

Assessment of the Microbial load of Vegetables and Fruits in Ethiopia

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Abstract

Background: Fruits and vegetables are widely accepted in the scientific community and are advised for daily dietary consumption. Although these food groups have more assents, their preparation and sanitation before consumption at the household level must receive proper attention.

Objective: To assess the microbial load of vegetables and fruits submitted to the public health microbiology laboratory of the Ethiopian Public Health Institute from 2008-2017.

Methods: To ascertain the degree of contamination, samples were examined for the presence of mould, yeast, mesophilic aerobic bacteria, total coliform, thermotolerant coliform, and indicator *E. coli* in accordance with the Nordic Committee for National Reference Laboratory methodology. The American Public Health Association methodology was followed for the viable bacteria count.

Result: Out of 3279 food samples submitted, 5.9% (195/3279) were raw and semi processed fruit and vegetables. Of these, 15% (29/195) of the samples revealed an intolerable microbial quality of the mesophilic aerobic plate count, followed by total coliforms 7.7% (15/195), thermotolerant coliforms 10.8% (21/195), *E. coli* 3% (6/195), and the Mold and Yeast counts observed in 1.5% of samples. Although *Staphylococcus* species and *S. aureus* were enumerated in 2% (4/195) and 1% (2/195) of samples, respectively, none of the samples had detectable numbers of *Salmonella* and *Shigella* species.

Conclusion: Microbial contamination levels in 24 food categories were found, with mixed vegetables and fruit cocktails being the most common. The contamination levels varied significantly, highlighting the need for health education and improved fruit and vegetable shelf life.

Keywords: Microbial quality, indicator, pathogens, vegetables, fruits

Introduction

Fruit and vegetables are crucial food sources for disease prevention, containing various vitamins, minerals, and fiber. Compared to emerging nations, the US saw increases in fresh fruit and vegetable consumption rates of 25.8 and 32.6%, respectively, between 1970 and 2004. Therefore, if it keeps up this tendency, it might surpass the consumption of processed fruits and vegetables. In every nation, there has been a recent upward trend in dietary choices and nutritional preferences. Nevertheless, it also stated that, in 1995, the US lost 19.6% of its edible food-related economic losses to rotting, or around 18.9 billion pounds of fresh fruits and vegetables yearly (European Commission 2002).

Because they are ecologically friendly, food sources like fruits and vegetables can readily become infected by a wide variety of microbes. Additionally, the polysaccharides that make up their tissue structures cellulose, hemicellulose, pectin, and starch can be broken down by a variety of bacteria, fungi, and external lytic enzymes like hemicellulase and pectinases (European Commission 2002). The main issue is direct consumption without heat treatment and the cleanliness of every tool used to chop and

squeeze produce. Another issue is the microbiological load of the source water used for washing and storing until it is used for home purposes. It is possible that some fruits and vegetables that are ready to eat (RTE) may still contain parasite and virus contamination after undergoing these processes. Additionally, the growth of psychoactive pathogenic and spoilage microbes may be promoted by chilled storage.

Mortality and morbidity rates related to food safety have been linked globally to microbiological hazards. Diseases connected to the microbial degradation of such food samples by microbial growth and their toxins are the cause of major epidemics. Different bacterial diseases (*Salmonella* spp., *E. coli* O157: H7, and *Shigella* spp.) can contaminate fruits and vegetables, according to reports (Victor et al. 2017; Mirtunjay et al. 2015). Mycotoxins produced by foodborne mould can infect humans and animals and cause illnesses. Microbes, primarily coliforms, have long been used as markers of the microbiological quality of food and water. Despite the widespread presence of signs, there was a seasonal trend in leafy vegetable groupings. Denis's et al. (2016) and Back et al. (2003) research revealed that the summer months had the highest levels of bacterial contamination.

Raw fruits and vegetables are known to house a wide range of bacteria and cause several outbreaks. Fresh vegetables sometimes include a variety of dangerous bacterial pathogens, including *Clostridium botulinum*, *Salmonella* species, *Shigella* species, *Bacillus* species, *Campylobacter* species, *Listeria monocytogenes*, and several toxin-producing *E. coli* strains. Additionally, it was shown that 49% of *Pseudomonas* species formed biofilms in vegetables that had hardly been treated (Merieles et al. 2017). According to FSANZ (Food Standards Australian New Zealand) in 2005, *Yersinia pseudotuberculosis* in carrots was identified as the biggest problem in Australia. 33% of the samples also included *Yersinia enterocolitica* along with other infections and markers. (Aleksiainen and others, 2016).

