Evaluation of the consumption and contamination level of Vegetables and Fruits in Ethiopia

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Abstract

Background; Vegetables and Fruits have got major acceptance in the modern scientific world nowadays and advisable to use more per daily food consumption. Although these food products have got more acceptances, their preparation and sanitation before consumption at the household level have to get proper attention.

Objective: The objective was to assess the microbial load of vegetables and fruits which had been submitted at the Public health microbiology laboratory of Ethiopian Public Health Institute, from the year 2008- 2017, (10 years Retrospective data).

Methods: Samples were tested for the presence of Mold, Yeast, Mesophilic aerobic bacteria, Total coliform, Thermo-tolerant coliform and the Indicator *E.coli*, to determine the contamination level based on the NMKL protocol. For viable bacteria count, APHA protocol was applied.

Result: One hundred ninety-five 5.9% (195/ 3279) raw and processed products had been received per ten years. 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), thermo-tolerant coliforms 10.8 % (21/ 195), *E.coli* 3 % (6/ 195), mold count 1.5% (3/195) and yeast count 1.5% (3/195) (ICMSF protocol).

Discussion and Conclusion: Although vegetables and fruits are currently proved to be the best healthy foods worldwide and are available with the low cost relatively, in the developing countries like Ethiopia, their consumption rate is overwhelmed by cereals and animal products based on the live status of the community. Therefore, joint efforts have to be exerted by different branches of MOH for encouraging the community to use vegetables and fruits as its main food source for better health and to reduce nasty illnesses like diabetes, obesity, heart diseases and so on. However, while doing so, series health education has to be provided on sanitation procedures like immediate cooking or disinfection before consumption to prevent the community from environmental risks.

1. Introduction

Fruit and vegetable cocktails are unfermented raw fruit or vegetable products prepared by cutting, mixing and/ or diluting (in case of fruits) of the product after removing the unwanted portion. Based on the global trend observed, unlike in the developing countries, fresh fruit and vegetable consumption rate increased by 25.8 and 32.6%, respectively in the United States (US) from the year 1970–2004. Thus it was predicted that it can be exceeded processed fruit and vegetable consumption if it continues with a similar tendency. It was very important to acknowledge such a positive shift in nutritional preference and diet selection, for every country. However, it was also described that, in the year 1995, 18.9 billion pounds of fresh fruits and vegetables were lost annually due to spoilage, which accounts for 19.6% of all US economic losses of edible foods for that particular year (European commission 2002).

Vegetables and fruits are easily invaded by many microbes since they grow friendly with the environment. Moreover, their tissue is composed of the polysaccharides cellulose, hemicellulose, pectin and starch which is their storage polymer. Therefore, they can be easily spoiled by bacteria and fungi species which have the particular extracellular lytic enzymes like pectinases and hemicellulase (European commission 2002, Asha ‘et al.’ 2014). The direct consumption by the consumers without heat treatment is the primary concern. The cleanness of

all utensils used for squeezing/ mixing, types of the water samples used and their microbial content and loads, storage conditions until serving at the household level are also another concern. These steps may not be efficient to eliminate contamination of ready to eat (RTE) vegetables and fruits from parasites and viruses. Besides, the storage under refrigeration may favour the growth of psychotropic pathogenic and spoilage microorganisms.

Globally, significant food safety concern (Mortality and Morbidity) has been linked with the microbiological hazards. Illnesses associated by microbial degradation of such food samples by the microbial proliferation and their toxins lead to major outbreaks. Such contamination of fresh fruits and vegetables by various bacterial pathogens (Salmonella spp., *E. coli* O157: H7 and Shigella spp.) was seen (Rajvan Shi A 2010, Victor 'et al.' 2017, Mirtunjay 'et al.' 2015). Food-borne mold can produce mycotoxins, and some yeasts and mold are responsible for human and animal infections (Jeddi 'et al.' 2014). Microbes, mainly the coliforms group has been used extensively as an indicator of the main indicators of microbiological quality of water and food. Their presence indicates improper treatment or post-disinfection contamination (Badasa 'et al.' 2008, Sewan 'et al.' 2012). Despite the overall prevalence of contamination seen, a notable seasonal trend was observed in the leafy vegetable groups and higher bacterial contamination rates were recognized in the summer. (Denis 'et al.' 2016, Back 'et al.' 2003).

