higher mean yeast and mold counts were found from guava fruit peel (1.85log10cfu/g) and avocado fruit surface (3.50log10cfu/g), respectively. There was also a significant difference in yeast and mold mean counts between avocado fruit surfaces and their peel ($p = 0.02$) and between guava fruit surfaces and their peel ($p= 0.02$).

The probable reasons for the discrepancy might be the presence of different variables among the fruits and juice houses that contribute for the differences. In most juice houses fruits were stored in an opened shelf without any covering materials; open display of fruits attracts the customers but encourages sporadic visits by flies [20]. The dusty, unhygienic storage environments coupled with the poor handling, storage conditions, distribution, marketing practices and transportation are factors contributing to the high microbial load including yeast and mold. Moisturized or cooler storage of fruits and deteriorated surfaces of fruits are also known to be the major pre-disposing factors of fruits to microbial attack both in transit and in storage [21, 22]. The common practice of using the same bucket of water to wash all the fruits and the use of the same holding materials (cross-contamination) could also be responsible for the microbial loads [23].

From the total of fruit samples, almost half 38 (47.5%) of the samples found to be contaminated by yeast and mold counts and the remaining 42 (52.5%) of the fruit samples were free from yeast and mold counts. Out of the contaminated fruit samples, 30 (75%) of avocado fruit surface, 18 (45%) of avocado peel, 30 (75%) of guava fruit surface and 5 (12.5%) of guava fruit peel samples have mean yeast and mold counts and categorized as unsatisfactory. While the remaining fruit samples fell into the maximum count permitted level in which only 10 (25%) of avocado fruit surface had mean yeast and mold counts and categorized as minimum count anticipated; 11 (27.5%) and 11 (27.5%) of avocado fruit peel categorized as minimum count anticipated and maximum count permitted level respectively. But in case of guava fruit sample, only 10 (25%) of guava fruit surface had mean

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yeast and mold counts and categorized as minimum count anticipated; 18 (45%) and 17 (42.5%) of guava fruit peel had mean yeast and mold counts categorized as minimum count anticipated and maximum count permitted level as well [9] (Table 2).

Table 2: Number and percentage of Gulf standard category levels of yeast and mold counts on the surface and in the peel of fruits in juice houses of Bahir Dar town, 2015.

Types of sample	Gulf Standard Categories			Total
	Minimum count anticipated no (%)	Maximum count permitted no (%)	Unsatisfactory no (%)	
Avocado surface	10 (25)	0	30 (75)	40
Avocado peel	11 (27.5)	11 (27.5)	18 (45)	40
Guava surface	10 (25)	0	30 (75)	40
Guava peel	18 (45)	17 (42.5)	5 (12.5)	40

Table 3: Mean and ranges of Microbial counts (log cfu/gm and MPN/gm) on the surface and in the peel of fruits collected from juice houses of Bahir Dar town, 2015.

Enumerated Microbes	**Parameters**	**Sample types and Microbial Counts (log cfu/gm)**			
		Avocado Fruit Surface	**Avocado Fruit Peel**	**Guava Fruit Surface**	**Guava Fruit Peel**
	No of Samples	**40**	**40**	**40**	**40**
AMC	Mean ± SD	5.24 ± 0.17	4.88 ± 0.45	4.98 ± 0.36	4.35 ± 0.63
	Range	4.32-5.57	4.11-5.24	4.16-5.35	3.11-5.17
	P - Value		0.00		0.00
TCC	Mean ± SD	272.08 ± 371.40	67.31 ± 88.45	183.80 ± 245.09	46.63 ± 38.07
	Range	15-1100	7.30-460	14-1100	9.10-150
	P - Value		0.00		0.04
FCC	Mean ± SD	59.45 ± 61.12	21.87 ± 22.72	48.77 ± 47.43	19.51 ± 20.60
	Range	11-210	3.60-93	6.10-150	3-93
	P - Value		0.00		0.01
SAC	Mean ± SD	4.91 ± 0.84	3.94 ± 0.83	2.91 ± 1.46	1.74 ± 1.28
	Range	0 -5.26	0 - 4.99	0 - 4.99	0 - 2.99
	P - Value		0.00		0.00
YMC	Mean ± SD	3.50 ± 2.07	2.39 ± 1.56	2.95 ± 1.78	1.85 ± 1.28
	Range	0 - 5.04	0 - 3.86	0 - 4.82	0 - 3.89
	P - Value		0.02		0.02

*AMC: Aerobic Mesophilic Count; *TCC: Total Coliform Count; *FCC: Fecal Coliform Count;

*SAC: *Staphylococcus aureus* Count and *YMC: Yeast and Mold Count.

