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Microbiological Quality of Avocado and Mango Fruit Juices Served in Juice Houses, Cafes and Restaurants in Debre Berhan Town, Ethiopia

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Abstract

Fresh fruits are essential components of the human diet due to nutritional benefits. However, during processing contamination of the final product of fruit juices results in illness. The study aimed to evaluate the microbiological quality of avocado and mango juices consumed in juice houses, cafes, and restaurants of Debre Berhan town. Twenty-four fruit juices samples have been collected from different juice houses, cafés, and restaurants of Debre Birhan town in 2019 and 2020. Results show that the mean TAVBC, ASFBC, TSC, TCC, EC, *E. coli* and yeast and mould count of avocado juice samples were 1.89×10^6, 3.21×10^2, 7.4×10^5, 2.08×10^6, 1.02×10^6, 1.31×10^6 and 1.25×10^6 cfu/ml respectively. The mean TAVBC, ASFBC, TSC, TCC, EC, *E. coli* and yeast and mould count of mango were 1.51×10^6, 2.76×10^2, 9.3×10^5, 9.2×10^5, 9.5×10^5, 8.4×10^5 and 7.7×10^5 cfu/ml respectively. The bacterial isolates were identified as *Staphylococcus aureus, Escherichia coli, Enterobacter* spp. from biochemical tests. The source of contamination of fruit was during harvesting, transporting, processing, and handling of fruits of fresh fruit juices. Therefore, regular supervision and training about harvesting fruit, safe processing, and handling of fruit juices and hygiene of venders could increase the quality of fresh fruit juices.

Keywords: *Avocado; Contamination; Fresh; Mango; Microorganisms*

Introduction

Fruits are important in human nutrition, supplying the necessary vitamins and essential minerals in daily diet. Juices are the aqueous liquids expressed or extracted usually from fruits or vegetables, purees of the edible portion of fruits or vegetables [1]. Juices are prepared by mechanically squeezing of the fruit or vegetable flesh without heating or adding solvents [2] and commonly consumed as a beverage or as flavoring in foods [3].

Fruit and fruit juice consumption could have both positive and negative effects on consumers. Fruit Juices have no fats and are cholesterol-free, rich in vitamins, minerals, and naturally occurring phytonutrients that contribute to good health maintenance. orange juice is rich in vitamin C, an excellent source of bio-available antioxidant photochemical, and significantly improves blood lipid profiles in people affected by hyper-cholesterolemia [1,4]. Fruit juices processed under hygienic condition could play important role in enhancing consumers' health through inhibition of breast cancer, congestive heart failure (CHF), and urinary tract infection [5].

In absence of good production practice, the nutritional richness of fruits and fruit juices makes the product a good medium for microbial growth, a vehicle of foodborne pathogens, and associated complications [6]. The microorganisms present in fruit juices frequently originate from the natural flora of the raw materials used for the preparation and introduced during the processing [7]. Several factors encourage, prevent, or limit the growth of microorganisms in juices; the most important ones are water activity (aw), pH, hygienic practice, and storage temperature and concentration of preservatives. The storage of products at refrigerator temperature is not always best for the maintenance of the desirable quality of some fruits [6].

There are reports of food borne illness associated with the consumption of fruit juices at several places [8-10]. Food borne diseases are harmful illness mainly affecting the gastrointestinal tract and are transmitted through consumption of contaminated food or drink with food borne pathogens. Studies showed that fruit juice might be potential source of pathogenic bacteria of *Bacillus cereus, Clostridium botulinum, Escherichia coli, Shigella spp, Salmonella spp, Vibrio parahaemolyticus, Staphylococcus aureus, Campylobacter jejuni, Streptococcus pyogenes, Mycobacterium bovis, Listeria monocytogenes, Klebsiella spp, Enterobacter spp, Staphylococcus spp* [11,12]. Frequently studied pathogenic food borne bacteria are *E. coli* 0157:H7, *Salmonella spp*, *Shigella spp* and *S. aureus* [2,13,14].

Disease causing microorganisms are introduced to fruits and vegetables via punctures, cuts and splits that occur during growing and harvesting [15]. Colonization of raw materials and equipments, processing conditions, improper handling and unhygienic conditions contribute to the entrance of bacterial pathogens to juices prepared from fruits or vegetables [16].

Food-borne disease is usually caused by bacterial cells themselves or their toxins, which are poisonous proteins produced by these bacteria. Contamination of juices with pathogenic microorganisms has caused various illness and even some fatalities [17,18]. In Debre Berhan town, there is always a great demand for fresh fruit juices while most of juice houses, restaurants and café serve juices in apparently hygienic conditions, their microbiological qualities still remain questionable.

Aim of the Study

The present study aimed to evaluate the microbiological quality of avocado and mango juices and to determine the loads of indicator microbial microorganisms in fresh fruit juices consumed in juice houses, cafes, and restaurants in Debre Berhan town, central Ethiopia.

