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CENTER FOR FOOD SCIENCE AND NUTRITION

MASTER THESIS ON:

BACTERIOLOGICAL QUALITY AND SAFETY ANALYSIS OF COMMONLY CONSUMED FRUIT JUICES AND VEGETABLE SALADS SOLD IN SOME SELECTED FRUIT JUICE HOUSES IN ADDIS ABABA

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STUDY OF BACTERIOLOGICAL QUALITY AND SAFETY ANALYSIS OF COMMONLY CONSUMED FRUIT JUICES AND VEGETABLE SALAD IN SOME SELECTED FRUIT JUICES HOUSES IN ADDIS ABABA

Msc. Thesis

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A thesis submitted to School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirement for the Degree of Master of Science in Food Science and Nutrition

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Declaration

I, the under signed, declare that this is my original work. It has never been submitted in any Institution and that all sources of material used for the thesis have been dully acknowledged.

Name : Fekadu Ketema Kechero

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Date : _____

Dedication

This thesis is dedicated to all those who try to work hard towards solving problems of societies in the field of food science and nutrition.

Table of Contents

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List of figures:

List of Tables:

List of Abbreviations and Acronyms:

AICR = Association of International Cancer Research

CDC = Center for Disease Control

CFU = Colony Froming Unit

ESA = Ethiopian Standard Agency

FAO = Food and Agricultural Organization

FCC = Fecal Coliform Count

FDA = Food and Drug Administration

GMP = Good Manufacturing Practice

HACCP = Hazard Analysis and Critical Control Point

HE = Hektoen Enteic

IARC = International Agency for Research on Cancer

ICMSF = International Commission on Microbiological Specification for Food

IMViC = Indole, Methyl read, Vogos-proscaure and Citric utillization test

LDL = Low-Density Lipoproteins

LI = Lysine Iron

MR-VP = Methyl read-Vogos Proscaure

ND = Not Detected

OF = Oxidase Fermentase

RDA = Recommended Dietary Allowance

RTE = Ready to Eat

SC = Selenite Cystein

TCC =Total Coliform Count

TSI = Triple Sugar Iron Agar

TT =Tetrathionate

TVC =Total viable count

UN = United Nation

WCRF = World Cancer Research on Food

WFP = World Food Program

WHO = World Health Organization

XLD = Xylone Lysine Deoxycholate

ABSTRACT

Fruit juices and vegetable salad are important components of healthy diet, an extraordinary dietary source of nutrients, vitamins and fiber for humans and thus vital for health and well-being. Contamination of fruit juices and vegetable salad by bacteria is a major food safety concern in developing countries. This study was conducted to assess the bacteriological quality and safety analysis of locally prepared unpasteurized fruit juices and vegetable salad sold in fruit juices houses in Addis Ababa city. Analysis of chemical treatment and resistance pathogenic bacteria in fruit juices and vegetable salad was also part of the investigation. Study samples were collected from 7 sub-sites in Addis Ababa and cross sectional study design was used. Accordingly, 61 samples of fruit juices and 21 samples of vegetable salad were randomly collected from fruit juice houses, analyzed by duplicate and mean values were reported. The mean total viable counts of Avocado, Mango, Mixed juice and Vegetable salad samples were 5.93 log cfu/ml, 5.88 log cfu/ml, 5.97 log cfu/ml and 6.06 log cfu/g respectively; showing higher bacterial load than the International Commission of Microbial Standard for Food (ICMSF). The mean total coliform count was highest in Avocado samples 1.17 log cfu/ml, and lowest in Mango samples 0.51 log cfu/ml. The mean fecal coliform counts were 0.09 log cfu/ml, 0.007 log cfu/ml, 0.04 log cfu/ml, and 0.07 log cfu/g for Avocado, Mango, Mixed juice and Vegetable salad samples respectively. Among the treatment chemicals used 0.1% sodium benzoate was the most effective in reduction of total viable count. Out of 84 samples pathogenic bacteria such as E. coli was detected in 32.1% samples and 3.6% samples were positive for Salmonella. The bacterial isolates were tested for their susceptibility to common antibiotics using disc diffusion method on Muller Hinton Agar and all of the pathogenic bacteria isolates were resistance to Vancomycine drug. Generally, 91.7% of juice and vegetable salad samples analyzed were high in their total viable count and 50% of all samples showed a total coliform count above gulf standard. Also all of the samples (44) collected in the afternoon were above but 7(15.9%) samples collected in the morning were below the maximum permissible limit.

Key Words: *Bacteriological quality, ICMSF, Antibiotic, Antibiotic susceptibility.*

1. Introduction

In a very broad sense the term fruit refers to the mature ovary of a plant, including its seeds, covering and connected tissue. This includes both fleshy and dry fruits (IARC, 2003). Vegetable refers to edible plants, commonly collected or cultivated for their nutritional value for humans. Botanically, vegetable is defined as "edible part of a plant" such as the stem, stalk, bulb etc. In addition to this classification of fruits and vegetables definitions should always be related be their nutritional quality and their health benefits (WCRF, 1997).

Fruit juice are defined in the most general sense as the extractable fluid contents or tissues of the fruit or aqueous liquid squeezed or extracted usually from one or more fruit fruits (Bello *et al*., 2014). Depending upon further processing fruit juices either unpasteurized or pasteurized. Unpasteurized juice does not undergo further treatment like thermal processing, it is simply made from fruits that are ground and/or pressed or squeezed to extract the juice. This is to maintain its original test and flavor. Unpasteurized fruit juice was considered nonhazardous due to its freshness, acidic nature (Gahan *et al*., 2006). Pasteurization is relatively mild heat treatment killing vegetative cells of pathogenic microorganisms that impact food safety. Food safety is the assurance that food will not cause any harm to the consumer when it is prepared and/or consumed according to its intended use. Fruit juice is pasteurized to kill those harmful microorganisms and to extend shelf-life (Health Canada, 2006).

According to Ankita (2010), vegetable salad can be defined as a food made primarily of mixture of raw vegetables and/or fruits. Like ready to eat fruit juices vegetable salad

requiring minimal or no further processing prior to consumption have been implicated as vehicles for transmission of infectious microorganisms.

Nowadays, ready to eat (RTE) foods like vegetable salad and fruit juices constitute a suitable and convenient meal for today's lifestyles, because they need no cooking or further preparation. As wall as being considered low-calorie food, they are rich in fiber and provide a great variety of vitamins, minerals, and other phyto-chemicals (Sarjo *et al.*, 2006). However fruits and vegetables are widely exposed to microbial contamination through contact with soil/dust and water and poor handling at harvest or during postharvest processing. They, therefore create favorable condition for diverse range of microorganisms including plant and human pathogens (Nguyen and Carlin, 1994).

Microorganisms initially observed on whole fruit and vegetable surfaces are soil inhabitants. For example, human and animal enteric pathogens (except soil-borne spore formers such as *Bacillus cereus* and *Clostridium perfringens*) are usually absent from fresh vegetables and fruits at harvest unless they have been fertilized with human and animal wastes or irrigated with contaminated water with such wastes (Bryan, 1979). Microbial profile of fruits and vegetables are direct reflection of the sanitary quality of the cultivation, harvesting, transportation, storage, and processing of the produce (Janisiewicz and Korsten, 2002; Andrew and Harris, 2000). The difference in the microbial profiles of fruits and vegetables also result largely from unrelated factors like resident micro-flora in the soil and nonresident micro-flora through animal manures, sewage or irrigation water, transportation and handling by sellers (Ray and Bhunia, 2007; Ofor *et al*., 2009).

Vegetable salads and fruit juices are mostly contaminated with *Staphylococcus aureus*, *Entrobactersp*, *Klebsiella sp*., *E. coli*, *Salmonella- typhi*, and *Serratia sp*. *Escherichia coli*

is one of the most common human pathogen that cause several diseases such as diarrhea, kidney failure, pneumonia, skin infection, respiratory disease, meningitis, food poisoning etc. This is mostly in immune-compromised people (Heaton and Jones, 2008).

In developing countries because of inadequate or even non existing systems for routine diagnosis and monitoring or reporting for many of the food-borne pathogens, most outbreaks caused by contaminated fruit and vegetables go undetected and the incidence of their occurrence in food during pre and postharvest is underestimated (Dorny *et al*., 2009). As an example in the developing countries, the diarrheic diseases of food or hydrous origin kill 2.2 million people annually (FAO, 2007). According to the study conducted in Addis Ababa, Ethiopia on lettuce and green paper, majority of lettuce and green paper samples had microbial load ≥ *log 6 cfu/g.* All of the *Salmonella* and 97% of *Shigella* isolates showed resistance to penicillin and Ampicillin resistance was also observed in 42% of *Salmonella* and 79% of *Shigella* isolates. On the other hand the aerobic micro-flora of the vegetables was dominated by a variety of *Bacillus* and *Micrococcus spp* (Biniam and Mogessie, 2010). In another similar study conducted around Jimma reported aerobic mesophilic count ≥ *log 5cfu/g* in lettuce, cabbage and carrot which could be attributed to various pre harvest and postharvest source of contamination (Alemayehu *et al*., 2014).

As we know Addis Ababa is the capital city of Ethiopia and the location of the head-quarter of Africa Union (AU). Because of this the city is growing in fast rate, so that most of citizens are migrating from rural area to urban area in search of better life. Now a day the city has around 3.5 million populations and becoming densely populated area.

However, to our knowledge, no investigation has been conducted on the bacterial quality and safety of commonly consumed fruit juices and vegetable salads in fruit juice houses of

Addis Ababa city. Different researches have been carried out on raw fruits and vegetables. But none of them focus on the bacteria quality and safety of fruit juices and vegetable salads served in the fruit juice houses. Due to lack of awareness fruit juice vendors thought that fruits and vegetables once washed and sanitized are free from microorganisms. In larger cities, like Addis Ababa due to change in lifestyle consumption of ready to eat foods like fruit juices and vegetable salads are becoming common in most parts of the country. Apart from increasing consumption of such susceptible product, safety and quality criteria for such product were not clear for most of the sellers and consumers.

1.1.Statement of the problem

Currently, fruit juice and vegetable salad are widely consumed by most people (wide age group) as meal and dessert. Fruit juices and vegetable salad are also consumed at fruit juice houses, cafeterias and restaurants. According to Ethiopian Investment Agency (1998), fresh and processed fruits and vegetables have a large domestic market in Ethiopia despite the fact that the bacterial quality and safety is lower in the preparation of fruit juices and vegetable salad. Even if no quantifiable scientific data is available at hand, it is possible to observe that raw fruit juice and vegetable salad consumption has been increasing and many new fruit juice houses have been emerged in the market at alarming rate. The shift in the lifestyle of the societies in the cities like Addis Ababa has increased the demand of such ready to eat foods like fruit juice and vegetable salad.

Because of lack of food safety starting from field to fork, microbiological contaminations including human pathogens like *E. coli* and *Salmonella* are expected to exist at a heavy load. This is due to unhygienic practices such as poor preparation method, treatment and handling of equipment, and bad hygienic environment of kitchen in the fruit juice houses.

In addition lack of waste disposal, and tape water interruption were the main cause of contamination of juices and vegetable salad. Also squeezing the juices in the morning for the whole day time sell and put it in the plastic joke and left on shelf without using refrigerator creates favorable condition for the growth of bacteria. Fruit juices and vegetable salad prepared and handled on such way are simply sold and served by the vendors without any good hygienic practice and quality assurance. The problem is also aggravated due to nonexistent criteria to open fruit juice house and controlling mechanism for sellers and also the contaminated water used for washing and dilution during the rainy season specially June - October.

In Ethiopia the risk associated with exposure to outbreak of food-borne illness like contaminated vegetable mainly due to lack of awareness on sanitation methods especially during the rainy season(June to October) (Alemayehu *et al*., 2014).According to different information sources like media outbreaks of food-borne illness is increasing in Addis Ababa seasonally. The report of current outbreak of cholera in Addis Ababa city related to raw contaminated foods including fruits and vegetable products and water can be considered as evidence (FMHE, 2016).This is because of the presence of pathogenic bacteria in the vegetable and other raw consumed products and due to the exceeded level of none pathogenic bacteria from the recommended specification (Girmaye *et al*., 2014). Besides, in Ethiopia especially in large cities no continuous survey/assessment of food safety has been implemented in fruit juice houses where fresh fruit juices and vegetable salad are sold.

Thus, this study was conducted to determine the bacteriological quality and safety of fruit juices and vegetable salad sold in fruit juice houses in Addis Ababa city.

1.2.Research Question

To what extent fresh fruit juices and vegetable salad sold in fruit juice houses are contaminated by bacteria?

Does sampling time affect total viable bacterial load?

Is there any contamination of pathogenic bacteria such as *E. coli* and *Salmonella?*

Which treatment chemicals (lemon, benzoic acid & sodium benzoate) are effective for immediate use in the reduction of total viable bacterial count?

Did *E. coli* and *Salmonella* isolates develop first line drugs resistance?

Do standards for total viable bacterial count, total coliform count and fecal coliform count exist in Ethiopia?

1.3.Significance of the study

This study helps vendor to have better understanding and awareness about microbial quality and safety and stay competent in the market and also they benefit consumer by preventing disease spreading through consumption of fruit juice and vegetable salad. Government regulatory bodies (Ethiopian Standard authority and Ethiopian Food, Medicine and Health Care Administration and Control Authority) can use the study result for designing appropriate disease prevention strategies. Moreover, the study will be used as an initial data for future researches on bacteriological safety of fruit juice and vegetable salad. It helps to aware people about the health risks that possibly associated with consuming unpasteurized fruit juice and vegetable salad from street vendors.

1.4.Objectives

General objective

To evaluate bacteriological quality and safety of commonly consumed raw fruit juices and vegetable salads sold in fruit juice houses in Addis Ababa City.

Specific Objectives:-

- To evaluate the hygienic-sanitary (way of personal hygiene and washing and treating raw fruits and vegetables, equipment handling) quality of commonly consumed raw fruit juices and vegetable salads in fruit juice houses.
- To examine the bacteriological load and also to isolate and identify the dominant bacteria from Avocado, Mango and Mixed juice juices and vegetable salad.
- To evaluate the difference in total viable bacterial load from Avocado, Mango, Mixed juice and Vegetable salad samples collected in the morning and afternoon time.
- To isolate, identify and characterize food-borne pathogens *E. coli* and *Salmonella*.
- To examine the effectiveness of treatment chemicals (sanitizers) such as lemon, benzoic acid and sodium benzoate in reducing total viable bacterial load.
- To investigate the antimicrobial susceptibility of isolated pathogenic microorganisms (*E. coli* and *Salmonella*).

2.Literature Review

Fruit refers to the mature ovary of a plant, including its seeds, covering and connected tissue. This includes both fleshy and dry fruits. Botanically, vegetable is defined as "edible part of a plant" (IARC, 2003). From nutritional point of view, fruits and vegetables are low energy-dense food relatively rich in vitamins, minerals and other bioactive compounds as well as good sources of fiber (WCRF, 1997).

2.1. Fruits

Fruit, in botanical terms is freshly or dry ripened ovary of a plant, which encloses the seed or seeds. The fleshy component, which is normally the portion eaten, serve to protect and eventually nourish the seed as part of the natural development of the original plant's progeny (FAO, 2001).Fruits, either fresh or processed, form an important part of our daily diet, and demand is increasing in all over the world. Recent advances in agricultural technology have contributed significantly to the production of fruits throughout the world. Fruits are very perishable in nature because they are living beings and carry out transpiration, respiration, ripening and other biochemical activities which adversely affect the quality. In addition, because of their high moisture content (in an average 85%) fruits are inherently liable to deteriorate, especially under tropical conditions, and finally become unmarketable (Titarmare *et al*., 2009).

