

Microbiological Safety and Quality of Homemade Foods among Jimma University Community Primary School Students, and Growth Potential of Isolated Pathogens on Some Traditional Sauces, Jimma Town, Southwest Ethiopia

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Abstract

The study was carried out between March–June, 2013 to assess the microbiological quality and safety of various ready-to-eat foods from Jimma University Community School Students. Among 170 food samples assessed 18(10.58%) were vegetables, 24(14.11%) were rice, 17(10%) were spaghetti, 76 (44.70%) were firfir, 26(15.29%) were legumes and the last 9(5.29%) were meat. The isolates were identified following the standard microbiological methods and data was analyzed using the one-way-ANOVA test. Bacterial growth was present in all the food types evaluated, high mean total aerobic count were observed in meat ($5.44 \log \text{CFUg}^{-1}$) followed by vegetables ($5.27 \log \text{CFUg}^{-1}$) while rice had the lowest count ($4.03 \log \text{CFUg}^{-1}$). The main identified organisms in this study were *Bacillus* (42.58%), *S.aures* (15.18%), *pseudomonas spp.*(3.79%), *micrococcus*(22.41%), *aeromans*(1.33%), *Entrobactor*(12.5%), *alcaligens*(1.61%), and *acintobactor*(0.56%). Fortunately, *Salmonella spp.* was not isolated from spaghetti, meat and rice. The results indicated that most of the ready-to-eat food examined in this study did not meet the NSW, 2009 bacteriological quality standards, therefore posing potential risks to students. It is concluded that parents, the school administrators and others responsible persons needs to take measures that ensures the food quality and safety standards to reduce public health hazards.

Keywords: contaminated foods, food safety, Hazards, pathogens, ready-to-eat foods

INTRODUCTION

Food borne illness is usually either infectious or toxic in nature, and caused by agents that enter the body through the ingestion of food. There for they are responsible for high levels of morbidity and mortality in the general population, but particularly for at-risk groups, such as infants and young children, elderly and immunocompromised. Cases of foodborne diseases (FBDs) occur daily throughout the world, from the most to the least developed countries. The consumption of foods contaminated by foodborne pathogenic microorganisms and toxins produced by the micro-organisms causes deaths, illnesses, hospitalization, and economic losses. In industrialized countries the percentage of the population suffering from FBDs each year has been reported to be up to 30% (WHO, 2007).

The global incidence of food borne illnesses is difficult to estimate but it has been reported that in 2000 alone 2.1 million people died from diarrheal diseases. A great proportion of these cases can be attributed to contamination of food and drinking water (Oranusi, *et al.*, 2013)

Homemade foods are often prepared by hand and this direct contact may lead to an increased incidence of contamination with potential food borne pathogens, such as *Staphylococcus spp* (Colombariet *al.*, 2007).

The microbial load and the presence of the bacterial pathogens in foods are a good indication of the food quality and the potential health risk they pose to consumers. Especially *Escherichia coli* O157:H7 and *Salmonella spp.* are among the most dangerous food borne bacterial pathogens in terms of human health and disease (Hosein, *etal*, 2008)

The microbial load and the presence of the bacterial pathogens in foods are a good indication of the food quality and the potential health risk they pose to consumers. According to one report from the United States Department of Agriculture Economic Research Service (USDA-ERS) “Food borne illnesses account for about 1 of every 100 U.S. hospitalizations and 1 of every 500 deaths” (Buzby, *et al.*, 2001). Another study conducted by Roberts (2007) estimates that social costs of all acute food borne illness are a total of US\$1.4 trillion.

The microbial load and the presence of the bacterial pathogens in foods are a good indication of the food quality and the potential health risk they pose to consumers, and use of unhygienic utensils and materials, consumption of raw or unsafe food, as well as cross-contamination via inanimate surfaces by raw food, are some of the factors and practices that have been implicated in food-borne outbreaks (Taulo, *etal.*, 2008).

As reviewed by sudershan, *etal.*, (2009), Food borne illnesses are a widespread public health problem globally. Particularly Developing countries bear the brunt of the problem due to the presence of a wide range of food-borne diseases. In India an estimated 400,000 children below five years age die each year due to diarrhea.

Several millions more suffer from multiple episode of diarrhea and still others fall ill on account of hepatitis A, enteric fever, etc. caused by poor hygiene and unsafe drinking water (sundershan, *et al.*, 2009).

On the other hand, 9.4 million people are sickened and 1350 deaths occur each year in the United States due to 31 major pathogens that contaminate food. The relative numbers of illnesses due to microorganisms makes microbiological quality to be the most important aspect of food safety. Thus, food safety primarily focuses on the control of contamination of foods by pathogens (Hanning, *et al.*, 2012).

Numerous studies in developing countries have shown that homemade foods prepared under unhygienic conditions are heavily contaminated with pathogenic agents and are a major risk factor in the transmission of diseases, especially diarrhea. It is generally recognized that contamination of foods may occur as a result of poor hygiene of food handlers, household equipments and the environment where the preparation of food takes place (Muhimbula and Issa-Zacharia, 2010).

As the opinion of the ACFDP (Advisory Committee for Food and Dairy Products) that ready-to-eat foods should be free from *Salmonella* spp, *Campylobacter* spp, and *E. coli* O157 and other Verocytotoxin producing *E. coli* (VTEC). Appropriate control measures during production, adequate hygiene standards, and appropriate cooking during final preparation should ensure that the end products are free from viable organisms and that the foods are therefore of good quality. Gilbert *et al.*, 2000, mentioned that Ready-to-eat foods containing salmonellas or other pathogens may not always cause illness but there is good microbiological and epidemiological evidence that small numbers of pathogens in foods have caused illness. The ACFDP takes the view that there is no justification for processed ready-to-eat foods being contaminated with these organisms and that their presence, even in small numbers, results in such foods being of unacceptable quality/potentially hazardous.