Material and Methods

Study area

The study was conducted in Debre Berhan town from October 2019 to February 2020. Debre Berhan town is located in Central Ethiopia, North Shewa zone of Amhara region about 130 km northeast of Addis Ababa. This town has a latitude and longitude of 9^0 41'N $39^0$32'E and an elevation of 2,840 meters [19]. The average annual rainfall is 965.09 mm with an average maximum temperature of 23.71ºC and an average minimum temperature of 4.07ºC, mean annual temperature of 13.3ºC of ten years (2010 to 2019) [19]. In the town, there are some juice houses, cafes, and restaurants that prepare and sale unpasteurized mango and avocado fruit juices [20].

Sample size

A random sampling technique was used to take the representative of fruit juices. The samples consisted of 48 fresh juice samples 24 each of Mango (*Mangifera indica*), and Avocado (*Persea americana*) from twelve sites (6 juice houses, 4 Cafes and 2 restaurants) in two rounds. On average of two rounds, twenty-four samples of avocado (12) and mango (12) of locally prepared in fruit juice houses, cafes and ristorantes were collected aseptically in sterile polyethylene bags. All the samples (500 ml of each) were labelled, and immediately transported to Debre Berhan University Microbiology Laboratory in an icebox where they were examined immediately.

Laboratory procedure

For analysis, 25 ml of fruit juice was measured using a measuring cylinder, transferred to 200 ml of sterile peptone water, and homogenized by shaking in an aseptic environment. Serial dilutions of (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}) were prepared by taking 1 ml of a homogenized sample and dishes retained in the first dilution, adding to a sterile test tube containing 9 ml of sterile peptone water and mixing properly [21]. The countable colonies were counted using colony counter from petri plates containing less than 300 and greater than 30 colony forming units (cfu) at two consecutive dilutions to calculate the mean colony forming units per millilitre (cfu/mL) using the formula:

$$N = \frac{n}{df} x\ V(ml)$$

Where N: Number of bacteria colony in original sample; n: Number of colony counted; df: Dilution factor; V: Volume in ml.

The result of cfu was rounded to two significant figures and expressed as a number between 1.0 and 9.9 multiplied by 10 x where x is the appropriate power of 10 [21]. For all laboratory experiments, the petriplates and test tubes were arranged in completely randomized design (CED) in incubators with three replications of two rounds (n = 6).

Microbiological analysis of fruit juice

The microbiological analysis was done using appropriate media designed for enumeration and identification of different microbial groups following standard procedures [22]. Plate count agar was used for bacterial colony counting and potato dextrose agar for fungi [23]. Specifically, Violet Red Bile Agar (VRBA) was used for enumeration of coliforms, whereas *staphylococci* was cultured on Mannitol Salt Agar (MSA). Moderately selective and differential medium for the isolation, cultivation and differentiation of *Salmonella* spp. and *Shigella* spp. SS Agar was used to assure the presence or absence of *Salmonella and Shigella* (SS) in the fruit juices. Absence of lactose fermentation, and presence of production of hydrogen sulfide (H_2S) gas and appearance of colorless colonies with black centers were the indication for presence of *Salmonella spp*. Absence of lactose fermentation, and absence of production of hydrogen sulfide (H_2S) gas and appearance of colorless colonies were indications for the presence of *Shigella spp*.

The total aerobic viable bacterial count

The total aerobic viable bacteria count (TAVBC) of bacteria was performed on plate count agar using the spread plate method. Plate count agar was prepared based on the manufacturer's instruction. Inoculated plates were then incubated at 37ºC for 24 - 48 hours and the total colony counts were determined. The colony developed on the plate was counted from incubated plate as colony-forming units per millilitre (cfu ml^{-1}).

Aerobic spore-forming bacterial

For the enumeration of aerobic spore-forming bacteria count (ASFBC) samples were heat-treated at 80^0C for 10 minutes to destroy vegetative cells and transferred to the plate count agar [9]. The colonies were counted using digital colony counter after incubation at 30ºC for 48 hours [21].

Detection and enumeration of total coliform

MacConkey agar contains four key ingredients (lactose, bile salts, crystal violet, and neutral red) that make it a selective and differential media. One ml sample from each sample of serial dilution was transferred into sterile Petri dishes and 15 ml of violet red bile agar (RVBA) medium (Oxoid company). Lactose-fermenting bacteria produce pink-red colonies, after fermenting the lactose to acids and dropping the pH of the indicator (neutral red) present in the medium. Since, non-fermenters can't utilize lactose, colonies appear colorless or transpar-

ent. Pink-red colonies that are 0.5 mm or larger in diameter and surrounded by a zone of precipitated bile were counted using the digital colony counter.

Detection and enumeration of coagulase positive *Staphylococcus aureus*

In order to detect and enumerate coagulase positive *Staphylococci* (CPS), initial suspension and serial dilutions were prepared. From each samples of previously serial dilution, 1 ml was transferred in to Mannitol salt agar (MSA) and incubated at 37ºC for 24 - 48 hours and growths of yellow and orange colonies surrounded by yellow zones due to mannitol fermentation was counted. Estimation of bacterial load was performed by Gulf standard method known as the recommended microbiological standard for fruit juices for all bacteriological analysis [8].

Coagulase test was performed by a tube coagulase test. The selected *Staphylococcus* was sub cultured into brain heart infusion (BHI) broth and incubated at 37°C for 24 hours. Then, 0.5 ml of broth culture and 0.5 ml of sterile rabbit plasma was put into a narrow sterile tube and placed in an incubator with a control tube containing a mixture of 0.5 ml of sterile brain heart infusion broth. The tubes were incubated at 37°C and examined after 24 hours of incubation for clot formation. Any sign of coagulation of plasma, compared to the control, was regarded as positive for the test.