Fruits are not only colorful and flavorful components of our diet, but they also serve as a source of energy, vitamins, minerals, dietary fiber and antioxidants. They are very low in fats and proteins but high in sugar as they contain large amount of glucose, fructose, and

sucrose. In addition, most fruits are often consumed fresh due to their cherished flavor/pat ability and they contribute immensely to nutrients intake (Adel and Deane, 2005).

The structure and functional aspects of fruits dictated the general fruit composition depending upon fruit, cultivar, cultivation, maturity and other factors.

Table 1: Fruit edible portion composition ranges (Fresh weight basis).

Component	Range (%)	Comments
Water	97 – 70	Influenced by cultivation and post-harvest conditions
Carbohydrate	25 – 3	Sugars and polymers – pectin, hemicelluloses, cellulose
Protein	5 – trace	More in oily fruit and seeds
Lipids	25 – trace	Traces in cell membrane, in seeds, high in avocado
Acids	3 – trace	Citric, tartaric, malic, lactic, acetic, ascorbic + minor
Phenolics	0.5 – trace	Tannins and complex phenols
Vitamins	0.2 – trace	Water soluble > fat soluble
Minerals	*0.2 – trace*	Soil and species dependent
Dietary fiber	<1 to > 15	Peel and core dependent
Pigments	0.1 - trace	Carotenoids, anthocyanins, chlorophyll

***Source*;** Principles and practices of small- and medium-scale fruit juice processing (FAO, 2001). *P.19.*

2.2.Fruit juices

Fruit juice are defined in the most general sense as the extractable fluid contents or tissues of the fruit or aqueous liquid squeezed or extracted usually from one or more fruits (Bello *et al*., 2014). Fruit juices are prepared mechanically by squeezing or macerating the pulp

of fresh fruits or vegetables without application of heat or solvent to give an unfermented cloud, un-clarified and untreated juice ready for consumption. A common practice like diluting or blending in fruit juices preparation determine the strength of acidity or flavor (Asha *et al*., 2014). Depending upon further processing fruit juices either unpasteurized or pasteurized.

2.2.1.Unpasteurized fruit juice

Unpasteurized juice does not undergo further treatment like thermal processing, it is simply made from fruits that are ground and/or pressed or squeezed to extract the juice. This is to maintain its original test and flavor. Often it can be prepared or purchased as freshly from local market, orchards, farmers and juice houses (Harris *et al*., 2003). Unpasteurized fruit juice was considered free from bacteria due to its acidic nature (Gahan *et al*., 1996).

2.2.2.Pasteurized fruit juicer

Pasteurization is relatively mild heat treatment killing vegetative cells of pathogenic microorganisms that impact food safety. Fruit juice is pasteurized to kill those harmful microorganisms and to extend shelf-life (Health Care Canada, 2006). Not only the locally prepared/fresh fruit juices but also some times pasteurized juices are important problem in resulting food borne illness. A study conducted in Kumasi, Ghana, on the fresh minimally processed fruit juices and vegetable salad, its' microbial profile indicate significant increase in bacteria load in the apple and mango fruit juices as they stayed for a long period in shelves (Abadias *et al*., 2008).

2.3.Vegetables

Vegetable refers to edible plants, commonly collected or cultivated for their nutritional value for humans.

2.3.1.Vegetable Salad

Accordingly Ankita (2010), vegetable salad can be defined as a food made primarily from mixtures of raw vegetables and/or fruits. Like ready to eat fruit juices and vegetable salad requiring minimal or no further processing prior to consumption have been implicated as vehicles for transmission of infectious microorganisms and also food borne outbreaks cause gastrointestinal illness (Health Canada, 2006). *Salmonella* and *E. coli* are most frequently linked to produce related and hygienic practice in street vending (Abadias *et al*., 2008).

2.4.Food Safety versus Food Quality

Due to progress in science and technology and the growing gobalization of production and trade of food, national and international legislations were recently developed. Safety differs from many other quality attributes like size or color since it is a quality attribute that is difficult to observe. Safety is defined as the condition of being safe from undergoing or causing hurt, injury or loss (Webster's Ninth New Collegiate Dictionary, 1990). Food safety is the assurance that food will not cause any harm to the consumer when it is prepared and/or consumed according to its intended use. Whereas food quality the quality characteristics of the food that is acceptable to consumer. This includes external factors as appearance (size, shape, color, gloss, and consistency), texture, and flavor (FAO/WHO, 1997).But both food safety and quality assurance in fresh produce should be ongoing processes that incorporate activities from the selection and preparation of the soil in agricultural operations through the final preparation and consumption of the food.

2.5.Bacteriological Quality of Fruit Juices and Vegetable Salad

Microorganisms (bacteria, virus, fungi, and parasites) are a group of naturally occurring living organism that can initially in all food crop plants starting from pre harvest up to consumptions. They are found in a wide range of foods around the world. Their presence or absence in the food is considered as one quality. This quality is sometimes affected by the presence of microorganisms that are resident and non-resident the soil. This mainly occurs in fruit and vegetables which grow with contaminated irrigating water and human and animal faces, animal grazing area etc. Studies reveled that bacterial qualities are fluctuating throughout most food commodities (Burnett, and Beuchat, 2001). This lead to food poisoning due to food-borne pathogens which is a major public health issue associated with food hygiene and overall food safety. In developing countries, bacterial quality problem is common for some foods that are important part of the diet. *Salmonella* and some strains *of E. coli,* such as *E.oli O157:H7*, the most common food poisoning bacteria (Mead *et al*., 1999). But the acidity of the juices and salad can affect their growth.

The intensity of acidity of a food expressed by its' pH value. The pH of a food is one of several important factors that determine the survival and growth of microorganism especially bacteria during processing, storage and distribution. The acidity of a food may occur naturally as in citrus fruits, apple, tomatoes and strawberries or it may be produced in foods through microbial fermentation. High acidic fruit juices (pH 3.0 – 4.0) could not support survival and growth of bacteria pathogens. However, a number of documented outbreaks of human infections associated with the consumption of raw fruits, vegetables, and unpasteurized fruit juices increased in recent years (Buck, 2003). Although growth is

unlike at low pH, it is well documented that pathogenic bacteria may survive in fruit juices and vegetable salad, become adapted to the acidic environment, and cause outbreaks of food borne illness (Parish, 2009).Mostly, fruits juice and vegetable salad can become contaminated while growing or during harvesting, postharvest handling, or distribution or preparation for consumption. This is because of direct contact with animal or human face, or indirect contact with contaminated water, soil, processing/preparation equipment, or infected food handlers (Mukherjee *et al*., 2006).

2.6.Bacterial profile of fruit juices and vegetable salad

Naturally most fruit juices and vegetable salads are rich in nutrients that could support microbial growth. Beside this there are several factors that encourage, prevent, or limit the growth of microorganisms in juices; the most important are quality of raw fruits and vegetables, a_w, pH, juice preparation, hygienic practice, and storage (Bates and Crandall, 2001).Preparation environment mainly make the fruit juice and vegetable salad unsafe for consumption and may play vital role in spreading of *Salmonella, E. coli, Vibro cholera, Shigella* and other bacteria. It should also be noted that change in the pH to neutral shifts food to support growth of pathogens (ICMSF, 1980). In the absence of good manufacturing and hygienic practice the nutritional richness of fruit juices and vegetable salad makes the product good medium for bacterial growth (Al-jedah, 2001).

Fruit juices and vegetable salad contaminated at any point of processing could be the source of infectious pathogen. Infection has been linked with consumption of freshly squeezed juices. Study conducted on the bacteriological safety of some fruit juices showed high prevalence of *E. coli* and *salmonella* in orange and apple juices (Chen *et al*., 20001). *E.*

coli 0157:H7 and *Salmonella* species are the prominent pathogens in unpasteurized juices and vegetable salad (Burnett and Beuchat, 2001).

Street vended fruit juices and vegetable salad play an important socioeconomic role in meeting food and nutritional requirements of city consumers at affordable prices to the lower and middle income people (Ihekoronye, 1995).Despite of the potential benefits offered by fruit juices and vegetable salad, concerns related their quality and safety have been raised; as freshly prepared juices and vegetable salad have no preliminary steps or process to minimize microorganisms if they are contaminated (Sarjio *et al*., 2006).

2.7.Nutritional Benefits and Safety of Fruit Juices and Vegetable salad

Regarding ready to eat (RTE) foods, like vegetable salad and fruit juices constitute a suitable and convenient meal for today's lifestyles because they need no cooking or further processing. As well as being considered low-calorie food, they are rich in fiber and provide a great variety of vitamins, minerals, and other phyto-chemicals (Sarjo *et al*., 2006). And also fruits and vegetables are important components of healthy diet, and their consumption could help prevent a wide range of disease.

Scientific evidences are increasing that consumption of fruit and vegetables decreases the risk of several chronic diseases.

To meet the daily recommended amount, fruits and vegetables consumed or added in different form such as juice, salad mixes, side dishes/dessert, snack or as an ingredients in our daily meal (Amoah *et al*., 2009; IARC, 2003). But most of the fruits and vegetables are

normally consumed without being cooked, so the possibility of food poisoning existence is high (Aycicek *et al*., 2006).

Microorganism are initially observed on whole fruit and vegetable surfaces are soil inhabitants, members of a very large and diverse community of microbes that are responsible for maintaining ecological dynamic with in most agricultural systems. These microorganisms, use soil particles, airborne spores, and irrigation water as vector for disseminating these microbes (Janisiewicz and Korsten, 2002). Some of this microorganism can enter fruits and vegetables through damaged surfaces, such as punctures, wounds, cuts and splits that occur during growing or harvesting (Oliveira *et al.*, 2006; Nicolas *et al.*, 2007). These means the microbial profile of fruits and vegetables are a direct reflection of the sanitary quality of the cultivation water, harvesting etc (Andrews & Harris, 2000). Therefore they harbor a diverse range of microorganisms including plant and human pathogens (Nguyen and Carlin, 1994). And also the difference in the microbial profiles of fruits and vegetables result largely from unrelated factors like resident microflora in the soil and nonresident microflora through animal manures, sewage or irrigation water, transportation and handling by sellers (Ray and Bhunia, 2007; Ofor *et al*., 2009).

According to WHO (2008) green leafy vegetables such as spinach, lettuce and cabbage and all verities salad leaves are identified as the commodity group of highest concern from a microbiological safety perspective. Vegetable salad are mostly contaminated with *staphylococcus aureous*, *Entrobactersp, Klebsiella sp. Salmonella typhi, E. coli, Salmonella sp, Serratiasp*, *P.aeruginosa, Providencia, listeria monocytogenes* and *Cryptosporidium oocyts* that causes several diseases such as diarrhea, typhoid fever,

kidney failure, paratyphoid fever, pneumonia, skin infection, respiratory disease, meningitis, food poisoning etc.

Washing, rinsing, good agricultural practices during growth and harvesting, and good postharvest practices including transporting and marketing reduce the number of microorganism and extend the shelf-life of fruits and vegetables. However, only a portion of pathogenic microorganisms removed with this simple treatment. Many studies investigated that thorough washing is not sufficient to reduce pathogen levels to safe limits in leafy vegetable types. For example, result obtained from study conducted by Parish (2003) on method of reducing pathogen on fresh fruit cuts stresses the importance of using a disinfecting substance such as salt and lemon since washing of vegetables in tap water for three times results only a 10-fold reduction of VBCs. Similarly *Salmonella* survived washing to a much greater extent when attached at cut surfaces of apple and green pepper disks than on unbroken external surfaces (Liao *et al*., 2001).

In countries, where street food vending is prevalent, there is commonly a lack of information on the incidence of food borne diseases related to the street vended foods. However, microbial studies on such foods in American, Asian and African countries have revealed increased bacterial pathogens in the food in American, Asian and African countries have revealed increased bacterial pathogens in the food.

2.8.Sources of Microbial Contamination:

Deterioration and spoilage of fresh produce may be due to the result of microbial, physiological/biochemical or physical factors acting on the products. Lack of proper training, inadequate storage structures unsuitable handling technologies, ineffective quality

control and adverse environmental conditions favor for this factors. Also, time is a key determinant of deterioration (Satine, 2011).

2.8.1.Pre Harvest Source

Vegetable salad and fresh produce do not naturally contain pathogenic microorganisms such as bacteria, viruses and parasites. Irrigation water or sewage, organic fertilizers, manures etc are some of pre harvest sources of pathogen that can cause food-borne illness. For example, salad vegetables such as lettuces, cabbage, tomato and spinach carry the risk of microbial contamination because of the usage of untreated irrigation water (Taban and Halkman, 2011). In study done by Alice (1997) manures used to promote the growth of vegetables and crops containing a large number of pathogenic microorganisms including *Salmonella*, *Eschercia coliO157:H7*,*Bacillus antracis*, *Yersinia*, *Clusteridum perfringes*, *Klebsiell aspp. M.paratuberculosis* and *Listeria monocytogenes*. Similarly fields on which wild animals or livestock grazed are more likely to be contaminated with enteric pathogens like *Salmonella* and *Lesteria monocytogenes* could survive in agricultural soils for many months. So the growing location is probably the initial contributing factor which affects vegetable safety (Brackett, 1999; Nguyen and Carlin, 1994). Pre-harvest parameters like selection of proper planting material, crop management, and disease and pest control must be geared to the direction of producing high quality product (Kumar, 2012).

2.8.2.Postharvest Source

Postharvest source of fruits and vegetables contamination may include pathogenic contamination during transportation, washing, peeling, slicing, trimming, packaging and handling (Oranusi and Olorunfermi, 2011). Similarly preparation environments are often contaminated with *Micrococcus spp*. and *Staphylococcus spp* (Mensah *et al*., 2005) which originated from the vendors hand when they touched the food preparation areas,

equipment, dishcloths, or the water during dish or hand washing. This indicates cross contamination between dishwater, food preparation surfaces, serving equipment, and the food itself (Mensah *et al*., 2005).Similar conclusion was done on cross contamination point of view when handlers suffer from specific diseases. This indicates that handling by individual vendors affects the level of microbial contamination of fruits and vegetables. In addition to this transportation from place to place by the consumer also affects microbial safety of salad vegetables (WHO, 2008).

Regarding food safety level of awareness, training and motivation can bring dramatic effect on microbiological safety. Study conducted in Nigeria, Sango Ota was related on level of workers awareness and the microbial load difference was observed in sliced ready to eat pineapples preparation from two different vendors Also similar difference also observed for the microbial load of carrot from another study result (Eni *et al*., 2010).

Table 2:- The common routes of microbial contamination of fruits and vegetables.

Pre harvest sources	**Postharvest source**
Contaminated irrigation water	Improper cooking and /or holding temperature after cooking
Animal waste fertilizers	Improper packaging and Improper storage
Wild and domestic animals	Contamination from other foods in food preparation area

Source;- *Sujeet Kumar Mritunjay and Vipin Kumar, Potential Hazards of Microbial Contamination Associated with Raw Eaten Salad Vegetables and Fresh Produces, Middle-East Journal of Scientific Research 23 (4): 741-749, 2015.*

2.9.Bacteria in fruits and Vegetables as a cause of disease

Nowadays, fruit and vegetable consumption is commonly a risk factor for infection with enteric pathogens (Heaton and Jones, 2008). This is due to the existence of high number of bacterial profile including *Escherichia coli*, *Clostridium botulinum*, *Salmonella*, *Listeria monocytogenes*, *Bacillus cereus* and *Staphylococcus aureus*, grow on lettuce, cucumber, carrot, cabbage, tomatoes and variety of vegetables salad and fruit (Kumar, 2012). In investigation done by Kim *et al*., (2013) *Staphylococcus aureus*, *Salmonella enteric*, *Listeria monocytogenes*, and *Escherichia coli* are known as common food borne pathogenic microorganisms.