Raw fruits and vegetables are known to have the potential for harbouring a wide range of microorganisms and causing several outbreaks (EC-SCF, 2002). Foodborne bacterial pathogens commonly detected in fresh vegetables are different toxin producing *E.coli* strains, Staphylococcus aureus enterotoxin, Salmonella species, Shigella species, Bacillus species, Campylobcter species, *Listeria monocytogenes*, *Clostridium boutlinium*. For example, Salmonella species were isolated from all the fruits samples tested in Sango Ota Nigeria (Eni 'et al.' 2010). *E.coli* 0157: H7, Salmonella species, Cryptosporidium spp and *Vibrio cholera* were reported as major outbreak causing organisms from fruit juices, in India (Asha 'et al.' 2014). *Aeromonas hydrophila, Citrobacter freundii, Enterobacter cloacae* and Klebsiella species have been isolated in lettuce and salad vegetables (Eni 'et al.' 2010, Chaturvedi 'et al.' 2013). 49% of Pseudomonas species with biofilm formation also reported in minimally processed vegetables (Merieles 'et al.' 2017). *Yersinia pseudotuberculosis* in Carrots identified as the biggest concern in Australia (Australian FMP, 2005). *Yersinia entercolitica* also reported in addition to other pathogens and indicators as higher as 33% (Nousiainen 'et al.' 2016).

The Canadian Food Inspection Agency conducted similar surveys in a wide range of products (local vs. imported, organic vs. conventional) and reported comparable findings (Denis 'et al.' 2016). *Escherichia coli O157: H7* infection, in bagged spinach resulted in almost 200 cases of food poisoning and three deaths in USA. Salmonella in apple and orange juice, *E.coli 0157* in apple juices were reported (Asha 'et al.' 2014, Australian FMP, 2005). Likewise leafy greens, such as lettuce and spinach, and fresh herbs, such as parsley and basil are well-recognized and reported as potential sources of bacterial infections (Nwachukwa 'et al.', WHO 2018, Bereger 'et al.'2010, Abadisa 'et al.' 2008).

With regard to Global transmission, in 2007 fresh herbs sold at retail in the UK were reported to cause international outbreak of Salmonella infection linked to contaminated basil from Israel and affected at least 51 individuals from England, Wales, Scotland, Denmark, the Netherlands and USA. Fresh cut and whole melons (Collazoa 'et al.'2017), watermelon from Brazil In late 2011/early 2012, Fresh tomatoes in USA, 11 outbreaks in Canada in sprouts, cantaloupe, lettuce and fresh herbs, Sprouts in the Check Republic (Vojkovslea 'et al.' 2017), Fresh produce in Italy (Caramone 'et al.'2015), vegetable salad in developing countries (Mira 'et al.' 2018) have

been registered. As the result of E. *coli O157: H7* in lettuce imported from the USA, affected 31 people in 2012 and one caused by Salmonella in domestic green onions, which resulted in 20 cases of foodborne illness (Denis 'et al.' 2016).

Although such outbreaks were infrequently reported in the country, the main objective of this evaluative study focused to assess the microbial quality of such vegetables and fruits by testing the common indicator and pathogenic organisms. Specifically, to look into their distribution and to estimate the level of consumption rate in the country.

2. Materials And Methods

a. Study Design and sites of the study

A retrospective study had been undertaken using a routine, previously analyzed and reported data. The study included all fresh vegetable and fruit samples which had been officially submitted at Food microbiology laboratory of EPHI for routine testing within the indicated period. Samples from manufacturers or service providers OR from shops or different markets were collected using sterile plastic bags, by the health professionals. The samples were kept in appropriate storage condition during transportation time and until it was processed. Subsequently, it was submitted for different laboratories of EPHI, which one of them is Food microbiology laboratory. Then, each sample was tested by homogenizing of its contents and a portion from every five units was taken into a sterile Petri dish (Composite). For detection of pathogens and indicators, 25 g of the composite representative sample was weighed and blended in 225 ml of sterile Buffer peptone and in 1% saline peptone solution under sterile conditions respectively.