Detection and enumeration of *Enterobacteriaceae*

Identification of *Enterobacteriaceae* in juice samples was done by using ISO 21528-2:2004 protocols through the following stages of preparation of initial suspension and serial dilutions. Ten ml of the Violet Red Bile Glucose (VRBG) agar at 45ºC was poured into each petri dish. The inoculum and the medium were carefully mixed by rotating the Petri dishes and allowed to solidify by leaving the Petri dishes standing on the horizontal surface of the working bench. After solidification of the mixture, a covering layer of about 10 ml of the VRBG agar was added onto Petri dishes to prevent spreading growth and to create semi-anaerobic conditions and then allowed to solidify again. Thereafter, the plates were inverted and incubated at 37ºC for 24 hours. After the incubation period, the plates were examined for typical and atypical colonies of *Enterobacteriaceae*. Typical colonies are pink to red or purple, with or without precipitation haloes or colorless mucoid colonies, with a diameter of 0.5 mm or more.

Detection of *Escherichia coli*

Escherichia coli were detected according to the procedures outlined by Food and Drug Administration [24]. Petri dishes with MacConkey agar media was labelled and divided into three equal parts. A sterile loop was dipped into a thawed juice sample and streaked onto MacConkey agar plates as a differential media for the identification of *E. coli*. Then, the plates were inverted and incubated at 37ºC for 24 hours. After the incubation period, the plates were examined for typical and atypical colonies. Typical colonies of *E. coli* grown on MacConkey agar are dry, medium in size, pink in color, and appeared singular or in groups.

Yeast and mold count

Each sample was then serially diluted 10 fold (i.e. 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}) in sterile peptone water. An aliquot of 1 ml of each sample was plated out on potato dextrose agar (PDA) and incubated at ambient temperature (26 - 28ºC) for 48 - 72 hours. The colonies were counted, multiplied by the inverse of corresponding dilutions, and reported as yeast and mold count (YMC) ml^{-1} [25]. During enumeration, yeast was distinguished from mold by using a microscope. Thus, smooth (nonhairy) colonies and without extensions at the periphery (margin) was quantified as yeasts, whilst big, spreading and hairy colonies at margin and surface was counted as molds.

Microbial characterization

For microbial characterization, 20 colonies with different morphology and color were picked randomly from countable plate and were purified by repeated plating and characterized to the genus and species level using the following tests like Gram's reaction, urease test, catalase test, oxidase test, indole test, nitrate reduction, citrate test, H_2S test and VP test.

Catalase test

A well-isolated colony was transferred to a clean slide and 1 drop of 3% H_2O_2 was added. Immediate bubble formation was observed for positive test while negative test did not show bubble formation. Catalase positive reaction was ensured by immediate effervescence (bubble formation) while catalase negative reaction was by no (effervescence) bubble formation or a few bubbles after 20 seconds. Used to characterize *Staphylococcus aureus* or *enterics* as catalase-positive.

Indole test

Peptone water was prepared and about three ml of it is dispensed in bijou tubes using a sterile pipette. Then, fresh sterile loops were used to pick isolated colony of bacteria and inoculated into bijou tubes, thereafter, the tubes were incubated at 37ºC for 48 hours. After the incubation period, 0.5 ml of Kovac's Indole Reagent was added to the inoculated bijou tubes. The tubes were subjected to gentle shaking and examined for the red colour in the surface layer within 10 minutes [26]. A red ring on top of the tube was the positive test for *E. coli*.

Oxidase test

The test was performed as described by Oxoid® Ltd., Basingstoke, Hampshire, England, Ref MB0266A, Lot 1284539. The presumed and well-isolated colonies were stickled and streaked onto the moistened oxidase detection strips using sterile plastic loops, and then the strips were observed for colour change within 10 seconds. The appearance of deep blue or purple colour, was a confirmation for an oxidase-positive reaction.

Glucose fermentation test

Using sterile loops, selected colonies that are negative on the Oxidase test was stickled and stabbed into tubes containing glucose agar and then, the tubes were incubated at 37ºC for 24 hours. After the incubation period, the tubes were examined for colour change. If a yellow colour develops throughout the tube and sometimes with gas production, it regarded as a positive reaction of Glucose fermentation.

Detection of microbial pathogens

Salmonella: For detection of *Salmonella*, 25 ml juice samples were added to 200 ml buffered peptone water, vigorously shaken and the suspension was incubated at 37ºC for 24 hours for metabolic recovery and proliferation of cells. From this, 1 ml of culture was transferred into separate tubes each containing 10 ml of Selenite Cystine Broth. The broth was incubated at 37ºC for 24 hours. After secondary enrichment, culture from enrichment broth was separately streaked on plates of Xylose Lysine Deoxycholate (XLD) (Oxoid) medium. Pink colonies with or without black centers from selective medium was picked, purified and tested biochemically [8].

***Escherichia coli*:** Some pathogenic bacteria such as *E. coli* were detected according to the procedures outlined by Food and Drug Administration [24].