Regarding studies on food-borne disease, fruits and vegetable consumption has been associated with outbreaks in many countries. From these enteric pathogens such as *Escherichia coli* and *Salmonella* are among the greatest concern during food related outbreaks (Buck *et al*., 2003). For example, Salmonellosis has been associated with consumption of cut watermelon causes outbreaks in the United States of America (Oranusi and Olorunfemi, 2011). These recent outbreaks in food borne infection result from increased consumption of contaminated fruits and vegetables outside homes as most people spent their long hours (Beuchat, 2002).

2.10.Incidence of Pathogens

Human infectious diseases traditionally are acquired via the ingestion of contaminated foods and drinks (Stine, 2011). This becomes an important issue when coupled with the trend of people consuming more vegetables and fruits for health and nutritional reasons.

Irrigation with poor-quality water is a major source of contamination to fruits and vegetables with food-borne pathogens (Nutt *et al*., 2003). According to Olaimat and Holley (2012) over the past few decade food-borne illness outbreaks and cases associated with fresh produce rapidly increased. Because of once pathogens attached it is very difficult to remove it from contaminated fruits and vegetables by washing. The presence of this pathogen on edible plants is a significant potential source of human illness. Based on its severity significant portion of enteric pathogens can persist on the surface and proliferate to cause disease (Mead *et al*., 1999).Mostly, outbreaks have been attributed to sprouted seeds, leafy vegetables, tomatoes, melons, berries, and unpasteurized juices (Nguyen and Carlin, 1994).

According to Chang and Fang (2007) investigation on Salmonellosis is the most common outbreak in the world caused by *Salmonella serovars* has been found in humans and animals. In the USA from 1973 through 1997 each year overall rate of *salmonella* infection ranged from 15 to 20/100,000 population, during each year in the USA. Similarly *E. coli O157:H7* causes 20, 000 infections and more than 100 deaths each year in the United States. For example from 1982 to 2002 and 2000 to 2004, 21% outbreaks are caused by *E.oli O157:H7*, this is due to fresh produce which was the second most identified vehicle causing *E. coli O157* food-borne illness outbreaks (Michino *et al*., 1999; Olaimat and Holley, 2012). The most recent *E. coli* outbreak in world was linked to contaminated fenugreek sprouts and contaminated vegetable resulted in over 50 deaths and over 4,000 hospitalizations in 16 countries mainly in north Germany from May to June 2011. This illness was characterized by bloody diarrhea with high frequency of serious complication including hemolytic-ureamic syndrome (HUS), a condition that requires uremic treatment

(GärtnerhofBienenbüttel, 2011). This is because epidemiological evidences found from Sao Paulo, Brazil and Afghanistan suggested that pathogens has the capacity to survive under stress conditions like low temperature, high salt concentration, and low pH (Jamali *et al*., 2013; Sant'Ana *et al*., 2012).

2.11.Comparative Evaluation of the Preservatives

Prescott *et al*., (2002) defined preservatives as a group of chemical compounds deliberately added to food or that appears in food as a result of pre-processing treatment, processing treatment or storage. This includes simple organic acids (propionic acid, sorbic acid, benzoic acid) and its salt product. So, mostly chemical preservatives play vital role in the shelf life extension. The shelf-life of a product is defined as the expected time of duration that a product will remain organoliptically acceptable. It is a function of holding temperature and the number of microorganisms remaining in it after processing or preparation. Benzoic acid has been widely employed as an antimicrobial agent in foods and it occurs naturally in cranberries, prunes, cinnamon and cloves. It is well studied for acidic foods such as fruit juices, carbonated beverages, pickles and sauerkraut. In Addis Ababa even if there is no documented study, lemon slice is most commonly used to reduce the bacterial load for an immediate use (Ashagrie *et al*., 2012).

2.12.Antibiotic Resistance of pathogens

Antibiotics are among the most commonly prescribed drugs used in human medicine. However, up to 50% of all the antibiotics prescribed for people are not needed or are not optimally effective as prescribed. Due to this reason prevalence of antimicrobial resistance among food borne pathogens has increased during recent decades. According to Alice

(1997) report each year in the United States, at least 2 million people acquire serious infections with bacteria that are resistant to one or more of the antibiotics designed to treat those infections. In developing countries because of poverty, inadequate or even non-existing systems for routine diagnosis and treatment of food-borne pathogens they develop resistance by exchanging genetic information (Amabile-Cuevas, 2003). Besides this antibiotic sensitivity of these bacteria has not been well studied (Davis *et al.*, 1999).

2.13.Related works done on fruit juices and vegetable Salad bacterial contamination around the world.

According to Addo *et al*., (2008) the total heterotrophic bacteria and yeast counts were taken to determine the overall contamination by mesophilic bacteria. However, certain levels may indicate serious case of poor hygienic condition and the food become unfit for consumption.

Total viable count is a measure of bacterial quality of fruit juices. Presence of bacteria in high numbers (TVC >4 log10 cfu/ml) is responsible for the spoilage of fruit juices (Gulf standards, (2000) and Codex standards, (2005).

In a recent studies higher levels of total viable counts (TVCs) in fruit juices were found in comparison with the previous studies (Bagde and Tumane, 2011; Lewis *et al*., 2006). According to Simforian *et al*., (2015) study the total viable count in avocado juice was reported 1- 5.98 *log* cfu/ml. In another study Al-Jedah and Robinson, (2002) reported that the total viable bacterial counts of avocado was 6.69 *log* cfu/ml. According to recent study

conducted in Bahir Dar, Ethiopia reported that mean total viable count was 7.49 *log* cfu/ml (Mekonen and Tadele., 2016). Another comparable study conducted in Jimma town reported 6 *log* cfu/ml (Ketema *et al*., 2008). Another study conducted on Mango juice in Ghana reported 3.76 *log cfu/ml* total viable bacterial count was detected (Addo *et al*., 2008), even if Mango juice had low pH (4). Another comparable study conducted in Bair Dar, Ethiopia reported 4.76 *log* cfu/ml mean total viable bacterial count in fresh Mango juices (Asmamaw and Mulugeta, 2012).

Study conducted on mixed juices (Mixed juice) in Nigeria reported that total viable bacterial count was 3.54 *log* cfu/ml (Ojukwu, 2015). However, another comparable study conducted on mixed citrus juice reported the growth range of total viable count between 3.0 - 4.0 *log* cfu/ml which is below the maximum limit (Eni *et al*., 2010).

George *et al*., (2014) studied the bacteriological quality of mixed vegetable salad in Accra, Ghana reported that total viable bacterial count ranged 3.87-5.6 *log* cfu/ml. In a similar related work it has been found that the bacterial contamination of salad samples in Kumasi, Ghana was 5.13 *log* cfu/ml (Ameko *et al*., 2012). Similarly the study conducted by Viswanatha and Kaur (2010) in India support this result. Another work on the prevalence of bacterial contamination on mixed vegetable salad reported that the total viable count was 5.17 *log* cfu/ml (Mensah *et al*., 2002). According Mohammed *et al.,* (2011) study the mean TVC of each juice types and vegetable salad were exceeded above the maximum permitted level (4 *logcfu/ml*) of Gulf Standard, but Tasnim *et al.,* (2010) reported the bacterial counts of fruit juices within the standard limits. Even if the time elapsed between preparing and serving locally vended fruit juice and vegetable salad was not long enough to allow microbial growth, such high counts may be due to cross-contamination from

improperly washed utensils or contaminated fruits (Lewis *et al*., 2006). In addition pH and moisture variation, water used for washing and dilution, time of sample collection, and hygiene, were another factor for cross-contamination (Yigeremu *et al*., 2001).

Coliforms are considered as indicators of quality and its' presence in high numbers (CC > 2 log10 cfu/ml) is health hazard causing spoilage of fruit juices and food borne diseases (Gulf standards, 2000).

Study conducted in Qatar reported that 3.97 *log* cfu/ml and in Nagpur city, India also reported the mean total coliform 4 *log* cfu/ml in avocado juices (Titarmare *et al*., 2009). Another similar work conducted in Bahir Dar, Ethiopia reported the mean total coliform count was 7.49 *log* cfu/ml in Avocado juices (Mekonen and Tadele, 2016). In addition the study conducted in Hawassa, Ethiopia reported that the mean 3.98±1.23 *log* cfu/ml in Avocado juice (Mesfine, 2011). But according to Bello *et al*., (2014) work done in Nigeria the mean total *coliform* count was $4.0x10^4$cfu/ml, which was against another reported study. This evidence was an indication that some of the unpasteurized juices sold in the street may be out of the standard range.

Mango juice was highly contaminated due to unhygienic preparation with total coliforms (Tambekar *et al*., 2009). On the other hand study conducted in Bahir Dar town, Ethiopia reported that the mean total coliform count in Mango juices was $1.7x10^5$cfu/ml (Mekonen and Tadele, 2016). However, another study in Qatar reported the total coliform in the range ND – 4.0 x 10^4 cfu/ml. This means total coliform was detected in some of the samples, which was different from the above reports (Al-Jedah and Robinson, 2002). This might be due to difference in handling and washing practices of mango (Asmamaw and Muluken, 2012).

In mixed juice case, Dushyant *et al*., (2015) reported that the mean total coliform count was within the range of 3.49 – 7.69 *log* cfu/ml. Another similar study reported that 6.2 *log*cfu/ml total coliform counts and this were clearly by human contact, and this level of contamination was totally unacceptable (Iqbal *et al*., 2015). In contrast, similar work done in Qatar on mixed juices exception of one sample the total coliform counts of all samples analyzed were not detected for coliform (Al-Jedah and Robinson, 2002).

According to Holt (1994) a number of genera within the coliform group are widely found on vegetable tissues and pose no hazard to humans, but it was the possible presence of human pathogens including *E. coli* and *Salmonella* spp. For the vegetable salad sample the mean total coliform count reported in Pakistan was 4.9 *log* cfu/g (Mohammad*et al*., 2011). Another similar study conducted in Nigeria reported that 46.66% of samples had coliform count more than 2 *log* cfu/ml which exceeded the standard (Iqbal *et al*., 2015). Tambekar *et al*. (2009) suggest that the main source of coliform contamination was mainly due to contaminated water supplies which were used in preparation of juice. The presence of coliform bacteria could be due to inadequate hand washing by juice handlers, poor processing practices, and unhygienic environment. In addition Chen *et al*., (2001) reported that the total coliform may still be present and can be transferred from washed hands to lettuce during chopping.

Fecal coliforms are the normal inhabitant of intestinal tracts of man and animals. They are not known to be found in nature in the absence of fecal contamination from the above sources. They are excluded out of animal body through excretion process, in the form of faeces.

Nguz *et al*., (2005) reported that fecal coliform counts were efficient indicators of sanitization, but the detection of fecal coliform counts does not completely indicate the presence of pathogen. But sometimes some of them are pathogenic and cause diseases. Thus, the presence of these organisms in water and fruit juices is dangerous for human consumption (Salle, 2000). Moushumi *et al*., (2009) explained the presence of fecal coliforms in freshly squeezed juices and explained the possible entry points of bacterial pathogens in juice.

As an example geographical source that could have undergone different pre-harvest practices and pretreatments during their postharvest and personal hygiene were the major factors that contributed to high fecal contamination in Nigeria (Jones *et al*., 2008). Another comparative study in Bangladesh reveled that most of the juice samples showed equal or slightly higher fecal count than the permitted count, these were unfavorable for consumption (Tasmina *et al*., 2011).

There are many factors which affect the proliferation of microorganism. James and Ngarmsak, (2011) reported that sampling time, storage temperature and pH are the three principal determinant factors for growth of food borne pathogens associated with fresh produce. Because these factors, create favorable environment for the growth of microorganism.

According to Oranusi and Olorunfemi, (2013) study conducted in Nigeria report there was significant difference between bacterial load in the samples collected in the morning time and afternoon time. Similarly Tamberkar *et al*. (2009) reported samples collected in the evening had high bacteria count. On the other hand study conducted in Accra, Ghana reported that bacteriological analysis on raw mixed vegetable salad indicate 20% of the

salad sold in the mornings was with bacterial load in excess of 5 x 10^4cfu/g (*log* 4.7 cfu/g), and this increased by 80% for the salad sold in the afternoons (Ameko *et al*., 2012).

There are lots of bacteria genera in fruit juices and vegetable salads. According to Lateef (2004) the presence of bacterial genera such as *Bacillus* sp, *Proteus* sp, *Micrococcus* sp and *Entrobacteriaceae*in fruit juices is considered a safety concern.

Staphylococcus and *Micrococcus* in fruit juices and *Bacillus* in vegetable salad are the dominant bacterial genus (Rajvanshi, 2010). Another comparative work done in Sudan on vegetable salad revealed that *Bacillus* (17%) was the third most dominant genus next to *Staphylococcus* (33%), *Entrobacteriaceae* (25%) and *Bacillus* in fruit juices (Mahmoud *et al*., 2013). But in some case bacteria belongs to the same genera were also isolated and identified by other researchers from fruits and vegetable in different countries (Osamwonyi *et al*., 2013; Eni *et al*., 2010).

Among the greatest concerns with human pathogens in fresh fruits and vegetables are enteric pathogens (e.g., *E. coli* O157:H7 and *Salmonella*) that have the potential for growth prior to consumption or have a low infectious dose (Buck *et al*., 2003).Food Safety Authority of Ireland, (2007) reported that human pathogens, like *E. coli* and *Salmonella* can survive for extended periods of time in low pH food and causes diarrhea, urinary infection, pyogenic infections etc. Specially, some strains of *E. coli* synthesize heat stable entero-toxin are responsible for diarrheal disease in humans and domestic animals.

Tambekar *et al*., (2009) reported the food borne illness associated with different consumption of road side freshly squeezed fruit juices at public places in Amaravati city, India. Similar work done on mixed juice in Delhi, India reported the presence of pathogenic

bacteria specially, *E. coli* were 40% (Dushyant *et al*., 2015). To the contrary, survey conducted in Ireland shown that only 0.2% of the unpasteurized juices contaminated with *E. coli* (Mohammed, 2011). Also similar study conducted by Ogbonna *et al*., (2011) reported that the contamination of cabbage by *E. coli* and *Pseudomonas species*. This was because contamination during preparation has shown potential sources of bacteria pathogens like *E. coli, Salmonella, Shigella*, and *Staphylococcus aureus* (Sandeep *et al*., 2001). In addition, unhygienic handling and processing increased potential for the invasion or growth of pathogenic bacteria and hence the risk to transmission of food borne illness (Little and Mitchell, 2004; Shakir *et al*., 2009).

Occurrence of *Salmonella* in fruit juices and vegetable salad

Outbreaks with identified etiology were predominantly bacterial origin, primarily *Salmonella*. More recently, *Salmonellosis* has been linked to tomatoes, seed sprouts, cantaloupe, mamey, apple juice, and orange juice (Beuchar, 2002). The presence of *E. coli* and *Salmonella* was reported in Sao Polo, Brazil (Mohammad *et al*., 2011).Similar study conducted in Delhi, India reported that *Salmonella* was detected in 13% samples collected for the analysis (Dushyant *et al*., 2015).Another study in Mexico reported that 14% of samples of juice were positive for *Salmonella* (Castillo *et al*, 2006). Similar comparative study in Bangladesh also reported that unpasteurized fruit juices were 7.89% positive for *Salmonella*s pp (Shakir *et al*, 2009).

More recently, *Salmonellosis* also linked to vegetables like tomatoes, lettuces and carrot. Study conducted in Nigeria reported that *Salmonella serovar* to be the major contaminant of vegetables obtained from farms and central market (Raufu *et al*., 2014). Similar research conducted in India again strongly support by 50% positive for *Salmonella* species in fruit

and vegetable, but 16% in street vended fruit juices (Titarmare *et al.*, 2009). Another work conducted on fresh vegetables in Sri Lanka reported that *Salmonella* was detected in 6% of the samples tested (Neusely *et al*., 2013). Contamination was mainly due to poor quality of water used for dilution as well as prevailing unhygienic conditions related to washing utensils, contaminated water, poor personal and domestic hygiene, peeling of fruits with unhygienic hands, shop in crowded places etc (Tambekar *et al*., 2009).

