# Addis Ababa University

# College of Natural Sciences

# Center for Food Science and Nutrition


**METAL LEACHING FROM TRADITIONAL COOKWARE: COULD IT BE A PUBLIC HEALTH CONCERN?**


**By**

**Binyame Tesfaye**


**A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN FOOD SCIENCE AND NUTRITION**

**March, 2015**

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**A THESIS SUBMITTED TO THE CENTER FOR FOOD SCIENCE AND NUTRITION**

**PRESENTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN FOOD SCIENCE AND NUTRITION**

Under the Supervision of

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March, 2015

**Addis Ababa University**

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This is to certify that the thesis presented by Binyame Tesfaye, entitled ‘Metal Leaching from Traditional Cookware: Could it be a Public Health Concern?’ submitted in partial fulfillment of the requirements for the degree of Master of Science (Food Science and Nutrition) complies with the regulations of the university and meets the accepted standards with respect to the originality and quality.

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DECLARATION

I the undersigned, declare that this thesis is my original work and has not been presentd for the award of a degree at any university and all the sources of materials used for this thesis have been duly acknowledged.

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**ABSTRACT**

Metal Leaching from Traditional Cookware: Could it be a Public Health Concern?

Binyame Tesfaye

Geogenic and anthropogenic activities are the bases for the contamination and entrance of metals into the food chain and poses health risk. Therefore, in the present work, leaching of some selected metals (Aluminium, Iron, Lead and Nickel) in traditional cooking pot manufactured in unstandardized condition was investigated. Factors that can influence metal leaching from cooking wares such as pH of food, cooking duration and cooking frequency were studied. Sauce was made simulating the usual preparation. Levels of metals were determined by Flame Atomic Absorption Spectrophotometer following high temperature assisted wet acid digestion in a closed model. In addition, same metals were determined in sauce prepared in a beaker as a control to rule out the effect of metal concentration in a sauce. To study the effect of pH and boiling duration on the level of metal leaching, buffers of Sodium citrate (pH 4.0) and Tris (pH 7.0 and 9.0) was boiled for 2 hrs. and samples were regularly drawn every 12 min. for the first one hour and every 30 min. for the next one hour of boiling. The mean (±SD) Al content of the foodstuffs was; onion (9.82±0.17 mg/kg); berbere, (18.8±0.35 mg/kg); *shiro* powder, (39.15±0.27 mg/kg); and tomato, (1.6±0.04 mg/kg). The Fe content of the foodstuffs was ranged from 15.2±0.2 to 30.51±0.4 mg/kg. Nickel content was found to be highest in *shiro* powder (3.42 ±0.66 mg/kg), followed by Berbere (2.80±0.66 mg/kg), tomato (0.92±0.08 mg/kg), and onion (0.64±0.23 mg/kg). The level of Al (38.38±0.3 mg/kg) and Fe (55.07 ± 2.11) in tomato sauce prepared in a beaker was found to be significantly different ($p<0.001$) as compared with first time (Al, 203.68±2.03

mg/kg; Fe, 112.62±1.1 mg/kg), second time (Al, 176.82±2.8 mg/kg; Fe, 109.79±3.4 mg/kg), third time (Al, 152.69±3.2 mg/kg ;Fe, 84.76 ± 1.23 mg/kg), and fourth time (Al, 142.94±2.7 mg/kg; Fe, 67.20±0.6 mg/kg) usage of the cooking pot. However, lead was not detected in any of the food. Acidic pH had higher ($p<0.001$) leaching effect on Al (17.4±1.9 mg/kg) and Fe (2.80±1.6) compared to leaching at alkaline (Al, 12.64±4.1 mg/kg Fe, 0.99±0.3 mg/kg) and neutral pH (Al, 1.5±0.5 mg/kg Fe, 0.5±0.3 mg/kg).

In addition, cooking duration had a significant increasing effect ($p<0.001$) on leaching of Al and Fe at all pH values. Similarly, the interaction of pH and boiling duration had significant ($p<0.001$) on leaching of Al and Fe. Results of the present investigation indicate that leaching of metals from unstandardized traditional cooking wares is significant and could be public health concern. In addition, acidic pH and cooking duration increases metal leaching.

**Keywords**: cooking pot; leaching; Metals; cooking frequency; pH; boiling duration

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| AOAC | Association of Official Analytical Chemists |
| ANOVA | Analysis of Variance |
| ATSDR | Agency for Toxic Substances and Disease Registry |
| CAC | Codex Alimentarius Commission |
| CSEM | Case Studies in Environmental Medicine |
| EPA | Environmental Protection Agency |
| FAO | Food and Agricultural Organization |
| FDA | Food and Drug Administration |
| FASS | Flame Atomic Absorption Spectroscopy |
| GMP | Good Manufacturing Practice |
| IDA | Iron Deficiency Anemia |
| IOM | Institute of Medicine |
| IPCS | International Programme on Chemical Safety |
| JECFA | Joint Expert Committee for Food Additives |
| LD | Lethal Dose |
| NAS | National Academy of Science |
| NCC | National Codex Committee |
| NRC | National Research Council |
| PTWI | Provisional Tolerable Weakly Intake |
| PMTDI | Provisional Maximum Tolerable Daily Intake |
| RDA | Recommended Dietary Allowance |
| TDI | Tolerable Dailey Intake |

| | |
|---|---|
| UL | Upper Level Intake |
| WHO | World Health Organization |

## 1. INTRODUCTION

The industrial applications of several metals have revolutionized the human environment. However, they have also been proven to be hazardous to health following their ingestion, intentionally or accidentally (Zohar, 1980).