As mentioned by Cairncross and curties (2003) diarrheal diseases are the second most common global illness affecting young children and a major cause of death in lower income countries, moreover they are closely linked with poor sanitation, poor hygiene, and lack of access to safe and sufficient supplies of water and food. Each year, nearly two million children under the age of five die of diarrheal diseases caused by unsafe water supplies, sanitation, and hygiene. Interventions such as simple hand washing have been shown to reduce sickness from diarrheal diseases by up to 47%, and could save up to one million lives.

In most cases parents might give attention only to the availability of food but not of its safety. As a result, those children may easily be threatened by foodborne diseases of the enteric pathogens and other disease causing agents which contaminating the food. In most developing countries including Ethiopia, sufficient statistics on food borne diseases are not available due to poor or nonexistent reporting systems (Kinfe and Abera 2007).

Therefore studying the microbial load of the homemade foods and the prevalence of these pathogens among the tested food could have a great importance in understanding the health status of the children. However, no previous study has been conducted on the study area; the present study was carried out to assess the microbiological quality of various ready-to-eat foods from randomly selected students at Jimma University Community School.

Methods and materials

Study design and population

Cross sectional study design was used. The total population of Jimma University Community Primary School first cycle students were 351 of these 307 brought their lunch frequently. So, the sample size was calculated by using Cochran (1977) formula. Accordingly, a total of 170 samples were included in the study.

Sampling technique

A systematic random sampling technique was used to address representatives of each grade (grade 1 to grade 4) of Jimma University Community primary School first cycle students. By using proportional calculation the appropriate sample were taken from the strata.

Sample collection

A total of 170 samples were collected from Jimma University Community School students between March, 2013–June, 2013. The samples were consisted of 76 firfir, 17 spaghetti, 9 meat, 26 legume, 24 rice and 18 vegetables. All samples were collected aseptically and immediately brought to the Microbiology laboratory Department of Biology, Jimma University for analysis. Microbiological analysis was conducted within an hour of sample collection. The food samples were kept in refrigerator at 4 °C until microbial analysis was conducted.

Sample preparation and Microbial Enumeration

Twenty five grams of each sample was weighed and homogenized by blending in 225 mL of sterile buffered peptone water. One milliliter of the homogenate was introduced into 9 mL of the buffered peptone water in a test tube, labelled 1:10 (10^{-1}) dilution and serially diluted to 10^{-2} , 10^{-3} , 10^{-4} , and 10^{-5} . One hundred microliters of each of the diluted sample was spread-plated on a pre-sterilized and surface dried PCA, MSA, MCA, VRBA, XLD and PDA agar plates. The plates were incubated aerobically for 24 h at 37 °C except detection of fungi,

which were incubated at 25°C for 5 days. *Staphylococcus* isolates and salmonella were confirmed by microscopic, cultural and standard biochemical tests (motility, catalase, coagulase, oxidase, urease, citrate utilization, indole, lysine iron agar, triple sugar iron agar tests). All discrete colonies were counted and expressed as the log₁₀ of colony forming units per gram (CFU g⁻¹)(Weil *et al.*, 2006).

Microbial Identification

The identification of a bacterial species is based on many factors, including cell colony, morphology, and Chemical composition of cell walls, biochemical activities, and nutritional requirements. In this study 10 to 15 colonies with distinct morphological differences were randomly picked from countable plates and Cultures were purified by repeated plating and organisms were identified depending up on their shape, cell arrangement, gram staining, endospores staining, and biochemical tests.

Growth potential of some food borne pathogens

The growth potential of *Salmonella typhimurium* (ATCC13311), *S.aureus* (ATCC25923) and *E. coli* (ATCC25922) were assessed in three different sauces (meat, shiro and cabbage) by which the majority of the students consumed for their lunch. Three hundred grams of each food items was separately homogenized using food processor (NM-343.nima LTD Osaka, Japan) and steamed at 80 °C for 10 minutes to kill any vegetative cells that might be present in the food items. One hundred grams of each food items was challenged separately with 1 ml overnight culture of the test strains to bring the final inoculums level of 10²-10³ CFUg⁻¹. The challenged food was incubated at 37°C for 24 hrs. To determine the initial inoculum level, 10 g of each freshly inoculated food was homogenized in 90 ml of BPW and 0.1 ml of appropriate dilution was spread plated on XLD for *S.typhimurium*, MSA for *S.aureus* and VRBA for *E. coli*. A portion of food sample was further sampled aseptically at 6 hrs interval from 0-24 hrs, while assessing growth potential, the pH of each food sample was measured using digital pH meter from 0 hr to 24 hrs at an interval of 6 hrs (Muleta and Ashenafi, 2001)

Result

Microbiological enumeration

The mean bacterial count of the isolates in the food samples were expressed as log₁₀ CFU g⁻¹ for easy computation. The mean value of aerobic bacterial count on firfir, spaghetti, rice, legume, vegetables and meat were 4.71, 4.34, 5.03, 4.9, 5.27 and 5.44 log₁₀ CFU g⁻¹ respectively (Table 6).