In contrast, Dannison (1996) reported there was no potential pathogenic strain like *Salmonella.* In case of Ethiopia similar study conducted in Hawassa 2.5% fruit juices were positive for *Salmonella* (Mesfin, 2011). Whereas, similar unpublished study conducted in Debre-Markose, North-Western Ethiopia reported that due to hygienic and proper preparation *salmonella* was not detected in fruit juice samples (Kindu, 2015).

Generally, improper washing of fruits and rotten fruits and vegetables adds these bacteria to juices and vegetable salads leading to contamination (Jones *et al*., 2008). In addition, lack of awareness of basic safety issues by vendors might contribute to augmentation of the microbial loads (Mahale *et al*., 2008). These include unavailability of running water for dilution and washing, prolonged preservation without refrigeration, unhygienic surroundings with swarming flies and airborne dust. (Lewis *et al*. 2006). It is contended that the unhygienic location of the shops like heavy vehicular traffic, heavily crowded market place are responsible for such huge contamination. In comparison canned and preserved fruit juices sold in the market showed no microbial contamination and appeared clean and safe for human consumption.

Due to such heavy microbial contamination, attempts were taken to decontaminate or to reduce the microbial load of the fruit juice. But the FDA, India (Food and Drug Association

of India, 2010) does not recommend using any soap, cleaning agents or detergent to wash fruits and vegetables but thorough washing with fresh water can help to reduce the number of microbial communities. Besides, more recently various thermal and non-thermal treatments were found to be effective in decontaminating the fruit juices (Raso *et al*. 1998; Yen and Lin 2003).

The elimination of bacteria from seeds by chemical or physical treatment is critical for reducing the risks of sprout borne disease outbreaks. Study conducted in Nigeria reported that even if the concentration were the main factors chemical treatments like benzoic acid were the most effective against reduction of bacterial load (Oladipo *et al*., 2010). Another similar study reported that juices treated with sodium benzoate recorded a decrease from 6.08 to 5.36 *log* cfu/ml and the addition of lime also reduce bacterial load and extended the shelf life (Nwachukwu and Ezeigbo, 2013).

Standard agar disc diffusion technique on Muller Hinton agar using commercial discs were mostly used for antimicrobial sensitivity testing. The following antibiotics with the disc strength in parentheses were Amoxicillin (30µg), Chloramphenicol (30µg), Tetracycline (30µg), Co- Trimoxazole 25µg), Cephradine 30µg), Ciprofloxacin (30µg), Cephalexin (30µg) commonly used for testing isolates of *E. coli* and *Salmonella* (Bauer *et al*., 1966).

Now a day many bacterial specious are becoming resistance to multiple drugs mainly because of selective pressure exerted by over-prescription of drugs in clinical settings and their heavy use as growth promoters in farm (Charpentier and Courvalin, 1999). This leads bacteria to develop multiple resistances but their degree of resistance varies with different isolates and time (Sharada *et al.*, 2011). Once antibiotic resistant bacteria get in the gastrointestinal tract of the consumer and can be a potential source for diseases (Osterblad

et al., 1999; Levy, 2001). Some authors reported antibiotic resistance of bacterial isolates against commonly used antibiotics has been increased from time to time (Vicas, 2010). Adetunji and Isola (2011) who reported that 40% and 70% resistance level in *E. coli* from abattoir. Similarly Lateef, (2004) reported that Amoxicillin were not active against the strain of *E. coli.* But Marwa *et al.,* (2012) reported that most *E. coli* isolates from food were sensitive to amoxicillin was disagree with the above report.

Salmonella strains isolated from fruit juices were resistant to multiple antibiotics (Jones *et al*., 2002 and Aditunji and Isolate, 2011). According to Nipa *et al*. (2011) multiple drug resistance was observed in 98.06% isolates with a resistance to two to seven antibiotic. Another similar comparable study reported 85% of the resistant isolates were multiple drug resistant where highest (89.1%) resistance was to the amoxicillin (Oluyege *et al*., 2009). Oppositely none of *Salmonella* isolates from salad were resistant to Ciprofloxacin and Chloramphenicol, but66.67% showed resistance against Cephradine and Cephalexin (*Nawas et al*. 2012).

2.14.Review of Ethiopian Studies related to chemical treatment of foods

Even if there is no trend of using preservatives in Ethiopia, benzoic acid, sorbic acid, and propionic acid are the most commonly used preservatives in foodstuffs in the world. The only report done on the preservative efficiency of the above chemicals in any of the traditional Ethiopian fermented foods was on injera. They are generally used to inhibit yeast and mould growth, being also effective against a wide range of bacteria. According to Ashagrie *et al*., (2012) it was shown that the chemical preservatives were effective in inhibiting moulds responsible for *injera* spoilage. This was shown by the reduction in

percentage of mould invasion of the samples containing preservatives as compared to the sample without preservative, the control.

3.Methods and Materials

3.1. Study Design

The design of the study was cross-sectional study involving structured questionnaire determining the factors related to bacteriological quality and safety of fruit juice and vegetable salad, and laboratory investigation to determine the bacteriological load, pathogenic microbes and antibiotic susceptibility of the isolated bacterial species from samples of fruit juice and vegetable salad collected from Addis Ababa city.

3.2. Description of the study site and Period:

The study was conducted in Addis Ababa from November 2015 to June 2016. The city has 10 sub cities each having different number of woredas (*Figure 1*). As a capital city, Addis Ababa is a major trade center for fruits and vegetables with many fruit juice houses that prepare and sell fruit juice and vegetable salad. The number of fruit juice houses clearly indicates that there is a high demand of fresh fruit juice and vegetable salad in Addis Ababa.

No	Sub city	Total Number of Weredas	Sub weredas	Sefers	Blocks
1	Addis Ketema	10	28	84	302
2	Arada	10	31	100	316
3	Lideta	10	27	75	228
4	Kirkos	11	41	146	474
5	Yeka	13	124	394	1344
6	Bole	14	152	552	1695
7	Akaki Kaliti	11	135	683	1660
8	Nifas Silk Lafto	12	128	397	1059
9	Kolfe Keranyo	15	103	392	1385
10	Gulele	10	73	200	546
	Total	116	842	3003	9009

***Figure 1*;-** *Addis Ababa city map (Source:-Addis Ababa city Administration web site)*

Samples were collected from seven randomly selected representative sub cities and analyzed at Addis Ababa University Food Science and Nutrition department microbiology laboratory.

3.3. Source of Sample

Fruit Juice houses that prepare and sell unpasteurized fruit juices and vegetable salad in Addis Ababa city.

3.4. Sampling and Sample Size

Avocado, Mango, mixed juice and vegetable salad samples were collected from fruit juice houses that prepare and sale unpasteurized fruit juices and vegetable salad in Addis Ababa city.

A total of 84 samples (63 fruit juice with three fruit juice t and 21 vegetable salads) from seven representative sub cities, i.e. 21 samples of fruit juice from each and mixed vegetable salad, were collected. This was achieved by collecting triplicate samples for each type of fruit juice.

In this study varieties of unpasteurized fruit juice types and vegetable salad were included, whereas pasteurized fruit juices were excluded. Therefore, unpasteurized Mango, Avocado, mixed juices and mixed vegetable salad samples were collected.

Samples of fresh fruit juices were selected from different fruit juice houses across Addis Ababa. A wide range of fruit juice house and fruit juice varieties were covered in order to ensure that the survey was representative of the supply of the products in Addis Ababa. Before sampling was performed, seven out of ten sub cities, namely: Gulele, Addis Ketema, Arada, Yeka, Kirkos, Bole and AkakiKaliti sub cities.

Regarding sampling unit, the amount of the each samples collected was equal to the amount sold in the fruit juice houses in a glass. Meaning about 250 ml of fruit juice from each type was collected and transported to the laboratory by sterilized juice collecting jar. Regarding the vegetable salad, a single serving of vegetable salad was taken as a sample unit and taken to the laboratory with the aid of sterilized dish.

3.5. Data Collection

Three basic data collection methods were used in this study:-Structured questionnaire, checklist and laboratory experiment.

3.5.1. Structured Questionnaire

Structured questionnaire was distributed to 21 juice makers, who prepare fresh juice in twenty one fruit juice houses. The questionnaire was aimed to obtain firsthand information on awareness of juice makers, sources of fruit, storage conditions, water source for juice preparation as well as for cleaning purpose, practice of washing of equipment and fruits before squeezing out the juice and whether or not the juice makers have had training in food hygiene and safety, awareness about microbial contamination and its health risks.

3.5.2. Laboratory-based experiment

The laboratory based experiment involved Mango, Avocado, mixed juice and vegetable salad sample collection, processing for analysis, isolation and identification of microorganism from the juices and salad samples; and testing the isolated pathogenic bacteria for their antibiotic sensitivity test.

Variables

A. Dependent Variable

- Quality of locally prepared fresh fruit juices and vegetable salad.

B. Independent Variable

- Storage environment of juices
- Way of washing of equipment used in the juice houses
- Environmental hygiene
- The quality and amount of water used
- Personal hygiene
- Educational status of juicer
- Health status of juicer
- pH of the lemon

3.6. Laboratory Analyses

3.6.1. Chemical and Physical Analysis

pH and moisture content determination were done as the chemical and physical analysis. The pH of each fruit juice type and vegetable salad was measured using a digital pH- meter. The pH of each fruit juice sample was determined by blending 25 ml fruit juice sample in separate beakers (100 ml). Before reading its pH, each sample was agitated manually for 1 min until a stable reading was obtained. Each fruit was tested three times to determine mean measurement. Between readings, the electrode was rinsed in distilled water to ensure that the reading is not affected by the previous sample.

Oven drying method was used for determination of the moisture content of each sample. Accurately 5 gram of well mixed fruit juice and vegetable salad sample was weighted in a previously dried moisture crucible (about 75 mm and 25 mm deep). The crucibles were placed in an air oven maintained at 105 ± 2 ^{0}C and dried for at least 3 hr. Then dried samples were cooled in desiccators and weighted. The process of heating, cooling and weighing was repeated until the same difference between two successive weightings less than 1 mg (Cornelius and Elizabeth, 2013).

3.6.2. Sample processing

The samples collected for microbial analyses were handled in a sterilized jar and put in cooling jar until they were transported to laboratory. Then after arrival, the analyses were executed immediately so as to avoid any change developed inside the samples. For microbial analysis, 25 ml of fruit juice and 25 g of vegetable salad was measured using measuring cylinder and weighing balance, respectively, and transferred to 225 ml of sterile distilled water and homogenized by manual shaking in an aseptic environment which was achieved by cleaning and disinfecting by different disinfectants and as well as using Bunsen burner flame (Robinson and Al-Jedah, 2001). Serial dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}) were prepared by taking 1ml from the homogenized sample and transferring to it sterile test tube containing 9 ml of sterile distilled water and properly mixing using Vortex (Biocote).

3.6.3. Bacteriological Counts

Bacteriological analysis including identification and enumeration of potential pathogen was carried out according to standard procedures (Buchanan and Gibbsons, 2004). The

total colony count was done by pour plate method using plate count agar for bacteria (Lateef, 2004).

3.6.3.1. Total aerobic viable bacteria count **(TAVBC)**

The total aerobic viable bacteria count was performed on plate count agar (Oxoid) in four replicates and each was duplicated then spread plating method was used. The media was used based on the manufacturer's instruction. From each of an appropriate dilution was transferred to plate count agar plates of the four replicates. Then the inoculated plates were then incubated at 35 ^{0}c for 24-48 hour and the total viable colony count determined. The result was expressed as colony forming unit per milliliter (cfu ml $^{-1}$). At the end of enumeration, the dominant bacterial specious were analyzed using gram staining and some colonies were randomly picked and identified based on the taxonomic schemes and described in FDA, (2001).

The plates containing fewer than 300 colonies at two consecutive dilutions were used to calculate the results from a counted mean. The number (N) of cfu/g or ml of test sample was calculated as follows:

$N=C/v\ (n1 + 0.1n2)\ d$ where: C is the sum of colonies on all plates counted;

V is the volume applied to each plate;

n 1 is the number of plates counted at the first dilution;

n 2 is the number of plates counted at the second dilution;

d is the dilution factor from which the first count was obtained

The result was rounded to two significant figures and expressed as a number between 1.0 and 9.9 multiplied by 10^x where x is the appropriate power of 10 (Robers and Greenwod, 2003).

3.6.3.2. Total Coliform Count (TCC)

Counts of coliforms were obtained by mostly accepted method called MPN (Most Probable Number) technique. One ml of each of the three consecutive dilution tubes was inoculated into tubes containing Lactose Broth (LB) with Durham's tubes and incubated at 35 ^{0}C for 48 hours (Uma *et al*., 2009). From positive cultures (determined by turbidity and gas production) a loop-full of suspension was transferred to tubes containing Brilliant Green Lactose Bile (BGLB) broth, 2% and then incubated at 35 ^{0}C for 48 hour. After incubation positive tubes for growth and gas production were considered positive to coliform. Then coliform count was calculated following the MPN method in the Bacteriological Analytical Manual (FDA, 2010).

3.6.3.3. Fecal coliform count (FCC)

Similarly, fecal coliforms count was performed using MPN method. Once one ml each of 10^{-3}, 10^{-4} and 10^{-5} dilution was inoculated into three test tubes of LB with Durham's tube and incubated at 35 ^{0}C for 48 hours. Presumptive positive tubes of lactose broth were gently mixed and using inoculating loop a loop-full of each positive culture was transferred to tubes of EC broth. Inoculated EC broth tubes were incubated for 48 hours at 45.5 ^{o}C. Production of gas in an EC broth culture was considered as positive fecal coliform. Those tubes, which were positive in gas production within 24 hours, were used in calculation of fecal coliform.

3.6.4. Identification of Micro-flora

Once bacterial load of the samples were determined, a loop-ful of 4 different colonies ranking from one up to four in their size and number were randomly picked from countable plates and purified by repeated streaking. The isolates of dominant aerobic viable bacteria

were subjected to different morphological and biochemical test and identified to species level (McCance *et al*., 1998).

3.6.4.1. Cell morphology

Cell morphology was used as one of the confirmation of dominant species. Gram staining was performed for each purified culture to determine cell shape and arrangement of dominant bacteria.

3.6.4.2. Catalase Test

Catalase test was used as a second confirmatory test in the isolation of dominant species. Fresh pure culture of the isolates were picked using sterile loop from the agar plate and mixed with a drop of 3% H_2O_2 solution on a clean glass slide. Liberation of oxygen in the form of bubbles within a few seconds was indicated as positive for catalase test. Isolates which did not produce bubbles considered as catalase negative.

3.6.4.3. Oxidase Test

Oxidase test was used as the third conformation of dominant species. The oxidase test was used to identify bacteria that produce cytochrome c oxidase that catalyse the transport of electrons between electron donors in the bacteria and reagent 1% tetramethyl-*p*-phenylene-diamone. First filter paper was soaked with the substrate tetramethyl-*p*-phenylene diamine dihydro chloride. The paper was moistened with sterile distilled water, and the colony to be tested was picked with sterile loop and smeared in the filter paper. The inoculated area of paper was observed for color change to intense deep blue or purple within 10-30 seconds, as a positive test.

3.6.4.4. Oxidative Fermentative (OF) Test

Oxidative fermentative test was used another method of confirmation of dominant specious. According to UK Standards for Microbiology Investigation (2015), the

oxidative-fermentative test is used to determine if bacteria metabolize carbohydrate by oxidation, fermentation, or have no ability to use the carbohydrate in the media. OF basal semi-solid medium were prepared in two test tubes. The two test tubes were heated in boiling water for 10 minutes to remove the oxygen and allowed to cool. Once the media solidified, two test tubes were stab-inoculated by inserting a straight wire vertically to approximately ¼ inch from the bottom and one test tube was immediately filled with liquid paraffin to create anaerobic conditions. The two test tubes incubated at 35 ^{0}C for 48 hour. The test tubes color changes were evaluated daily.

