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Research Article

Physical Quality and Microbiological Safety of Some Fruit Juices Served in Cafes/Juice Houses: The Case of Hossana Town, Southern Ethiopia

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Abstract

In general, fruit juices are considered as microbiologically safer than other food stuffs. Nevertheless, numerous infections of human epidemics have been related with the intake of fruit juices, which are contaminated. The objective of the current study was to assess the microbiological safety and quality of juices being served in Cafes/ Juice houses in Hossana town, Southern Ethiopia. Overall of 90 juice samples (30 samples each for avocado, mango and papaya), collected from six purposively selected cafes and/or juice houses in Hossana town, were examined. Additionally, the juices physico-chemical parameters, for instance pH and Titratable acidity were analyzed following standard protocols. The average aerobic mesophilic bacteria count (CFU/ml) of avocado, mango and papaya were respectively 2.2×10^4, 1.3×10^4, and 7.4×10^3. The pH of juices was ranged from 4.05 - 5.79 and that of TA from 0.021 - 0.140 (g lactic acid/100g sample). Mango juice was observed more acidic (pH = 4.05 ± 0.120) than papaya juice (pH = 5.33 ± 0.140) and avocado juice (5.79 ± 0.021). The main bacterial groups isolated from the fruit juices included *Klebsiella, Enterobacter,* and *S. aureus* species. The microbial masses of the fruits juices examined were greater than the specifications set for fruit juices vended in the other areas of the world. To the writers' level of understanding, there is no requirement set for the acceptable level of microbes in fruit juices being served in the study area. Since main isolates were colonies of microorganisms, the reduced hygienic condition of the fruit juice makers and absence of information of using disinfection during processing, also the promising physico-chemical settings of the fruit juices could be contributed to the high microbial concentrations. Thus, great level of workers sanitation is necessity and the use of decontaminators would be better applied for the betterment the microbial quality, safety, and shelf life.

Keywords: Contamination; Fruit Juice; Hossana; Microbiological Safety

Introduction

Fruit juices are well known drinks in various parts of the world. Fruit juices are not fermented but can be fermentable liquid or beverage designed for direct drinking, got from the eatable portion of properly matured and fresh fruit. In hot weather, cafes, and street sideway shops have local services to extract the juice from fruits and then serving the juice abundantly to the thirsty consumers. Fruit juices are food products of great nutritional value, rich in vitamins, mineral salts, simple sugars and organic acids which are easily assimilated by the human. Juice is a fluid drink usually restricted in fruit or vegetable tissues. Juice is made by physically crushing fresh fruits or vegetables without the use of heat or solvent [1].

The drinking of fruit juices might have both positive and negative consequences on the part of customers. Fruit juices handled under germ-free condition can play significant role in increasing consumers' well-being through mitigation of breast cancer, congestive heart failure, and urinary area infection. However, in case of lack of good processing condition, the dietary value of fruit juices makes the product good culture for microbial growth, vector for food borne pathogens and connected problems [2].

Disease causing microbes can get into fruits and vegetables through injured surfaces, such as wounds, scratches and ruptures that happen through growing to harvesting [3]. Contamination from raw processing tools and, processing conditions, inappropriate handling, and incidence of insanitary circumstances result considerably to the entrance of pathogens into fruit juices [3-5].

The survival of different of disease causing organisms in low pH atmospheres has been recognized at distance [6]. Fruit juices contaminated during processing might be the basis of infection. Consequently, it is not amazing that unpasteurized fruits juices, such as avocado, mango, papaya, orange and apple juices have been identified as the vehicle of food borne pathogens in many epidemics. Investigation done on the bacteriological safety of certain juices showed *Salmonella* and *E. coli* O157:H7 infection have been related with orange and apple juices drinking. The most important pathogens strains that contribute to outbreaks in un-pasteurized juice have been indicated as *E. coli* O157:H7, *Salmonella* spp. and *Cryptosporidium* [7].

In Ethiopia, predominantly in urban parts, fruit juices are accessible in shops as bottled forms. Moreover, fruit juice selling facilities, which have been serving diverse types of fruit juices in fresh forms, are booming. However, the scholarly evidence on microbiological safety of fruit juices prepared and consumed in many parts of the country including Hossana town is inadequate. To this end, the present study was initiated with the objective to evaluate the physical quality and microbiological safety of some fruit juices being served in cafes/juice houses in Hossana town.