***Staphylococcus aureus*:** For detection of *S. aureus*, golden yellow colonies from Mannitol Salt Agar (MSA) during *staphylococci* count were picked, purified and preserved. Coagulase test was done by two ways: slide coagulase test and tube coagulase test [26].

Data analysis

Analysis of variance (ANOVA) was performed to analyze the bacterial count data using Statistical Analysis System (SAS) version 9.1.3 software. Data analysis was performed averagely for the two seasons since homogeneity of error variances was tested positive using the t-test as described by [27]. The t-test had shown homogeneity of the variances of the two seasons for all parameters. Thus, a combined analysis of variance was computed and results were presented accordingly. Fisher's Least significant difference (LSD) was used to separate treatment means at a probability level of $P < 0.05$.

Results

Enumeration of microorganisms

Total aerobic viable bacterial count

The total aerobic viable bacterial count (TAVBC) was significantly influenced ($P \leq 0,05$) by the type of fruits used and the samples. The mean of total viable count for avocado juice was higher (1.89×10^6 cfu ml^{-1}) compared to that of mango juice was (1.51×10^6 cfu ml^{-1}). In avocado juice, the highest TAVBC (2.67×10^6 cfu ml^{-1}) was obtained from sample 11 followed by 2.58×10^6 and 2.45×10^6 cfu ml^{-1} from sample 5 and sample 12, respectively while the lowest TAVBC (0.91×10^6 cfu ml^{-1}) was obtained from sample 3 followed by 1.04×10^6 and 1.06×10^6 cfu ml^{-1} from sample 2 and sample 11 respectively (Table 1). In mango juice, the highest TAVBC (2.73×10^6 cfu ml^{-1}) was obtained from sample 10 followed by 2.62×10^6 and 2.29×10^6 cfu ml^{-1} from sample 9 and sample 11, respectively while the lowest TAVBC (0.571×10^6 cfu ml^{-1}) was obtained from sample 6 followed by 0.60×10^6 and 0.80×10^6 cfu ml^{-1} from sample 8 and sample 7 respectively (Table 1).

Aerobic spore-forming bacterial count

The aerobic spore-forming bacterial count (ASFBC) was significantly influenced ($P \leq 0,05$) by the type of fruits used and the samples. The mean spore-forming bacterial counts ranged from 0.00 to 7.73×10^2 cfu ml^{-1} and 0.00 to 7.37×10^2 cfu ml^{-1} for avocado and mango, respectively. The mean aerobic spore-forming bacterial count (ASFBC) for was higher (3.21×10^2 cfu ml^{-1}) compared to that of mango juice was (2.76×10^2 cfu ml^{-1}). In avocado juice, the highest ASFBC (7.73×10^2 cfu ml^{-1}) was obtained from sample 8 followed by 4.47×10^2 and 4.30×10^2 cfu ml^{-1} from sample 1 and sample 9, respectively while no growth was recorded from sample 6, sample 10 and sample 12 (Table 1). In mango juice, the highest ASFBC (7.37×10^2 cfu ml^{-1}) was obtained from sample 8 followed by 4.47×10^2 and 4.30×10^2 cfu ml^{-1} from sample 1 and sample 9, respectively while no growth was recorded from sample 4, sample 5 and sample 11 (Table 1).

Sample No	TAVBC		ASFBC	
	Avocado	Mango	Avocado	Mango
1	2.47 ± 0.03	2.08 ± 0.03	5.00 ± 0.00	4.47 ± 0.02
2	1.04 ± 0.05	1.17 ± 0.03	3.87 ± 0.04	3.37 ± 0.03
3	0.91 ± 0.01	1.89 ± 0.02	4.10 ± 0.04	3.17 ± 0.02
4	2.23 ± 0.02	1.05 ± 0.06	3.43 ± 0.04	0.00 ± 0.00
5	2.58 ± 0.03	1.23 ± 0.04	4.23 ± 0.05	0.00 ± 0.00
6	1.70 ± 0.01	0.57 ± 0.03	0.00 ± 0.00	3.17 ± 0.02
7	1.97 ± 0.02	0.80 ± 0.03	7.73 ± 0.06	0.00 ± 0.00
8	1.87 ± 0.02	0.60 ± 0.01	3.33 ± 0.02	7.37 ± 0.03
9	1.75 ± 0.05	2.62 ± 0.03	3.60 ± 0.02	4.30 ± 0.01
10	1.06 ± 0.06	2.73 ± 0.04	0.00 ± 0.00	3.43 ± 0.02
11	2.67 ± 0.03	2.29 ± 0.04	3.23 ± 0.03	0.00 ± 0.00
12	2.45 ± 0.05	1.08 ± 0.08	0.00 ± 0.00	3.87 ± 0.04
Total mean	1.89 ± 0.61	1.51 ± 0.75	3.21 ± 0.54	2.76 ± 0.22

Table 1: *The (mean ± standard deviation X106) of total aerobic viable bacterial count (TAVBC) and mean ± standard deviation X102) of Aerobic spore-forming bacterial count (ASFBC) for avocado and mango juices at Debre Berhan Town in 2019 and 2020 (n = 6).*

ASFBC means Aerobic spore-forming bacterial count; TAVBC means total aerobic viable bacterial count.