All samples had been analyzed and checked for the presence of Mold/yeast count, Aerobic plate count, Total coliform count, thermotolerant coliform count and *E.coli*, based on the Nordic Committee for National Reference laboratory (NMKL) protocol. For viable bacteria count, the American Public Health Association (APHA) protocol was applied; using a pour plate enumeration culture technique. Pathogen detection (Salmonella and Shigella species) were processed following the instructions of NMKL protocol on XLD media after inoculating on the primary (Buffer peptone broth) and secondary (Selenite cysteine broth) enrichment broth.

2.1. Method used

Plate count agar (Oxoid CM463) was used for detection of the total aerobic bacterial count after incubation at 30 °C for 48 h. Both total coliforms and *E.coli* were determined using Violet Red Bile Agar (Oxoid, CM463) followed by incubation at 37 °C and 44 °C respectively. Typical colonies on the plates were enumerated and colony counts per 1 g sample were determined. Only typical red colonies were included for the calculations after confirming the formation of gas production in Brilliant Green broth (BGB) and EC broth respectively. To detect the presence of mould and yeast, Baird Parker selective agar with chloramphenicol supplement per 0.1 ml of sample suspension was used by streak plate method and after preparing a 1:10 dilution. The final count then reported by multiplying with a total dilution factor. Staphylococcus and Staph aureus were also counted similarly using Mannitol salt agar after 1:10 dilution prepared with 1% Saline peptone. To isolate Salmonella and Shigella species 25 g of each vegetable and fruit samples were transferred to sterile plastic bags and homogenized with 225 ml of 1% (w/v) buffered peptone water (BPW) (Oxoid) and kept at room temperature for 30 min followed by incubation at 37 ^{0}C. The analyses of pathogenic bacteria like Salmonella, Shigella were performed using primary

and secondary enrichment broth and confirmed by isolation, purification and identification on XLD selective media and Nutrient Agar. Results were obtained as presence or absence of Salmonella species in 25 g of vegetables and fruit samples.

2.3 Data quality and Statistical analysis

Suspected colonies from each category were picked and further checked using different biochemical media for confirmation and the report had been made per final volume. For determining the testing efficiency of each culture media/ broths, *S. aureus and E.coli* standard strains were used as a positive control for Gram-positive and Gram-negative organisms' detection respectively. SPSS version 20.00 had been used for statistical analysis and to test the values of the significance of the results respectively. Unacceptable food types over the total were calculated to find the single proportion value (P) followed by calculating the standard error of the proportion using the following formula. SE = square root of (P x (1-P)/ N). The P values < 0.05 was taken as statistical significance. This research was ethically cleared by EPHI Scientific and Research Ethical Clearance Committee (SERC).

3. Results

A total number of one hundred ninety-five freshly eaten vegetables and fruits were received per ten years, which is around 5.9% (195/ 3279), of all food types which had been submitted during the indicated period. Twenty-four solely served or combined commonly used vegetables and fruits were tested for determining their microbiological contamination level (Table 1).

Table 1: Frequency distribution of bacteriological and mycological contaminants of fruits and vegetable samples (ICMSF reference protocol was used per this food type category)

Types of food samples tested	Frequency N	Mold $>10^4$	Yeast $>10^4$	TVC $>10^5$	Total Coliform $>10^4$	Thermo-tolerant Coliform $>10^2$	*E.coli* $>10^2$	Total
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 (Keysir, Spinach, sweet potato, Apple, Cavachi fruit, Bell pepper)	6							0
Total	195	3 (1.5%)	3 (1.5%)	29 (14.9%)	15 (7.7%)	21 (10.8%)	6 (3%)	49 (25.1%)

A total number of 141 (72.3%) vegetables, 13 (6.7%) fruit salad (mixture) and 41 (21%) fruit samples were received in the lab. Of which 38 (27.5%), 6 (46.1%) and 5 (12.2%) were significantly contaminated respectively.

Around 90% of the samples were collected from Addis Ababa city, six samples from SNNPR and one from Harar town.