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*SD: Standard Deviation.

3.2 Isolation and Characterization of Salmonella and Shigella spp.

In the study *Salmonella* spp. were isolated in 12 (15%) fruit samples containing both of the fruit sample types including their peel; of which 5 (12.5%) avocado fruit surface, 2 (5%) avocado fruit peel, 4 (10%) guava fruit surface and 1 (2.5%) guava fruit peel.

Shigella spp. were also isolated from 5 (6.3%) in both of the fruit sample types except guava fruit peel; of which 3 (7.5%) avocado fruit surface, 1 (2.5%) avocado fruit peel and 1 (5%) from guava fruit surface. The maximum and minimum number of contaminated fruit samples by the two pathogens were identified from juice house 01 (JH01) and 05 (JH05) (5 each) followed by juice house 03, 07 and 08 (2 samples each) and juice house 06 (1 sample), respectively.

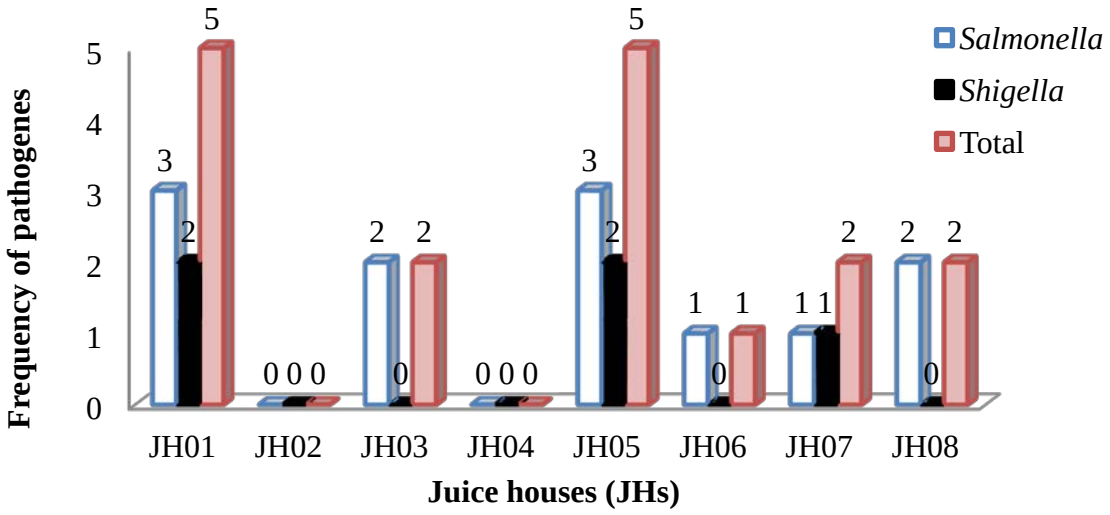

Figure 1: Frequency of isolated *Salmonella* and *Shigella* spp. from surface and peel of avocado and guava fruit samples across juice houses of Bahir Dar town, 2015.

The detection of pathogens like *Salmonella* and *Shigella* spp. in 25gm of food samples examined is regarded as potentially hazardous to consumers and is unacceptable for consumption [27]. This also indicates the necessity for observing hygienic conditions during production and preparation. Because such type of contamination can occur from water, soil, waste and humans who can be carriers of pathogenic species like *Salmonella* and *Shigella* that eventually transfer these foodborne hazards to consumers [24]. So, it is suggested that regular monitoring of hygienic conditions and the quality of fruits that used for preparation of juices and raw consumption must be introduced to protect any future pathogen outbreaks and/or to decrease the density of microbial contaminants from the surface of the fruits [25].

3.3 Assessment of Risk Factors for Fruit Contaminations

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The socio-demographic profile of respondents is presented in (Table 4), altogether there were 40 volunteer personnel in eight juice houses (5 in each juice house) were interviewed; majority 33 (82.5%) of them were females and 7 (17.5%) of them were males. All the respondents were less than 35 years old. Almost half of the respondents 18 (45%) completed secondary school, 9 (22.5%) of the respondents no schooling, 9 (22.5%) completed primary school, 4 (10%) were completed vocational and above. Majority of the respondents 31 (77.5%) did not get training on fruit management systems. However, only 9 (22.5%) of the respondents had professional trainings related to safe fruit management and handling practices that takes its own part to reduce high foodborne contaminations [26].