The presence of metals in food can be caused by different sources such as through direct contamination during production, with metal-rich soil, air, or contaminated water as well as from the use of pesticides or fertilizers (Fishbein, 1981; Arora *et al*., 2008). Food can also be contaminated during processing, transportation and storage (Wang *et al*., 2006).

According to International Occupational Safety and Health Information Centre (CIS), (1999) there are 35 metals that are concern to humans due to occupational or residential exposure and it has been estimated that around one billion people worldwide suffer from some form of diseases attributed to those metals (WHO, 2011). Regardless of their effect, many of them play a crucial role in all life forms. For example, arsenic (As), copper (Cu), iron (Fe) and nickel (Ni) are considered essential at low concentrations but are toxic at high levels (Mertz, 1981). However, elements like aluminium (Al), beryllium (Be) and lead (Pb) have no biological significance (Trichet & Defarge, 1995).

Although metal toxicity depends on the amount ingested, chronic exposure to certain metals, such as cadmium (Cd) and lead (Pb), can cause severe toxic effects, even in low amounts. Humans are exposed to metals through different exposure pathways, the most common being inhalation of contaminated air and ingestion of products such as water, medicinal herbs, and food (Nowak & Chmielnicka, 2000; Abou-Arab, 2000).

Metals tend to bioaccumlate thus their concentration increases in a biological system over time. This is because they are stored faster than being metabolized or excreted (Hare, 1992). Unlike organic molecules, metals do not require bioactivation or undergoes enzymatic modification that produces a reactive chemical species for detoxification process (Waalkes, 1995). However, metals use other mechanisms, such as long-term storage (e.g., Iron) and biliary and/or urinary excretion (Waalkes, 1995).

Generally, metals disrupt basic metabolic functions in two ways; first, when stored in the soft tissue of the body, they compete with other ions. For instance, heavy metals like Pb, Hg and Cd have affinity to sulphur and attack the thiol groups in the active site of enzymes, thus inactivating and disrupt their function (Ademoroti, 1996; Alka, 2000). Secondly, they antagonistically compete with essential ions and prevent them from fulfilling their biological functions. For instance, Pb replaces Ca in bones (Pounds, 1983).

One of the sources of food contamination by metal includes techniques and materials used in food processing (Dabonne *et al.,* 2010). The domestic preparations of food as a potential source of metal contamination have been overlooked for long time. However, there are reports that indicate that certain kitchen utensils used for food preparation can represent a significant risk because they are manufactured with materials that can be hazardous or contaminated by toxic metals (DM *et al.*, 1985; Hight, *2001*).

The problem with the presence of metals in kitchen based equipments lies in the fact that these contaminants can be transferred to food and beverages by a leaching process, which is again influenced by physical and chemical conditions of the food, such as boiling duration and pH (zález *et al.*, 1996; Baba *et al.*, 2004; Dadd, 2009).

Various kinds of utensils are hand crafted and used for the preparation, storage and consumption of foods. These utensils can be source of metal contamination since some of the raw materials used in their manufacturing contain heavy metals, such as clay and enamel (Dabonne *et al.,* 2010). The use of these utensils can release toxic metals into food substances during processing, sometimes in amounts high enough to constitute health hazard (Dadd, 2009). For instance, studies conducted by Dabonne *et al.,* (2010) in Cote d'Ivoire shows that traditional utensils made out of clay and traditional Aluminum pots are the potential sources of metals like aluminium and iron. Another study by Omolaoye *et al.,* (2010) in Nigeria also shows that food items and beverages prepared in ceramic products imported from China are likely to show high levels of lead.

Similarly, cottage industries in our country are producing cooking utensils from metallic Scraps. Therefore, the present study was aimed at determining the leaching of selected metals from unstandardized cookwares.

## 2. OBJECTIVES

### 2.1. General objective

To determine the leaching of selected metals from locally made cooking pot into the food matrix during preparations

### 2.2. Specific Objectives

- To evaluate the natural metallic content of the various food ingredients
- To determine the leaching of some selected metals (Al, Fe, Ni and Pb) from traditional cooking pot
- To determine the influence of pH, cooking duration and cooking frequency on metal leaching

# 3. LITERATURE REVIEW

## 3.1. Metals

Metals play significant biochemical and physiological roles in living organisms, and they are recognized as essential elements for life. However, some metals are toxic to the body and others could cause a health risk if present in excess (Simkiss and Mason, 1983). Concentrations of several toxic metal and metalloids have been largely increased as a result of human activities and lead to threat for the health of plant and animals (Duruibe *et al.*, 2007).

## 3.2. Classification of Metals

The most appropriate manner to classify metals of interest by their impact on health effects are: nutritionally essential, nonessential but with a possible beneficial effect, or nonessential with no beneficial effects (Gloub *et al.*, 2004).

Nutritionally essential metals: Metals that are generally regarded as nutritionally essential for humans are cobalt (Co), chromium III (Cr III), copper (Cu), iron (Fe), molybdenum (Mo), selenium (Se), and zinc (Zn) (Goyer and Clarkson, 2001).

Metals with No Known Essential or Beneficial Effects: The most potentially toxic metals in the environment are arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg) as well as their inorganic compounds. They have no known nutritional or beneficial effects on human health but they are present in air, water, and soil, so that some level of exposure is not readily preventable. Other metals of concern to Environmental Protection Agency (EPA) include aluminum (Al), antimony (Sb), barium (Ba), beryllium (Be), silver (Ag), strontium (Sr), and thallium (Tl). These metals have many industrial uses, which increases the probability of human exposure. Industrial activities may also convert the metallic forms of the metals to compounds that may be more soluble in various media, with a resultant increase in risk for exposure and toxicity. Because these metals have no known essential or beneficial effect, guidelines for regulatory activity might limit human exposure to the lowest level known to have a possible adverse health effect (Goyer, 2001).