Staphylococcal count

The *staphylococcal* count was significantly influenced ($P \leq 0,05$) by the type of fruits used and the samples. The mean of *staphylococcal* count for avocado juice was lower (0.74×10^6 cfu ml^{-1}) compared to that of mango juice was (0.93×10^6 cfu ml^{-1}). In avocado juice, the highest *staphylococcal* count (1.09×10^6 cfu ml^{-1}) was obtained from sample 10 followed by 0.89×10^6 cfu ml^{-1} from sample 1 while the lowest *staphylococcal* count (0.57×10^6 cfu ml^{-1}) was obtained from sample 5 followed by 0.61×10^6 cfu ml^{-1} from each sample 4 and 6 (Table 2). In mango juice, the highest *staphylococcal* count (1.65×10^6 cfu ml^{-1}) was obtained from sample 1 followed by 1.34×10^6 and 1.09×10^6 cfu ml^{-1} from sample 9 and sample 7, respectively while the lowest *staphylococcal* count (0.55×10^6 cfu ml^{-1}) was obtained from sample 3 followed by 0.59×10^6 cfu ml^{-1} from each sample of 6 and 11 (Table 2).

Total coliform count

The total coliform count was significantly influenced ($P \leq 0,05$) by the type of fruits used and the samples. The mean of total coliform count for avocado juice was higher (2.08×10^6 cfu ml^{-1}) compared to that of mango juice was (9.2×10^5 cfu ml^{-1}). In avocado juice, the highest total coliform count (2.73×10^6 cfu ml^{-1}) was obtained from sample 9 followed by 2.68×10^6 and 2.67×10^6 cfu ml^{-1} from sample 2 and sample 7, respectively while the lowest coliform count (5.8×10^5 cfu ml^{-1}) was obtained from sample 3 followed by 1.36×10^6 and 1.51×10^6 cfu ml^{-1} from sample 5 and sample 12, respectively (Table 2). In mango juice, the highest total coliform count (2.15×10^6 cfu ml^{-1}) was obtained from sample 1 followed by 1.28×10^6 and 1.04×10^6 cfu ml^{-1} from sample 9 and sample 12, respectively while the lowest coliform count (5.7×10^5 cfu ml^{-1}) was obtained from sample 5 followed by 5.8×10^5 and 6.1×10^5 cfu $ml^{-}$from sample 8 and sample 10, respectively (Table 2).

Sample	*Staphylococcal* count		Total coliform count	
	Avocado	Mango	Avocado	Mango
1	0.89+0.04	1.65+0.05	1.87+0.03	2.15+0.05
2	0.65+0.01	0.96+0.06	2.68+0.03	1.03+0.07
3	0.57+0.05	0.55+0.06	0.58+0.03	0.67+0.07
4	0.61+0.04	0.80+0.03	2.65+0.02	0.63+0.02
5	1.04+0.07	0.64+0.01	1.36+0.01	0.57+0.03
6	0.61+0.02	0.59+0.02	1.57+0.02	0.80+0.03
7	0.72+0.33	1.09+0.09	2.67+0.10	0.99+0.12
8	0.72+0.35	1.00+0.15	2.64+0.01	0.58+0.02
9	0.72+0.33	1.34+0.05	2.73+0.03	1.28+0.03
10	1.09+0.06	1.03+0.03	2.40+0.05	0.61+0.04
11	0.66+0.05	0.59+0.03	2.36+0.04	0.71+0.02
12	0.65+0.05	0.87+0.11	1.51+0.04	1.04+0.05
Total mean	0.74+0.22	0.93+0.33	2.08+0.68	0.92+0.44

Table 2: *The (mean + standard deviation X106) of Staphylococcal count and total coliform count for avocado and mango juices at Debre Berhan Town in 2019 and 2020 (n = 6).*

Total *Enterobacteriaceae* count

The total *Enterobacteriaceae* count was significantly influenced ($P \leq 0,05$) by the type of fruits used and the samples. The mean of total *Enterobacteriaceae* count for avocado juice was higher (2.08×10^6 cfu ml^{-1}) compared to that of mango juice was (9.2×10^5 cfu ml^{-1}). In

avocado juice, the highest total *Enterobacteriaceae* count (1.37 X 10^6 cfu ml^{-1}) was obtained from sample 1 followed by 1.22 x 10^6 and 1.21 X 10^6 cfu ml^{-1} from sample 10 and sample 9, respectively while the lowest *Enterobacteriaceae* count (6.2 x 10^5 cfu ml^{-1}) was obtained from sample 11 followed by 6.6 x 10^5 and 7.3 X 10^5 cfu ml^{-1} from sample 3 and sample 2, respectively (Table 3). In mango juice, the highest total *Enterobacteriaceae* count (2.10 x 10^6 cfu ml^{-1}) was obtained from sample 1 followed by 2.05 x 10^6 and 1.23 x 10^6 cfu ml^{-1} from sample 9 and sample 4, respectively while the lowest *Enterobacteriaceae* count (5.7 x 10^5 cfu ml^{-1}) was obtained from sample 5 followed by 5.2 x 10^5 cfu $ml^{-}$from each sample 5 and 11 (Table 3).

