

Microbiological Quality of Freshly Prepared, Packaged Fruit and Milk Juices Sold in Cafés, Shops, and Supermarkets in Hargeisa, Somaliland

Mohamud Ahmed Farah^{1,2,3*}, Ahmed Hussein Esa¹, Helmi S. Abdelaziz⁴

¹Department of Health Science, Faculty of Medicine and Health Science, Abaarso Tech University, Hargeisa, Somaliland ²College of Applied Science, University of Hargeisa, Hargeisa, Somaliland

³Hargeisa Water Quality Control Laboratory, Hargeisa Water Agency, Hargeisa, Somaliland

⁴Department of Microbiology, Faculty of Medical Laboratory Science, Haldoor Medical University, Hargeisa, Somaliland Email: *abucarafaad228@gmail.com

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Abstract

Background: Due to their delicious taste, high nutritional content, and health benefits, fruit juices are well-known drinks in many countries and are now an essential component of the modern diet. Objective: Determining the microbiological quality of both packaged and freshly made fruit and milk juices. Method: The spread-plate approach was employed to isolate and count the bacteria. 90 ml of sterile peptone water were blended with 10 ml of wellmixed, packed, and freshly made fruit juices. The samples were sequentially diluted (10¹ - 10⁵) in accordance with the Indian Manual of Food Microbiological Testing Methods. Results: From eight samples of imported packaged fruit and milk juice, the average of total coliform, staphylococci, and viable bacterial counts were zero, 1.39×10^2 , and 2×10^2 CFU/ml, respectively. In contrast, from three samples of locally produced fruit and milk juice, the average of total coliform, staphylococci, and viable bacterial counts were zero, 5.83×10^2 , and 2.73×10^3 CFU/ml, respectively. Four samples of handmade prepared fruit and milk juices had a mean of total coliform, staphylococci, and viable bacterial count of 1.441×10^4 , 4.1×10^3 , and 2.35×10^5 CFU/ml, respectively. Conclusion: 33.3% of the results from microbiological analysis of freshly made fruit and milk juices met the permissible range of the Revised Microbiological Standards for Fruit and Vegetables and Their Products, which were published in 2018 and as well as the Hong Kong Center for Food Safety, whereas 66.7% of the microbiological analyses of freshly prepared fruit and milk juices were above the permissible reference range of GSO standard 2000. 12.5% of the investigated imported and packed fruits and milk juices had one failed test (TSC), which was above the acceptable limit, 87.5% of the tested samples of fruit and milk juices fulfilled the necessary standards of TCC, TVBC, and TSC. 100% of the tested locally manufactured fruit and milk juices complied with TSC, TCC, and TVBC requirements. All investigations showed that freshly made fruit and milk juices were heavily contaminated (Total viable bacterial count, total coliform count, and total staphylococcus count).

Keywords

Total Viable Bacterial Count (TVBC), Total Coliform Count (TCC), Total Staphylococci Count (TSC), Colony Forming Unit (CFU), Gulf State Organization (GSO), Food and Drug Agent (FDA)

1. Introduction

Fruit juices are famous drinks in many nations and are now a vital part of the modern diet due to their brilliant taste, nutritive value, and health advantages [1]. In tropical nations, the consumption of both freshly prepared and packaged fruit juices throughout the year except the coldest months of winter is highly demanded by all classes as well as any age group of the people [2]. Fruit drinks assist in the prevention of different deficiency disorders via the supply of vitamins and minerals [3]. Juices from different fruits lack fat and have different vitamins, flavonoids, carotenoids, polyphenols, and tocopherols, which are important sources of antioxidant phytochemicals that can reduce the chances of arteriosclerosis, cardiovascular diseases, and some types of cancer [4] and [5].

Fruit drinks consist of several vital therapeutic characteristics that may minimize the risk of different disorders such as diabetes, loss of bones, stroke, and neural tube defects like spina bifida and anencephaly during fetal development [6]. However, unless it is prepared safely and in better hygienic situations, fruit juices can be important sources of different infectious agents that can cause outbreaks of foodborne infections across the universe [7]. Insufficiently hygienic situations make fruit drinks a good medium for the multiplication of microbes [8]. Previous research showed that fruit juices may contain pathogenic bacteria such as Salmonella spp., S. aureus, E. coli 0157:H7, Enterobacter species, Klebsiella species, and Listeria monocytogene. Recently, foodborne infections have become worldwide public health concerns, leading to both health and economic effects [9]. In response to health problems associated with foodborne disorders, the Department of Health and other concerned institutions are promoting their efforts to develop the quality and safety of fruit products. However, in thirdworld countries, including Somaliland, there are no regular evaluations of food safety and quality where fresh fruit drinks are prepared and sold [10].