There was statistically significance difference (p<0.05) among the mean count of AMB, *Enterobacteriaceae*, Aerobic bacterial spore (ABS), *Staphylococci*, *Yeasts* and *Molds* in all food samples However no significant difference was observed in mean count of LAB and coliforms (p>0.05)

Plate count of aerobic mesophilic microorganisms found in foods is one of the microbiological indicators for food quality. The presence of aerobic organisms reflects existence of favorable conditions for the multiplication of microorganisms. In this study, of all the evaluated samples rice, vegetable and meat had mean contamination levels of ≥5.0 log₁₀ CFU g⁻¹ (table 6).The mean counts of *Staphylococci* in meat sauce and spaghetti were 3.06 and 2.84 log CFU g⁻¹.

The mean count of *Enterobacteriaceae* was highest (3.42logs CFUg⁻¹) in spaghetti followed by legumes (2.36logs CFUg⁻¹). The mean count of *staphylococcus* was higher in vegetable followed by meat (Table 6).Even if the isolated *S.aures* in the samples were few but their mean counts were above the detectable level. Except in vegetables the mean count of aerobic spore former bacteria was below log 4(Table 6).

Table.6. Mean counts (log₁₀ CFU g⁻¹) of microbial groups in different food samples, Jimma University Community School, Jimma town, Ethiopia, 2013.

Food item	AMB	ENTRO	Coliforms	ABS	STAPH	LAB	Yeast	Mold
Firfir	4.71	2.95	2.02	3.13	2.85	2.85	2.25	2.04
Spaghetti	4.34	2.91	3.42	3.54	2.84	3.16	1.23	1.23
Rice	4.03	2.46	1.66	2.63	3.23	3.60	1.64	1.72
Legume	4.9	3.23	2.36	3.81	3.05	3.28	2.14	1.96
Vegetable	5.27	3.39	2.34	4.07	3.65	2.62	2.62	1.90
Meat	5.44	2.73	1.89	3.95	3.06	3.92	2.76	1.32

Where: AMB=Aerobic Mesophilic Bacteria; ENTRO=Enterobacteriaceae; ABS=Aerobic Bacteria Spore; STAPH=Staphylococci; LAB=Lactic acid bacteria

Firfir- stands to means food (Injera or bread) broken into smaller pieces and mixed with spice or sauce. Usually it is fast meal made of [injera](#) (pancake like bread) mixed with different sauce

*The word legume in the present study stands for Ethiopian popular sauce shiro wat and lentil (misir wat) which was made from the family of legumes.

Food category

The food samples obtained from the students were catagorized as satisfactory, intermidiate ,unsatisfactory and potentially hazardous based on the standared manual(Table.7).

Table.7. Food safety categories of samples collected from Jimma University community primary school students. Jimma town, Ethiopia, 2013

Food item	Number No(%)	Satisfactory No (%)	Intermediate No (%)	Unsatisfactory No (%)	Hazardous No (%)
Firfir	76(44.70)	63(37.05)	4(2.35)	3(1.76)	6(3.52)
Spaghetti	17(10)	14(8.23)	2(1.17)	1(0.58)	0(0)
Meat	9(5.29)	6(3.52)	0(0)	3(1.76)	0(0)
Legume	26(15.29)	17(10)	4(2.35)	3(1.76)	2(1.17)
Vegetable	18(10.58)	6(3.52)	3(1.76)	4(2.35)	5(2.94)
Rice	24(14.11)	21(12.35)	1(0.58)	2(1.17)	0(0)

Microbiological analysis

From the total of food samples analyzed, 1423 bacterial isolates were characterized and grouped in to their respective genera Using standard manual (John, 2012). The aerobic micro flora of firfir was dominated by a variety of Gram positive and Gram negative bacterial groups. *Bacillus* and *Micrococcus* species dominate the microflora. Among the Gram positive isolates bacillus was dominant on firfir samples followed by legumes than other samples. In contrary, Gram negative isolates dominated more of the spaghetti samples. *Pseudomonas* isolates were the dominant among Gram negative isolates (table 9)

Table .8.Frequency distribution of dominant bacteria in some homemade foods obtained from Jimma University Community School. Jimma town, Southwest Ethiopia, 2013

Food type	No of isolate	<i>Bacillus</i>	staph	<i>Micrococcus</i>	entro	<i>Pseudomonas</i>	<i>Aeromonas</i>	<i>Acintobactor</i>	Alkaligens
Firfir	655	271	110	159	91	15	2	0	7
Spaghetti	80	34	26	7	3	3	4	0	3
Meat	135	59	23	29	11	9	3	1	0
Legume	260	113	17	85	26	13	1	4	1
Vegetable	145	77	19	16	18	4	0	2	9
Rice	148	52	21	23	29	10	9	1	3
Total	1423	606	216	319	178	54	19	8	23
percentage	100	42.58	15.18	22.41	12.50	3.80	0.63	0.56	1.62

The Prevalence of *Staphylococcus* and *Salmonella* species

From the overall food samples analyzed in this study 41(24.11%) were positive for *S.aureus*,but the frequency distribution varied among the food samples. Its prevalence was hihgest in firfir 17(41.46%),followed by vegitable11(26.82 %),and the least was in spagetti 2(4.87 %) accordingly,it was 5(12.19%) in legume and 3(7.31 %) in both meat and rice(Table 10.)On the other hand 13(7.64%) were positive for *salmonella spp.* However the frequency distribution varied among the food samples. Its prevalence was the highest in firfir 6(46.16%) followed by vegetables 5(38.46%) and the least in legumes 2(15.38%). However, *Salmonella* spp.were not isolated from rice,meat and spaghetti food samples (Table.9).