Materials and Methods

Description of the study Area

The study was conducted at Hossana town, Southern Ethiopia. Hossana town is located at 230 km from Addis Ababa. The altitude of the town is 2200 meter above sea level with mean annual rainfall of 1100mm and mean annual temperature of 18°C. Geographically, the Town is located between 7^0.42'-7.75'N latitude and 37^0.80'-38.07'E longitude [8].

Sample Collection techniques and Survey

Altogether, 90 fruit juice samples (30 each of avocado, mango and papaya) were collected from 6 purposively selected cafes/juice houses in Hossana town from February 2017 to June 2017. Some of the cafes/ Juice houses providing fruit juices for more consumers and serving either one, two or three types of the fruit juices, only those serving maximum number of were considered and six of them were carefully chosen for sampling purpose.

25 ml of each sample of these fruit juices were independently collected in germ-free flask kept at 4oC and sent to laboratory and were analyzed 4 - 6 hours after collection. Initial data on demographic features of the fruit juice producers, servers, and cares being taken throughout storing and processing of the fruit juices was obtained through questionnaire. Individuals involved in the handling and/or serving of the fruit juices in the selected cafes/juice houses were involved.

Sample Processing and Microbial Analysis

Twenty-five milliliters (25 ml) of the fruit juices were distinctly drained and mixed in 225 ml of sterile physiological saline solution (0.85% NaCl). The samples were normalized, and suitable dilutions were plated in replica on pre-dried surfaces of individual media for microbial count: aerobic mesophilic bacteria (AMB) were counted on Plate Count Agar (PCA) after incubation at 32°C for 48 hours; Violate Red Bile Agar (VRBA) were used to count coliforms after incubation for 48 hours at 32°C. Purplish red colonies surrounded by reddish zone of precipitated bile were counted as coliforms. MacConkey agar was used to count Enterobacteriaceae after incubating at 32°C for 48 hours. Associates of Enterobacteriaceae counts were showed Pink to red purple colonies with or without haloes of precipitation. Staphylococci were counted on Mannitol Salt agar (MSA) after incubation at 32°C for 48 hours [9].

Likewise, the counts of yeasts and molds were made on Sabouraud agar plus 0.1g chloramphenicol incubated at 25 - 28°C for 2 - 5 days. Yeasts counted as smooth (non-hairy) colonies without extension at margin and hairy extension colonies at margin were counted as molds.

After inventory, ten colonies from PCA, MSA, VRBA, and MacConkey Agar plates were arbitrarily picked from countable plates and further purified by recurrent plating on PCA.

Determination of pH

After standardizing 10 ml of the fruit juices in 90 ml of distilled water, pH was measured by using digital pH meter (Erkmen and Bozkurt, 2004; Ferrati, 2005).

Titratable acidity Determination

To measure Titratable acidity, the fruit juice sample (5 ml) was normalized in distilled water (20 ml) and sieved through what man No.1 filter paper. 2-3 drops of phenolphthalein were added to 20 ml of the filtrate as indicator and titrated against 0.05M NaOH to the end point of phenolphthalein. Titratable acidity was articulated as gram lactic acid/100g of juice and calculated using the recipe:

$$TA = \frac{\text{MNaOH x ml NaOH x 0.09 x 100}}{\textit{ml juice sample}}$$

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

Experimental Design and Data Analysis

Each experiment was carried out using Completely Randomized Design (CRD) with four replications. Analysis of variance (ANOVA) and significant difference on different parameters was performed using Statistical Software for Social Sciences (SPSS) software package, version 16. Based on the significant results obtained in ANOVA, mean separation was employed using Least Significant Difference (LSD) mean separation method.

Results

Fruits Storage Conditions

From the eighty (80) fruit juice makers/ servers interviewed 59 (73.75%) were females and 21 (26.25%) were males and 61 (76.25%) of them were younger than 25 years. The largest age and smallest age ranges of participants were 49 and 15 respectively, and their average age was 22.78. Even though, 49 (61.25%) of them had completed or were attending high school education, none of the fruit juice makers/ servers 80 (100%) had any experience to professional training on food hygiene and safety related to their contemporary job.

Furthermore, the majority of fruits used for juice making 66 (82.25%) were bought from open markets in Hossana Town and nearby areas/ localities with first choice to the ripened fruits 62 (77.5%). The practice of using disinfectant or any other method of disinfection in most of the studied cafes/Juice houses during processing of juice depend on just on tap water for all purposes. The practice of using disinfectant was unknown. Most of the juices making house/Cafes 67 (83.75%) were temporarily store fruits before juice making in open ground. In all studied Juice houses/cafes there was no knowledge that microorganisms can contaminate fruits/ fruit juices (Table 1).