Metals That May Have Some Beneficial Effect: Some metals are not known to be essential to human health at larger dose but may have some beneficial effects at low levels of exposure. These include silicon, nickel, boron, and vanadium. Some have said arsenic may have beneficial effects (NAS/NRC, 1999), but a recent critical review does not support this view for human exposure (NAS/IOM, 2003). Even though, the possible beneficial metabolic functions of As for humans have not been established, carcinogen at extremely low levels of exposure, some organic arsenic compounds have been used as growth factors in different animals; (NAS/NRC, 1999). boron (B), nickel (Ni), silicon (Si), and vanadium (V) have been shown to have biological functions in plants and some animals but essentiality for humans has not been demonstrated (NAS/IOM, 2003).

For some of the metals in this group, therefore, it must be concluded that there are no rigorously defined limits or levels that might have a particular beneficial human health effect, but upper safe levels are defined. In terms of a framework for assessment of metals and inorganic metal compounds, potential beneficial human health effects at low levels might be considered, but as yet these metals cannot be regarded as essential for humans. Also, one of the metals in this group, nickel, is regarded as a human carcinogen by inhalation.

*Carcinogenic Metals*: Due to their complex nature of metals' interaction in biological nature the following five transition metals emerge as an important class of human carcinogens in one form or another. These are —As, Cd, Cr VI, Be, and Ni—are accepted as human carcinogens (NTP, 2002). This is probably the result of similar binding preferences between a carcinogenic metal and nutritionally essential metals (Clarkson, 1986).

Table 1. Summary of Classification of Metals Based on Characteristics of Health Effects (Source: EPA, 2004)

| Nutritionally essential metals | Metals with possible beneficial effects | Metals with no known beneficial effects |
|---|---|---|
| Cobalt | Boron | Aluminum |
| Chromium III | Nickel | Antimony |
| Copper | Silicon | Arsenic |
| Iron | Vanadium | Beryllium |
| Manganese | | Cadmium |
| Molybdenum | | Lead |
| Selenium | | Mercury |
| Zinc | | Silver |
| | | Strontium |

### 3.3. Sources of Metals in Food

The presence of metals in food in our days are caused by direct contamination during production as well as from the use of pesticides or fertilizers (Fishbein, 1981; Arora *et al*., 2008), industrial processing, during transport, or during storage (Wang *et al*., 2006). The domestic preparation of food as a potential source of metal contamination has been afforded little importance. However, there are reports that indicate that certain kitchen utensils used for food preparation can represent a significant risk because they are manufactured with materials that can be hazardous or contaminated by toxic metals (Wallace *et al*., 1985). Generally, metals are introduced into the environment either by natural means or human activities (Anthropogenic).

Natural sources: excessive levels of trace metals may occur naturally by geological phenomena like volcanic eruptions, weathering of rocks (acid rock drainage) and leaching into rivers, lakes and oceans due to action of winds and precipitational erosion and find their way into plants and animals as a result of direct input (Jones and Denton, 1984).

Anthropogenic sources: In modern times, anthropogenic sources of trace metals, i.e. pollution, have been introduced to the ecosystem. People have been exposed to metals in the environment. Metallic constituents of pesticides and therapeutic agents are additional sources of hazardous exposure. The burning of fossil fuels containing trace metals, the increase in industrial applications of metals, such as metal plating factories, mining industries, tanning, dye and chemical manufacturing industries, etc., have made heavy metal poisoning a major source of environmental pollution (Pacyna & Pacyna, 2001).

### 3.4. Toxicity Mechanism of Metals

Metals are known to disturb redox homeostasis by stimulating the formation of free radicals. A free radical is any atom (e.g. oxygen, nitrogen) with at least one unpaired electron in the outermost shell, and is capable of independent existence. A free radical is easily formed when a covalent bond between entities is broken and one electron remains with each newly formed atom (Karlsson, 1997).

Free radicals are highly reactive due to the presence of unpaired electron(s) and disturb redox homeostasis. Free radical involving oxygen can be referred to as reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), superoxide radicals ($O_2^{\bullet -}$), hydrogen peroxide ($H_2O_2$), and hydroxyl radicals ($^{\bullet}OH$) (Stohs & Bagchi, 1995) and methylglyoxal (MG), a cytotoxic compound, also found to increase in response to various stresses (George & Goyer, 1978; Stohs & Bagchi, 1995).

Inhibition of Enzymatic Activities: metals such as Pb, Hg and Cd have affinity for sulphur and therefore attack sulphur bonds in enzymes, thus immobilizing them. Other site of attack include the free amino ($-NH_2$) and carboxyl (-COOH) groups in protein (Alka, 2000).

Attacks on Cell Membrane and Receptor: The heavy metals bind to cell membrane and receptor, thereby altering their structures. This affect transport and other inter or intra cellular processes in the body. Cd inhibits oxidative phosphorylation in the body (Shukla & Singhal, 1984).

Interference with Metabolic Cations: Heavy metals interfere with the metabolism of essential cations such as absorption, transportation, decomposition and storage. Cd follows the pathway of Zn and Cu metabolisms. Pb replaces Ca in bones (Habermann *et al.*, 1983). This is the most crucial aspect of metals that leads to manifestation of toxic effect.