Table.9. Frequency of isolation of *Staphylococcus spp* and *Salmonella spp.* from different food item, Jimma town,Southwest, Ethiopia 2013.

Food Item	<i>Staphylococcus spp.</i>	<i>Salmonella spp.</i>
	Frequency(%)	Frequency(%)
Firfir	17(41.46)	6(46.16)
Spaghetti	2(4.87)	0(0)
Legume	5(12.1)	2(15.38)
Vegetable	11(26.82)	5(38.46)
Meat	5(7.31)	0(0)
Rice	3(7.31)	0(0)

Growth potential of some standard bacteria on some traditional sauces

Growth potential and PH of some selected traditional sauces challenged with *S. aureus*.

The mean count *staphylococcus aures* in each food sample was 1.8 to 3.29 logs CFUg⁻¹ at 0 hr. At 6 hour there was a slight increase rate in all foods but the growth was faster in shiro followed by meat. At 18 hrs the highest growth was in meat and shiro (5.42 and 5.14 log CFUg⁻¹) followed by cabbage (4.61CFUg⁻¹) (Fig 2.)

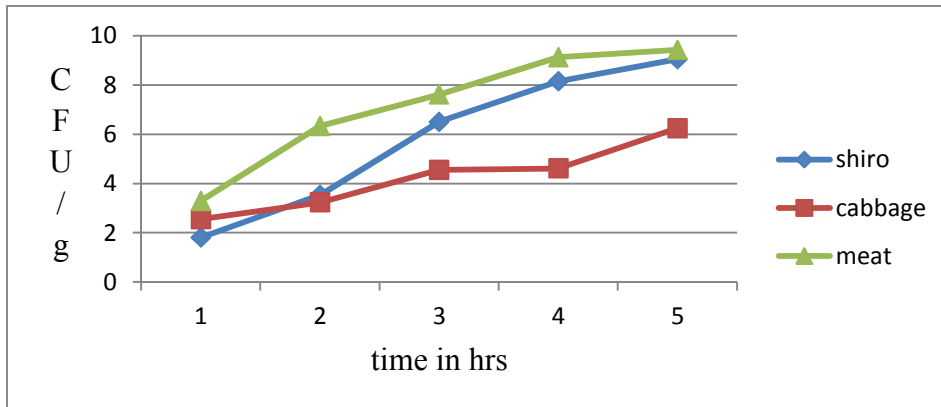


Fig.2. Growth potential of *S. aureus* in some selected traditional sauces Jimma town, 2013

The pH of food samples challenged with the isolates of *S. aureus* was above 5.5 at 0 hour. In the next two 6 hours it was increased in all food items. The pH drops down at 18 hrs except shiro. In the last 24 hours the pH of all the food items was approaches to neutral (Fig.3)

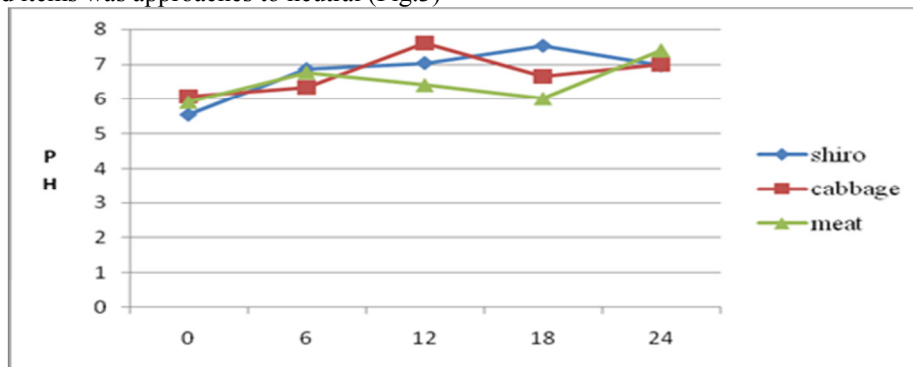


Fig.3. Change in pH of some traditional foods challenged with *S. aureus*, Jimma town, Southwest Ethiopia, 2013
 Growth potential and pH of some selected traditional sauces challenged with *S. typhimurium*.

The growth potential of *S. typhimurium* was analyzed in shiro, meat and cabbage over a period of 24 hours. In this study, the highest count of *S. typhimurium* were 8.9 log CFUg⁻¹ with in 24hrs in meat sample (Fig. 4) and the lowest were 6.5 log CFUg⁻¹ in cabbage with in 24 hrs. The mean count of test strains were increased by >2 log units in the first 6 hrs in shiro and meat. In the third 6 hrs, the growth rate of test strains were increased by three logs CFUg⁻¹ in cabbage followed by meat and shiro (Fig.4).

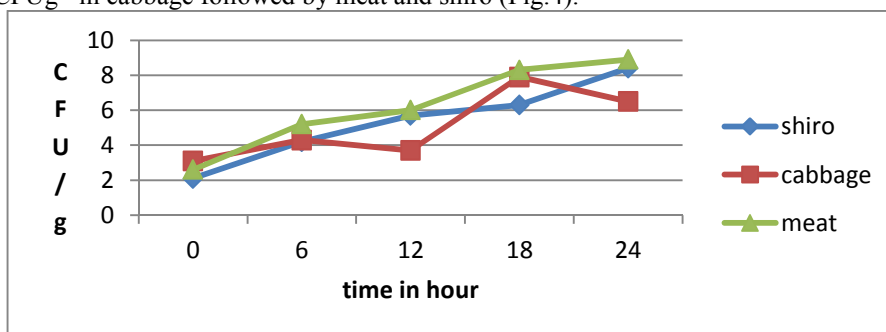


Fig.4. . The growth potential of *S. typhimurium* in traditional sauces, Jimma town, Southwest Ethiopia, 2013