Foodborne diseases are caused by the consumption of fruit juices containing pathogenic bacteria such as Shigella, Salmonella, and *Staphlococcus aureus*.

Contamination of fruit juices originates from raw materials, processing conditions, equipment, contaminated water, inappropriate handling, and prolonged storage at room temperature [10] and [11]. According to research carried out by Callejo *et al.*, which was about the reports of foodborne diseases in the European Union and the United States between 2004 and 2012, Salmonella was the predominant bacterial pathogen. The second predominant bacterial infectious agent was *E. coli*. One of the main outbreaks was associated with the intake of spinach in the United States in 2006. Almost 200 individuals got ill with the *E. coli* 0157:H7 strain, and three individuals passed away. In 2016, a huge outbreak of *E. coli* 0157:H7 occurred in the United Kingdom, where the main transmission mode was blended salad leaves. In 2020, two foodborne outbreaks were noticed in the US. The first was related to green leafy vegetables polluted by *E. coli* 0157:H7, and the second outbreak, which was severe, was due to the same strain of *E. coli* detected in Romaine lettuce. Consequently, fifteen individuals had a kind of renal failure known as hemolytic uremic syndrome [12].

2. Objectives of the Study

To determine the microbiological quality of packaged and freshly prepared fruit juices.

3. Materials and Method

3.1. Study Area

This study was carried out in Somaliland, Hargeisa; capital city of Somaliland. It is situated in the Maroodi-Jeex province, which is one of the country's western regions. Hargeisa's population is estimated to be over 1.2 million individuals. Its latitude and longitude are 9.56 N and 44.077 E, respectively. It has eight districts in which the samples were collected from the two largest and most densely populated districts: Koodbuur and 26th June. This study took two months, from November to December 2022.

3.2. Study Design

A cross-sectional study method was used to assess the microbiological quality of both packaged and freshly prepared fruit drinks in cafes, supermarkets, and shops.

3.3. Sampling Method

The study areas were selected as cafés, shops, and supermarkets by the purposive random sampling method. The selection of study areas was based on the community that they served. Samples were taken from five main areas in two districts of Hargeisa.

3.4. Sample Size Determination

The two largest and densest populated districts in Hargeisa were used to select a total of 15 freshly made and packaged fruit and milk juices. The purposive sam-

pling technique was used to choose seven distinct fruit juices (mango, banana, paw-paw, guava, apple, orange, and banana-flavored milk) based on consumer demand and commonly purchased fruit juices from stores, marketplaces, and cafés. Samples were examined two hours after being obtained. There were five samples of mango, three samples of milk with banana taste, two samples each of banana and apple juice, and one sample of each of the remaining items.

3.5. Sample Collection

11 samples of packaged fruit and milk juices were taken from shops and supermarkets, while 4 freshly prepared fruit juices were taken from a café and collected in sterilized glass bottles following aseptic methods. During transportation of the samples to the laboratory, they were kept in an ice box.

3.6. Coliforms, Staphylococci, and Viable Bacteria Isolation

After samples were bought and brought to the laboratory, their conditions were checked. The microbiological quality of the samples was concentrated on three tests: total viable bacterial count, total coliform count, and total staphylococcal count. The spread plate method was used to isolate and count bacteria. 10 ml of well-mixed, packaged, and freshly prepared fruit drinks were added and sequentially diluted into 90 ml of sterile peptone water. According to the Indian manual of food microbiological testing methods [13], 1 ml of each diluted juice sample was spread on plate count agar, m-Endo agar, and mannitol salt agar for total viable bacterial count, total coliform count, and total staphylococcal count, respectively. After incubation, plates with 25 to 300 colonies were selected and their colonies were enumerated. The results were expressed as colony forming units per milliliter (CFU/ml).