Action on the Artery: Heavy metals can increase the acidity of the blood. The body draws Ca from the bones to help restore blood pH. Further toxic metals set up conditions that lead to inflammation in arteries and tissues, causing more Ca to be drawn to the area as a buffer. The Ca, coats the inflamed area in the blood vessel but creating another by the hardening of the artery walls and its progressive blockage of the arteries. This leads to osteoporosis (Berglund *et al*., 2000)

## 3.5. Characteristics, Sources and Health Effects Some Metals

### 3.5.1. Aluminium

Aluminium is the third most abundant and widespread element in the earth crust. Inorganic compounds of Al normally contain Al (III). The mechanical strength of pure Al is low. However, the high ductility and malleability properties make Al to be used in alloys (Beliles, 1994). Due to its reactive nature Al is not found free in nature. Many of its natural-occurring compounds are insoluble at neutral pH. For instance, the concentration of Al in water is < 0.1 mg/l (Beliles, 1994).

Food is one of the source of Al. Al concentration in unprocessed food can contain 0.1-20 mg/kg. About 0.1 mg/kg of Al is found in unprocessed aliments like egg, apple, raw cabbage, corn, and cucumber (Agency for Txic Substances and Disease Registry (ATSDR), 1997) and 4.5 mg/kg in tea powder (Pennington and Jones, 1989). Much higher values are found in some industrially processed foods with aluminum salts added as food additives. However, in the EU e.g. the use of aluminum salts as food additives is limited to certain products like scones but it is accepted for decoration of confectionary (Directive 95/2/EC). Al is also used in medical therapy in amounts up to 5 mg/person/day (Codex, 1995; Elinder & Sjögren, 1986).

Significant amount of Al can be transferred into the food through food contact materials contact materials such as saucepans, Al lined cooking utensils, coffee pots, and packaging products such as food trays, and cans (Elinder and Sjögren,1986; Codex,1995). Aluminium compounds used in pigments of utensils also the other sources of Al contamination in the food (Elinder and Sjögren, 1986).

Al metal and its alloys develop a thin film of aluminium oxide ($Al_2O_3$) almost immediately when it exposed to air. The formation of colorless, tough and non-flaking $Al_2O_3$ film helps the material to be high resistant to corrosion and preventing further oxidation or chemical reaction (Beliles, 1994). Nonetheless, few chemicals can dissolve it. For instance; pure Al is attacked by most dilute mineral acids. At neutral pH, aluminum hydroxide has limited solubility. However, the solubility increases markedly at pH below 4.5 and above 8.5 (Elinder and Sjögren, 1986).

Al can migrate in to the foodstuff unless the material is glazed (coated) appropriately (Burleson, 2003). Uptake of Al from uncoated food contact materials depends to a large extent on the acidity of the food. High salt concentrations (over 3.5% NaCl) can also increase the migration. Use of uncoated Al sauce pans, aluminum-lined cooking utensils and containers may increase the content of Al in certain types of foodstuffs, especially during long-term storage of strongly acidic or salty and liquid foodstuff (Pennington and Jones, 1989). Al was previously regarded as non-toxic (Metwally and Mazhar, 2007). However, Al as a chelator has the ability to capture and prevent the uptake of essential elements and thereby disrupt the proper use of many of them such as Ca, Zn or Cu (Couzy and Mareschi, 1988).

Al is not known to be of any nutritional value to human beings. However, over the past 20 years more evidence has come to light to link Al with certain neurodegenerative diseases (Gray, 2002). Al affects skeletal and bone tissue as well as the neural system (Sedman *et al.*, 1987). Some of the diseases implicated in Al toxicity include osteomalacia, Fe inadequate microcytic anemia, Alzheimer's disease and a number of respiratory allergic effects (UNEP *et al.*, 1997).

Even though, the mechanism by which this toxicity is exerted has not been fully established, some literature describes the formation of free radical and disruption of cell metabolism leading to cytotoxicity, lipid peroxidation, and changes in serum essential elements (Metwally and Mazhar, 2007).

### 3.5.2. Iron

Iron is the fourth most abundant element and it constitutes about 5% of the earth's crust. The principal ions of Fe are ferrous, $Fe^{2+}$ and ferric, $Fe^{3+}$. Fe is a mineral present in many foods naturally, added to some food products, and as a dietary supplement (Beliles, 1994). Fe is an essential mineral necessary for the production of heme of hemoglobin in the blood for the transfer of oxygen from the lung to the tissues, myoglobin that provides oxygen to muscles, and supports metabolism (Erdman *et al*., 2012). The iron-sulfur proteins are another important group of iron-containing proteins. Fe-S cluster are found in a variety of metalloproteins, such as the ferredoxins, as well as NADH dehydrogenase, hydrogenase, coenzyme Q- reductase and nitrogenase (Lippard, 1994). Fe is also necessary for growth, development, normal cellular functioning, and synthesis of some hormones and connective tissue (Erdman *et al*., 2012).

Under normal conditions the body contains about 4 g of Fe (Beliles, 1994). Hemoglobin contains the greatest amount of body Fe (67%) and this is largely in the red blood cells (Bellies, 1994). Much of the remaining iron is stored in the form of ferritin or hemosiderin (a degradation product of ferritin) in the liver, spleen, and bone marrow or is located in myoglobin in muscle tissue (Abbaspour *et al*., 2014)

The principal dietary sources of Fe are classified into two main forms: heme and non-heme depends on their bioavailability. Plants such as green leafy vegetables, whole grains, cereals and iron-fortified foods contain non-heme iron only, whereas meat, fish, and poultry contain both heme and non-heme iron tissue (Erdman *et al*., 2012). In general, to the order of 30-150 mg/kg Fe concentrations are available in liver, kidney, beef, ham, egg yolk, and soybeans. Iron deficiency is generally acknowledged to be the most common, single nutritional deficiency in both developing and developed countries (Pettersson & Sandstrum, 1995). Iron deficiency affects around 500 million people around the world. The consequences of iron deficiency can range from anemia to mental retardation in growing children (Valko *et al*., 2005).