Detection of *Escherichia coli*

The total *Escherichia coli* count was significantly influenced ($P \leq 0,05$) by the type of fruits used and the samples. The mean of total *Escherichia coli* count for avocado juice was higher (1.31 x 10^6 cfu ml^{-1}) compared to that of mango juice was (8.4 x 10^5 cfu ml^{-1}). In avocado juice, the highest total *Escherichia coli* count (2.66 x 10^6 cfu ml^{-1}) was obtained from sample 7 followed by 2.64 x 10^6 and 2.62 X 10^6 cfu ml^{-1} from sample 4 and sample 2, respectively while the lowest *Escherichia coli* count (6.8 x 10^5 cfu ml^{-1}) was obtained from each sample 10 and 11 (Table 3). In mango juice, the highest total *Escherichia coli* count (1.56 x 10^6 cfu ml^{-1}) was obtained from sample 9 followed by 1.46 x 10^6 cfu ml^{-1} from sample 1 while the lowest *Escherichia coli* count (5.3 x 10^5 cfu ml^{-1}) was obtained from sample 4 followed by 5.4 x 10^5 cfu $ml^{-}$from sample 5 (Table 3).

Sample	*Enterobacteriaceae* count		*Escherichia coli*	
	Avocado	Mango	Avocado	Mango
1	1.37+0.04	2.10+0.06	0.98+0.02	1.46+0.05
2	0.73+0.02	0.66+0.05	2.62+0.03	0.73+0.03
3	0.66+0.13	0.98+0.10	0.71+0.04	0.53+0.03
4	1.07+0.02	1.23+0.03	2.64+0.06	0.65+0.09
5	1.07+0.06	0.52+0.01	0.78+0.01	0.54+0.04
6	1.06+0.05	0.53+0.01	0.88+0.23	0.65+0.03
7	1.08+0.07	0.67+0.03	2.66+0.06	1.01+0.03
8	1.08+0.02	0.53+0.02	1.57+0.04	0.95+0.05
9	1.21+0.01	2.05+0.05	0.73+0.05	1.56+0.05
10	1.22+0.03	0.90+0.09	0.68+0.01	0.60+0.03
11	0.62+0.02	0.52+0.01	0.68+0.01	0.66+0.13
12	1.12+0.05	0.66+0.06	0.78+0.02	0.69+0.13
Total mean	1.02+0.23	0.95+0.56	1.31+0.81	0.84+0.34

Table 3: *The (mean + standard deviation X106) of total Enterobacteriaceae count and total Escherichia coli count for avocado and mango juices at Debre Berhan Town in 2019 and 2020 (n = 6).*

Yeast and mould count (YMC)

The yeast and mould count (YMC) was significantly influenced ($P \leq 0,05$) by the type of fruits used and the samples. The mean of total yeast and mould count for avocado juice was higher (1.25 x 10^6 cfu ml^{-1}) compared to that of mango juice was (7.7 x 10^5 cfu ml^{-1}). In avocado juice, the highest total yeast and mould count (2.18 X 10^6 cfu ml^{-1}) was obtained from sample 2 followed by 1.86 x 10^6 and 1.83 X 10^6 cfu ml^{-1} from sample 8 and sample 11, respectively while the lowest yeast and mould count (5.9 x 10^5 cfu ml^{-1}) was obtained from sample 5 followed by 6.2 x 10^5 and 6.6 x 10^5 cfu ml^{-1} from sample 6 and sample 12, respectively 11 (Table 4). In mango juice, the highest total yeast

and mould count (1.27 x 10^6 cfu ml^{-1}) was obtained from sample 1 followed by 9.7 x 10^5 cfu ml^{-1} from each sample of 3 and 11 while the lowest yeast and mould count (5.3 x 10^5 cfu ml^{-1}) was obtained from sample 5 followed by 5.6 x 10^5 cfu $ml^{-}$from sample 7 (Table 4).

Sample	Avocado	Mango
1	1.19 ± 0.04	1.27 ± 0.03
2	2.18 ± 0.02	0.70 ± 0.10
3	0.92 ± 0.02	0.97 ± 0.03
4	1.37 ± 0.05	0.60 ± 0.01
5	0.59 ± 0.01	0.53 ± 0.02
6	0.62 ± 0.04	0.67 ± 0.01
7	0.80 ± 0.05	0.56 ± 0.02
8	1.86 ± 0.06	0.58 ± 0.02
9	1.75 ± 0.05	0.84 ± 0.07
10	1.21 ± 0.01	0.92 ± 0.04
11	1.83 ± 0.07	0.97 ± 0.06
12	0.66 ± 0.04	0.63 ± 0.02
Total mean	1.25 ± 0.54	0.77 ± 0.22

Table 4: *The (mean ± standard deviation X 106) of yeast and mould count of avocado and mango juices in Debre Berhan town (n = 6).*

Morphological tests of fruit juices

Twenty (20) isolates were selected from 10 avocado and 10 mango samples for morphological and biochemical characterization. The average size and colour of the colonies, configuration, and gram reactions of those isolates were analysed and presented in the following table 5. Out of 20 isolates, 15 (75%) were large, and 5 (25%) were medium in size. Out of 20 isolates, 9 (45%) were yellow, 6 (30%) were grey, and 5 (25%) were white in color. 11 (55%) isolates were circular, and 9 (45%) were round in colony shape. Out of 20 characterized isolates, 11 (55%) had gram negative reaction while 9 (45) isolates had gram positive reaction (Table 5).