According to Denis et al. (2016), the Canadian Food Inspection Agency produced equivalent results from similar studies it did on various food products (local versus imported, organic versus conventional). The United States of America saw nearly 200 cases of food illness and three fatalities due to *Escherichia coli* O157:H7 contamination in bagged spinach. Reports have been made of *E. coli* O157 in apple juice and salmonella in apples and orange juice (FSNAZ 2005). According to WHO (2018), Berger et al. (2010), Abadisa et al. (2008), and other sources, leafy greens like lettuce and spinach as well as fresh herbs like parsley and basil are known to be possible sources of bacterial infections.

Regarding worldwide transmission, in 2007 it was reported that fresh herbs sold in retail settings in the United Kingdom (UK) were responsible for an international *Salmonella* outbreak that affected at least 51 people from England, Wales, Scotland, Denmark, the Netherlands, and the USA. The outbreak was linked to contaminated basil from Israel. There have been reports of 11 outbreaks in Canada involving sprouts, cantaloupe, lettuce and fresh herbs; fresh produce in Italy (Caramone et al. 2015); watermelon from Brazil in late 2011/early 2012; fresh tomatoes in the USA; sprouts in the Check Republic (Vojkovslea et al. 2017); and vegetable salad in developing nations (Mira et al. 2018). In addition, in 2012, 31 individuals were afflicted by *E. coli* O157:H7 in lettuce imported from the USA, while 20 occurrences of foodborne illness were attributed to *Salmonella* in domestic green onions (Denis et al. 2016).

There are little data that demonstrate the microbial load of fruits and vegetables across the country Ethiopia, despite similar trends being noted. Thus, this study was aimed to assess the microbial load of fruits and vegetables using common indicators and pathogenic organisms.

Materials and Methods

The study participants were customers (regulatory bodies/ Producers/ Hotels and Restaurants/ catering service providers, consumers ...) who officially submitted fresh vegetables and fruit samples to the Food Microbiology Laboratory of the Ethiopian Public Health Institute (EPHI) for routine microbial testing within the indicated period from 2008 to 2017. The samples were kept in the cold chain (Icebox) during transportation until submission. Samples were tested using the standard protocol (NMKL), where 25 g of the composite representative sample was weighed and blended in 225 ml of sterile Buffer peptone water (BPW) for *Salmonella* and *Shigella* isolation. In addition, 1:10, 1:100 and 1:1000 dilutions of the samples were prepared using 1% saline peptone solution to enumerate indicators, yeast and mold and *Staphylococcus* species per 1 g of the samples .

All samples were tested for mold/yeast count, aerobic plate counts, total coliform count, thermotolerant coliform count and *E. coli*, based on the Nordic Committee for National Reference Laboratory (NMKL) protocol. For viable bacteria count enumeration (pour plate enumeration culture technique), the American Public Health Association (APHA) protocol was applied. *Salmonella* and *Shigella* species were tested based on the NMKL protocol on xylene lysine decarboxylase (XLD) media after enrichment steps on primary buffer peptone water and secondary selenite cysteine broth.

Plate count agar (Oxoid CM463) was used for total aerobic bacterial count at 30°C for a 48 hours incubation period. Both total coliforms and *E. coli* were determined using violet red bile agar (Oxoid, CM463) within 37°C and 44°C selective growth temperatures, respectively. Typical colonies on the plates counted per 1 g of sample were determined. Suspected pink colonies taken for the calculations after confirming the formation of gas production in Brilliant Green Lactose broth (BGLB) and *E. coli* (EC) broth. The presence of mold and yeast was tested using Rose Benegal selective agar with chloramphenicol supplement. *Staphylococcus* species and *S.aureus* were enumerated using mannitol salt agar. For *Salmonella* and *Shigella* species isolation, 25 g of each vegetable and fruit sample was transferred to sterile plastic bags and homogenized with 225 ml of 1% (w/v) BPW (Oxoid) and kept at room temperature for 30 min followed by incubation at 37° for 24 hrs. The analyses of pathogenic bacteria such as *Salmonella* and *Shigella* were performed using primary and secondary enrichment broth and confirmed by isolation, purification and identification on XLD selective media and nutrient agar. Then, *Salmonella* and *Shigella* species were verified using

biochemical tests such as sugar fermentation tests (TSI, LIA), enzymatic tests such as urea and citrate, motility tests, hydrogen sulfide and antisera, particularly for suspected species. The results were interpreted as the presence or absence of *Salmonella* species in 25 g of vegetables and fruit samples. Similarly, *Staphylococcus aureus* was checked by its particular golden yellow appearance on mannitol salt agar (after transferring the suspected colonies from Baired Parker agar) and the coagulase test.