Table 4: Association of socio-demographic profiles with isolation of *Salmonella* spp. (n = 40).

Parameters	Frequency (%)	*Salmonella* spp.		X^2 (P-value)
		Positive No (%)	Negative No (%)	
Sex				
Male	7 (17.5)	2 (28.6)	5 (71.4)	0.008 (0.93)
Female	33 (82.5)	10 (30.3)	23 (69.7)	
Age				
≤19	7 (17.5)	1 (14.3)	6 (85.7)	1.076 (0.73)
20-29	27 (67.5)	9 (33.3)	18 (66.7)	
≥30	6 (15)	2 (33.3)	4 (66.7)	
Educational Status				
No schooling	9 (22.5)	4 (44.4)	5 (55.6)	2.698 (0.44)
Primary school	9 (22.5)	3 (33.3)	6 (66.7)	
Secondary school	18 (45)	5 (27.8)	13 (72.2)	
Vocational and above	4 (10)	0	4 (100)	
Training on fruit management				
Trained	9 (22.5)	0	9 (100)	4.977 (0.03)*
Not trained	31 (77.5)	12 (38.7)	19 (61.3)	

*= Statistically significant association

Regarding fruit management systems and handling practices presented in (Table 5), most 27 (67.5%) of the respondents reported that they purchased fruits from whole sellers, 10 (25%) also from open markets retailers and the remaining 3(7.5%) directly from producers. After purchasing fruits, majority 37 (92.5%) of the respondents temporarily stored their fruits on shelf outside the juice house in a condition that exposed to temperature abuse and dust. The other 3 (7.5%) of the respondents were stored in refrigerator that inhibit microbial growth. It was also observed that, most 24 (60%) of the respondents stored fruits in bulk for the next more days, 6 (15%) of the respondents also stored fruits in bulk for the coming one day and other 5 (12.5%) of them stored in bulk for the day's use only. Such type of fruit storage systems may contribute to microbial cross-contamination between and/or among fruit types. Only 5 (12.5%) of the respondents reported that they stored fruits separately in type for the day's use that can reduce cross-contamination [27]. Regarding the shelf-life of fruits, 22 (55%) of the respondents disclosed that avocado fruits can't stay for long period of time followed by mango and orange 12 (30%) and 6 (15%), respectively.

Table 5: Association of fruit management systems with isolation of *Salmonella* spp. (n = 40).

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Parameters	Frequency (%)	*Salmonella* spp.		X^2 (P-value)
		Positive No (%)	Negative No (%)	
Sources of fruits				
Open market	10 (25)	3 (30)	7 (70)	0.018 (0.99)
Whole seller	27 (67.5)	8 (26.6)	19 (70.4)	
Producers	3 (7.5)	1 (33.3)	2 (66.7)	
Temporary storage of fruits				
Shelf	37 (92.5)	12 (32.4)	25 (67.6)	1.390 (0.24)
Refrigerator	3 (7.5)	0	3 (100)	
Ways of fruit storages				
In bulk for the day's use	5 (12.5)	1 (20)	4 (80)	11.43 (0.00)*
In bulk for the next day's use	6 (15)	1 (16.7)	5 (83.3)	
In bulk for more days use	24 (60)	10 (41.7)	14 (58.3)	
Separately for day's use	5 (12.5)	0	5 (100)	
Fruits can't stay for long period of time				
Avocado	22 (55)	9 (40.9)	13 (59.1)	3.961 (0.14)
Mango	12 (30)	3 (25)	9 (75)	
Others (Orange)	6 (15)	0	6 (100)	

*= Statistically significant association

Concerning fruit handling and washing practices as presented in (Table 6), majority 30 (75%) of the respondents used fruits for juice preparation without washing that might be responsible for the transmission of pathogens to the consumers or products [28]. However, 10 (25%) of the respondents disclosed that they washed fruits before juice preparation. Hence, washing of fruits before juice preparation is the best means to remove microbial loads, dusts and soil on the surface of fruits. Most 29 (72.5%) of respondents responded that fruits handled by hand only to move fruits from storage to preparation site that can increase the probability of cross- contamination [29]. The remaining 11 (27.5%) responded fruits handled by handling materials used as barrier between hands and fruit contact. However, none of the respondents were used glove to move fruits in juice houses. Majority 32 (80%) of the respondents reported that they did not wash their hands before and after every fruit handling services, this can contribute for microbial contaminations. only 8 (20%) of the workers washed their hands before and after fruit handling.