Characteristics	Number (n)	Percent (%)
Source of Fruits		
Open markets	66	82.25
Growers	14	17.75
Nature of fruits used		
Not ripen	6	7.50
Ripen	62	77.50
Over ripen	12	15.00
Storage condition of fruits		
Shelves	7	8.75
Refrigerators	2	2.50
Baskets	4	5.00
Open ground	67	83.75
Training on food hygiene and safety		
Yes	-	-
No	80	100.00
Total	80	100.00

Table 1: Fruit juice processing conditions in the cafes /juice houses.

Total Heterotrophic Bacterial Count

In avocado fruit juice samples, the total heterotrophic bacteria count ranged from 3.4 x 10^2 CFU/ml to 5.3 x 10^4 CFU/ml. Mango juice samples had total heterotrophic bacteria count of 4.1 x 10^3 CFU/ml to 3.5 x 10^4 CFU/ml. Total heterotrophic bacteria count of papaya fruit juice samples differed from 4.2 x 10^2 CFU/ml to 2.8 x 10^4 CFU/ml as indicated in table 2.

Juice house/ Café code		Fruit Juice	
	Avocado	Mango	Papaya
A	3.4 x 10^2	5.6 x 10^3	2.8 x 10^4
B	5.3 x 10^4	4.1 x 10^3	3.3 x 10^3
C	4.2 x 10^3	3.5 x 10^4	4.2 x 10^2
D	3.5 x 10^4	2.1 x 10^4	5.3 x 10^3
E	2.5 x 10^3	5.4 x 10^3	8.1 x 10^2
F	3.9 x 10^4	7.2 x 10^3	6.5 x $1^0$3

Table 2: Total heterotrophic bacteria count (CFU/ml) of the studied fruit juice samples.

Microbiological Analysis

In papaya fruit juice samples, the mean count of AMB was the maximum (6.8 log cfu/ml). All the studied fruit juices took closer counts of Enterobacteriaceae (5.9 to 6.6 log cfu/ml), although the count was relatively higher (6.3 log cfu/ml) in juice made of avocado. Likewise, the mean counts of staphylococci, yeasts and molds were the highest in avocado (5.61 log cfu/ml), thus, the mean microbial counts were beyond obvious level in all the fruit juice samples studied.

Excluding mango fruit juice sample (pH = 4.05), the mean pH of avocado and papaya juices was in a range that provide the progression of most bacteria and molds. The highest titratable acidity was documented in mango juice that is in agreement with its low pH, (Table 2).

Some members of the Enterobacteriaceae family were observed in almost all the three fruit juices types. Of the ninty fruit juice samples examined, 59 (65.5 %) yielded enteric bacteria, where all avocado and papaya samples were positive. Most often found enteric bacteria were *Klebsiella oxytoca, K. pneumoniae, Enterobacter aerogenes, E. cloacae, E. sakazaki, Serratia liquefaciens, S. odoifaction and S. marcescens, Penicillium* spp (Table 3 and 4).

Type of fruit juice	Sample size	pH	TA
Avocado	30	5.79 $\pm$ 0.15^a	0.021
Mango	30	4.05 $\pm$ 0.02^c	0.222
Papaya	30	5.33 $\pm$ 0.14^{ab}	0.120

Table 3: pH and Titratable acidity (TA) of fruit juices served in cafes/ juice houses.

TA: Titratable acidity (g lactic acid/100g fruit juice). Means followed by the same letter(s) within the rows are not signifi

Discussion

The results obtained in the study indicated that majority of the fruit makers/servers were with inadequate knowledge about whether fruits cause food born disease, cause of spoilage, the effect of spoiled fruits on the healthy fruits and cross contamination of fruits. This might be due to the educational background and lack of information. Containers used to handle fruits, Storage conditions of fruits and type of fruits used could be a source of potential contamination [10].