Data quality and statistical analysis: The testing efficiency of each culture medium was determined using *S. aureus* and *E. coli* standard strains and

Salmonella known isolates applied as positive controls. SPSS version 20 was used for data entry. The research proposal was submitted under the Study of Food Microbiology Retrospective Data and ethically cleared by the EPHI Scientific and Ethical Research Clearance Committee (SERC).

Results

A total of one hundred ninety-five freshly eaten vegetables and fruits were received per ten years, which is approximately 5.9% (195/3279). The samples were then classified into twenty-four solely served or combined categories.

Table 1: Frequency distribution of fruits and vegetable samples with high microbial load

Types of food samples	Frequency	Mold >10 ⁴	Yeast >10 ⁴	APC >10 ⁵	TCC >10 ⁴	TTCC >10 ²	<i>E.coli</i> >10 ²	Total samples Exceedingly contaminated
Mushroom	13	1		2	2	2	1	5 (38.5%)
Potato	12			1		1		2 (16.7%)
Carrot	3			2		1		2 (66.7%)
Lentil	3							0
Moringa	3				1	1		2 (66.7%)
Onion	5							0
Broccoli	3			1				1 (33.3%)
Cassava	4							0
Fossolia with carrot	2							0
Salad	20		1	3	3	3	1	6 (30%)
Cauliflower	1			1				1 (100%)
Mixed bean salad	21			4		1		5 (23.8%)
Mixed vegetables	45			8	5	8	2	14 (31.1%)
Fruit Salad	13	2	2	3	4	2	1	6 (46.1%)
Papaya	2					1		1 (50%)
Strawberry	8							0
Fruit cocktail	16			2		1	1	2 (12.5%)
Mango mousse	15			2				2 (13.3%)
*Others	6							0
Total contamination	195	3 (1.5%)	3 (1.5%)	29 (14.9%)	15 (7.7%)	21 (10.8%)	6 (3%)	49 (25.1%)

N.B: APC-aerobic plate count (total viable count), TC-total coliform count. TTC- Total thermotolerance (Fecal coliform) count, *others (beet root, spinach, sweet potato, apple, cavachi fruit, bell pepper)

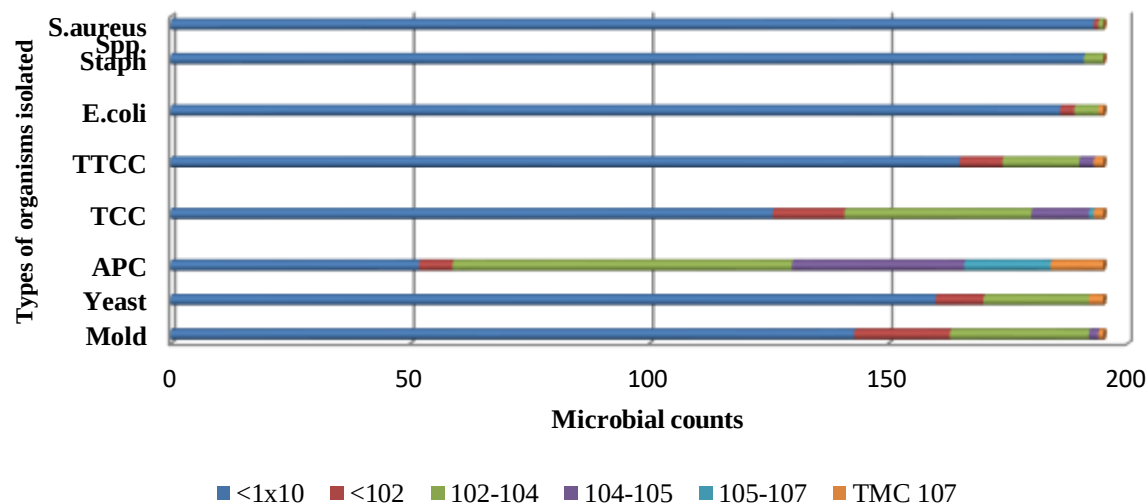
Table 2: Comparison of mean differences and significant values using the independent samples statistical test tool