Fe overload is a less frequent condition, and involves defects in Fe absorption, transport and secondary iron disorders. Toxicity and chronic iron toxicity is a status that can be associated with: (i) primary haemochromatosis, a genetic disorder related to increased intestinal absorption of iron, (ii) high dietary iron intake and (iii) frequent blood transfusions. Cases of acute Fe toxicity are relatively rare and mostly related to hepatoxicity (Tenenbein, 2001). A high content of Fe has been associated with several pathological conditions, including liver and heart disease (Rasmussen *et al*., 2001), cancer (Valko *et al*., 2001), neurodegenerative disorders (Berg *et al*., 2001), diabetes (Lao and Ho, 2004), hormonal abnormalities (Fraga and Oteiza, 2002), and immune system abnormalities (Walker, 2000).

### 3.5.3. Lead

Lead is a bluish-white metal which occurs naturally in harmless trace amount of soil, rock and water. The principal ions of Pb are $Pb^{2+}$and $Pb^{4+}$. According to Agency for Toxic Substances and Disease Registry (ATSDR, 2005), Pb is the second to As leading toxic heavy metal. It is a very soft metal and was used in pipes, drains, and soldering materials for many years. Millions of homes built before 1940's still contain Pb (e.g., in painted surfaces), leading to chronic exposure from weathering, flaking, chalking, and dust. Every year, industries produce about 2.5 million tons of Pb throughout the world. Most of it is used for batteries. The remainder is used for shielding, crystal glass production, and pesticides (ATSDR, 1993).

According to 'code of practice for the prevention and reduction of Pb contamination in foods': Chronic exposure to Pb at relatively low levels can result in damage to the kidneys and liver, and to the reproductive, cardiovascular, immune, hematopoietic, nervous, and gastrointestinal systems (Schwartz, 1994). Short-term exposure to high amounts of Pb can cause gastrointestinal distress, anemia, encephalopathy, and death. Young children are particularly vulnerable to Pb poisoning because of their tendency to put chips of peeling paints into their mouth or sucking of fingers contaminated with lead-laced dust. Studies revealed that children absorb Pb into their bodies at a higher rate than adult just as children naturally absorb more nutrients from their diet. They absorb more toxicant than a mature body could flush away. The most critical effect of low-level Pb exposure is reduced cognitive and intellectual development in children (Lanphear *et al*., 2005).

The effect of Pb differs with age, gender and nutritional status. Those with malnutrition, anemia and colic etc are prone to Pb poisoning with symptoms like low intelligent quotient (IQ) (Munoz *et al.*, 1993), impaired speech and hearing, hyperactivities, decreased verbal activities, decrease learning and memory activities. Higher doses generate fatigue, lethargy, abdominal discomfort, irritation, headache, vomiting and constipation (Bellinger *et al.*, 1994).

### 3.5.4. Nickel

Nickel is the 24$^{th}$ most abundant element, which can exist in valence states of 0, +1, +2 and +3 and form a varity of compounds primarily as oxides and sulfides (Poonkothai and Vijayavathi, 2012). Ni naturally occurs as one constituent of soil, and also emitted from volcanoes. The Ni concentration in soil and drinking water ranges from 10–1000ppm and 6 μg/l respectively. The level of Ni in seawater and river is approximately 0.5-2 ppb and 0.3 ppb respectively (Poonkothai and Vijayavathi, 2012).

Ni is probably an essential element for organisms (ATSDR, 1995) and found in small quantities in many foodstuffs and vegetables usually contain higher concentration of nickel than other food items do; higher levels have been found in legumes, spinach, lettuce and nuts which is up to 0.8 mg/kg (National Food Agency of Denmark, 1995; Grandjean, 1983). In the diet it is found as complex bound $Ni^{2+}$ -ions (Codex, 1995).

Due to the strong corrosion-resistance Nickel-containing stainless steel equipments such as kitchen utensils, processing equipments and food transportation tracks are the most important food contact material (Beliles, 1994). Certain products, such as baking powder and cocoa powder, have been found to contain excessive amounts of nickel, perhaps related to nickel leaching during the manufacturing process (Grandjean, 1983). Leaching or corrosion processes may contribute significantly to the oral nickel intake, occasionally up to 1 mg/day. Soft drinking-water and acid beverages may dissolve $Ni^{+2}$ from pipes and containers (Salniko *et al*., 2003). Scattered studies indicate a highly variable dietary intake of Ni, but on average it is about 200-300 micrograms/day (Grandjean, 1983).

Nickel plays a major role in helping the body to absorb the iron it needs. Additionally, Ni helps to prevent conditions such as anemia and helps towards building strong skeletal frames by strengthening bodies. Ni is also presente in DNA and RNA which means it is found in every cell of the human body. It assists in breaking down glucose, helps in creating energy for daily use and even contributes to the production of certain enzymes that initiate important chemical reactions such as the development of nucleic acids (Shirali *et al.*, 1991).

Ni deficiencies are rare and people at a high risk of developing Nickel deficiency are people suffering from kidney problems, cirrhosis of the liver or Vitamin B6 deficiency. Symptoms of Ni deficiency can range from urinary tract infections to severe allergic reactions, most often seen in the form of skin rashes. In severe cases, Ni deficiency may result in paralysis alongside inflammation of the liver and lungs (Anke, 1995).

Although Ni is vital for the function of many organisms, concentrations in some areas from both anthropogenic release and naturally varying levels may be toxic to living organisms. Ni induces embryo-toxic and nephrotoxic effects, allergic reactions and contact dermatitis (EPA, 2002). Ni sensitization also occurs in general population from exposure to coins, jewellery, watchcases, and clothing. It causes conjunctivitis, eosinophilic pneumonites, asthma and local or system reactions to nickel containing prostheses such as joint replacement, cardiac valve replacements, and cardiac pacemaker wires and dental inlays (Hostynek and Maibach, 2002).