3.7. Enumeration of Bacteria

Coliforms, staphylococci, and viable bacteria were counted using a colony counter after incubation. Pink and greenish-sheen colonies on m-Endo agar were enumerated as coliforms; yellowish and pink colonies grown on mannitol salt agar were counted as staphylococci; while colonies cultivated on plate count agar were enumerated as viable bacteria in both freshly prepared and packaged fruit juices. Gram staining of bacteria, primarily coliforms and staphylococci, was performed following enumeration. Other biochemical tests were not done due to two factors: the purpose of the study, which was to find out the microbial quality of both freshly prepared and imported packaged fruit juices, and the limited resources of the study.

4. Results

4.1. Comparative Distribution of Consumability of Freshly Prepared, Locally Manufactured, and Imported Packaged Fruit and Milk Juices

This study found that one sample of imported fruit and milk juice (6.6%) failed

one test (TSC) but passed the acceptable criteria for two other tests, seven samples of imported packaged fruit and milk juices (46.7%) met the standard reference range of GSO (TVBC and TCC). When the results of three locally manufactured fruit juices (20%) were compared to the GSO standard reference range, they all passed all three tests (TVBC, TCC, and TSC). **Table 1** shows that 4 samples of freshly made fruit juice (26.7%) did not meet Gulf State Organization requirements.

4.2. The Count of Total Viable Bacteria, Coliform and Staphylococci in Imported Packaged Fruit and Milk Juices

According to **Table 2**, the average of total coliform and staphylococci counts were zero and 1.39×10^2 CFU/ml, respectively, while the mean of total viable bacterial count from eight samples of imported packaged fruit and milk juices was 2×10^2 CFU/ml. While 87.5% of the outcomes from the analysis of eight samples met the Gulf State Organization standard's reference range, 12.5% of the findings from that analysis failed one test (TSC) but passed two other tests (TVBC and TCC), meeting the GSO standard's acceptable range.

 Table 1. Comparative distribution of consumability of freshly prepared and imported packaged fruit juices.

Fruit juices	Standard	Non-standard	Total
Imported packaged fruit juices	7	1	8
Locally manufactured packaged fruit juices	3	0	3
Freshly prepared fruit juices	0	4	4
Total	10	5	15

Table 2. The count of total viable bacteria, coliform and staphylococci in imported packaged fruit and milk juices.

Juices	Total viable bacterial count (CFU/ml)	Total coliform count (CFU/ml)	Total Staphylococci count (CFU/ml)
JJ	0	0	0
RFD	0	0	0
FMJ	3×10^2	0	0
Rp	4×10^2	0	0
OC	0	0	0
FPQ	2×10^2	0	0
BFM-1	4×10^2	0	0
BFM-2	3×10^{2}	0	1.11×10^{3}

4.3. The Count of Total Viable Bacteria, Coliform and Staphylococci in Locally Manufactured Packed Fruit and Milk Juices

Table 3 shows that the means of the total coliform and staphylococci counts were zero and 5.83×10^2 CFU/ml, respectively, the average of the total viable bacterial count from three samples of locally produced fruit and milk juices was 2.73×10^3 CFU/ml. Three locally made fruit and milk juices underwent microbiological investigation, and the results fell within the GSO Standard 2000's permissible reference range.

4.4. The Count of Total Viable Bacteria, Coliform and Staphylococci in Freshly Prepared Fruit and Milk Juices

Table 4 shows that the mean of total coliform and staphylococci counts were 1.441×10^4 and 4.1×10^3 CFU/ml, respectively, while the mean of total viable bacterial count from four samples of freshly made fruit and milk juices was 2.35×10^5 CFU/ml. Three newly prepared milk and fruit juice samples had TVBC levels that were beyond the permitted range; all four samples failed the TCC test, and one sample had a TSC value that was above the acceptable upper limit. 33.3% of the results met the permissible range of the Revised Microbiological Standards for Fruit and Vegetables and Their Products, which were published in 2018 by the Food Safety and Standard Authority of India, the Hong Kong Center for Food Safety, and FDA circular No. 2013-010 [14], [15], and [16] respectively. 66.7% of the microbiological analyses of freshly made fruit and milk juices were above the permissible reference range of GSO standard 2000.

Table 3.	The	count	of total	viable	bacteria,	coliform	and	staphylococci	in locally	y manu-
factured	packe	ed frui	t and mi	ilk juice	es.					