	<1x10 ¹	1-10 ²	10 ² -10 ⁴	10 ⁴ -10 ⁵	10 ⁵ -10 ⁷	>10 ⁷	Fisher EVO (Sig)	T-test EOM 2 tailed (Sig)	95%CI (Lower-Upper)
Mold	143	20	29	2	0	1	0.018	0.187	-172.9-46.9
Yeast	160	10	22	0	0	3	0.019	0.252	-196.6-70.6
APC	52	7	71	36	18	11	0.169	0.348	-78.3-34.9
TCC	126	15	39	12	1	2	0.039	0.180	-149.1-39.1
TTCC	165	9	16	3	0	2	0.017	0.292	-202.9-79.6
<i>E.coli</i>	186	3	5	0	0	1	0.016	0.349	-232.8-104.1
Staphyl-occus Species	191	0	4	0	0	0	0.016	0.361	-239.9-109.9
<i>S.aureus</i>	193	1	1	0	0	0	0.016	0.367	-242.7-112.7

CI: Confidence Interval, APC= Aerobic Plate Count, TC= Total Coliform count, TTCC= Thermotolerant Coliform Count, Sig=Significant, F= Fisher Test statistics t Test= T Student test statistics, Sig= Significant, EO= Equality of Variance, EOM=Equality of Means

A total of 141 (72.3%) vegetables, 13 (6.7%) fruit salad (mixture) and 41 (21%) fruit samples were collected. Of these, 38 (27.5%), 6 (46.1%) and 5 (12.2%) were contaminated, respectively. Approximately 90% of the samples were collected from Addis Ababa city. Only six samples from SNNPR and one from Harar town were enrolled. The

following figure shows the microbial types and loads in the tested food samples (Figure 1). Although majority of the tested fruits and vegetables harbor tolerable or acceptable limits of the indicator and spoilage organisms per this study, some tested samples exhibited high microbial loads.



N.B: Horizontal legend entry read as $<1 \times 10^1$, $< 10^2$, 10^2 - 10^4 , 10^4 - 10^5 , 10^5 - 10^7 , 10^7 (TMC- Too many to count); APC- Aerobic plate count, TCC-Total coliform count, TTCC-Thermotolerant coliform count

Figure 1: Microbial load of contaminants in vegetables and fruits

Ten-years retrospective data were assessed to examine the submission trend of vegetables and fruits compared to other food samples. The following figure compares and reveals this trend.

