ORIGINAL ARTICLE

MICROBIOLOGICAL SAFETY OF FRUIT JUICES SERVED IN CAFES/RESTURANTS, JIMMA TOWN, SOUTHWEST ETHIOPIA

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ABSTRACT

BACK GROUND: ***Traditionally, fruit products have been regarded as microbiologically safer than other unprocessed foods. However, many outbreaks of human infections have been associated with the consumption of contaminated fruit juices. The objective of this study was to evaluate the microbiological safety and quality of fruit juices being served in Jimma town, Southwest Ethiopia.***

MATERIALS AND METHODS: ***The microbiological safety of different fruit juices being served in cafés/ restaurants in Jimma town were assessed based on the isolates API-20E (Analytical Profile Index used to test for twenty substrates to differentiate Enterobacteriaceae) profile from February, 2005 to July, 2006. A total of 90 samples (30 samples each for avocado, papaya and pine-apple), collected from six randomly selected cafés and/or restaurants in Jimma town, were analyzed. In addition, some physico-chemical parameters of the juices, such as pH and Titratable acidity were determined following standard procedures***.

RESULT: ***The mean aerobic mesophilic bacteria counts (CFU/ml) of avocado, papaya and pine-apples were 8.0 x10^6, 3.1x10^7, and 7.9x10^6, respectively. The counts of yeasts were relatively higher in avocado (4.5x10^{5}CFU/ml) and pine-apple (5.0 x10^6 CFU/ml) as compared to that of papaya (6.2x10^{3}CFU/ml). The pH and Titratable acidity (TA) of all fruit juices were 4.0-5.84 and 0.08-0.223 (g lactic acid/100g sample), respectively. Pine-apple was more acidic (pH= 4±0.001) than avocado (pH= 5.84 ±0.14) and papaya (5.23±0.02). The dominant bacterial groups isolated from the fruit juices included two Klebsella , three Enterobacter , and three Serratia species.***

CONCLUSION: ***The microbial loads of most of the fruits juices were higher than the specifications set for fruit juices sold in the Gulf region and other parts of the world. To the authors' knowledge, there is no specification set for the permissible level of microbes in fruit juices being served in Ethiopia. As dominant isolates were colonies of organisms, the poor hygienic practice of the fruit juice handlers and lack of sound knowledge of using disinfectant during processing, besides the conducive physico-chemical profiles of the fruit juices, might have contributed to the high microbial load. Thus, high level of workers hygiene should be enforced and the use of disinfectant better practiced to improve the microbial quality, safety, and shelf-life of the final product.***

KEY WORDS: ***Fruit juice, Jimma, Microbial safety, physicochemical parameters***

INTRODUCTION

Fruit juices are common beverages in many countries of the world. In hot climate areas, cafés, restaurants and road side stalls have local facilities to extract the juice from fresh fruits and then serving the juice liberally dozed with ice, to the thirsty customers (1).

The consumption of fruit juices could have both positive and negative effect on the part of consumers. 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 (2,3). In absence of good manufacturing practice, however, the nutritional richness of fruit juices makes the product good medium for microbial growth, vehicle of foodborne pathogens and associated complications (1).

Fruit juices contaminated at any point of processing could be the source of infectious pathogens. Study conducted on the microbiological safety of some fruit juices showed *Salmonella* in apple and orange juices (4). *E. coli* O157:H7 infection has been linked with consumption of apple juices (5). The prominent pathogens involved in un-pasteurized juice outbreaks have been identified as *E. coli* O157:H7, *Samonella* spp and *Cryptosporidium* (6).

Although scanty on Ethiopian side, some countries of the world have set standards for the maximum permissible level of microbes in fruit juices and related products (1).

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The setting of standards with respect to microbial load and physico-chemical parameters of products could have paramount important not only for regulatory bodies, but also to the consumers' health.