3.6.4.5. Spore Staining Test

Spore staining test was used as differential stain to selectively differentiate dominant bacterial spore formers, and to differentiate spore formers from non-spore formers. First smear was made on clean slide and air dried and fixed the organism on the glass slide and covered with a square of blotting paper. Then, the blotting paper was saturated with malachite green stain solution and steamed on boiling water for 5 minutes. Next, the slide was washed with sterile distilled water and counterstained with 0.5% safranin for 30 seconds. Finally, the slide was washed with distilled water and dried to examine under microscope for the presence of spore. The spores were seen as bright green and the vegetative cells as brownish red to pink.

3.6.5. Detection of *E. coli* and *Salmonella spp*

Some pathogenic bacteria such as *E. coli* and *Salmonella* were detected according to the procedures outlined by Food and Drug Administration (FDA) (2001).

3.6.5.1. Detection of Salmonella

Sample was prepared based on the analysis of a 25 ± 0.5 g analytical unit at a 1:9 sample/broth ratio. The test sample was prepared in duplicate for each sample.

3.6.5.1.1. Pre enrichment media

Sterilized Buffered peptone water (BPW) was used for as pre enrichment media. Representative and homogenate sample of 25 g was weighted in sterile Erlenmeyer flask (250 ml volume) then buffered peptone water was added to it. The test sample was inoculated with previously sterilized buffered peptone water for 24 hr ± 2 hrs at 37 ^{0}C to favor the repair and growth of stressed or sub lethally injured *Salmonella* arising from exposure to heat, freezing, desiccation, preservatives, high osmotic pressure or wide temperature fluctuations.

3.6.5.1.2. Selective enrichment and plating

After incubating the sample for 24 hr ± 2 hr at 35 ^{0}C in appropriate pre-enrichment medium, then 1 ml sample homogenate was transferred on Tetrathionate (TT) broth and incubated TT at 35 ^{0}C for 24 hr ± 2 hr. The analyses were duplicated for each sample in each step.

Once cultured on enrichment media then proceeded by streaking onto selective differential agar. Xylose lysine desoxycholate (XLD) agar was used for the isolation of *Salmonella* after incubated for 24 hr ± 2 hr at 35 ^{0}C.

Detection of *Salmonella* was checked by typical colony characteristics depicted in each agar. In case of any observation of typical colony characteristics, further confirmatory tests were performed (as indicated in screening test).Triple Sugar Iron Agar (TSI), Lysine Iron Agar (LIA), and XLD Agar, were used in the screening procedure. The media that showed typical colony characteristics was inoculated in TSI and LIA for 24 hr ± 2hr at 35 ^{0}C. The presence of alkaline slant and acidic (yellow) butt with or without blackening was checked in TSI agar. In LIA the purple slant or purple butt was also checked. Typical or suspicious

Salmonella colonies were isolated and biochemical confirmatory test were conducted. Media used in isolation step was XLD as indicated in selective plating. Media used in biochemical confirmatory test were: MR-VP broth and Simon's citrate agar. MR-VP and Simon's citrate were incubated for 96 hr at 35 ^{0}C. The typical colony characteristic in biochemical confirmatory test was red or pink color on the surface in MR-VP broth.

3.6.5.1.3. Urease Test

Sterile urea broth was prepared with test tubes. Two loop-full of growth was inoculated from presumed-positive TSI slant culture with sterile needle and incubated for 24 hour at 35^{0}C. The change of the broth to purple-red color is considered as positive. This is conventional test for confirmation of *Salmonella*.

3.6.5.1.4. *Salmonella* Polyvalent Agglutination test

Serological tests principle is based on the fact that antibodies in serum produced in response to exposure to bacterial antigens, will agglutinate with bacteria carrying homologous antigens. This test was distinguished by *salmonella* antigenic characteristics. Two separate drops of saline were prepared on a glass slide and portion of test cultures were emulsified in each drop of saline to give a smooth, fairly dense suspension. To one suspension, one drop of saline was added as a control and mixed, and one drop of undiluted antiserum was added to the other suspension and mixed. Rock slide for one minute and observe for agglutination. Finally, the positive result was compared with known positive culture like *salmonella typhimurium.*

3.6.6. Treatment of Juices and vegetable salad with different chemicals

This was to measure the effect and effectiveness of different chemicals such as squeezed lemon, benzoic acid and sodium benzoate on bacterial load in the test samples. 0.1% concentration with an amount of 5 ml benzoic acid and sodium benzoate, and the lemon

fluid squeezed from one slice were added respectively for each juice and vegetable salad samples analyzed. This because 0.1% concentration is the most advised concentration for human consumption. The pH was measured before and after treatment. Then the effect on total viable count was analyzed by counting the total viable count on plate count agar for each treated sample type and compared with that of the total viable count obtained from untreated samples.

3.6.7. Antimicrobial Susceptibility Testing

In-vitro test was used to confirm susceptibility of isolates to chosen antimicrobial agents, or to detect resistance in the isolated human pathogen by means of a disc diffusion method on Mueller-Hinton Agar. The test was performed by adjusting suspensions (bacterial culture inoculums and sterile liquid glucose) turbidity to 0.5 McFarland standards which was assumed approximately equivalent to 1-2x10^8 CFU/ml on the surface of Muller-Hinton agar plate. Sterile cotton swabs were dipped into the suspensions and spread evenly over the entire agar surface. Ten commercially prepared fixed concentration paper antibiotic (Antibiotics impregnated) discs were used in the experiment for each isolate. Plates were incubated for 16-24 hour at 35 ^{0}C. The diameters of zone of inhibition were measured to the nearest whole millimeter using the transparent rule interpreted as susceptible, intermediate and resistant based on the recommendations of Alice (2008).

3.6.8. Data analysis

Data from all questionnaires were verified, rechecked and filtered. All collected data were recorded and entered into MS-Excel sheet. Also the TVC, TCC and FCC values were log transformed before statistical analysis in order to make the frequency distribution more symmetrical. Next the data were statistically analyzed and the differences in bacterial

counts among fruit juice type as well as vegetable salad samples were analyzed by analysis of variance and means separated (ANOVA), using SPSS software version 16.0. Significance was determined at the 5% level and the coefficient of variance was determined at greater than 10 values to indicate that the tested factor were significant.

4.Results

4.1.Findings of the questionnaire survey

A total of twenty one juice makers were interviewed to obtain primary data on fruit juice processing, source of fruits, storage of fruits and practice of hand washing from randomly selected fruit juice houses. Among 21 respondents, 6 (29%), 8 (38%) and 7 (33%) were illiterate, elementary and high school and above in their educational status, respectively. None of the juice makers had training related to food safety management and fruit juice processing. Open market served up to 20 (95%) as a source of raw material for juices and salad preparation and 1 (5%) which was found far from the center of the city took from the primary source. Comparatively more vendors 10(47.6%) use shelf as temporary storage for the fruits and also for the squeezed juices. In case of fruits and vegetables used for squeezing mostly they were sorted out when they reach near to rancidity. But only 9 (43%) vendors were using refrigerator for temporary storage of the fruit but the remaining were storing on shelves and in baskets. All of the respondents were using tap water for juice dilution purpose. Regarding cleaning habit, all respondents clean fruits and vegetables before preparation. In connection to this, 19(90.5%) respondents used only water as a cleaning agent. Only 2(9.5%) respondents used water and soap depending upon the quality of raw fruits and vegetables supplied. But the percentage of frequency of cleanings were once 13(61.9%), twice 7(33.3%) and three or more 1(4.8%) within a day. Regarding habit of cleaning their hands after toilet at work, all respondents replied of having habit of cleaning their hands after using toilet. As far as cleaning agent is concerned, however, more than half of the respondents (52.4%) used only water, whereas 10 (47.6%) respondents used water and soap as cleaning agent (Table 3).

Table 3: Respondents' level of awareness towards personal hygiene, microbial contamination & food safety in Addis Ababa city, fruit juice houses, 2016.

Data collected by questioner from vendors

Parameter assessed	Category	Percentage (%)
Educational status	Illiterate	28.6
	Elementary	38.1
	High school and above	33.3
Types of Fruit Juices and salad prepared	Avocado, mango &Mixed juice only	23.8
	mixed salad only	38.1
	Both	38.1
Source of juices and vegetable	Open market	95.2
	Directly from producer	4.2
Temporary storage site	Shelf	47.6
	Basket	9.5
	Refrigerator	42.9
Water source used for preparation	Tape	100
	Well	0
	Spring	0
Cleaning habit of fruit & vegetable during preparation	Yes	100
	No	0
Cleaning agent during preparation	Water Only	90.5
	Water and Soap	9.5
	Other	0
Frequency of cleaning	Once	61.9
	Twice	33.3
	Three or more	4.8
Cleaning habit of hands after using toilet	Yes	100
	No	0
Cleaning agent used in hand washing	Water and soap	47.6
	Water only	52.4

4.2. pH and Moisture Content of fruit juices and vegetable salad

In the present study, a total of eighty four fruit juices and vegetable salad samples were analyzed for their pH. The mean pH values were as follows:- Avocado 5.8, Mango 4, Mixed juice 4.6 and Vegetable salad 4. The pH of Avocado was found to be the highest among all the results. And also there is no significant difference between the pH of fruit juices and vegetable salad.

In addition, the average moisture content of the samples Avocado (83.9%), Mango (85.6%), Mixed juice (84.4%) and Vegetable salad (87.6%). However, there is no significant difference between the moisture contents among all sample types (Table 4).

Table 4: The Average pH and moisture content of fresh fruit juices and vegetable salads sold in fruit juice houses in Addis Ababa, 2016.

Sample Type	No	pH			P-value	Moisture			P-value
		Mean PH	Lower Boundary	Upper Boundary		Ave. Moist(%)	Lower Boundary	Upper Boundary	
Avocado	21	5.8	5.51	6.07	0.19	83.9	83.24	84.51	0.343
Mango	21	4	3.85	4.09		85.6	85	86.3	
Mixed juice	21	4.8	4.6	5.04		84.4	83.7	85.1	
V. salad	21	4	3.86	4.18		87.6	87.2	88	

4.3.Total Viable Count (TVC), Total Coliform Count (TCC), and Fecal Coliform Count (FCC)

From the total of eighty four (84) locally prepared fresh fruit juice and vegetable salad samples the mean total viable count of vegetable salad was the highest (6.06 *log cfu/g*). Whereas the total viable count for Avocado, Mango and mixed juice were 5.92 *log cfu/ml*,

5.88 *log cfu/ml*, and 5.97 *log cfu/ml,* respectively. The difference in total viable bacterial count among fruit juices and vegetable salad were not statistically significant (P≤0.05) (Table 5).

Similarly, a total of eighty four (84) fresh fruit juices and vegetable salad were analyzed in order to determine total coliform count (TCC). The mean count of total coliform of all samples was 2.88 *log cfu/ml*. Whereas Avocado, Mango, Mixed juice and Vegetable salad were 1.89 *log cfu/ml*, 3.*43 log cfu/ml*, 2.69 *log cfu/ml*, 3.52 *log cfu/g,* respectively. The total coliform count was not statistically significant between fruit juices types and vegetable salad (P≤0.05) (Table 5).

The highest fecal coliform count was recorded in the Avocado juices and found to be 0.1 *log cfu/ml*. In comparison to other sample types the lowest count was obtained in Mango juices with the result of 0.04 *log cfu/ml.* The fecal coliform count among fruit juices and vegetable salad were statistically significant between all fruit juices and vegetable salad. (P≤0.05) (Table 5). For all count tables [Log 1 cfu/ml(g) = 10,640.63 cfu/ml(g)]

Table 5: The mean total viable bacterial counts (TVC), total coliform counts(TCC) & fecal coliform counts(FCC) from fruit and vegetable salad sold in fruit juice houses in Addis Ababa, 2016. [log_{10} cfu/ml(g)]

			TVC				**TCC**				**FCC**			
	Sample Type	No of sample	Mean TVC	L. Boundary	U. Boundary	P-Value	Mean TCC	L. Boundary	U. Boundary	P-Value	Mean FCC	L. Boundary	U. Boundary	P-Value
Avocado		21	5.93	5.73	6.12		1.17	0.12	2.21		0.09	0.004	0.2	
Mango		21	5.88	5.72	6.05	0.5	0.51	0.24	0.78	0.6	0.01	0.001	0.002	0.01
Mixed juice		21	5.97	5.67	6.26		0.86	0.36	1.36		0.04	0.01	0.1	
V.salad		21	6.06	5.83	6.3		0.68	0.33	1.04		0.07	0.006	0.1	

TVC: total viable count, TCC: total coliform count and FCC: fecal colifromcount.

4.4.Effect of Sampling Time on Total Bacterial Load of Fruit Juices and Vegetable salad.

In order to analyze the effect of sampling time on the total viable bacterial count sampling time was separated into two sections which were morning and afternoon section. From the overall 84 samples 44 were collected in the morning time and its mean total viable bacterial count for Avocado, Mango, Mixed juice and Vegetable salad were 5.87 *log cfu/ml*, 5.83 *log cfu/ml*, 5.82 *log cfu/ml*, and 5.89 *log cfu/ml,* respectively. But from 40 fruit juices and vegetable salad collected in the afternoon time the total viable bacterial count were 5.98 *log cfu/ml*, 5.95 *log cfu/ml*, 6.11 *log cfu/ml* and 6.22 *log cfu/ml* for Avocado, Mango, Mixed juice and Vegetable salad, respectively. Comparatively the mean of total viable bacteria count from samples collected in the morning were lower than the samples collected in the afternoon. As listed in the table below, the mean total viable count difference between samples collected in the morning and afternoon did not show significant difference in all sample types ($P \leq 0.05$) (Table 6).

Table 6: Comparative effect of sampling time on the mean total viable count (TVC) of fruit juices & vegetable salad sold in fruit juice houses in Addis Ababa city. [log_{10}cfu/ml(g)]

		Morning samples			Afternoon samples			
Sample Type	No	Mean TVC	Upper Boundary	Upper Boundary	Mean TVC	Lower Boundary	Upper Boundary	P-value
Avocado	21	5.87	5.48	6.24	5.98	5.75	6.21	0.572
Mango	21	5.83	5.55	6.1	5.95	5.72	6.18	0.444
Mixed juice	21	5.82	5.16	6.49	6.11	6.02	6.2	0.362
V. salad	21	5.89	5.62	6.17	6.22	5.82	6.61	0.159

4.5.Bacterial species prevalent in Avocado, Mango, Mixed juice Juices and Vegetable salad

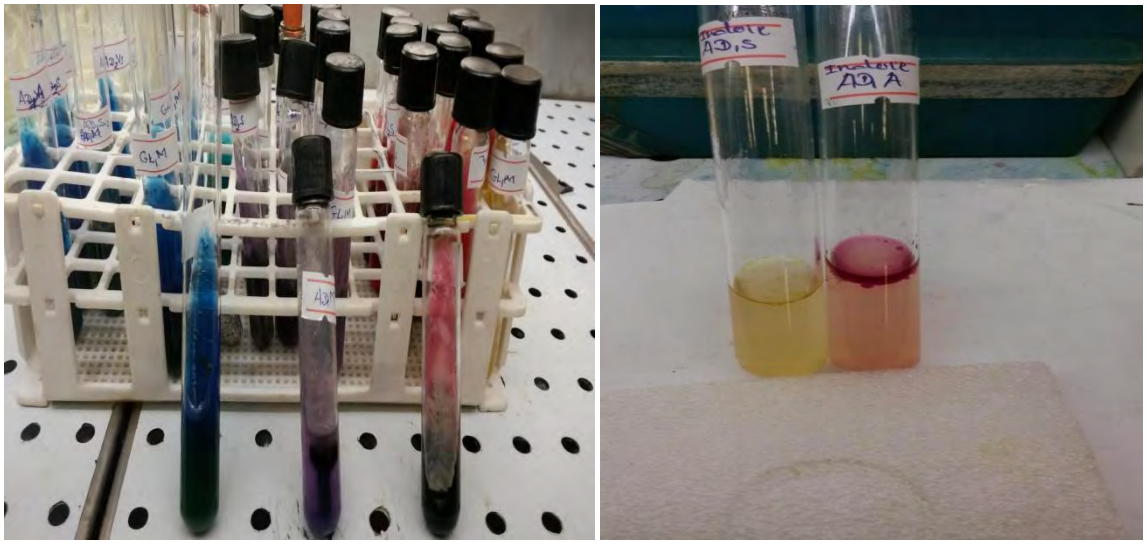
In the present study from the overall samples a total of eleven bacteria genus were isolated. But 33% *Staphylococcus* was identified as dominant genus in Avocado juices, 22% and 29% *Micrococcus* was identified in both Mango and mixed juice juices and also 33% *Bacillus* was the most dominant genus in vegetable salad.