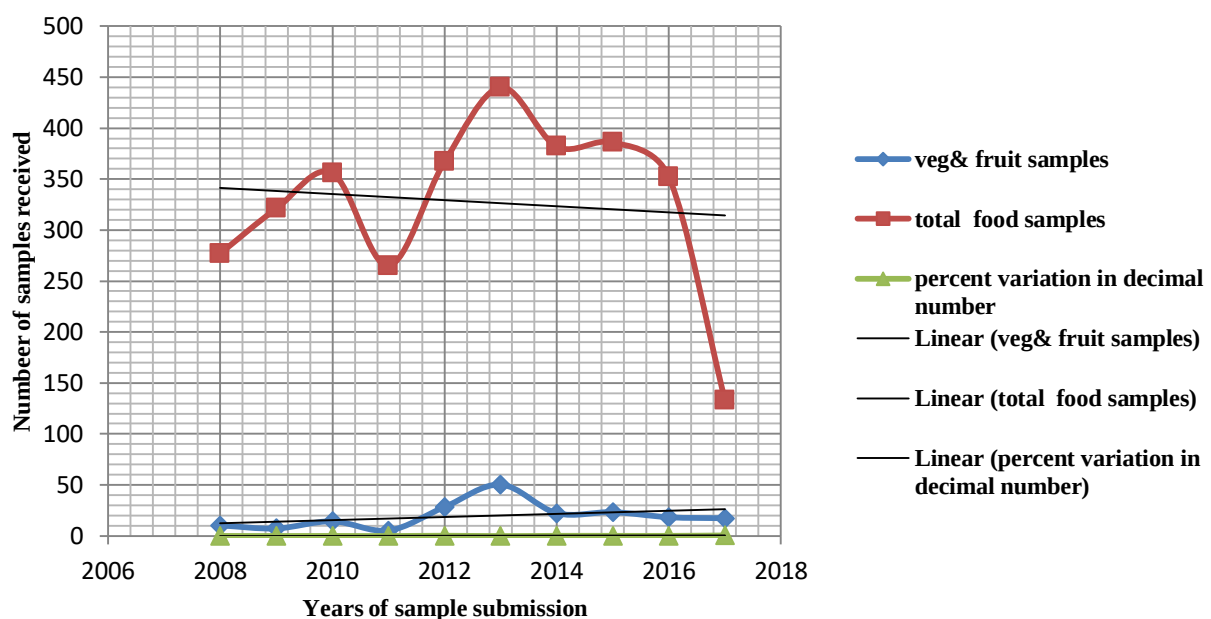


Figure 2: Trends of Vegetables and Fruit samples submitted in comparison to other food samples per ten years

Discussions

Vegetables and fruits are relevant food constituents that contain vitamin and fiber sources. They are currently proven to be the best healthy foods worldwide, although their consumption rate is overwhelmed by cereals and animal products. Approximately 5.9% (195/3279) of study samples were submitted to the laboratory in ten years for quality monitoring by the regulatory body. On the other hand, approximately 90% were collected from Addis Ababa city, which indicates that the food regulation and monitoring system is active in the central part of the country. Of course, most regions do not have a food microbiology testing laboratory. These might be the reason for such low coverage in the decentralized regions.

Unacceptable bacterial contamination levels were observed in the twenty-four food categories and estimated to be in the low to medium range. Samples that prevailed with a greater microbial load of bacterial contamination demonstrated an elevated value for each parameter. For instance, a mesophilic aerobic plate count of $> 5 \log \text{CFU/g}$ was recorded in 29 (14.9%) of the samples. Similarly, a total coliform count of $> 4 \log \text{CFU/g}$ in 15 (15%), thermotolerant coliform count of $> 2 \log \text{CFU/g}$ in 21 (10.8%), *E. coli* count of $> 2 \log \text{CFU/g}$ in 6 (3%), mold count of $> 4 \log \text{CFU/g}$ in 3 (1.5%) and yeast count of $> 4 \log \text{CFU/g}$ in 3 (1.5%) of the samples were reported as unacceptable results in descending order. *Staphylococcus* species and *S. aureus* enumerated in 4 (2%) and 2 (1%) of the samples, respectively, with a log equal to 4 microbial loads. However, none of the samples had detectable levels of *Salmonella* and *Shigella* species per 25 gm of the samples.

When compared to the Brazilian study, the results of this investigation indicate a lower prevalence rate of food quality indicators. With about 8 to 9 Log microbial loads for a total viable count in 96.7% of the samples, they showed a significant level of contamination. The reported values for total coliform, faecal coliform, and *E. coli* were 132 (81.5%), 107 (66%), and 86 (53.1%), respectively. Pathogenic organisms such as *Listeria* and *Salmonella* were also isolated from the vegetable samples that were analysed. 2011; De Oliveira et al. Given that the sample size in the current study was smaller than in the previous one, a discrepancy of this kind may be seen. Alternatively, this article may be limited by the examples that were provided by high-end standard clients like hotels and restaurants. Only official samples provided by the regulatory agency and experts in the field were tested for this investigation.

Mixed vegetables (45/141, 31.9%) and fruit cocktails (16/141, 8.2%) were the most frequently submitted food samples per category, in addition to the fruit salad samples, which consisted of a mixture of the two. The level of microbial contamination also differed from [14, 31.1%] to (2, 12.5%). Similar to the Brazilian research, in our study, only one vegetable sample revealed visible spoilage, which underlines that the concept of external and internal appearance does not guarantee the contamination level of food samples (De Oliveira et al. 2011).

Even though the prevalence rate in both vegetables and fruits seems to be low to medium, the contamination level varies significantly, particularly for the Mold and Yeast count, Total Coliform Count, Thermotolerant Coliform Count, *E. coli*, *Staph* species and *Staph aureus*, based on Fisher's exact test, where the P value was calculated as $\alpha < 0.05$ at a 95% confidence interval with the asymptomatic distribution. In contrast, the t test statistics showed insignificant variations for all test parameters. The current study exhibited similarity only in the statistically significant differences in thermotolerant coliforms among samples tested in Maryland, USA. All the other microbiological parameters reported insignificant variations (Konir et al. 2016). These results revealed that the contaminants in these food categories were mainly derived from sanitation defects/poor storage conditions and fecal sources of contamination. In contrast to these findings, fungal contaminants revealed lower microbial loads (Vojkovslea et al. 2017).