The pH of meat challenged with *S. typhimurium* was 5.21 at 0 hour which was the lowest pH as compared as cabbage and shiro (Fig.4) in addition there were a fluctuation of pH throughout the period of 24 hours. Furthermore the pH was relatively stable in shiro remaining almost around neutrality (Fig. 5)

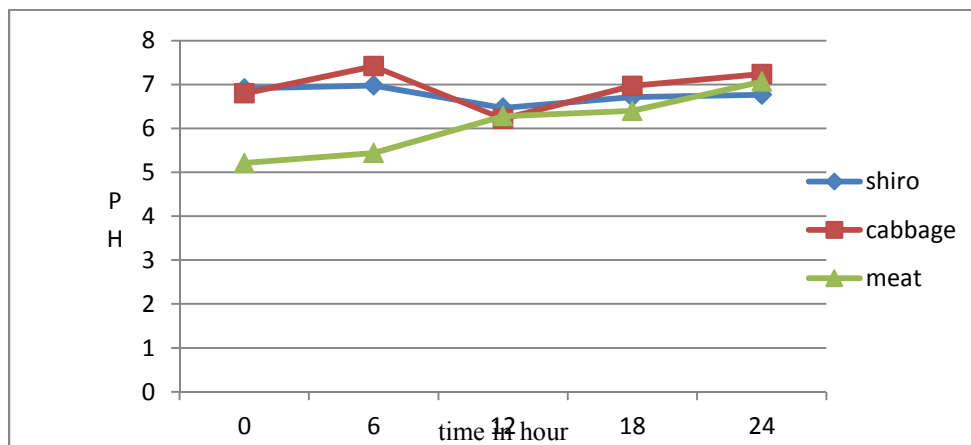


Fig.5 Change in pH of some traditional foods challenged with *S.typhimurium*, Jimma town, Southwest Ethiopia, 2013

Growth potential and ph of some selected traditional sauces challenged with *E.coli*

The growth of *E. coli* in the first 6 hrs was higher in cabbage. Although the pattern was the same for all sauces. In the second 6 hrs (at 12hrs) the growth rate increased by greater than 2 log units in meat sauce followed by shiro, but in cabbage its growth decreases by 0.5log CFUg⁻¹. at 24 hr the growth was very high in meat (9CFUg⁻¹). Accordingly mean colony forming unit in all food items were >7log CFUg⁻¹(Fig.6)

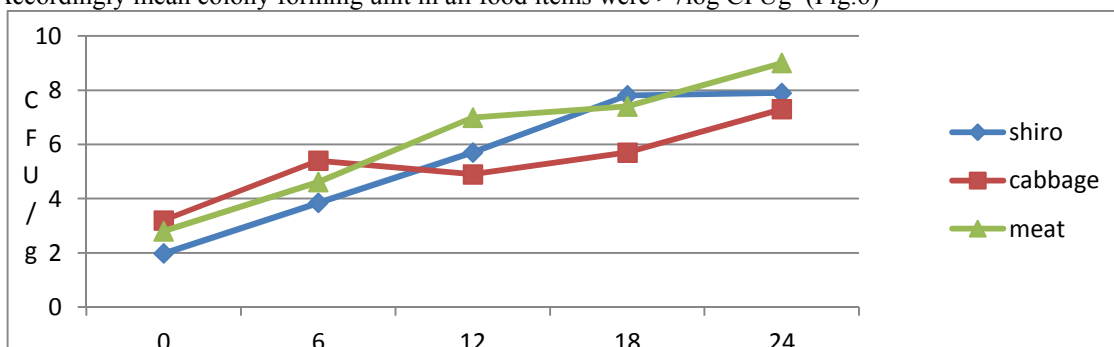


Fig.6. The growth potential of *E.coli* in traditional sauces, Jimma town, Southwest Ethiopia, 2013

At the beginning (0 hour) the pH of meat challenged with the test strain *E.coli* was 5.69 in the next two 6 hrs the pH falls down to 4.11 and 4.01 at six and 12 hrs respectively. Finally it rise up and approaches to neutral. Accordingly the pH of shiro and cabbage was around neutral throughout a period of 24 hrs (Fig 7).

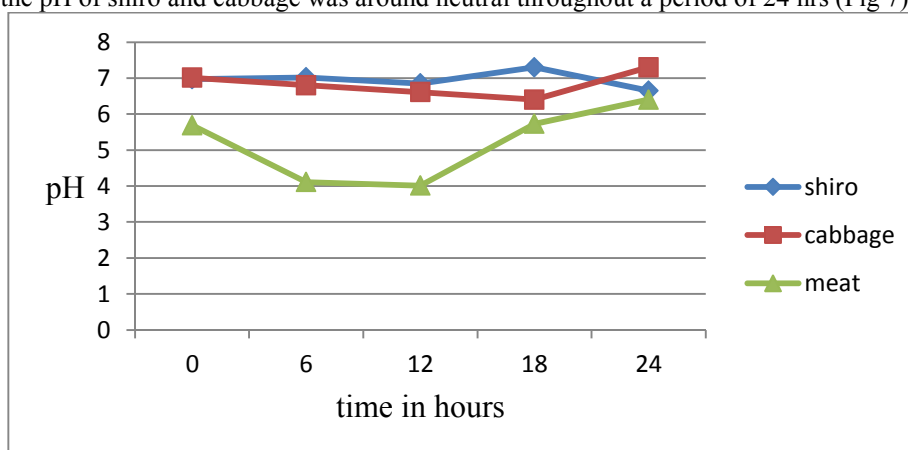


Fig.7. Change in pH of some traditional foods challenged with *E.coli*, Jimma town, Southwest Ethiopia, 2013