Table 6: Association of fruit handling and washing practices with isolation of *Salmonella* spp.

(n = 40)

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Parameters	Frequency (%)	*Salmonella* spp.		X^2 (P-value)
		Positive No (%)	Negative No (%)	
Washing of fruits before juice preparation				
Yes	10 (25)	0	10 (100)	5.714 (0.02)*
No	30 (75)	12 (40)	18 (60)	
Handling practices of fruits				
Hand only	29 (72.5)	12 (41.4)	17 (58.6)	6.502 (0.01) *
Hand with glove	0	0	0	
Handling materials	11 (27.5)	0	11 (100)	
Washing of hands before and after fruit handling				
Yes	8 (20)	0	8 (100)	4.286 (0.04)*
No	32 (80)	12 (37.5)	20 (62.5)	

* = Statistically significant association

Concerning the sanitary condition of juice houses and personal hygiene, it was observed that most 27 (67.5%) of the respondents used open space to dispose liquid wastes that provides nutrients for flies and other microbes which may carry foodborne pathogens [30]. Only 11 (27.5%) of the respondents used proper septic tanks with receptacle. However, 2 (5%) did not use liquid waste disposal, instead they used toilet for liquid wastes that may expose for fecal contamination. Like liquid wastes, majority 24 (60%) of the respondents had no specific and proper solid waste disposal, only 13 (32.5%) had proper solid waste disposal with lid and 3 (7.5%) did not use solid waste disposal. Majority 29 (72.5%) of juice house personnel did not wear clean apron and hair cover, this may expose for body contact between fruits and increase cross-contamination. The remaining 11 (27.5%) of the workers wore apron and hair cover. Majority 27 (67.5%) of the respondents did not trimmed their fingernails for the purpose of fruit safety but 13 (32.5%) trimmed their fingernails. Finally, only 8 (20%) followed regular medical checkup for knowing their health status related to foodborne outbreaks.

Table 7: Association of sanitary conditions with isolation of *Salmonella* spp. (n = 40).

Parameters	Frequency (%)	*Salmonella* spp.		X^2 (P-value)
		Positive No (%)	Negative No (%)	
Liquid waste disposal				
Open space	27 (67.5)	10 (37)	17 (63)	10.018 (0.01)*
Septic tank	11 (27.5)	0	11 (100)	
Note available	2 (5)	2 (100)	0	
Solid waste disposal				
Proper with lid	13 (32.5)	3 (23.1)	10 (76.9)	2.225 (0.33)
Improper without lid	24 (60)	7 (29.2)	17 (70.8)	
Note available	3 (7.5)	2 (66.7)	1 (33.3)	
Wearing of apron and hair cover				
Yes	11 (27.5)	1 (9.1)	10 (90.9)	3.159 (0.08)

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No	29 (72.5)	11 (37.9)	18 (62.1)	
Cutting of fingernails short				
Yes	13 (32.5)	2 (15.4)	11 (84.6)	1.959 (0.16)
No	27 (67.5)	10 (37)	17 (63)	
Regular checkup of the health status				
Yes	8 (20)	2 (25)	6 (75)	0.119 (0.73)
No	32 (80)	10 (31.3)	22 (68.7)	

*= Statistically significant association

In this study, all of the risk factors mentioned for isolation of *Salmonella* spp. showed various frequencies in isolation of *Shigella* spp. as well in both fruit surfaces and peel parts of each fruit types from each juice houses. However, they did not show statistically significant association with isolation of *Shigella* spp. all ($p > 0.05$).

4. Conclusion

The study revealed that the microbial quality of avocado and guava fruits including their peel used for preparation of different juices had high counts of aerobic mesophilic bacteria, total coliform, fecal coliform, *S. aureus* and yeast and mold. This indicates that most fruits are exposed for microbial contamination and unsafe for human consumption in raw. It can also be concluded that avocado fruits and their peel had higher microbial loads than guava fruits and their peel. The microbial loads of each fruit surfaces were higher than the microbial loads of each fruit peel as well; this indicates the need of proper washing of fruits prior to juice preparation and raw consumption. Most of the microbial loads of each fruit samples were higher than the microbial limits set for ready-to-eat foods including fruits and vegetables in Gulf regions and other parts of the world. So, juice vendors that produce freshly squeezed juices from these fruits should be aware that preventative measures through food safety control strategies is important. Further study should be recommended on other fruit types in different juice houses together with isolation of other pathogens.

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