Table 7: Biochemical identification test for dominant genus.

Sample Type	No	%	Grams'	Shape	Arrangement	Spore	Catalase	Oxidase	O/F	Dominant Genus
Avocado	21	33%	†	Cocci	Clustered	–	†	–	F	*Staphylococcus*
Mango	21	22%	†	Cocci	Chained	–	†	–	O	*Micrococcus*
Mixed juice	21	29%	†	Cocci	Chained	–	†	†	O	*Micrococcus*
V. salad	21	33%	–	Rode	Chained	†	†	–	F	*Bacillus*

4.6.Occurrence of *E. coli* and *Salmonella* in fruit juices and vegetable salad

Out of 84 samples, the highest percentage of *E. coli* was found from Vegetable salad sample which was 52.4%. And also the lowest (9.5%) was isolated from Mango samples. Regarding *salmonella,* only 4.7% mixed juice and 9.5% Vegetable salad samples were positive. Generally, in this study the prevalence of *E. coli* and *Salmonella* was 27 (32.1%) and 3 (3.6%) out of 84 fruit juices and Vegetable salad, respectively (Table 8).



A Simmon's citrate agar (deep blue color), Lysine Irone agar (blackening) and Triple sugar Iron Agar (blacking) for salmonella

B (Indole test for *E. coli)*

Figure 2: Typical colony characteristic of pathogenic bacteria on different selective agar media.

Table 8: Detection of *E. coli* and *Salmonella* in fruit juices and vegetable salads sold in fruit juice houses in Addis Ababa city, 2016. [cfu/ml(g)]

Sample Type	No of sample	Positive *E. coli*	Positive *Salmonella*	Total positive *E. coli*	Total positive *Salmonella*
Avocado	21	7(33.3%)	0	27(32.1%)	3(3.6%)
Mango	21	2(9.5%)	0		
Mixed juice	21	7(33.3%)	1(4.7%)		
V. salad	21	11(52.4%)	2(9.5%)		

4.7.Chemical Treatment of fruit juices and vegetable salad

Even if the effectiveness of treatment chemical was dependent upon concentration, investigation of antibacterial activity of the chemical treatments tested in this study revealed that the chemical treatments were effective against bacterial load in fruit juices and vegetable salad. This was shown by comparison of total viable count between untreated samples (control) and different chemical treated samples. From the present study, results of the mean total viable bacterial count, for samples of Avocado, Mango, Mixed juice and Vegetable salad treated with lemon were 3.18 *log cfu/ml*, 2.69 *log cfu/ml*, 3.23 *log cfu/ml* and 3.37 *log cfu/g* respectively. This is to evaluate and compare the common practice of using slice of lemon with benzoic acid and sodium benzoate. Similarly, total viable count obtained from samples of Avocado, Mango, Mixed juice and Vegetable salad treated with benzoic acid were 2.87 *log cfu/ml*, 2.62 *log cfu/ml*, 2.85 *log cfu/ml* and 2.96 *log cfu/g,* respectively. Whereas, the mean total viable bacterial count, samples treated with Sodium benzoate showed 1.95 *log cfu/ml* for Avocado*,* 1.43 *log cfu/ml* for Mango*,* 1.51 *log cfu/ml* for Mixed juice and 1.23 *log cfu/g* for Vegetable salad*.* Comparatively from the three treatment chemicals sodium benzoate was the effective in the reduction of total viable bacterial count (Table 9).

Table 9: Effect of chemical treatment on total viable counts load of fruit juices and vegetable salads sold in fruit juice houses in Addis Ababa city, 2016.[log_{10}cfu/ml(g)]

Sample Type	No	Lemon				Benzoic acid				Sodium Benzoate			
		Mean TVC	L. Boundary	U. Boundary	P-Value	Mean TVC	L. Boundary	U. Boundary	P-Value	Mean TVC	L. Boundary	U. Boundary	P-Value
Avocado	5	3.18	3.07	3.28		2.87	2.7	3.03		1.87	1.78	1.95	
Mango	5	2.69	2.56	2.82	0.18	2.62	2.5	2.74	0.4	1.39	1.36	1.43	0.4
Mixed juice	5	3.23	3.07	3.4		2.85	2.78	2.92		1.45	1.39	1.51	
V. salad	5	3.37	3.27	3.48		2.96	2.88	3.02		1.12	1.01	1.23	

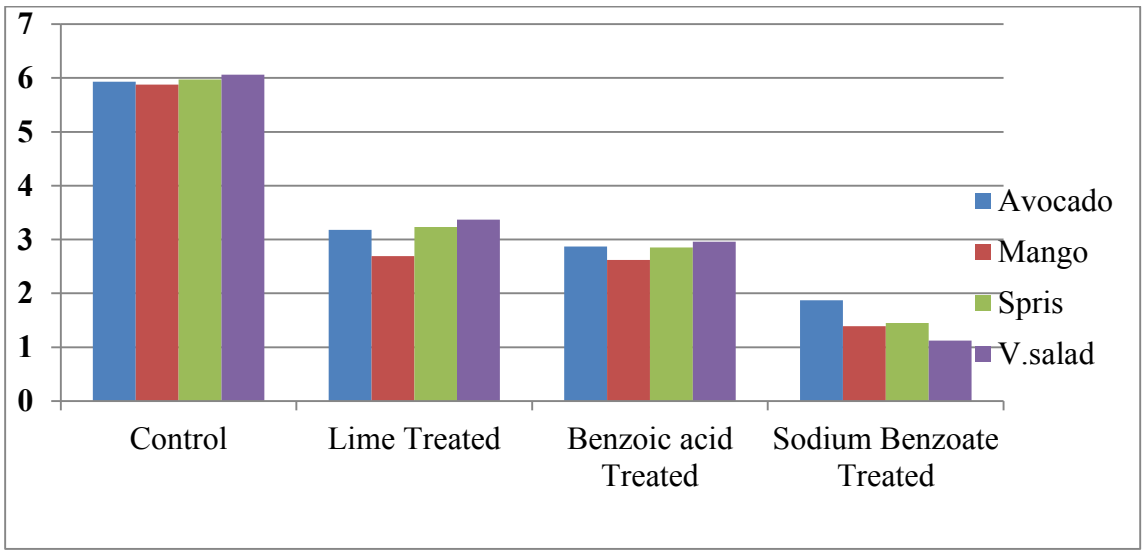


Figure 3: Graphical comparison of total viable bacterial counts between control and treated samples.

4.8.Antibiotics Sensitivity Testing of *E. coli* and *Salmonella* Isolates

In this study, thirty bacteria isolate (27 *E. coli* and 3 *Salmonella*) from the total of eighty four Avocado, Mango, Mixed juice and Vegetable salad samples were subjected to antibacterial sensitivity testing. Isolates were tested against 10 common antibacterial drugs by the disc diffusion assay on Muller Hinton Agar. The results of antibiotic sensitivity testing were interpreted and presented as the resistant, intermediate and susceptible of bacterial isolates to the antibiotics (Table 10). All *E. coli* isolates were completely resistance (100%) to vancomycin and most isolates were moderately resistance to penicillin 78%, ampicillin 67%, sulphonamides 52%, nitrofurantoin 63%, sulphonamides 70%. ciprofloxacin, oxytetracycline 96%, chloramphenicol 96%, and trimethoprim 85% were moderately susceptible to *E. coli*.

Regarding *Salmonella* isolates all of them were completely resistance (100%) penicillin, ampicillin and vancomycin. However, they were complete susceptible (100%) to ciprofloxacin, oxytetracycline, chloramphenicol, and trimethoprim. But all of them were moderately intermediate (67%) to amoxicillin trimethoprim and nitrofurantoin antibiotics (Table 10).

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Table 10: Antibiotic susceptibility pattern of E. coli and Salmonella isolates from fruit juices and vegetable salads sold in fruit juice houses in Addis Ababa city, 2016.(n=30).

		n = 27			*n = 3*		
		E. coli isolate			***Salmonella isolate***		
No	**Drugs/Antibiotics**	**R**	**I**	**S**	**R**	**I**	**S**
1	PEN (10µg)	78%	7%	15%	100%	0	0
2	AMP(10µg)	67%	11%	22%	100%	0	0
3	CIP(5µg)	0	19%	82%	0	0	100%
4	AML(25µg)	52%	11%	37%	33%	67%	0
5	VAN	100%	0	0	100%	0	0
6	OT (30µg)	4%	0	96%	0	0	100%
7	C30(30µg)	4%	0	96%	0	0	100%
8	W(5µg)	4%	11%	85%	33%	67%	100%
9	F50(50µg)	63%	11%	26%	33%	67%	0
10	S300(300µg)	70%	19%	11%	67%	33%	0

n= number of isolate bacteria

All the experiments have been done two times and the results were reproducible. One representative data have been shown.

PEN=Penicillin,AMP=Ampicillin,CIP=Ciprofloxacin,AML=Amoxicillin,VAN=Vancomycine,OT=Oxytetracycline,C30=Chloramphenicol,W5=Trimethoprim,F50=Nitrofurantoin, S30=Sulphonamides. N=Number of isolates, R=Resistant, S=sensitive

5.Discussion

5.1.Questionnaire

Questionnaire was used to obtain preliminary information on hygienic and safety practices of fruit juice makers and handlers. A total of twenty one juice makers were interviewed to obtain primary data on fruit juice processing, source of fruits, storage of fruits and practice of hand washing from randomly selected fruit juice houses. None of the juice makers had training related to food safety management and fruit juice processing. According to Kindu (2015) assessment; on similar study conducted in north western Ethiopia 87.5% had education higher than primary school education, 9.17% primary school education and only 3.33% were illiterate. But from the present study 28.6% illiterate, 38.1% elementary and 33.3% high school and above. The percentage of illiterate in this study was comparatively higher than the similar study conducted by Kindu. This was may be due migration of illiterate to the cities in need of better opportunity for work including in the fruit juice houses. Similarly, study conducted in Nigeria reported that from those vendors in street due to their low educational status and awareness on food safety 47.62% handled food with bare hands and 52.38% wore no hair covering while 61.90% handled money while serving food (Chukuezi *et al*., 2010).

Open market served up to 95% of the demand and primary source contribute only 5% of raw material required for the juices and salad preparation. In similar comparable study conducted in Hawassa reported open market was the only source of fruits (Mesfin, 2011). The significant of buying fruits and vegetables from open market aggravate bacterial contamination rather than buying from farm. In an open market due to storage and handling problem the rate of exposure to bacterial contamination and rancidity in the fruit and

vegetable value chain increases. The percentage of the respondents on most of possible factors affecting the quality of juices was in line with the work of Bello *et al*., (2004) who reported that sources of fruits used for the processing of juices was mainly from the open market.

In case of temporary storage site, comparatively more vendors (47.6%) use shelf as temporary storage for the fruits and also for the squeezed juices. In case of fruits and vegetables used for squeezing mostly they were sorted when they reach near to rancidity. But only (43%) vendors was using refrigerator for temporary storage of the fruit but the remaining were stored on shelves and in baskets.

All of the respondents were using tap water for juice dilution purpose. According to cleaning habit all respondents clean fruits and vegetables before preparation. Whereas most of respondents (90.5%) answered that they use only water as cleaning agent. Only (9.5%) respondents used water and soap depending upon the quality of raw fruits and vegetables supplied. But the percentage of frequency of cleanings was once (61.9%), twice (33.3%) and three or more (4.8%). All juice producers lacked especial training in food hygiene and safety as it is indicated in this study and some (71%) had awareness on the consequences of contamination of food.

The last questions address their personal habit after using toilet at work time but before data collection all respondents informed to be open not to baize data. All respondents answered they washed their hands after using toilet. As far as cleaning agent is considered, more than half of the respondents (52.4%) use only water, whereas (47.6%) respondents used water and soap as cleaning agent. According to Ekanem (1998) report, vendors who pass out body waste in nearby hidden places and often, they return to business without

washing their hands properly. It has been discovered that in countries where street vending of foods is common, there is usually death and are frequently not investigated (Bryan, 1988).

In addition, none of the fruit juice makers experienced using additional material like antiseptic before preparation of fruit juices. Around 70% of the respondents had better awareness, but 30% of the respondents do not. According to the survey questionnaire, most of the vendors focus on profit maximization rather than safety related issues. In the case of housing, some of them were very concerned about the dining room, but most of them do not care about the preparation room (kitchen) which was the most important place for microbial contamination. Elevated levels of contamination in the juice and vegetable salad samples collected from all sites might be associated with the location where environmental micro flora harboring over the fruit juices and vegetable salad. Moreover, unhygienic food handling practices in the form of inadequate washing of fruits and tumblers before juice extraction, preparation and storage possibly increase the extent of contamination, resulting in high bacteriological contamination like total viable bacterial counts. Similar findings were reported in a study on fresh fruit juices in Dhaka city, Bangladesh by Shakir *et al*., (2009).

5.2.pH and Moisture Content Measurement

pH and moisture content are the most important factors in the bacterial survival and growth in the fruit juices and vegetable salad. That is why low pH and moisture content of the fruit juices and vegetable salad greatly limits the number and types of bacteria that can survive or grow (Oranusi *et al*, 2011). Margaret (2009) reported that most bacteria grow in the pH≥4.5. That means according to this study both avocado (5.8) and Mixed juice (4.8) have

the pH range that support growth of most bacteria (Table 2). The pH results indicated for fruit juices and vegetable salad obtained from this study was comparable with study conducted in Jimma of, Ethiopia and Nagpus city of, India (Ketema *et al*., 2008; Titarmare *et al*., 2009). According to Ghenghesh *et al*., (2005) and Tasmina *et al*., (2010), it was suggested that although most of the microbes do not survive low pH of juices but certain spores of *Bacillus spp*. survive and pose a serious threat to the consumers. But those which survive acidic pH were able to survive indicates the acidophilic nature of the organisms. It was also suggested that quality of fruit juices should be monitored on regular basis to avoid any future outbreaks (Tasnim *et al*., 2010).

Regarding moisture analysis, 83.9% for Avocado, 85.6% for Mango, 84.4% for Mixed juice and 87.6% for vegetable salad were obtained as average moisture content. This was comparable with the report of Margaret (2009), where the average moisture content of fruits and vegetable were 85% and 88%, respectively and this amount of moisture content together with its food content support the growth of bacteria. Another study conducted by Addo *et al*., (2008) reported that high moisture content in fruit juices and vegetable salad promotes the growth of bacteria, as it is evidenced from this study, higher bacterial count was observed in the fruit juices and vegetable salad with higher moisture content and high pH.

5.3.TVC, TCC and FCC

Bacteriological quality is very important measure of the safety of food intended for human consumption. The presence of certain bacteria such as *E. coli*, *Salmonella* and *Shigella* indicate that food is hazardous and should not be used for human consumption. On the other hand the presence of non-pathogenic bacteria in the food does not necessitate

unfitness for consumption, but may indicate the hygienic status of the raw fruits, vegetables, preparation and processing. According to Addo *et al*., (2008) the total heterotrophic bacteria and yeast counts were taken to determine the overall contamination by mesophilic bacteria. However, certain levels may indicate serious case of poor hygienic condition and the food become unfit for consumption.