In Ethiopia, particularly in large urban areas, fruit juices are available in supermarkets in canned or bottled forms. In addition, fruit juice vending houses, which have been serving different types of fruit juices in fresh forms, are proliferating. However, the scientific information on the safety of fruit juices prepared and consumed in Ethiopia is scanty. The objective of this study was, therefore, to evaluate the physico-chemical quality and microbiological safety of some fruit juices being served in Jimma town, southwest Ethiopia.

MATERIAL AND METHODS

A total of 90 fruit juice samples (30 each of avocado, papaya, and pine-apple) were collected from six randomly selected cafés/restaurants from among 30 cafés/restaurants in Jimma from February 2005 to July 2005. As some of the fruit juice vending cafés/restaurants were serving either one, two or three types of the fruit juices, only those serving the maximum number of fruit juices were considered and six of them were selected for sampling following lottery method. The maximum types of fruit juices encountered in the study area were juices made from avocado, papaya, and pine-apple. Samples (250ml each) of these fruit juices were collected in sterile flask (500ml) separately and transported to laboratory at Jimma University, Biology Department, using cold chain. Samples were processed within maximum of an hour after its collection and arrival at laboratory.

Questionnaire was used to obtain preliminary information on the demographic characteristics of the fruit juice makers, servers, and cares being taken during processing of the fruit juices. All the personnel's involved in the processing and/or serving of the fruit juices in the six selected cafés/restaurants were included.

Twenty five milliliters (25ml) of the fruit juices was separately drawn and blended in 225ml of sterile physiological saline solution (0.85% NaCl). The samples were homogenized and appropriate dilutions were plated in duplicate on pre-dried surfaces of respective media for microbial count: aerobic mesophilic bacteria (AMB) were counted on Plate Count Agar (PCA) after incubation at 32 ^{o}C for 48 hours; Violate Red Bile Agar (VRBA) was used to count coliforms after incubation for 48 hours at 32^{o}C. Purplish red colonies surrounded by reddish zone of precipitated bile were counted as coliforms. Enterobacteriaceae were counted on MacConkey agar after incubated at 32^{o}C for 48 hours. Pink to red purple colonies with or without haloes of precipitation were counted as member of Enterobacteriaceae. Staphylococci were counted on Mannitol Salt agar (MSA) after incubation at 32^{o}C for 48 hours. Similarly, Yeasts and molds were counted on Sabouraud agar plus 0.1g chloramphenicol incubated at 25-28^{o}C for 2-5 days. Smooth (non-hairy) colonies without extension at periphery (margin) were counted as yeasts. Hairy colonies with extension at periphery were counted as molds.

After enumeration, ten colonies were randomly picked from countable plates of PCA, MSA, VRBA, and MacConkey Agar plates and further purified by repeated plating on PCA. Cell morphology, Gram reaction, colony characterization and broth features were determined following standard microbiological methods: KOH test, a test for lipopolysaccharide, was made to distinguish between gram-positive and gram-negative bacteria (7). Catalase test was performed by adding few drops of 3% H_2O_2 on plates of an overnight culture of the pure isolates (8).

Isolates were purified by repeated plating on appropriate media. Rod shaped, Gram-negative, non-spore forming bacteria were considered as members of Enterobacteriaceae and were biochemically characterized using API 20E kit (Biomeriuex, Marcy I'Etoile, France). Biochemical profiles of the isolates were analyzed to species and subspecies level using APIWEB® Stand Alone V 1.1.0 software (Biomeriuex, Marcy I'Etoile, France).

pH was measured using digital pH meter (Nig 333, Naina Solaris LTD, India) after homogenizing 10ml of the fruit juices in 90 ml of distilled water (9). Standard method was used to measure Titratable acidity (10). The fruit juice sample (5ml) was homogenized in distilled water (20ml) and filtered through whatman No.1 filter paper. Two-three drops of phenolphthalein were added to 20ml of the filtrate as indicator and titrated against 0.05M NaOH to the end point of phenolphthalein. Titratable acidity was expressed as g lactic acid/100g of juice and calculated using the formula:

$$TA = \frac{MNaOH \times ml\ NaOH \times 0.09 \times 100}{ml\ juice\ sample}$$

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

To check the reliability of the test, API System was used. The standard reference strain used in this study was *Escherichia coli* ATCC 25922 (Oxoid).