In addition, skin contact to a multitude of metal objects may be of significance to the large number of individuals suffering from contact dermatitis and Ni allergy. Finally, Ni alloys are often used in nails and prostheses for orthopaedic surgery, and various sources may contaminate intravenous fluids. Thus, human Ni exposure originates from a variety of sources and is highly variable. Occupational nickel exposure is of major significance, and leaching of Ni may add to dietary intakes and to cutaneous exposures. Preventive efforts should mainly be directed towards adequate control of these exposure sources (Grandjean, 1983)

### 3.6. Metals Leaching from Utensils

Leaching of toxic elements from cooking utensils is a long recognized problem, and covers a wide range of elements, the traditional examples include materials such as Pb (mainly from earthenware) and Cu (typically from coffee pots with defective tin-plating) ( Baba *et al.*, 2008). The problem with the presence of metals in kitchen based equipments lies in the fact that these contaminants can be transferred to food and beverages by a leaching process, which is directly related with the physical and chemical conditions of the food, such as temperature and pH (Murphy and Müller, 1996).

Al is used in the manufacture of domestic cooking utensils, because it can be bent or pressed into various shapes. Its conductivity for heat is useful in cooking vessels (Minamida, 1985). Al metal and its alloys develop a thin film of $Al_2O_3$ almost immediately when exposed to air. The formation of colorless, tough and non-flaking $Al_2O_3$ film provides a relatively high resistance to corrosion and preventing further oxidation or chemical reaction (Beliles, 1994). However, uncoated and un-anodized Al can react with acidic foods to change the taste of the food and acts as a chelator in human physiological system. Studies suggested that 10 to 15 minutes of boiling of tap water with aluminum pan can result in Al migration into the foodstuff to the level of 1.5 mg/l depending on the acidity of the water and the chemical composition of the aluminum utensils (Gramiccioni *et al*., 1996; Nagy *et al*, 1994) but studies conducted by Liukkonen-Lilja and Piepponen (1992) have reported that values can goes up to 5mg/l.

Fe is used in a great variety of kitchen utensils together with some other metals like Cr, Mn, and Mo as well as Ni. Also, iron as cast iron is used for pots and pans (Elinder, 1986). Cast iron can chemically react with high acid foods such as wine or tomatoes and enhance the uptake of iron (Livingston, 1991). In addition, one of the novel strategies to control Iron Deficiency Anemia (IDA) is preparation of foods using iron cooking utensils (Adish *et al*., 1999).

Stainless steel is an iron alloy containing a minimum of 11.5% Cr. Blends containing 18% Cr with either 8% Ni, called 18/8, or with 10% Ni, called 18/10, is commonly used for kitchen equipment. Stain less steel equipments are resistant to corrosion, non-reactive with alkaline or acidic foods, and resistant to scratching and denting (Truman, 1976). However, longer use of this chromium metal forms a layer of $Cr_2O_3$ over the steel when exposed to $O_2$. This layer is impervious to water and air, protecting the metal beneath. This layer quickly reforms when the surface is scratched, so when food or beverage comes in contact with stainless steel, Cr contamination occurs. The release of $Ni^{2+}$ form Ni containing stainless steel cooking pots is generally < 0.1 mg/kg (Kuligowski & Halperin, 1992).

Clay or ceramics may be considered to be materials made from naturally occurring clay or earth. Scientifically, these utensils are compounds of metallic and non-metallic elements. Such utensils are produced either industrially or handcrafted as a cottage industry. Due to the fact that, metals are natural components of the earth crust and used as decorative and protective enamel, these utensils have a great chance to be contaminated. Food items and beverages prepared in such utensils are likely to show high levels of Pb (Lenntech *et al*., 1998). Some ceramic food wares have been found to leach significant quantities of Pb from the potential food contact surfaces (Sheets, 1997).

### 3.7. Factors leading to the Enhancement of Leaching of Metals from Utensils

Metals can accumulate in foods stored or cooked in uncoated metallic utensils. The amount and types of metal that accumulate in foods during preparation depend on the pH of the foods, the length of cooking periods and the types of utensils. Acidic foods had a great ability to transfer metals into the food according to a research conducted in Mexico by Valadez-Vega, 2011, examining containers obtained from four regions' in the Mexican state of Hidalgo.

Another study on leaching of heavy metals from traditional clay pots showed high concentration of heavy metals during preparation of tomato sauce and boiling of 4% acetic acid (leachability test provided by Food and Drug Administration (FDA)). From these point of view acidic foods and temperature have a great influence on the leach ability of metals from cooking ware. Acidic foodstuffs such as tomatoes, cabbage, rhubarb and many soft fruits are among the most that frequently take-up more Al from the containers (Lenntech *et al*., 2009).

The other influential factors for the migration of metals into foodstuff are temperature and storage. In a migration study with 3% acetic acid (Gramiccioni *et al*., 1996), the migration was approximately 10 fold higher at 40°C compared to 5°C after 24 hours. Typical values for migration of Al from foil were < 0.05 $mg/dm^2$ at 5°C and correspondingly 6 $mg/dm^2$ at 40°C. However, after 10 days, the migration was considerably higher: 0.5 $mg/dm^2$ at 5°C compared to 96 $mg/dm^2$ at 40°C (Gramiccioni *et al.*, 1996).

The other factor that greatly contributes to the migration of metals from utensils into the food is cooking time. Greger *et al*., (1985) reported that, tomatoes heated in aluminum pans for a few minutes accumulated only 0.02- 0.03 mg Al/100 g serving, while tomato sauces cooked for 3 hours in aluminum pans accumulated 5.7 mg Al/100g serving. Greger (1985) estimates that the amount of Al added to the daily diet through the use of aluminum pans, tray, and foil during food and beverage preparation to be about 3.5 mg/day.