Sample code and Type	TAMC	TFC	TCC	Microorganisms isolated
A-Avocado	2.6 x $10^{3\ def}$	1.5 x $10^{4\ cde}$	1.9 x $10^{4\ def}$	*S. aureus, Saccharomyces spp. Bacillus spp.*
A-Mango	1.7 x $10^{2\ fg}$	3.1 x $10^{4\ bc}$	-	*S. aureus, Enterobacter spp, Rhizopus*
A-Papaya	2.7 x $10^{2\ ef}$	6.1 x $10^{2\ ij}$	-	*Bacillus spp, Saccharomyces spp.*
B-Avocado	1.5 x $10^{5\ cd}$	3.6 x $10^{2\ kl}$	2.5 x $10^{4\ cd}$	*Saccharomyces spp, Aspergillus*
B-Mango	7.4 x $10^{3\ de}$	1.5 x $10^{3\ ghi}$	5.2 x $10^{3\ gh}$	*Bacillus spp, Aspergillus spp.*
B- papaya	4.3 x $10^{5\ c}$	3.7 x $10^{3\ fgi}$	-	*Enterobacter spp, Penicillium spp, Rhizopus*
C -Avocado	2.4 x $10^{4\ bc}$	-	2.6 x 10^{4} bcd	*Klebsiella oxytoca, Saccharomyces spp.*
C-Mango	8.3 x $10^{5\ b}$	5.5 x $10^{3\ fg}$	4.1 x $10^{5\ bc}$	*Enterobacter spp, Rhizopus*
C-Papaya	6.3 x $10^{5\ bc}$	5.8 x $10^{3\ ef}$	6.4 x $10^{3\ gh}$	*S. aureus, Lactobacillus spp.*
D-Avocado	4.2 x $10^{4\ cd}$	7.3 x $10^{5\ a}$	2.1 x $10^{3\ ij}$	*B. subtils, Penicillium spp.*
D-Mango	7.5 x $10^{6\ a}$	3.9 x $10^{3\ fgi}$	-	*Bacillus spp, Saccharomyces spp.*
D-Papaya	6.3 x $10^{5\ bc}$	3.6 x 10^{4} bcd	7.4 x 10^{3} fgh	*Rhizopus S. aureus, Enterobacter spp*
E-Avocado	3.8 x $10^{4\ bc}$	7.8 x $10^{4\ b}$	5.3 x $10^{5\ a}$	*S. aureus, Lactobacillus spp, Aspergillus spp.*
E-Mango	6.5 x $10^{6\ ab}$	5.7 x 10^{4} bcd	7.4 x $10^{3\ fgh}$	*S. aureus, Saccharomyces spp. Bacillus spp.*
E-Papaya	4.3 x $10^{5\ c}$	6.3 x 10^{4} bcd	3.3 x $10^{3\ hij}$	*Saccharomyces spp, Aspergillus*
F-Avocado	8.2 x $10^{3\ de}$	4.3 x 10^{4} bcde	4.5 x $10^{3\ hi}$	*Enterobacter spp, Rhizopus, S. aureus*
F-Mango	5.6 x $10^{3\ def}$	7.2 x $10^{4\ bc}$	4.8 x $10^{3\ gh}$	*Enterobacter spp, Penicillium spp, Rhizopus*
F-Papaya	7.3 x 10^{4} bcd	5.7 x $10^{3\ ef}$	8.3 x $10^{2\ kl}$	*S. odoifaction, Aspergillus spp*

Table 4: Total microbial counts (cfu/ml) and microbial isolates of fruit juices.

TAMC: Total Aerobic Mesophilic Count; TFC: Total Fungal Count; TCC: Total Coliform Count; -: No Growth. Means followed by the same letter(s) within the rows are not significantly different from each other, LSD at p = 0.05.

The results of experiment by Mosupye and van Holy [11] on Microbial Quality and Safety of Street vended fruit juices pointed out that conditions of fruit juices preparation and selling raise many concerns for consumer's health. In most cases, running water is not available at selling cafes/juice houses; hands and utensils washing are usually done in one or more buckets, and sometimes without soap. Wastewaters and garbages are discarded nearby, providing nutrients for insects and rodents. Some of the juices are not efficiently protected against flies, which may carry food borne pathogens. Safe food storage temperatures are rarely applied to juices. In addition, there is potential health risks associated with initial contamination of fruits by pathogenic bacteria as well as subsequent contamination by juice makers/ servers during preparation, handing, and cross contamination. Similar results are obtained in the present study.

Microbial contaminants of the fruit juice were below 106 cfu/ml thus within acceptable limit for human consumption (ICMSF, 1974). The existence of Benzoic acid together with the low pH of the juice might be accountable for keeping the microbial concentration in check in a tolerable limit. The occurrence of microbial contaminants in all the samples could be a likeness of the quality of the raw materials, processing equipments, environment, handling and serving samples and the workers in the making process. This finding confirms the result of Rahman., *et al.* [12].