Juices	Total viable bacterial count (CFU/ml)	Total coliform count (CFU/ml	Total Staphylococci count (CFU/ml)
UFM-1	5×10^3	0	7×10^2
UFM-2	2×10^3	0	$8 imes 10^2$
UFM-3	1.2×10^{3}	0	$2.5 imes 10^2$

Table 4. The count of total viable bacteria, coliform and staphylococci in freshly prepared fruit and milk juices.

Juices	Total viable bacterial count (CFU/ml)	Total coliform count (CFU/ml)	Total Staphylococci count (CFU/ml)
YAL-1	4.1×10^{5}	$1.31 imes 10^4$	$1.6 imes 10^4$
YAL-2	5×10^5	$4.4 imes 10^4$	2×10^2
YAL-3	4×10^3	3.4×10^2	0
YAL-4	$2.6 imes 10^4$	2×10^2	0

4.5. Morphological and Biochemical Characteristics of Coliforms and Staphylococci Isolates from Fruit and Milk Juices

15 samples of different fruit and milk juices were analyzed microbiologically. On m-Endo agar, bacterial colonies showed two main characteristics: a pink color developed by most of the colonies and a green sheen characteristics. The pink color seen with colonies on m-Endo agar indicates that these bacteria are lactose fermenters, while green-shaded colonies point to E. coli bacteria. On mannitol salt agar, pink and yellow colonies were seen. Pink colonies are coagulase-negative staphylococci: Staphylococcus epidermitis and Staphylococcus saprophyticus, and they don't ferment mannitol sugar in the media. Yellow colonies on mannitol salt agar are coagulase positive (Staphylococcus aureus), and it ferments mannitol sugar in the media. After an enumeration of colonies, bacterial smears of coliforms and staphylococci were prepared, heat fixed, and stained, then they were examined under the microscope to confirm the gram negativity of coliforms and the gram positivity of staphylococci. Other biochemical tests were not done due to two reasons: the purpose of the study, which was to find out the quality of packaged fruit and milk juices only, and limited resources.

5. Discussion

Total viable bacterial count of imported packed fruit and milk juices ranged from 0 to 4×10^2 CFU/ml while their mean was 2×10^3 CFU/ml. The average of TVBC from locally produced, packaged fruit and milk juices was 2.73×10^3 CFU/ml whereas their total viable bacterial count ranged from 1.2×10^3 to $5 \times$ 10⁵ CFU/ml. However the mean of total viable bacterial count from handmade fruit and milk juices was 2.35×10^5 while their TVBC ranged from 4×10^3 to 5×10^5 10⁵ CFU/ml. Freshly prepared fruit and milk juices displayed the highest TBVC when compared to both imported bottled fruit and milk juices as well as locally manufactured and packaged fruit juices. Freshly made pawpaw juice had the highest TVBC, while banana juice had the lowest. A study conducted in Bangladesh [5] found similar results of higher TVBC of handmade fruit juices. Results from microbial analysis of some packed fruit and milk juices from either locally produced or imported juices displayed better quality than freshly made fruit and milk juices, as shown in a study that was performed in Northwest Ethiopia [2]. Some of the factors causing severe contamination of freshly made fruit and milk juices are poor food hygiene and sanitation, contaminated water and utensils, dust particles, and a lack of insect and rodent control in the cafes.

Microbes in freshly prepared fruit and milk juices are reduced by good food hygiene and sanitation. Cleaning fruits before grinding washes microbes away from the fruits' surfaces and minimizes the number of microbes that may contaminate extracted juices. Wearing gloves also prevents contamination of fruit and milk juices from the vender because there are microbes on the palms and as well as under the nails, some of which originate from feces while others are natural inhabitant of the human integumentary system. Water quality in Hargeisa is also poor, particularly that from boreholes and shallow wells, because it has higher coliform and *E. coli* counts, as confirmed in a study carried out in 2021 [17]. Dust particles and insects also cause contamination of fruit and milk juices after falling and landing as dust particles and flies in the grinder, respectively. A similar study in Vietnam discovered higher total viable bacterial counts in freshly prepared fruit juices [18]. 73.3% of the analyzed samples had a total viable bacterial count that met the acceptable limit of the GSO standard for pasteurized fruit juices, while 26.7% of the samples had a higher TVBC than the accepted limit of the GSO standard. Packed fruit and milk juices, whether they are imported or produced locally, and meet international standards.