### 3.8. Safety Aspects of Utensils

Incompliance with Article 2 of Directive 89/109/EEC metallic materials under normal and foreseeable conditions, should meet the following conditions: They should be manufactured in accordance with good manufacturing practice and they should not transfer their constituents to foodstuffs in quantities, which could: endanger human health and/or bring about unacceptable change in the composition of the foodstuffs or deterioration in the organoleptic characteristics. In principle, the maximum quantity of metals which can be absorbed by individuals from all sources should not exceed daily or amount of weekly the TDI's and the PTWI's (multiplied by a bodyweight of 60 kg) established by JECFA or the Scientific Committee for Food of the EU (SCF) or any other international bodies.

### 3.8.1. Aluminium

According to JECFA (1989) established the Provisional Tolerable Weekly Intake (PTWI) is 7 mg/kg bodyweight for the total intake, including food additive uses of aluminum. Even if there is no health based guideline a certain value is recommended by WHO (1993) : Its directive 98/83/EC intended to set a standard value of 0.2 mg/l ion in drinking water for human consumption considering the practical use of aluminum for treatment and discoloration of distributed water can be seen as a guideline.

According to the Agency for Toxic Substance and Disease Registry (1997), exposure to aluminum is usually not harmful. Al is excreted by the kidneys, and only a small amount of Al is absorbed (JECFA, 1989). However, soluble aluminum salts are more easily absorbed. Patients with impaired renal function treated by dialysis could show a higher aluminum blood level. In the past, some of these dialyzed patients have shown neurological symptoms of Al intoxication due to an inappropriate treatment that is no longer used; these symptoms have sometimes been mistaken for those of Alzheimer's disease. WHO (IPCS, 1997) has concluded that Al is not the origin of Alzheimer's disease.

### 3.8.2. Iron

Iron is an essential trace metal (JECFA, 1983). Concerning health, Fe is mainly a deficiency problem and not a toxicological problem. Iron deficiency is generally acknowledged to be the most common, single nutritional deficiency in both developing and developed countries (Nordic Council of Ministers, 1995). JECFA (1983) has established a PMTDI at 0.8 mg/kg bodyweight. The value applies to Fe from all sources except for iron oxides used as coloring agents, supplemental Fe taken during pregnancy, lactation and for specific clinical requirements. The value is 8 times lower than the acute toxic dose (guidelines on metals and alloys used as food contact materials, 2002).

### 3.8.3. Lead

Due to the low safety factor, all use of lead in food contact materials should be abandoned or avoided**.** JECFA (1993), has established a PTWI at 0.025 mg/kg bodyweight. SCF at its 91$^{st}$ meeting held on December 9-10, 1993 agreed with the JECFA conclusion. Estimated daily dietary intake for adults range from 0.015-0.1mg, depending on the composition of the diet and where the consumer lives (Codex, 1995). According to the guidelines on metals and alloys used as food contact materials (2002), Parts made wholly or partly of lead and lead solder for repair should not be used in materials and articles intended to come into contact with foodstuffs including the use of lead in soldered cans and utensils.

### 3.8.4. Nickel

Although there is no specific evaluation on Ni, it seems that soluble Ni released from contact material is more readily absorbed than complex-bound Ni from food. Therefore, the contamination of aliment and drink from Ni containing food materials, e.g. kitchen utensils and electric kettles should be reduced (guidelines on metals and alloys used as food contact materials, 2002).

JECFA has not evaluated nickel. WHO (1997) has given a TDI of 0.005 mg/kg bodyweight. However, the migration of Ni to food stuffs should be as low as reasonably achievable and no more than: 0.1 mg/kg as a general limit of migration into foodstuffs and 0.05 mg/L from electric Kettles (NiDI, 1994). In the case of stainless steel, these values can safely be reached if, before initial cooking (first use of new items), the food contact items are exposed to boiling water and the water is discarded (guidelines on metals and alloys used as food contact materials, 2002).

# 4. MATERIALS AND METHODS

## 4.1. Study Design

In a laboratory based work, the leaching of Al, Fe, Pb and Ni from the repeated use of the traditional cooking pot "*Senegal Dest*" were analyzed using FAAS by preparing two kinds of food tomato sauce and *Shiro wot* (It's a homogenous legume based Ethiopian stew whose primary ingredient is powdered chickpeas or broad bean meal (*Shiro* powder). It is often prepared with the addition of minced onions, garlic (optional) and, depending upon regional variation, ground ginger or chopped tomato and chili-peppers (Selinus, 1971)). The same sauces were also cooked in a beaker to control the effect of metal leaching from the cookware. Further, to determine the effect of pH and boiling duration on leaching of metals, the method described by Semwal *et al.*, (2006) was used with some modification. Briefly, Sodium citrate buffer (pH 4.0) and Tris buffer (pH 7.0 and 9.0) was boiled for 2 hr. and 30 ml aliquot of samples from the buffer solution were taken for analysis. In addition, to see the effect of boiling duration on metal leaching from the cooking pot, samples were regularly drawn every 12 minutes for the first one hour and every 30 minutes for the next one hour of boiling and their metal (Al, Fe, Pb and Ni) content was determined.

Sample preparation for instrumental analysis and moisture quantification value was done at research laboratory of the Center for Food Science and Nutrition, and Department of Chemistry of Addis Ababa University. The determination of metal level was done at the Center for Environmental Science, Addis Ababa University.