Microbiological and some physico-chemical data were expressed as average of the total samples for each fruit juice analyzed. The significance of differences ($P<0.05$) of the mean microbial count among the fruit juices was evaluated with one-way ANOVA using SPSS for windows version 10.0.

RESULTS

All the ninety fruit juice makers interviewed were females and 54 (60.0%) of them were younger than 30 years. Although 45 (50%) of them had completed or were attending high school education, none of the fruit juice makers had any exposure to professional training related to their current career. Moreover, the fruits used for juice preparation were all bought from open markets in Jimma Town with preference to the ripened fruits. In all studied cafés/restaurants, there was no experience of using disinfectant or any other method of disinfection during processing of juice and relied solely on tap water for all purposes (Table 1).

Table1. Fruit juice processing conditions in cafés /restaurants in Jimma town, 2005 - 2006.

Characteristics	Number of respondents	Percent
Source of fruits		
Open market	75	83.3
Directly from producers	15	16.7
Nature of fruit used:		
Ripened	75	83.3
Over ripened	15	16.7
Temporary storage site of fruit		
Shelf	45	50.0
Basket	20	22.2
Refrigerator	15	16.7
No special storage	10	11.1

The mean count of AMB was the highest (6.6 log cfu/ml) in papaya fruit juices. All the three fruit juices had closer counts of Enterobacteriaceae (5.4 to 6.1 lof cfu/ml), although the count was relatively higher (6.1 log cfu/ml) in juice made of avocado. Likewise, the mean counts of staphylococci, yeasts and molds were the highest in avocado (5.41 log cfu/ml), pine-apple (5.75 log cfu/ml) and avocado (5.9 log cfu/ml), respectively, with counts ranging between 5 to 6 log cfu/ml in all cases. Thus, the mean microbial counts were above detectable level in all the fruit juice samples examined (Fig. 1).

Except for pine-apple (pH=4.0), the mean pH of juices made from avocado and papaya was in a range that support the growth of most bacteria and molds. In agreement with its low pH, the highest titratable acidity was recorded in pine-apple juice (Table 2).

Table 2 pH and Titratable acidity (TA) of fruit juices served in cafés/ restaurants in Jimma town, 2005 – 2006.

Types of fruit juices	Sample size	pH	TA
Avocado	30	5.84±0.14	0.081
Papaya	30	5.23±0.01	0.110
Pine-apple	30	4.00±0.001	0.223

Where, TA= Titratable acidity (g lactic acid/100g fruit juice)

Some members of the Enterobacteriaceae family were encountered in almost all the three fruit juices types. Of the ninety fruit juice samples examined, 65 (72.0 %) yielded enteric bacteria, where all avocado and papaya samples were positive. The most frequently found enteric bacteria were *Klebsiella oxytoca, K. pneumoniae, Enterobacter aerogenes, E. cloacae, E .sakazaki, Serratia liquefaciens, S. odoifaction* and *S. marcescens* (Table 3).

Table 3. API-20E profile of Enterobacteriaceae isolate from fruit juices, Jimma town, 2005- 2006.