According to **Table 5**, eleven samples of both imported and locally manufactured packed fruit and milk juice were tested, and their total coliform counts were zero and met the GSO standard. According to the results of locally manufactured fruit and milk juices, some of Somaliland's fruit juice producing industries use good manufacturing practices. According to research that was performed in Tirana, Albania, they also found similar results [19]. An analysis of four samples of freshly made fruit and milk juices revealed higher total coliform bacteria, whose mean was 1.441×10^4 CFU/mL while their TCC ranged from $2 \times$ 10^2 to 4.4×10^4 CFU/mL. TCCs of four freshly prepared fruit and milk juices did not meet the GSO standard. According to two researches which were carried out in Southwest Nigeria and Ethiopia also showed higher coliform counts which were beyond acceptable limit of GSO [20] and [21].

Juices	TVBC (CFU/ml)	TCC (CFU/ml)	TSC (CFU/ml)
JJ	0	0	0
RFD	0	0	0
FMJ	3×10^2	0	0
Rp	4×10^2	0	0
OC	0	0	0
FPQ	2×10^2	0	0
BFM -1	3×10^2	0	0
BFM -2	4×10^2	0	1.11×10^{3}
UFM-1	5×10^{3}	0	7×10^2
UFM-2	2×10^3	0	8×10^2
UFM-3	1.2×10^{3}	0	$2.5 imes 10^2$
YAL-1	4.1X 10 ⁵	$1.31 imes 10^4$	2×10^2
YAL-2	5×10^5	$4.4 imes 10^4$	$1.6 imes 10^4$
YAL-3	4×10^{3}	3.4×10^2	0
YAL-4	2.6×10^{4}	2×10^{2}	0

Table 5. Microbiological evaluation of various types of fruit and milk juice samples.

66.7% of the microbiological analyses of freshly made fruit and milk juices were above the permissible reference range of GSO standard 2000 while 33.3% of the results met the permissible range of the Revised Microbiological Standards for Fruit and Vegetables and Their Products, which were published in 2018 by the Food Safety and Standard Authority of India and the Hong Kong Center for Food Safety. Seven out of eight imported packed fruit and milk juices had zero colonies of staphylococci except one sample with 1.11×10^3 CFU/ml, so the average of total staphylococci for eight imported and packed fruit and milk juices was 1.39×10^2 CFU/ml, while the mean of total staphylococci count of three locally produced fruit juices was 5.83×10^2 colonies. The mean of total staphylococci counts for four samples of freshly prepared fruit and milk juices were 4.1×10^3 CFU/mL. According to two studies conducted in Ethiopia and Bangladesh, freshly prepared fruit and milk juices contained higher staphylococci counts [22] and [23].

6. Conclusion

Generally, this research indicated that some of the freshly prepared fruit and milk juices were contaminated with various bacterial species. Based on colony characteristics, and Gram staining, coliforms and staphylococci were isolated from handmade fruit and milk juices which exceeded the acceptable range of GSO standard 2000. Certain coliforms such as Enterotoxigenic *E. coli* and *Staphylococcus aureus* can cause food borne infections. Lack of training on food safety and hygiene including incorrect fruit storage and preparation of fruit juices may worsen the contamination. 87.5% of tested samples from imported packed fruit and milk juices met the TCC, TVBC and TSC standards of GSO 2000 while 12.5% of analyzed imported packed fruit and milk juices had a single failed test (TSC) which was above the acceptable standard. Analysis of locally produced packed fruit and milk juices demonstrated that their results completely met the required standards of TSC, TCC, and TVBC, although fewer samples of locally manufactured fruit and milk juices that were analyzed

7. Recommendation

These are some of the recommendations that came out of this study after packed and freshly prepared fruit and milk juices were tested microbiologically.

1) Imported fruit and milk juices should be analyzed both chemically and microbiologically to find out whether they are fit or not for human consumption.

2) Cafes should be monitored by the local government officers from the public health department of Hargeisa municipality to assess sanitation and hygienic conditions.

3) A similar study with more samples should be done by the Somaliland Quality Control Commission.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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