Furthermore, the submitted food products were minimally processed and ready to use for human consumption. In addition, approximately 10.8% (21/195) of them harbor fecal coliform indicators. These can be of the following genera: *Klebsiella*, *Enterobacter*, *Escherichia* or *Citrobacter*. The presence of one of the above four genera can also indicate the possibility of getting *Salmonella* and *Shigella* species in the food elements. In particular, those samples identified as positive for thermotolerant coliforms and *E. coli* tell us the possible presence of other pathogens, although they had not been isolated. Likewise, the best sanitation defect indicator, a mesophilic aerobic plate count (TVC) calculated as approximately 15%, also proves that for every thirteen samples/batches, one sample/batch was poorly handled without proper sanitation. Therefore, the probability of getting an infection from consuming such foods by chance could not be disregarded, especially for immuno-debilitated patients, children and adults. On another view, the trend of vegetable and fruit sample submissions for monitoring purposes showed slight increments over

ten years. A relatively high submission number in the year 2013 was 50. Similarly, the overall food sample submission record revealed the highest number in 2015, 440, compared to the lower number registered in 2017 (133), exceeded by 307 (Fig 2).

Generally, the overall result revealed that a low to medium number of vegetable and fruit samples presented poor microbiological quality. However, the enumerated indicators gave a hint for the presence of pathogens although not detected and could cause illnesses in those local regions when the condition is favorable to them. Furthermore, the risks of foodborne pathogens (outbreaks) through such kinds of foods could easily be transmitted to other locations. Therefore, vegetable and fruit processing companies and consumers should be aware of such facts and adopt hygienic practices to minimize unnecessary risks (Ssemandaab et al. 2017; Castro-Ibanez et al. 2017; Ricoa et al. 2007). On the other hand, the trend of food sample submission did not show a direct or indirect increment, although a straight rise was expected. Among the twenty-four individual samples submitted, Salad/Cauliflower from vegetables and Mango mousse/Papaya were the highest/lowest numbers submitted from the two categories. The proportion of vegetables to fruits was calculated as 135:47 (2.87:1). The other anticipated result was only a small number of samples received outside Addis Ababa, which is under the expectation.

Different factors, such as pH, moisture content and storage conditions, can be associated with low or high microbial growth depending on the type of microorganisms. Vegetables and Fruits may harbor pathogenic *L.monocytogenes*, *Campylobacter jejuni*, *Escherichia O157* and *Salmonella* species, which are involved in large foodborne outbreaks worldwide, causing symptoms of gastroenteritis and even chronic infections (De Oliveira et al. 2011). From a report from Brazil, packing under a modified atmosphere extends the shelf life of RTE vegetables and is used as a protective barrier against insects, rodents and microorganisms. Other technologies with the potential to increase the quality and safety of these products have been studied, such as ionizing radiation, ultrasound decontamination and the use of bio preservative cultures and their metabolites (De Oliveira et al. 2011; Maffei et al. 2013; Song et al. 2005; Seymou et al. 2002; Atribst et al. 2009). Enhancing the shelf life using different techniques, such as modified atmospheric conditions and refrigeration, to control microbial pathogens should be considered. (Mira et al. 2018; Castro-Ibanez et al. 2017)

According to the Food and Drug Administration (FDA) published guidelines of 1998, there is an agreement to minimize microbial food safety hazards for fresh fruits and vegetables by applying good agricultural practices. All of them address how to minimize the microbiological contamination level by tackling different associated factors at every stage during growing, packing, and shipping, especially on the selection of potable water for use, reducing the impacts of human and animal factors and so on (European Commission 2002; Atribst et al. 2009).

Conclusions and Recommendation

Unacceptable bacterial contamination levels were observed in some of the 24 food categories, with a higher microbial load indicating an elevated value for each parameter. Mixed vegetables and fruit cocktails were the most frequently submitted food samples per category, with varying levels of microbial contamination. The contamination level varies significantly, particularly for Mold and Yeast count, Total coliform Count, Thermo-tolerant coliform Count, *E. coli*, Staph species and Staph aureus per Fisher Statistical parameter calculation. Health education on sanitation procedures and enhancing shelf life of fruits and vegetables is crucial to protect the community from microbial effects.

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Conflict of Interest

The authors declared that they have no conflict of interest to this article.

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