Fruit juices have high attraction to redox potential. Having such a high redox potential is a sign for presence of adequate amount of free oxygen accessible to aerobic microorganisms. Therefore, the survival and growth of aerobic bacteria and molds in such products are mostly possible, with the same microbial groups being accountable for contamination of the products [13]. Countless loads coliforms, Enterobacteriaceae and other different microbial groups were recorded from the fruit juices examined in the present study. The variety of microbial counts recoded in the fruit juices examined (1.7×10^2 - 7.5×10^6 CFU/ml) was comparatively higher than the microbial load (1.1×10^2 - 10^5 CFU/ml) described in some past woks [14]. In our understanding, there is no specification set for the acceptable level of microbes in fruit juices being served generally in Ethiopia and specifically in the study site. Nonetheless, the mentioned specifications for fruit juices served in the Gulf region (1×10^4, 100, and 1×10^3 CFU/ml, respectively) recommend that the maximum count acceptable for total colony count of coliforms, yeast and molds [15]. It is clear that colony counts of most of the microbial groups in the present study indicated that our fruit juices surpassed the standard by significant boundary on the basis of the gulf standards. On the other hand, the high court obtained in this study might possibly not certainly pose risk to the well-being of consumers as long as there are no likely disease-causing kinds such as for instance E. coli and Salmonella types in the fruit juices to be used [16].

Even though, some molds and yeasts could withstand the acidity, the pH of fruit juices is generally low with good probability of hindering the growth of disease causing microbes. The large size of coliforms and other Enterobacteriaceae in the juices examined in this study may possibly be because of the high-water action of juices prepared to be served. The results of this study are in line with results obtained by Antony and Chandra [17]. Foodstuffs with high water activity have huge amount of un-bound water molecule that maintenances growth and existence of microbes. But, the low acidity (i.e. higher pH) and thickness of avocado, apart from its nutrient content, makes it a good culture for progression of pathogens.

The mean microbial counts showed that Mango juices were significantly different ($P < 0.05$) from both avocado and papaya juices. Thus, the avocado and papaya juices had more microbial loads than the mango juice. This might be resulted chiefly due to the very low pH seen in mango juice (4.04). Moreover, situations under which the juice was handled, kept, and/or served could contribute to the improvement of the juice product. Actually, its low pH did not prevent the growth of acid withstanding yeasts and permitted their propagation to level as high as 6 log CFU/ml. The decay of acidic foods is most frequently because of contamination of the foods with aerobic acid tolerant bacteria in addition to yeasts and moulds. *Serratia*, *Klebsiells* and *Enterocobacter* were examples of aerobic bacteria types obtained in the present study. Similar results have been reported by Hatcher *et al.*, [12].

The existence of *Bacillus* spp, *Enterobacter* spp, *Lactobacillus* spp, *Penicillum* spp, *Rhizopus* spp, *S. aureus, Aspergillus* spp. and *Saccharomyces* spp. identified in most of isolates known to cause agent of food poisoning and intoxication [18]. The existence of some of these microorganisms are not amazing since most of them are recognized to flourish in medium rich in fermentable substrates such as sugars which often led to the production of acids after fermentation [19]. Insanitary environmental circumstances may result in the occurrence of these bacteria. These findings are in agreement with the work of Nygozi and Adenji [20], on Microbial Analysis of some fruits juice in Port Hacourt Metropolis, Nigeria.

Conclusions and Recommendations

The fruit juices examined in the present study had more microbial concentration than the conditions set for fruit juices in other areas of the world. It is clear that the colony amounts of the microbial groups in our fruit juices surpassed the standard by substantial boundary based on the gulf standards. These high counts, though, could pose danger to the health of customers particularly if disease causing strains are exist in the fruit juices to be used.

Fruit Juice retailers were mostly uneducated of good hygienic practices and causes of diarrhea diseases which could increase the risk of contamination. They were also ignorant of food inspections in addition to lacking supportive services such as clean water supply of adequate quality, waste dumping systems which improve their ability to deliver harmless juice.

As compared to most other areas of the world, in general speaking, most of the fruit juices being served in the study area had higher microbial load than the condition set for fruit juices in some parts of the world. These products could be the cause of health problems and likely carriers of food borne epidemics in high level of workers and consumers.

The habit of consuming fruit and vegetable juices cannot be stopped on unsanitary basis, and juice makers cannot also be prohibited from selling such substances, since it is a source of their income. However, concerned bodies should implement actions to educate the fruit juice suppliers on food safety and sanitation practices. There should be a need to educate the juice makers and retailers on the hazards associated with the cultivation and attitudes to hygienic processing and display of these juices, control measures should be inculcated during providing to consumers. There should also be regular training/retraining and health education of handlers in all aspects of food hygiene and safety, high level of workers hygiene should be required, and the use of disinfectant should be better practiced improving the microbial quality, safety, and shelf-life of the ultimate product.

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