N0	Isolate	Size	Color	Configuration	Gram Reaction
1	AVJ1	Large	Yellow	Round	+
2	AVJ2	Medium	Yellow	Round	+
3	AVJ4	Medium	Yellow	Round	+
4	AVJ5	Large	Grey	circular	-
5	AVJ7	Large	Grey	circular	-
6	AVJ8	Large	Grey	circular	-
7	AVJ9	Large	Grey	circular	-
8	AVJ10	Large	Grey	circular	-
9	AVJ11	Large	White	circular	-
10	AVJ12	Large	White	circular	-
11	MAJ1	Large	Yellow	Round	+
12	MAJ2	Large	White	circular	-
13	MAJ4	Large	White	circular	-

14	MAJ5	Large	Yellow	Round	+
15	MAJ7	Large	White	Circular	-
16	MAJ8	Large	Yellow	Round	+
17	MAJ9	Medium	Yellow	Round	+
18	MAJ10	Large	Grey	circular	-
19	MAJ11	Medium	Yellow	Round	+
20	MAJ12	Medium	Yellow	Round	+

Table 5: *Morphological characteristics and gram reaction of bacterial colonies collected from Debre Berhan juice houses during 2019 and 2020.*

AVJ: Avocado Juice; MAJ: Mango Juice.

Biochemical analysis of bacterial isolates from juices

Based on the biochemical and gram staining reaction, from 20 isolates 9 (45%) were grouped under the genera *Staphylococcus*, 6 (30%) were classified as *Escherichia coli* and the remaining 5 isolates (25%) were classified as *Enterobacter spp* (Table 6). Based on the finding of this research, the bacterial isolates found in the fresh juices of Avocado and Mango at Dereberhan town were *Escherichia coli, Enterobacter spp.,* and *Staphylococcus spp.* The current study showed that all the juice samples tested were free from sever food born pathogens of *Salmonella* and *Shigella.*

Isolate	Gram Reaction	Catalase	Motility	Indole Test	Oxidase	Gas Production	Organism
AVJ1	+	+	-	-	-	-	*Staphylococcus* spp.
AVJ2	+	+	-	-	-	-	*Staphylococcus* spp.
AVJ4	+	+	-	-	-	-	*Staphylococcus* spp.
AVJ5	-	+	+	+	-	-	*Escherichia coli*
AVJ7	-	+	+	+	-	-	*Escherichia coli*
AVJ8	-	+	+	+	-	-	*Escherichia coli*
AVJ9	-	+	+	+	-	-	*Escherichia coli*
AVJ10	-	+	+	+	-	-	*Escherichia coli*
AVJ11	-	+	+	+	-	+	*Enterobacter* spp.
AVJ12	-	+	+	+	-	+	*Enterobacter* spp.
MAJ1	+	+	-	-	-	-	*Staphylococcus* spp.
MAJ2	-	+	+	+	-	+	*Enterobacter* spp.
MAJ4	-	+	+	+	-	+	*Enterobacter* spp.
MAJ5	+	+	-	-	-	-	*Staphylococcus* spp.
MAJ7	-	+	+	+	-	+	*Enterobacter* spp.
MAJ8	+	+	-	-	-	-	*Staphylococcus* spp.
MAJ9	+	+	-	-	-	-	*Staphylococcus* spp.
MAJ10	-	+	+	+	-	-	*Escherichia coli*
MAJ11	+	+	-	-	-	-	*Staphylococcus* spp.
MAJ12	+	+	-	-	-	-	*Staphylococcus* spp.

Table 6: *Biochemical characteristics of bacterial isolates of different juices collected from Debre Berhan juice houses during 2019 and 2020. AVJ, Avocado Juice; MAJ, Mango Juice.*

Discussions

In the present study, the result showed that all of the fruit juice samples had higher viable bacterial counts more than the acceptable limit. There was significant different between the types of fruits from which juices were prepared and between samples collected from different sites. The Gulf region specifications for fruit juices recommend that the maximum count permitted for the total aerobic bacterial count, coliforms, yeast and molds should be 1.0×10^4, 1.0×10^2, and 1.0×10^3 cfu ml^{-1}, respectively [28]. Even if there was significant differences between fruits and samples, the mean total aerobic viable bacterial count of the two samples was higher than the gulf standards. The possible reason for the variation between types of fruits and samples in viable bacterial count might be the source of fruits, geographical variation, seasonal variation, pH and moisture variation, water used for washing and dilution, time of sample collection, hygiene, and incubation time [18].