Table 2; the Standard error of the outlier (Unacceptable) results per 95% confidence interval of all tested vegetable and fruit samples. (single-arm phase II trial of single proportion calculation was used)

	$<1x10^1$	$<10^2$	10^2-10^4	10^4-10^5	10^5-10^7	TMC 10^7	Prop value	SE	95% CI
Mold	143	20	29	2	0	1	0.015	0.008	0.00-0.030
yeast	160	10	22	0	0	3	0.015	0.008	0.00-0.030
APC	52	7	71	36	18	11	0.149	0.025	0.1-0.198
Total colif	126	15	39	12	1	2	0.077	0.019	0.04-0.114
Fecal Colif	165	9	16	3	0	2	0.108	0.022	0.065-0.151
E.coli	186	3	5	0	0	1	0.031	0.012	0.008-0.054
Staphyl Spp.	191	0	4	0	0	0	0	0	0
S.aureus	193	1	1	0	0	0	0	0	0

4. Conclusions And Recommendation

Consumption of fruits and vegetables provide more antioxidants and vitamins for the body and support in preventing health risk diseases associated with consuming more heavy and fatty foods (animal products). These make them to be selected to use more recently. Most people become vegetarian or Lacto vegetarian to avoid such general risks especially when they become around adulthood ages.

Although, such food products have got more acceptances on a daily basis, their preparation and sanitation before consumption at household level has to get proper attention. Consideration has to be given to the dangerous habit of the Ethiopian custom of consuming raw food without treating with insufficient heat or detergents as

nationwide. This is because vegetables and fruits which are sold in the market can bring other side health risks since it mostly acts as a reservoir for many microorganisms. In most of the study, it was reported that higher microbial loads as the result of using different water sources, from the rivers, ponds or other similar sources which have easily in contact with animal fecal matters. Their contact with dust and soil has also played a major role as a contamination source since soil harbours millions of parasites and bacteria which are capable of surviving these environments, like roundworms and several bacterial pathogens.

On the other hand, the existing regulatory system of food products in Ethiopia has to be actively engaged in all regions like that of Addis Ababa. Capacitating the laboratory facilities and skills of the personnel in regional Food microbiology laboratories throughout the country has to get series devotion.

In summary, joint efforts have to be exerted by different branches of MOH for encouraging the community to use vegetables and fruits as its main food source for better health and to reduce nasty illnesses like diabetes, obesity, heart diseases and so on. However, while doing so, series health education has to be provided on sanitation procedures like immediate cooking or disinfection before consumption to prevent the community from environmental risks. Enhancing the shelf life using different techniques like modified atmospheric conditions and refrigeration to control the microbial pathogens should be taken into consideration. (Mira ‘et al.’ 2018, Castro-Ibanez ‘et al.’ 2017)

Summary declaration of interest statement & Consent for Publication

This article has not published previously in any journal and not under consideration for publication elsewhere. Its publication is approved by all authors and the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder.

Abbreviations

MOH; Ministry Of Health

NMKL; Nordic Committee for National Reference laboratory

APHA; the American Public Health Association

ICMSF; International Commission of Microbiology Standard for Food

Declarations

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Availability of Data and materials

All the data processed and analyzed were available in the logbook and SPSS 20 version software.

Ethical Approval and consent to participate

This research was ethically cleared by EPHI Scientific and Research Ethical Clearance Committee (SERC). All Authors were agreed to participate and accomplish what was assigned for the as part of the research Activities.