Sources	Biochemical Tests for utilization of different substrates																				Isolates possible identity	Percent identification
	ONPG	ADH	LDC	ODC	CIT	H_2S	URE	TDA	IND	VP	GEL	GLU	MAN	IND	SOR	RHA	SAC	MEL	AMY	ARA	NO_3-NO_2	
Avocado	+	-	+	-	+	-	-	-	+	+	-	+	+	+	+	+	+	+	+	+	*Klebsiella oxytoca*	97.0
Avocado	+	-	-	-	+	-	+	-	-	+	-	+	+	+	+	+	+	+	+	+	*Klebsiella pneumonia*	98.0
Avocado	+	+	-	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	*Enterobacter cloacae*	90.0
Avocado	+	-	+	-	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+	+	*Klebsiella oxytoca*	97.4
Avocado	+	-	-	-	+	-	+	-	-	-	-	+	+	+	+	+	+	+	+	+	*Klebsiella pneumoniae*	93.8
Avocado	+	-	+	-	+	-	+	-	-	+	-	+	+	+	+	+	+	+	+	+	*Klebsiella pneumoniae*	97.6
Avocado	+	+	-	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	*Enterobacter cloacae*	98.4
Avocado	+	+	+	-	+	-	+	-	-	+	-	+	+	+	+	+	+	+	+	+	*Klebsiella pneumoniae*	97.5
Avocado	+	+	-	+	+	-	+	-	-	+	+	+	+	-	+	+	+	+	+	+	*Enterobacter sakazaki*	78.0
Avocado	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	*Klebsiella oxytoca*	97.0
Papaya	+	-	+	+	+	-	-	-	-	+	-	+	+	+	+	+	+	+	+	+	*Enterobacter aerogenes*	96.6
Papaya	+	+	+	+	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	*Serratia odoification*	99.9
Papaya	+	-	+	-	+	-	-	-	+	+	-	+	+	+	+	+	+	+	+	+	*Klebsiella oxytoca*	97.7
Papaya	+	-	+	+	+	-	-	-	-	+	-	+	+	+	+	+	+	+	+	+	*Enterobacter aerogenes*	96.0
Papaya	+	-	+	+	+	-	-	-	+	-	-	+	+	+	+	+	+	+	+	+	*Klebsiella spp*	85.2
Papaya	+	-	+	-	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+	+	*Klebsiella oxytoca*	97.4
Papaya	+	-	+	+	+	-	-	-	+	+	-	+	+	+	+	+	+	+	+	+	*Enterobacter aerogenes*	96.0
Papaya	+	+	-	+	+	-	+	-	-	+	-	+	+	+	+	+	+	+	+	+	*Enterobacter cloacae*	96.6
Papaya	+	+	-	+	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+	+	*Enterobacter sakazaki*	90.4
Pine apple	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	*Serratia liquefaciens*	67.4
Pine apple	+	-	+	+	+	-	-	-	-	+	+	+	+	+	-	+	+	+	-	+	*Serratia marcescens*	85.5

ONPG= ß-galactosidase production, ADH= arginine dihydrolase, LDC= lysine decarboxylase, ODC= ornithine decarboxylase, URE= urease, TDA= tryptophane deaminase, CIT= Citrate, H_2S= production of hydrogen sulphide, and IND= indole, GLU= glucose, MAN= mannitol, INO= inositol, VP= Vogus prausker (acetoin production), GEL= Gelatin liquefaction, SOR= sorbitol, RHA= rhaminose, SAC=saccharose, MEL= melibiose, AMY= amylase, and ARA=arabinose

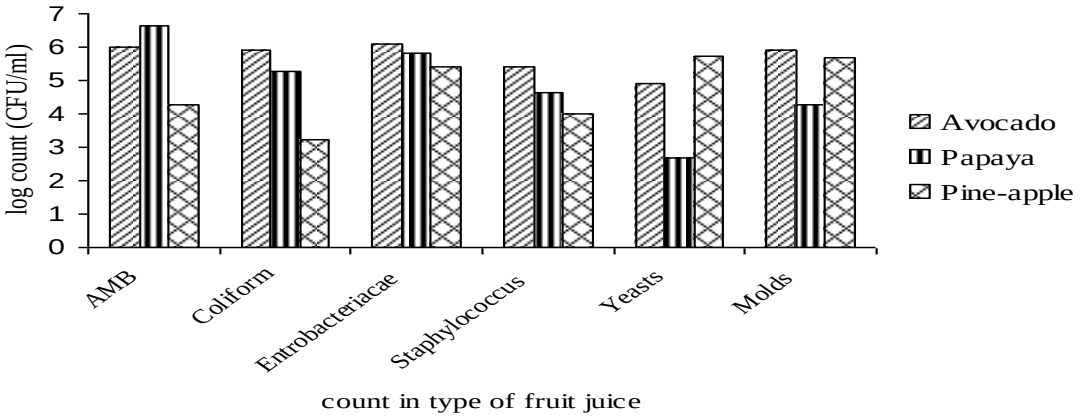

Figure1. Microbial load of fruit juice served in cafés/restaurants in Jimma town, 2005 – 2006. Where, AMB=aerobic mesophilic bacteria.