The study conducted in Bangladesh showed that the mean total viable count in all freshly prepared fruit juices ranges from 8.00×10^3 to 8.05×10^8 cfu ml^{-1} for mango juices and from 3×10^2 to 9.6×10^8 cfu ml^{-1} for all freshly prepared fruit juices [28]. The mean bacterial count of avocado and mango juices of the present study was within the range, which was studied by Shakir., *et al* [28]. The mean total viable bacterial count of both fruits of the present study revealed microbial load ranging from 5.7×10^5 to 2.73×10^6 cfu ml^{-1}. In Jimma town, [18] also reported that the mean aerobic mesophilic bacterial counts of avocado, papaya, and pineapples were 8.0×10^6, 3.1×10^7, and 7.9×10^6 cfu ml^{-1}, respectively. In this study, spore forming bacteria in fruit juices were higher than the standards of Gulf countries. A few reports have shown the prevalence of aerobic spore-forming bacteria in fruit juices. According to the study conducted in Dhaka city, Bangladesh, 64.91% of the samples exhibited the presence of *Bacillus cereus* [28].

The mean *Staphylococcal* count of avocado was 7.4×10^5 cfu ml^{-1} and that of mango was 9.3×10^5 cfu ml^{-1}. Higher counts of *Staphylococcus* spp were exhibited in all juices samples. The result of this study is inline with findings of earlier work done in Nagpur city, India by Bagde and Tumane [29]. The presence of *Staphylococcus* spp. in almost all the juice samples can be attributed to contamination via handling. This may be due to poor personal and household hygiene indicating a lack of knowledge of hygienic practices and the safety of food products [11]. The study conducted in Nigeria revealed that the lowest number of *Staphylococcus* species (3.5×10^4 cfu ml^{-1}) was observed in avocado juices [3]. The difference in colonial count between the studies may attribute to different factors such as geographical variation, seasonal variation, hygiene, incubation time, sample transportation time, handling, processing, and storage.

In the present study, the mean total *Escherichia coli* count was 1.31×10^6 cfu ml^{-1} for avocado juice and 8.4×10^5 cfu/ml for mango juice. The study conducted in India by Bagde and Tumane [29] reported that *E. coli* was heavily contaminated the fruit juices.

The mean yeast and mould count for avocado juice was 1.25×10^6 cfu ml^{-1} and that of mango juices 7.7×10^5 cfu ml^{-1}. The presence of yeast and moulds in many of the juices suggest that handling of the fruits and their extraction methods might be below the acceptable standards [30]. The result of this study is in agreement with the findings of research conducted in Jimma town by Ketema., *et al*. [18] revealed that the yeasts count of 4.5×10^5 cfu ml^{-1} in avocado. Shakir., *et al*. [28] showed that the presence of fungi in all the freshly prepared fruit juices in the range from 1.00×10^2 to 8.05×10^4 and 1.05×10^{2} 8.05×10^4 for mango juices.

Based on the morphological and biochemical characteristics, the bacterial isolates found in the fresh juices of Avocado and Mango at Dereberhan town were *Escherichia coli, Enterobacter spp* and *Staphylococcus spp.* The current study showed that all the juice samples tested were free from sever food born pathogens of *Salmonella* and *Shigella.* The research results conducted by Wedajo and Kadire, [31] revealed that *E. coli, Salmonella* and *Staphylococcus aureus* were analyzed.

Conclusion and Recommendation

Fruit juices are a vital diet of all age groups due to the associated health benefits. Improperly prepared fresh fruit juices that could be contaminated with microorganisms are recognized as an emerging cause of foodborne illnesses. Contamination of fruit juices vended in

restaurants, cafes, and juice houses are sometimes unacceptable for human consumption and create major health problems. There is no available information about the microbiological safety of the most popular juices, consumed in Debre Birhan town in particular. Therefore, this experiment aimed to evaluate the microbiological safety of avocado and mango juices consumed in cafes and/or restaurants. Almost all samples of avocado and mango juices showed a higher count of microorganisms above the Gulf region standard. All samples of both avocado and mango juice were contaminated with aerobic viable bacteria with a total mean count of 1.89 x 10^6 cfu/ml and 1.51 x 10^6 cfu/ml, respectively. The total mean aerobic spore former bacteria counts were 3.21 x 10^2 cfu/ml, and 2.76 x 10^2 cfu/ml for avocado and mango juice respectively. The total mean of *Staphylococcal* species was 7.4 x 10^5 cfu/ml, and 9.3 x 10^5 cfu/ml for avocado and mango juice respectively. The mean count of total *Enterobacteriaceae* and coliform of avocado juice (1.02 and 1.31) x 10^6 cfu/ml was higher than the mean count of mango juice, which was (9.5 and 8.4) x 10^5 cfu/ml, respectively. The total mean count of yeast and mould counts ranges from 1.25 x 10^6 cfu/ml, as in the case of avocado juice to 7.7 x 10^5 cfu/ml in mango juice.

Based on these data of the assessed fruit juices, the avocado was heavily contaminated with bacteria related to mango juices that could cause health problems. Lack of training (orientation) on food hygiene and safety; improper storage and poor processing and handling of fruit juices might be accredited to contamination of fruit juices. There should be a specific standard for the quality of fruit juices in Ethiopia to avoid bacterial pathogen outbreak. Since the current study was conducted on small sample size, it is also recommended that further study be made using a large sample size with a variety of juices made from different fruits. The food handlers should get training with the aspects of food safety usually in principles of good hygienic practice, the awareness of microbial contaminants, the concept of using pure and sterilized water, the use of safe, clean and better storage of fruits and the practice of using disinfectants.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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