In the present study the overall assessment of the fruit juices and vegetable salad samples analyzed for bacteriological quality indicated high counts including total viable count, total coliform count and fecal coliform count. From the overall 84 samples 77 (91.7%) of the juices and vegetable salad samples analyzed for bacteriological quality in the present study were poor and total viable count, total coliform and fecal coliform count were detected but only 7 (8.3%) of the samples were below the maximum permitted load of Gulf standard (2000). Vegetable salad showed highest contamination of total viable count which was 6.06 *log cfu/g* and Mango showed the lowest total viable count5.88 *log cfu/ml.* There was significant difference in total viable count between sample types (Table 5). The mean of each juice types and vegetable salad were deemed above maximum permitted level (4 *log cfu/ml*) of Gulf Standard for fruit juices and vegetable salad (Tasnim *et al*., 2010; Gulf Standard, 2000).

From the present study the mean total viable bacterial count of Avocado sample was 5.93 *log* cfu/ml, which was the second largest next to vegetable salad count. This finding was similar to the findings of Simforian *et al*., (2015) who reported mean total viable count 5.98 *log* cfu/ml, but lower than findings of Mekonen and Tadele, (2016) who reported mean total viable count of 7.49 *log* cfu/ml. Moreover, the total viable bacterial count of Avocado in present study is also closer with the study of Al-Jedah and Robinson, (2002)

who reported 6.69 *log*cfu/ml. In addition, the results of the current study were in agreement with findings which were reported from Jimma town for the Avocado (6 *log* cfu/ml) (Ketema *et al*., 2008). The possible reasons for the high contamination may be due to relatively rise of pH (>4.5) for both varieties of samples, as higher pH create conducive environment for growth of microbes (Margaret, 2009).

The mean total viable count from Mango juice was 5.88 *log* cfu/ml, which was the lowest from the four varieties. Similar study conducted in Ghana reported 3.76 *log cfu/ml* of total viable bacterial count was detected in Mango juice (Addo *et al*., 2008). Another comparable study conducted in Bair Dar, Ethiopia reported 4.76 *log* cfu/ml of mean total viable bacterial count in fresh Mango juices (Asmamaw and Mulugeta, 2012). Higher log count was recorded in the samples of mango of this study which is still above allowable limits of Gulf standard and Codex standard. In addition, the rise in log count of the mango may be due to unhygienic handling of juice makers and water used for dilution purpose.

The mean total viable count of Mixed juice was 5.97*log*cfu/ml. Similar work reported that the mean total viable count of Mixed juice juices was 6.80 ± 1.91 *log* cfu/ml which was greater than permissible limits (TVC > 4 *log* cfu/ml) (Gulf standard, 2000). In contrary, study conducted by Ojukwu (2015) on mixed juices (Mixed juice) reported the total viable bacterial count of up to 3.54 *log* cfu/ml in Nigeria which contrasts with present study in terms of permissible level. Similarly, comparable study conducted on mixed citrus juice reported the growth range of total viable count of between 3.0 and 4.0 x 10^3cfu/ml, which is below the maximum limit (Edit and Nedie, 2015).The rise of total viable count in mixed juice of this study is probably due to the unhygienic handling and processing of mixed juice during preparation as justified from questionnaire.

In the case of Vegetable salad the mean total viable count finding was 6.06 *log* cfu/ml, which was the highest from the remaining three varieties. In a similar related work it has been found that the bacterial contamination of salad samples in Kumasi, Ghana was 5.13 *log* cfu/ml (Ameko *et al*., 2012). Another work conducted in Accra, Ghana total viable bacterial count ranged 3.87-5.6 log cfu/g (George *et al*., 2014). Similarly, the finding 6.02 *log* cfu/g reported by Viswanatha; and Kaur (2001) in India supports this result (6.06 *log* cfu/g).Another work on the prevalence of bacterial contamination on mixed vegetable salad reported that the total viable count was 5.17 *log* cfu/ml (Mensah *et al*., 2002). Generally, in the present study mean of each juice types and vegetable salad were deemed above maximum permitted level (4 *log cfu/ml*) of Gulf Standard. From the present study the p-value, which was equal to 0.5 total viable bacterial counts between sample types, shows statistically significantly difference (P>0.05) (Table 5).

Coliforms are considered as indicator of quality and health hazard causing spoilage of fruit juices and vegetable salad. Total coliforms and fecal coliforms counts were done for assessing the fecal pollution. From the total 84 samples 64 (76.2%) were shown above the maximum level (100 *cfu/ml*) of the gulf region standard (2000), (WHO, 2008) and International Commission on Microbiological Specifications for Food (ICMSF, 1980) standards (10 to 100 coliforms g/l) fruit juices and wet weight vegetables. Out of twenty one samples for each sample type, 13(61.9%), 14(66.7%) and 16(76.2%) of Avocado, Mixed juice and Vegetable salad samples were above the maximum level of gulf standard by their coliform count. Whereas only 9(42.9%) of Mango samples were contaminated with total coliform above the specification.

The mean total coliform counts recorded under this study were higher in most samples analyzed. In the present study, the mean quantitative analysis of fruit juices for total coliform count was highest in Avocado which was 1.17 *log* cfu/ml. The total coliform count of current study for avocado was comparable with similar study conducted in Qatar 3.97 *log*cfu/ml and in Nagpur city, India also reported 4 *log* cfu/ml (Titarmare*et al*., 2009). But results of this study are lower than similar work conducted in Hawassa that reported the mean value of 3.3.98±1.23 *log* cfu/ml in Avocado. The total coliform count of current study in Mango was 0.51 *log* cfu/ml which was lowest from all other varieties. Similar comparable study conducted in Qatar found total coliform count 2.91 *log* cfu/ml. Another similar comparable study conducted in Hawassa town, Ethiopia reported that the mean total coliform count 2.54 *log*cfu/ml. But similar study conducted in Bahir Dar town, Ethiopia reported that the mean total coliform counts were 9.2 to > 1100 MPN/ml in Mango juices, which opposes the above mentioned studies. In this study, the mean total coliform count for Mixed juice was 0.86 log cfu/ml. In similar study conducted in Delhi Ncr, India the total coliform count was reported within the range of $3.1x10^2 – 4.9x10^6$ cfu/ml (Dushyant *et al*., 2015). For the vegetable salad samples the mean total coliform count was 0.68 log cfu/g. This was less than the finding reported with similar work conducted in Pakistan 4.9 *log* cfu/g on vegetable salad (Khiyami *et al*., 2011; Iqbal *et al*., 2015). Another similar study conducted in Nigeria reported that 46.66% of samples had coliform count more than 2 *log* cfu/ml (Iqbal *et al*., 2015). According to Tambekar *et al*., (2009) the main source of coliform contamination might be through contaminated water supplies which were used in processing of juice or the personnel. The result from the present study showed the mean total coliform count above the maximum limit. Notably these samples were prepared under

poor sanitation practices and stored in inappropriate storage conditions. Besides, contamination rate of raw material, growing area, geographical location, hygienic practice etc were considered as cause for contamination.

To get additional information the study was extended to determine the presence of fecal coliforms in ready to eat fruit juices and vegetable salad served in fruit juice houses. Fecal coliform used an indication of fecal contamination. Also might be pathogenic and may cause serious diseases in human beings. *For example*, *Klebsiella pneumonia* species cause urinary tract infection, chronic broncho-pulmonary disease pneumonia, septicemia, meningitis etc (Ananthanarayan and Jayaram Panikel, 1996). In the present study, the analysis of fecal coliforms showed that, the overall mean were 0.09 *log* cfu/ml, 0.007 *log* cfu/ml, 0.04 *log* cfu/ml, and 0.07 *log* cfu/g for Avocado, Mango, Mixed juice and Vegetable salad respectively. Thus, out of twenty one samples, 23.8% Avocado, 14.3% Mango, 23.8% Mixed juice and 57.1% Vegetable salads were contaminated with fecal coliforms. Similarly Moushumi *et al*., (2009) reported the presence of fecal coliforms in freshly squeezed juices and explained the possible entry points of bacterial pathogens in juice. Generally, these counts did show significant difference between juice types and vegetable salad ($P \leq 0.05$) (Table 5). Comparatively several researchers contributed similar type of investigations in different places with different street vended fruit juices and vegetable salads. One of the comparative studies made with fruit juices and vegetable salad in Nigeria clearly showed that geographical source could have undergone different pre-harvest practices and pretreatments during their postharvest and personal hygiene were the major factor that could contributed to high fecal contamination (Jones *et al*., 2008). Another study in Bangladesh revealed that most of the juice samples showed equal or

slightly higher fecal count than the permitted count, these were unfavorable for consumption (Tasmina *et al*., 2010). In addition Nguz *et al*., (2005) reported that fecal coliform counts were efficient indicators of sanitization, but the detection of fecal coliform counts does not indicate the presence of pathogen. The overall mean total coliform counts in fruit juices and vegetable salad samples were significantly different with the gulf and ICMSF standard permissible counts (Table 5). According to safe food consumption standard the presence of coliforms is not allowed in food such as fruit juice. As far as reports related with fecal contamination, most of the potential causes were mainly due to exposure of fruits and vegetables to feces during growth, poor quality of water used for washing and dilution as well as unhygienic conditions related to improper washing of fruits, and utensils, inadequate storage of fruits and vegetables, and personal hygiene of vendors.

Generally, higher viable bacterial count of fresh fruit juice and vegetable salad reflect poor agricultural and postharvest practices. Variation in total viable bacterial count of fruit juices and vegetable salad may be due to unhygienic conditions practiced in the preparation and handling of the juices. Even if the time elapsed between preparing and serving locally prepared fruit juice and vegetable salad was not long enough to allow bacterial growth, such high counts may be due to cross-contamination from improperly washed utensils or contaminated fruits (Lewis *et al*., 2006). Failure to apply good hygienic practices during juice making leads to high bacterial loads, thus reducing the quality of freshly squeezed fruit juices and vegetable salad. In addition to this the probable reason for the variation in the mean total sample viable bacterial count may be source of fruit and vegetable salad, geographical variation, microclimate change, seasonal variation, pH and moisture

variation, water used for washing and dilution, time of sample collection, hygiene, and incubation time (Yigeremu *et al*., 2001). Also the location by the side of a busy road with heavy vehicular traffic (airborne particles) and overcrowding seem to add to the contamination.

5.4.Effect of Sampling Time on Total viable Bacterial Count

According to Tamberkar *et al*., (2009) report samples collected in the evening had high microbial count which agrees with the present study. In comparison to samples collected in the morning and afternoon major difference in the mean total viable count was observed in Vegetable salad samples with 0.33 *log* cfu/ml deviation. While lowest deviation was observed in Avocado which was 0.11 *log* cfu/ml. Similar study conducted in Nigeria reported that there was significant difference between microbial load in the samples collected in the morning time and afternoon time (Osamwonyi *et al*., 2013). All of the samples collected in the afternoon were above the maximum level in their total viable bacterial count. But out of 44 samples collected in the morning 7(15.9%) were below the maximum permissible limit (4.9×10^6 aerobic count g/l) (WHO, 2008). Another comparative study conducted in Accra, Ghana reported that bacteriological analysis of raw mixed vegetable salads indicate that 20% of the vendors had the salads that they sold in the mornings with bacterial loads in excess of 5×10^4 cfu/g (*log* 4.7 cfu/g), and this increased to 80% of the vendors in the afternoons (Ameko *et al*., 2012). Generally, from this study the bacterial counts of the fruit juices and vegetable salad collected during the afternoon time (4:00pm) were higher than the bacterial counts observed for the fruit juices and vegetable salad collected from the fruit juice houses at the morning time (before 10:00am).

Higher bacterial load in samples collected in afternoon could suggest that the fruit juices and vegetable salad were stored at holding temperatures that favored the proliferation of bacterial load of respective fruit juices and vegetable salad. James and Ngarmsak, (2011) reported that storage temperature and PH are the two principal determinant factors for growth of food borne pathogens associated with fresh produce. Due to those factors the dominance of *Staphylococcus, Micrococcus* in fruit juices and *Bacillus* in vegetable salad (Table 7) was not surprising as Goja and Mahmoud (2013) and Rajvanshi (2010) reported that majority of bacteria found on the fruit juices and vegetable salad. The probable reason for this difference might be storage habit, ambient temperature, overcrowding, and more polluted environment or dust in the afternoon than in the morning. More specifically, based on the questionnaire results, the absence of refrigerator in most fruit juice houses can lead to the proliferation of microbes during pick hot time.

5.5.Dominant Bacterial Genera in Fruit Juice and Vegetable Salad

Present study also identified dominant bacteria at genera level. According to the cultural, morphological and biochemical characteristics of the organisms isolated *Staphylococcus*, *Micrococcus* and *Bacillus* were the three most dominant genera groups from all sample types. The percentage of genera in each fruit indicated that 33% of *Staphylococcus* in Avocado, 22% of *Micrococcus* in Mango, 29% of *Micrococcus* in Mixed juice and 33% of *Bacillus* in Vegetable salad were the most dominant genus (Table 7). The same bacterial genera were also isolated and identified by other researchers from fruits and vegetable in different countries (Osamwonyi *et al*., 2013; Eni *et al*., 2010; Tasmina *et al*., 2010). However, study done in Sudan on vegetable salad revealed that *Bacillus* (17%) was the

third most dominant genus next to *Staphylococcus* (33%), *Entrobacteriaceae* (25%) and *Bacillus* in fruit juices (Goja and Mahmoud., 2013).

5.6.Detection of *E. coli* and *Salmonella*

Generally unpasteurized juices and vegetable salad were considered as non-hazardous due to its freshness and acidic nature. But sometimes human pathogens, like *E. coli* and *Salmonella* can survive for extended periods of time in low pH food and causes diarrhea, urinary infection, pyogenic infections etc(Food Safety Authority of Ireland, 2007). Several researches showed similar type of investigation in different places with different street vended fruit juices and vegetable salad. Tambekar *et al*., (2009) reported the food borne illness associated with different consumption of freshly squeezed fruit juices at road side in public places of Amaravati city, India, and samples were *Escherichia coli* (40%), *Pseudomonas aeruginoas* (25%), and *Salmonella spp* (16%). This study also identified occurrence of *Entrobacteriaceae* members mainly *E. coli* and *Salmonella* ashuman pathogen. In this research some of the fruit juices and vegetable salad were found to be unfavorable for consumption because they showed the presence of coliform especially *E. coli*. In the present study from 21 Avocado juices 7(33.3%) isolates of *E. coli* were detected.