DISCUSSION

Hand-manipulated foods are among the most consumed products worldwide. They are also liable to high microbiological contamination due to their manufacturing process. One of the most basic ways to show that we care about children is to feed them nourishing safe food (Benjamin, 2012). Because feeding children healthy

food is important to be active, to think, and to grow. Therefore safely prepared food helps children avoid food borne illness and they can develop lifetime habits through what they eat in childhood. In addition when children eat with others; they develop social and communication skills. There for feeding microbially safe food should be the thing that parents think first (Benjamin, 2012).

The category ready-to-eat food can be considered as high risk foods because they do not require any heating or process prior to consumption. Therefore it has to be safe, but food workers may transmit pathogens to food from a contaminated surface, from another food, or from hands contaminated with organisms from their gastrointestinal tract (Weil, *et al.*, 2006). Thus hand contact with ready-to-eat foods represents a potentially important mechanism by which pathogens may enter the food supply (British Medical Journal, 1990). Food workers' poor personal hygiene is an important contributor to foodborne illness outbreaks. For example Guzewich and Ross found that in 89% of outbreaks caused by food contaminated by food workers, pathogens were transferred to food by workers' hands (Laura, *et al.*, 2007). When food handlers do not practice proper personal hygiene or correct food preparation, they may become vehicles for microorganisms, through their hands, cuts or sores, mouth, skin and hair, among others. In this study food workers had a habit of hand washing but the presence of fecal coliforms in tested food samples indicates a post-sanitization or post-process contamination, often caused by a lack of hand hygiene on the part of food handlers (Ana, *et al.*, 2008).

Numeration of the total aerobic bacteria on food samples examined in the present investigation showed high microbial contamination in some foods. Cenci-Goga, *et al.*, (2005) pointed out that the total aerobic bacteria count was a good indicator of food safety. A similar study was carried out in Lagos (Uzeh, *et al.*, 2009) and the total aerobic bacteria count ranged from 3.3×10^3 to 5.9×10^6 CFUg⁻¹. The same findings were reported Hanashiro, *et al.*, (2013) in Sao Paulo for coliforms load. These reflect the existence of favorable conditions for the multiplication of microorganisms. In this study, of all the food sample types tested rice, vegetable and meat had mean contamination levels of $\geq 5.0 \log_{10}$ CFU g⁻¹. The New South Wales Food Authority (NSW, 2009) recommends the standard limit for bacterial count of fully cooked ready-to-eat foods to be $< 5.0 \log_{10}$ CFU g⁻¹. Hence these foods could be of high risk in transmitting enteric pathogens. In their study, Mensah, *et al.*, 2002, found a bacterial count of 6.3 ± 0.78 in ready to eat food of Accra, which is greater than this finding ($5.27 \log_{10}$ CFU g⁻¹). This study is in line with the study conducted in Nigeria which was less than $6.3 \log_{10}$ CFU g⁻¹ (Odu and Ameweiyee, 2013). But a contrast count ($8.4 \log_{10}$ CFU g⁻¹), was reported from Misurata City, Libya (Abdalhamid, *et al.*, 2013)

According to food guide line all food samples investigated in the present study belongs to level one, which means all food samples are fully cooked. Specifically the mean count of AMB in rice, vegetable and meat samples in the present study were $> 5 \log_{10}$ CFU g⁻¹. Hence they belong to unsatisfactory, but a comparison made from the results, shows that meat sample had more bacterial contamination than the pastry. This may be because meat offers a rich nutrient media for microbial growth (Clarence, *et al.*, 2009).

The counts of coliforms varied between 1.66 – 3.42 logs CFU g⁻¹. This is in line with a research conducted in Tirumala, India which was between 0.28-3.99 logs CFU g⁻¹ (Suneetha, *et al.*, 2011). According to the NSW guide line the mean count of coliforms $< 2 \log_{10}$ CFU g⁻¹ is considered as acceptable. In the present study the mean count of coliforms in all foods except meat and rice were above this standard. Thus most of the food items tested was in unacceptable level. Existence of coliforms on ready to eat food products reflected the recontamination caused by secondary processing and poor personal hygiene, or probably due to water used for cooking and serving which could be contaminated with fecal coliforms (Weil, *et al.*, 2006). This implies that contamination was mainly due to poor quality of water used for preparation as well as prevailing unhygienic conditions related to improper washing of utensils, inadequate storage of these at ambient temperatures in unhygienic places, and personal hygiene of food makers (Suneetha, *et al.*, 2011).

Most ready-to-eat foods in Kumasi, Ghana were reported to be contaminated with enteric bacteria, and had bacterial counts higher than the acceptable levels (Feglo and Sakyi, 2012).

In contrast the mean count of enterobacteriaceae in this study was in between 2.46 and 3.39 log CFUg⁻¹ which was below the standard level of the guide line; therefore all the food items in relation to the present study were considered as satisfactory (NWS, 2009).