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Figures

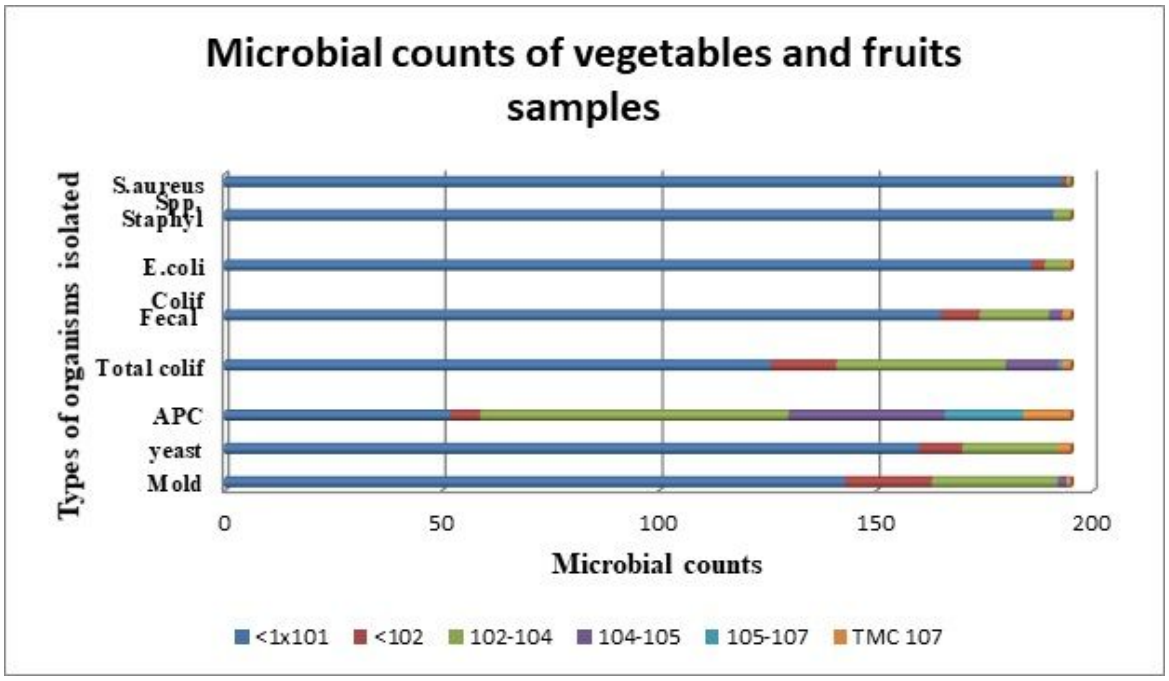

Figure 1

Unacceptable number of bacteriological and mycological contaminants of fruits and vegetable samples (ICMSF reference protocol was used per this food type category)

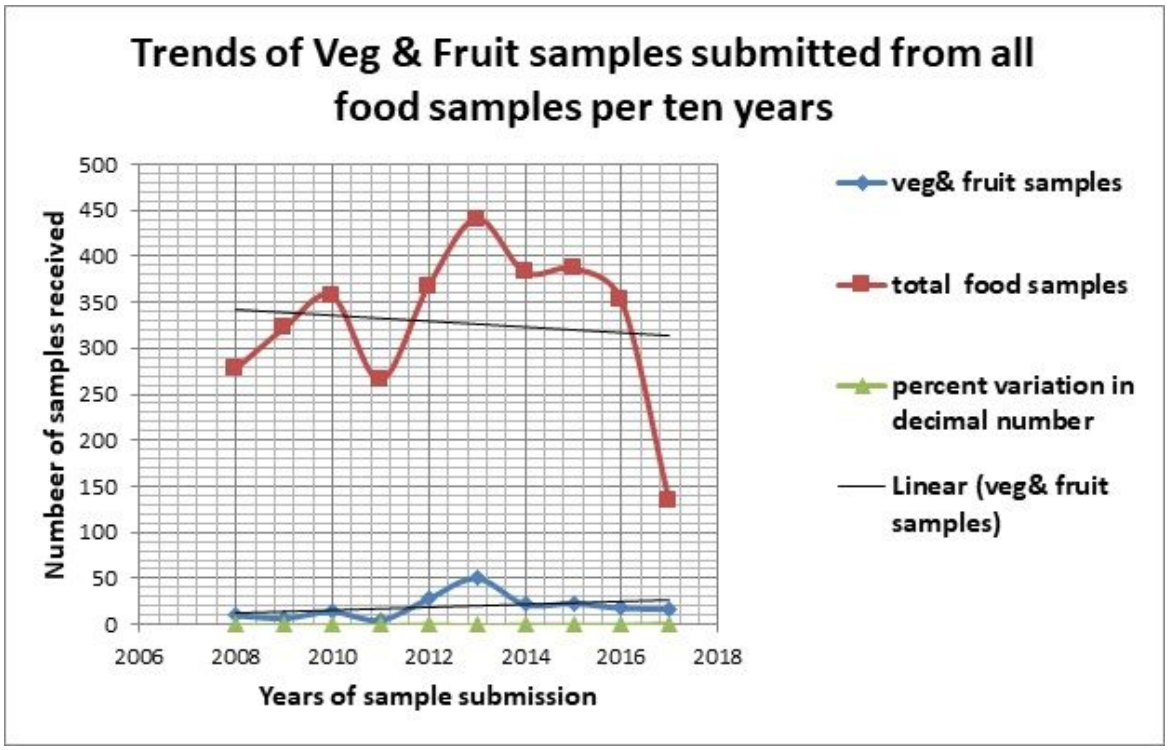

Figure 2

Trends of Veg & Fruit samples submitted in comparison with other food samples per ten years

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