In this study the *E. coli* detected from Mango juice was 2(9.5%), because the acidic property of some juices does not always prevent the survival of organisms like *E. coli*. Contamination of juices has shown to be potential sources of bacteria pathogens like *E. coli, Salmonella, Shigella*, and *Staphylococcus aureus* (Sandeep *et al*., 2001). The percentage of *E. coli* detected was 7(33.3%) from Mixed juice. Similar work done on mixed juice in Delhi, India reported the presence of pathogenic bacteria especially *E. coli* were

40% (Dushyant *et al*., 2015%). And also 11(52.4%) Vegetable salads were positive for *E. coli*. Also similar study conducted by Ogbonna *et al*., (2011) reported the contamination of cabbage by *E. coli* and *Pseudomonas species*. From the present study majority of vegetable salad were the mostly contaminated with *E. coli,* whereas Mango is the list contaminated. Generally, from the overall 84 samples 27(32.1%) of the samples were *E. coli* positive. This can affect many individuals who consumed those contaminated fruit juices and vegetable salads. The result obtained not in argument with codex standard (2005) and gulf standard (2000). This may be attributed to poor hygienic practice starting from pre-harvest to postharvest including of juice makers practice. Moreover preparation site may also contribute its own part to the occurrence of organism in juice and salad samples. From this study the presence of *E. coli* indicate cross-contamination of juice and vegetable samples and may be correlated with vendors' awareness. The finding from the questioner about hand washing, out of 21 respondents 11(52%) wash their hand only with water after using toilet and 20(95%) respondents used open market as their raw material sources strongly support this. Similar comparative study reported that unhygienic handling and preparation of fresh fruit juices like increase potential for the invasion of pathogenic bacteria and hence the risk to transmission of food borne illness (Little and Mitchell, 2004). Another similar research conducted in India reported that *E. coli* were the predominant bacteria about 40%. Another similar study conducted in Bangladesh showed that all of the samples of Papaya (100%), Mango (100%) and Pineapple (100%) were positive for *E. coli* (Shakir *et al*., 2009). In contrast, survey conducted in Ireland shown that only 0.2% of the unpasteurized juices were contaminated with *E. coli* (Melbourne, 2005). The incidence of *E. coli* in the current study does not agree with the above mentioned studies. The main reason for the

detection of *E. coli* may be due to geographical variation, pre-harvest and postharvest practice, sanitary habit of juice makers or procedure of incubation.

In the current study, probable incidence of *Salmonella* species was 3(3.6%) from the 84 fruit juices and vegetable salad (one from Mixed juice and two from vegetable salad). The probability of detecting seems small, but it can affect large number of individual who consumed these contaminated juice and vegetable salad. Another similar study conducted in Delhi, India reported the presence of 13% *Salmonella* (Dushyant *et al*., 2015).

Even if the percentage of occurrence varies, there was similar finding reported the presence of *E. coli* and *Salmonella* in Sao Polo, Brazil (Moushumi *et al*., 2011). Another study in Mexico reported that 14% of samples of juice were positive for *Salmonella* (Castillo *et al*, 2006). Similar comparative study in Bangladesh also reported that unpasteurized fruit juices were 7.89% positive for *Salmonella* spp (Shakir *et al*, 2009). Similarly a study in Nigeria reported *Salmonella serovar* to be the major contaminant of vegetables obtained from farms and central market (Raufu *et al*., 2014). Similar research conducted in India reported 50% positive for *Salmonella* species in fruit and vegetable, but 16% in street vended fruit juices (Titarmare *et al.*, 2009). According to study conducted on fresh vegetables in Sri Lanka *Salmonella* was detected in 6% of the samples tested (Silva *et al*., 2013). In contrast, Dannison (1996) reported no potential pathogenic strain like *Salmonella.* In case of Ethiopia similar study conducted in Hawassa 2.5% fruit juices were positive for *Salmonella* (Mesfin, 2011). Despite this unpublished study conducted in Debre-Markose, North-Western Ethiopia reported that *salmonella* was not detected in fruit samples (Kindu, 2015). The 3.6% finding in the present study indicates low rate of incidence of *salmonella* in Ethiopia as compared with the 50% finding reported in Asia

(Titamare *et al*., 2009). The main reason for this difference may be geographical variation, pre-harvest and postharvest practice, sanitary habit of juice makers, population demography and high rate of urbanization.

5.7.Chemical Treatment of fruit juices and vegetable salad

From the three treatment chemicals sodium benzoate was the most effective in the reduction of bacterial load followed by benzoic acid and lemon. In comparable study conducted in Nigeria reported chemical treatments like benzoic acid as the most effective against reduction of bacterial load (Oladipo *et al*., 2010). In our country because of different reasons sodium benzoate and benzoic acid were not used in all fruit juice houses. But lemon is the most common treatment chemical in all fruit juice houses in Addis Ababa city. From this study all treatment chemicals has significant effect on total bacterial load, but highly reduces total viable counts ($P \leq 0.05$) (Table 9).

5.8.Antibiotics Sensitivity Test of Bacterial Isolates

Furthermore, this study investigated effectiveness of 10 different common antibiotics on 30 *E. coli* and *salmonella* isolates. According to Levy (2001), it has been reported that major epidemics in the world have been linked with resistance pathogens. As evidence some authors reported antibiotic resistance of bacterial isolates against commonly used antibiotics has been increased from time to time (Vicas, 2010).Even though bacteria develop multiple resistances but their degree of resistance varies with different isolates and time (Sharada *et al.*, 2011). The present study analyzed antibacterial sensitivity of two species of pathogenic bacteria isolates (*E. coli* and *Salmonella*) on ten antibiotics and the

results were interpreted as resistance, intermediate and susceptible according to drug resistance chart. The drugs were penicillin, ampicillin, ciprofloxacin, amoxicillin, vancomycine, oxytetracycline, chloramphenicol, trimethoprim, nitrofurantoin, and sulphonamides. From this research out of 27 *E. coli* isolates all of them were 100% resistant to vancomycine but most of them were 78%, 67%, 52%, 63%, 70% resistant to penicillin, ampicillin, amoxicillin, nitrofurantoin, and sulphonamides. This study was comparable with Adetunji and Isola (2011) who reported 40% and 70% resistance level in *E. coli* from abattoir. Similarly Lateef, (2004) reported that Amoxicillin were not active against the strain of *E. coli.* But Marwa *et al.,* (2012) reported that most *E. coli* isolates from food were sensitive to amoxicillin was disagree with this result. Whereas all the 27 isolates of *E. coli* were 82%, 96%, 96% and 85% susceptible for ciprofloxacin, oxytetracycline, chloramphenicol, and trimethoprim antibiotics respectively. In general, from those ten antibiotics relatively oxytetracycline and chloramphenicol should be drugs of choice in the treatment of *E. coli* infections as noted from this study.

Regarding *Salmonella,* the 3 isolates of *salmonella* were shown to have complete resistance to penicillin, ampicillin, and vancomycine. Similar study indicates *Salmonella* strains were resistant to multiple antibiotics (Jones *et al*., 2002 and Aditunji and Isolate, 2011). According to Nipa *et al*., (2011) multiple drug resistance was observed in 98.06% isolates with a resistance to two to seven antibiotics. Oppositely ciprofloxacin, oxytetracycline, chloramphenicol, and trimethoprim were completely (100%) susceptible. But all the remaining three antibiotics amoxicillin, trimethoprim, and nitrofurantoin were moderately intermediate (67%). Similar study reported 85% of the resistant isolate were multiple drug resistant where highest (89.1%) resistance was to the amoxicillin (Oluyege *et al*., 2009).

From the present study ciprofloxacin, oxytetracycline, chloramphenicol, and trimethoprim should be drugs of choice in the treatment of *Salmonella* infection as noted from this study (Table 9). The results suggest the necessity to follow the hygienic practices in fruit juices and vegetable salad preparation and the raw materials might have important role as a source of multiple antibiotic resistant bacteria.

6.Conclusion and Recommendations

6.1.Conclusion

In the current study, fruit juices and vegetable salads prepared for human consumption in selected vendor shops were assessed for hygienic status of the preparation; bacteriological load; effect of sampling time on total bacterial count; the presence of *E. coli* and *Salmonella;* effectiveness of treatment chemicals and antibiogram profiles of the isolates.

Generally, the results in this study clearly indicate the poor hygienic conditions of juices and vegetable salad consumers for the risk of food borne infections. Lack of training (orientation) on food hygiene and safety including improper storage and preparation of fruit juices and vegetable salad may aggravate the contamination.

The majority (91.7%) of juice and vegetable salad samples analyzed were high in their total viable bacterial count, ranged 4 *log cfu/ml* to 7.38 *log cfu/ml,* above maximum permitted level (4 *log cfu/ml*) for fruit juices and vegetable salad.

In addition, more than 50% of all sample types showed a total coliform count of above maximum level of seated Gulf standard.

Fecal coliform contamination was also observed in samples. Out of twenty one samples, 23.8% Avocado, 14.3% Mango 23.8% Mixed juice and 57.1% Vegetable salads were contaminated with fecal coliforms.

Sampling time was found to be an important factor that affected total viable bacterial count. The finding revealed that all of the samples collected in the afternoon were above the maximum level in their total viable bacterial count. But out of 44 samples collected in the morning 7(15.9%) were below the maximum permissible limit.

A total of eleven bacterial isolates were identified to their genera. *Staphylococcus*, *Micrococcus* and *Bacillus* were the most dominant.

Furthermore, *E. coli* has been isolated from 32.1% of the samples; this correlates with the washing practice of vendors, who use only water after toilet use. However, only 3.6% of samples were positive for *Salmonella*.

Chemical treatments were used for immediate reduction of total viable bacterial counts. Three chemicals used were lemon, benzoic acid and sodium benzoate. From all the three chemical treatments sodium benzoate was the most effective in the reduction of bacterial load followed by benzoic acid and lemon.

Finally, based on the antibiogram resistance of the isolates on 10 commonly used antibiotics, the effective drugs were oxytetracycline and chloramphenicol for *E. coli,* and ciprofloxacin, oxytetracycline, chloramphenicol, and trimethoprim for *Salmonella.*

6.2. Recommendations

Contamination especially in fruits, vegetables and ready to eat juices and vegetable salad implies that the prevailing pre-harvest and post-harvest handling practices are insufficient in controlling contamination. The absence of awareness about bacterial contamination and traditional practice of using manure as fertilizer worsen the problem. Hence, several cultural related pre and postharvest practices should be improved.

Regular monitoring of the quality of fruits, vegetables and its products for human consumption must be introduced to avoid any future bacterial pathogen outbreak. The fruit juices house owners' should focus on food safety practices by giving trainings (orientations) and other safety related issues besides focusing profit maximization.

Vendors in corporation with Ethiopian Standard Agency should adopt rules and regulations on RTE foods and take regular fruits and vegetable inspection and its product handling. Food safety awareness should be improved in the fruit juice houses. Unless strict handling of fruit juices are used, fruit juices need to be prepared while customer is there to use, if not refrigerator use for storage should be mandatory. Besides, vendors should focus on use of antiseptics for cleaning equipment and hands.

There should be rules and regulations for the opening new fruit juice houses including the standardized preparation area and dining rooms.

There is a need for awareness creation at all levels of the fruits and vegetable value chain, especially for final consumers, campaigns to raise vendor awareness for safe, high-quality food.

More research should be done on the effectiveness of treatment chemicals used in foods, especially on the ready to eat fruit juices and vegetable salad to reduce bacterial load and its' side effect on human health.

Due to the occurrence of drug resistance pathogenic bacteria related with exposure of repeated food poisoning health workers should do further study on the effectiveness of antibiotics.

Since current study was conducted on small sample size, it is also recommended that further studies should be made using large sample size of variety of juices and vegetable salad sold in the fruit juice houses.

Furthermore, it is advisable to use processing technology to prepare pasteurized juices to prevent food related contamination.

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APPENDCIES

APPENDEX I

QUESTIONNAIRE

ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
CENTER OF FOOD SCIENCE AND NUTRITION

Name of data collector: _____

Sub-city of vender: _____

Questionnaire format sheet to assess safety and quality of locally prepared fruit juices and vegetable salads to be filled by fruit juice and vegetable salad makers.

1. What is the educational status of juice maker?

   - Illiterate ☐
   - Elementary ☐
   - High school and above ☐

2. What type of fruit juices and vegetable salad prepared?

   - Avocado, Mango and Mixed juice only ☐
   - Mixed salad only ☐
   - Both type ☐

3. Source of fruit and vegetable

- Open market ☐

- Directly from producers ☐

4. Temporary storage site
   - Shelf ☐
   - Basket ☐
   - Refrigerator ☐
5. Water source for juice and salad preparation
   - Tap water ☐
   - Well ☐
   - Spring ☐
6. Cleaning habit of juice and salad maker during processing
   - Yes ☐
   - No ☐
7. Cleaning agents used during processing
   - With water Only ☐
   - With water and soap ☐
   - Other ☐
8. Frequency of cleaning ☐

- Once

- Twice ☐

- Three and more ☐

9. Cleaning of hand after using toilet
   - Yes ☐
   - No ☐
10. Cleaning of hand after using toilet
    - With water and soap ☐
    - With water only ☐

THANK YOU

APRIL 2016

APPENDEX II

Grams' Reaction

Grams' reaction was a primary identification procedure used to determine whether the dominant microorganism were Gram positive or negative. Using a sterile loop light suspension of organism prepared in sterile distilled water on a leaned microscopic slide. The film dried by air and then fixed by passing through a gas flame. Then Four gram reagents (Crystal violet, Gram's iodine solution, acetone/ethanol, and safranin or fuchsin solution) were used following the steps. Following Roberts and Greenwood (2003) procedure Gram positive organisms retain the stain but Gram negative organisms were decolorized.

Table 11.Result Interpretation of OF Test

Open (Aerobic) Tube	***Covered (Anaerobic) Tube***	***Metabolism***
Acid (Yellow)	Alkaline (Green)	Oxidative
Acid (Yellow)	Acid (Yellow)	Fermentative
Alkaline (Green)	Alkaline (Green)	Non saccharolytic (glucose not metabolised)

(Source; UK Standards for Microbiology Investigations, Public Health England, p10)

Biochemical Characterization

The twenty seven isolates were characterized on the basis of biochemical tests. The tests performed to characterize the isolates were Indole, methyl red, vogesProskauer, citrate utilization, catalse, and triple sugar iron test were used for the confirmation according to standard procedure described in Roberts and Greenwood (2003).

Some pathogenic bacteria such as *E. coli* and *Salmonella* were detected according to the procedures outlined by Food and Drug Administration (FDA) (2001).

Escherichia coli Confirmatory Test

E.coli is gram negative non spore former rods or cocci. Most commonly IMViC test is used as confirmatory test for *E.coli* and gives IMViC patterns ++-- (biotype 1) or -+-- (biotype 2), Neusely Da Silva *et.al* (2013).

Indole Test

Indole test is used in the classification and identification of bacteria. This was based on the ability of microorganisms to break down the amino-acid tryptophan, with the production of indole. First 0.03% treptone water containing tube was inoculated with pure culture of the test organism and incubated at 37 ^{0}C for up to 48 hr. Then 5-10 drops (0.2 ml) of indole reagent (e.g. kovac's) was added and allowed to stand for up to 10 min. The presence of indole was indicated by pink coloration at the surface (Roberts and Greenwood, 2003).

Methyl Red (MR) Test

For Methyl Red (MR) test, methyl red was used to determine acidity when an organism ferments glucose. Since all Entrobacteriaceae ferment glucose, acidic metabolic by products were initially formed. Further incubated for 2-5 days, MR-positive organisms continued to produce more acids (Roberts and Greenwood, 2003)

Voges-Proskauer (VP) Test

Vogesproskauer test used for certain bacteria which produce neutral-reacting end products (e.g. acetyl-methyl carbinol /acetoin) when particular bacteria ferments glucose. Once the end product was produced, VogesProskauer (VP) test was carried out according to standard procedure described in Roberts and Greenwood (2003).

Citrate Test

Simon's citrate media, containing bromo-thymol-blue, was used to determine if a bacterium can grow utilizing citrate as its sole carbon and energy source. Un-inoculated agar was deep forest green color. But proceeding inoculation and growth of bacteria on the slant surface the media was changed to an intense Prussian blue otherwise it remains deep forest green color of the media (Harley, 2002).

APPENDEX III

Table 12.The recommended microbiological standards for any fruit juice; all numbers are as per ml of juice consumed (Gulf standard, 2000 and ICMSF, 2005).

Standard	Level	Total Viable count	Total Coliform	Fecal Coliform
Gulf	Maximum Bacterial load anticipated	5.0×10^3	10	0
	Maximum Bacterial load permitted	1.0×10^4	100	0
ICMSF	Maximum Bacterial load anticipated		10	0
	Maximum Bacterial load permitted	4.9×10^6	100	10

ICMSF= International Commission on Microbiological Specifications for Food

APPENDEX IV

Date of Pictures taken:-Sunday, 31/07/2016