S.aureus are found on the skin, nose and throat of most healthy people; they are also widespread in untreated water, raw milk and sewage. When *S.aureus* is allowed to grow in foods, it can produce a toxin that causes illness. Although, cooking destroys the bacteria, the toxin produced by *S.aureus* is heat stable and may not be destroyed even by heating, let alone by refrigeration. Foods that are handled frequently during preparation are prime targets for *Staphylococci* contamination (Ghosh, *et al.*, 2004)

In this study the highest and the lowest *S. aureus* count detected were $3.65 \log_{10}$ CFUg⁻¹ and $2.84 \log_{10}$ CFUg⁻¹. This result is in agreement with the finding of Sina, *et al.*, (2011) who reported $3.5 \log_{10}$ CFUg⁻¹ and $2.9 \log_{10}$ CFUg⁻¹ the highest and the lowest respectively. In contrast to this investigation Alyaaqoubi *et al.*, (2009) reported in forty eight ready-to-eat foods studied in Malaysia there was no *S.aureus*. Generally the presence of *S.aures* in these foods is probably because the hands which handled them were contaminated

(Amissah and Owusu, 2012). Thus the presence of *S. aureus* in food is an indication that such food is potentially hazardous (Amissah and Owusu, 2012). Moreover, these organisms may take the chance to multiply in the product during storage & produce their enterotoxins which constitute a public health hazard (Staphylococcal food poisoning) to the consumers (Abdalhamid, 2013).

The mean aerobic spore count of the present study is higher in vegetables ($4.07 \log \text{CFUg}^{-1}$) and lower in rice ($2.63 \log \text{CFUg}^{-1}$) on the other hand firfir, spaghetti, legume and meat had $3.13 \log \text{CFUg}^{-1}$, $3.54 \log \text{CFUg}^{-1}$, $3.81 \log \text{CFUg}^{-1}$ and $3.95 \log \text{CFUg}^{-1}$ respectively. In many types of food soil can be considered as the initial source of contamination for spore formers. Usually, when direct transfer from soil is involved, levels of these spore formers in foods, ingredients, or feeds are too low to cause problems but spores can germinate and grow during storage, which leads to enzyme formation and metabolism. Microbial spoilage enzymes such as proteases, lipases and lecithinases are often responsible for off-flavour and structural defects (Witthuhn, *et al.*, 2011). This can happen on the primary production level, in the processing line, during distribution. These proliferation steps enable the endospore former such as *B. cereus* provokes food quality or safety problems (Heyndrickx, 2011). For instance in England and Wales in the period 1992–2006, 4% of the outbreaks associated with prepared salads were caused by *Bacillus spp* (Little and Gillespie, 2008). The presence of these spore formers in this study is probably due to contamination during preparation, storage at home or it may be in the school because of the absence of an appropriate storage and feeding room.

The presence of *Salmonella* in 25 g of a sample examined is regarded as potentially hazardous to consumers, and is unacceptable for consumption. Rajkowski and Fan (2008) also isolated *Salmonella* from vegetable samples and suggested that contamination with human pathogen could occur when bovine manure used as fertilizer, contaminated water or cross contamination.

Even though ready-to-eat foods containing *Salmonella* or other pathogens may not always cause illness, there is good microbiological and epidemiological evidence that small numbers of pathogens in foods causes illness (Amissah and Owusu, 2012). Therefore according to Gilbert *et al.*, (2000) the presence of these organisms, even in small amounts, they make the food unacceptable. Therefore, in this study non detection of *Salmonella* in spaghetti, rice and meat makes these foods acceptable (Foskett *et al.*, 2003). This is in agreement with the study reported by Soriano *et al.*, (2001) who found no *Salmonella* in ready to eat food samples from Valencia, Spain. In addition, no *salmonella* was also reported from Hulu, Malaysia (Alyaaqoubi, *et al.*, 2009). The absence of *Salmonella* indicated that good handling practices during the cooking process and good storage facilities were available when these food items (spaghetti, rice and meat) were used. However a total mean count of $1.08 \log \text{CFUg}^{-1}$ of *Salmonella* was found in firfir, vegetable and legumes which were 7.64% of the overall samples tested in this study. This is in line with Sudershan, *et al.*, (2012) who found $2.6 \log_{10} \text{CFUg}^{-1}$ *Salmonella* from chicken. Bukar, *et al.*, (2010), also reported that about 10.0% *salmonella* positive ready-to-eat foods from Kanometropolis, Nigeria. Thus these food items are considered as potentially hazard (Abdalhamid, 2013).

In the present study the mean count of LAB in firfir, spaghetti, rice, legume, vegetable and meat was 2.85, 3.16, 3.60, and 3.28, 2.62 and $3.92 \log \text{CFUg}^{-1}$ respectively. Lactic acid bacteria (LAB) are a group of related bacteria producing lactic acid as the result of carbohydrate fermentation (Ali *et al.*, 2009). In addition to flavor development and food preservation, they also produce variety of compounds with antimicrobial activity, including organic acids, hydrogen peroxide and bacteriocin. Bacteriocin produced by LAB could inhibit not only closely related species but also the growth of pathogenic bacteria (Hajar and Hamid, 2013). Many LABs have important roles in the production of fermented foods, and some of the bacteria were capable of inhibiting the growth of a wide variety of food spoilage microorganisms (Lindgren and Dobrogosz, 2006). Thus, LABs are an attractive source of inhibitory compounds with promising natural food preservatives for improved food quality and safety. In contrast to the present study, the study conducted in ready to eat food for infants from Nigeria reported that the count of LAB ranging between 4.5 to $9.2 \log \text{CFUg}^{-1}$.