DISCUSSION

Plant foods, especially plant juices, tend to have redox potential (Eh) values from 300 to 400 millivolt. Having such a high redox potential is an indication for availability of sufficient amount of free oxygen accessible to aerobic microbes. Thus, the survival and growth of aerobic bacteria and molds in such products are highly likely, with the same microbial groups being responsible for spoilage of the same products (5). High loads of different microbial groups, including coliforms and other Enterobacteriaceae were recorded from the fruit juices examined in this study. The range of microbial counts recoded in the fruit juices analyzed in this study ($6.2x10^3$ - $3.1x10^7$CFU/ml) was relatively higher than the microbial load (10^2-10^5 CFU/ml) reported in some earlier woks (11). To the authors' knowledge, there is no specification set for the permissible level of microbes in fruit juices being served in Ethiopia. However, the recommended specifications for fruit juices served in the Gulf region suggests that the maximum count permitted for total colony count of coliforms, yeast and molds are $1x10^4$, 100, and $1x10^3$CFU/ml, respectively (12). On the basis of the gulf standards, it is clear that the colony counts of almost all the microbial groups in our fruit juices exceeded the standard by considerable margin. These high counts ,however, may not necessarily pose hazard to the health of consumers provided that there are no potential pathogenic strains such as strains of *E. coli* and *Salmonella* species within the fruit juices to be consumed(2).

The pH of fruit juices is usually too low with good potential of inhibiting the growth of pathogenic bacteria (5, 13) although some molds and yeasts could tolerate the acidity. Thus, the high magnitude of members of coliforms and other Enterobacteriaceae in the juices examined in this study could be due to the high water activity of ready- to -serve -juices (10). Products with high water activity possess good amount of un-bound water molecule that supports growth and survival of microorganisms. However, the low acidity (i.e., higher pH) and viscosity of avocado, besides its nutrient content, makes it good medium for growth of microorganisms.

The mean microbial counts of pine apple juices were significantly different ($P<0.05$) from that of both avocado and papaya. Pine-apple juice had lower microbial loads than the other two. This could be attributed mainly to the very low pH observed (3.94- 4.04). In addition, conditions under which the juice was processed, stored, and/or served might have contributed to the betterment of the product. In fact, its low pH did not inhibit the growth of acid tolerant yeasts and allowed their proliferation to level as high as 6 log CFU/ml. The spoilage of acidic foods is most often due to contamination of the foods with aerobic acid tolerant bacteria as well as yeasts and moulds (5). *Debaryomyces*, for example, are among the frequently reported genera of yeasts responsible for spoilage of fruit juices (5).

Aerobic bacteria isolated in this study were species of *Klebsiells*, *Serratia*, and *Enterocobacter*. Although reports on the microbiology of fruit juices were scanty, *Serratia* and *Enterobacter* spp, were reported

to dominate the early phases of fermentation of Nigerian palm wine (5).

In addition to the fruit, the equipment used for processing of the juices could contribute to the number of bacterial and fungal species. Regulating the microbial safety of facilities to be used for processing and the use of good quality fruits and surface disinfection besides cleaning with pure water will certainly improve the microbiological quality of these juices. For longer shelf-life and safety of the juices against fungi and molds, the use of an approved food additives could be another best option. Many organic acids with Generally Regarded as Safe (GRAS) status have been currently used for preservation of many foods and juices.

In general, most of the fruit juices being served in Jimma had higher microbial load than the specification set for fruit juices in some parts of the world. As these products could be the cause of health problems and potential vehicle of foodborne outbreaks, high level of workers hygiene should be enforced and the use of disinfectant better practiced to improve the microbial quality, safety, and shelf-life of the final product.

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