In the present study the mean count of molds and yeasts were ranging between 1.23 and 2.04 $\log \text{CFUg}^{-1}$ and 1.23 and $2.76 \log \text{CFUg}^{-1}$ respectively. It was the lowest microbial load for fungi when compared to the result obtained from Benign, Nigeria (Wogu, *et al.*, 2011). The highest food item contaminated by these fungus groups were vegetables and firfir. In Brazil cooked foods were considered as acceptable when the mean count of molds and yeasts is $\leq 5 \times 10^4 \text{CFU g}^{-1}$ in line to this guide all food items in this study could in acceptable range (Beatriz and Eliana, 2000). As Momoh, *et al.*, (2011) presented, Yeasts and molds are problematic in foods in that they discolor food surfaces, cause off odors and off flavors in certain instances. Contamination of foods by yeasts and molds can result in substantial economic losses to producer, processor, and consumer. Several foodborne molds, and possibly yeasts, may also be hazardous to human or animal health because of their ability to produce toxic metabolites known as mycotoxins. Most mycotoxins are stable compounds that are not destroyed during food processing or home cooking. Although most foodborne fungi are not infectious, some species can cause infection, especially in immunocompromised populations (Valerie, *et al.*, 2001) and some others are known to cause allergies when they are able to produce large numbers of conidia (Seo, *et al.*, 2010).

The microorganism in ready to eat homemade food in the present study was dominated by bacillus

(42.58 %) followed by micrococcus (22.41 %) and staphylococcus (15.17%). In contrast to this finding a research conducted in Nigeria revealed that *Bacillus sp* (25.0%) was second dominant bacterial isolates, next to *S.aureus* (35.7%) (Odu and Ameweiye, 2013). In ready-to-eat foods that are fully cooked, the presence of these microbes are used as an indication of either post processing contamination or inadequate cooking (NSW, 2009). The presence of *bacillus cereus* from food samples implicated the ubiquitous nature of bacterial spores. The predominance of *Bacillus* isolates on aerobic plate count plates was possibly due to the presence of spores in the raw material as they are spore forming bacteria. In general, the presence of *Bacillus cereus* in food is of great significance since this organism produces heat-sensitive (diarrheal) and heat-stable (emetic) toxins associated with food poisoning (Suneetha, *et al.*, 2011). In line with this study similar findings by Hanashiro suggested that ready to eat foods sold on the street of São Paulo city, Brazil, were considered unsuitable for consumption due to higher load of *Bacillus cereus* (Hanashiro, *et al.*, 2005).

The growth potential of standard strains of *S.aures*, *Salmonella* and *E.coli* examined in this study showed that the maximum count was recorded in meat challenged with the standard strains within 24 hours. This is similar as that of the result reported by Muleta and Ashenafi, (2001). This is probably due to meat provides bacteria with an ideal medium on which they can grow. Because it has ample nutrients, available water and a moderate pH. The infective dose for *S.aures* is $6 \log \text{CFUg}^{-1}$ (schelien, *et al.*, 2011). In this study the growth potential of *S. aures* reached to this infective dose within 6 hours, 12 hours and 24 hours in meat shiro and cabbage respectively. In this infective dose the *S.aures* populations may produce toxins which may produce illness to consumers (Bent and Monday, 2003).

The maximum bacterial count ($>8 \log \text{CFUg}^{-1}$) of *S. typhimurium* was recorded at the last 18 hrs in meat and 24hrs in shiro and cabbage. The PH increased as the time increased in meat till reaching neutral but almost similar in shiro and cabbage throughout 24 hrs. The change in PH could be because of changing of source of carbon and nitrogen. As Lee, (2011) explanation, some microbial cultures generate enzymes to utilize new carbon and energy substrate when a small amount of the original carbon and energy substrate is present. This is not saying that PH is the only required criteria for the growth of organisms but other intrinsic and extrinsic parameters should be full filled (Jay, *et al.*, 2005).

For causing illness the least infective dose for *salmonella typhimurium* is $\log 5$ (Toder, 2005). In the present study *salmonella* reaches to this dose within 6 hours in meat, 12 and 18 hours in shiro and cabbage respectively.

On the other hand the growth potential of *E.coli* was also examined in the present study. The infectious dose and the dose response are dependent upon the strains used, and the age and physical condition of the individuals, and can therefore show wide variations and the infectious dose for *E. coli* was large ($>5 \log \text{CFUg}^{-1}$). The result from this challenged study revealed that *E.coli* reached to the infective dose ($>5 \log \text{CFUg}^{-1}$) at 6hrs in cabbage and at 12 hrs in meat and shiro (Mahendra, *et al.*, 2007).

CONCLUSION

- ✦ In conclusion, Firfir which is the main food that students used for their daily lunch is the most contaminated foodstuff. Although food is cooked (boiled) at high temperature which could be enough to inactivate pathogens, post-contamination and cross-contamination that is being promoted by unhygienic food handling, and incorrect storage practices might made the food unsafe or potential hazardous.
- ✦ In this study high percentage of indicator organisms as well as food borne pathogens were identified, which showed unhygienic condition of handling and processing of food at household level.
- ✦ In addition this study confirmed the presence of drug resistant food borne pathogens; particularly *S.aureus* which are methicilin resistant and other multidrug resistant isolates.
- ✦ The predominance of *Staphylococcus* species and the presence of other microorganisms might leads to unexpected foodborne diseases outbreaks unless the sanitary facilities of the school and personal hygiene of the food makers are improved.
- ✦ The isolation of *enterobacteriaceae* in cooked ready to eat foods is good indicator of the risk of transmission of fecally contaminated pathogens from infected individuals to the healthy children.

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