## 4.2. Collection of Cookware

Traditional cooking pots (*Senegal Dest*) were purchased from five different utensil markets located in Taiwan, Dire Dawa. All the pots were washed with detergent and a commercial dish scrubbing fiber (Scotch Brite), simulating everyday use. The cooking pot was rinsed with deionized water and air dried for 24 hr (following the laboratory conditions established by De Mejía and Craigmill, 1996).

Figure 1. *Senegal Dest* display in the market

## 4.3. Sample Preparation

### 4.3.1. Sauce Preparation

Foods (*shiro wot* and tomato sauce) were cooked in new traditional pots to determine the leaching of metals. Parallelly, the same types of sauce were prepared in a glass beaker as a control with the assumption of no leaching. In addition, the same foods were also cooked repeatedly in the same cookware to determine the leaching of metals during the repeated use. The metallic (Al, Fe, Ni and Pb) contents of the ingredients for the preparation of the sauce were also determined.

*Shiro wot*: a traditional Ethiopian dish based on the proportion stated on Federal Democratic Republic of Ethiopia Ministry of Health; Nutrition Extension Package, (2003) were prepared. Briefly, the sauce was standardized and prepared by cooking a mix containing 60gm of onion (*Allium cepa*), 25 ml of oil and 125g of *Shiro* powder and 1lt. of deionized water in an open pot for 1hr and 50 min. pH value of the sauce was recorded at the end of cooking.

Tomato Sauce: The sauce was standardized and prepared by mixing 130g of onion (*Allium cepa*), 60ml of oil and 30g of Berbere (*Piper nigrum mixed* with different spices), 300g of tomato (*Lycopersicon esculentum*) and 150ml of deionized water in an open pot for 1hr and 30 min. pH value of the sauce was recorded at the end of cooking.

Ingredients: Samples of the ingredients used to cook the foods were taken for wet digestion and analyzed for the presence of metals. These are; Berbere (*Piper nigrum* based mixture of different spices), Red onion (*Allium Cepa*), *shiro* powder (Legume based Mixture), Tomato (*Lycopersicon esculentum*).

To compare and decide whether the metals are from the pots or ingredients the method described by Odularu *et al*., (2013) was used. Briefly, both the *shiro wot* and tomato sauces were also prepared in a beaker using the same procedure as used for traditional pot.

### 4.3.2. Preparation of Buffer Solution

To study the effect of pH on leaching of the metals, various buffer solutions (pH 4.0, 7.0 and 9.0) were prepared. These solutions (1200 ml each) were individually boiled in different pots for 2 hr., 5 aliquots of 30 ml of the solutions were sampled at 12 min intervals for 1 hr. and another 3 samples were taken randomely for the determination of metallic (Al, Fe, Ni and Pb) content by Flame Atomic Absorption Spectrometry (Vario 6 spectrometer, Analytik Jena, Germany).

Preparation of sodium citrate buffer with a ph value of 4: The stock solution of each was prepared by mixing 21 g citric acid in 1 L distilled water, and 29.4 g sodium citrate in 1 L distilled water in a separate volumetric flask. From the stock, 82 ml of the citric acid solution with 18 ml of the sodium citrate solution were mixed and distilled water was filled up to 1 L in a volumetric flask. 1M sodium hydroxide (NaOH) was added to adjust the pH of the mixture to 4 while gently stirring the solution using a magnetic stirrer (Phillips, 2014)

Preparation of Tris-HCl buffer with a pH Value of 7 and 9: 121.14 gm of Tris base was measured in a 1 lt beaker and 750 ml of deionized water was added. The mixture was stirred for a minute with a magnetic stirrer until completely dissolved and 12 M HCl was added in to the beaker slowly until the solution reaches a pH value of 7. The same procedures were used to prepare Tris-Hal buffer with a pH value of 9 except that 12M HCl was added in to the beaker slowly until pH value of 9 (Helmenstine, 2014).

### 4.4. Laboratory Analysis

All glasswares were soaked overnight in 10 % (v/v) nitric acid and rinsed with deionized water and dried before using. The analytical grade chemicals including ultra pure $HNO_3$ and $H_20_2$ have been used during the entire analysis. The equipments were cleaned and made ready before use (AOAC 986.15).

### 4.4.1. Digestion of *Shiro Wot* and Tomato Sauces

The food sample needs to be brought into clear solution for analysis. Samples were digested according to the method described by the Association of Official Analytical Chemists (AOAC, 2006). Briefly, 1g of each sample was weighed into 250 ml conical flask, 10ml aquaregia ($HNO_3$ and HCl in a ratio of 1:3) was added, and the mixture was evaporated on a hot plate in a fume cup board until the black fume disappeared leaving the white fume. The resulting sample was then made up to 50 ml using distilled deionized water and then filtered into a clean universal bottle for atomic absorption spectrometric analysis (AOAC, 2006).

To extract and concentrate the heavy metals, AOAC method (AOAC, 1990) was used. In short, all the ingredients and the cooked food were homogenized thoroughly in a food blender with a plastic spoon. Triplicates of 1.00 ± 0.001 g of the homogenized *shiro wot* and tomato sauce was sampled in pre-washed acid sterilized and dried 100 ml round bottom flax. Mixed 10 ml $HNO_3$ and 2 ml $H_2O_2$ (Rankem, RFCL-New Dalhi, India) were added to the samples. Afterwards, they were digested on hot plate for four hours at 240 $^0$C in a digestion hood to vaporize the organic constituents till it changed to light yellow. Then the resulting solutions were cooled and filtered by Whatman filter paper in volumetric flask and made-up to 50 ml volume with distilled water.

### 4.4.2. Instrumental Conditions

Maximum absorbance was obtained by adjusting the cathode lamps at specific slit and wavelengths:

Table 2. Instrumental conditions

| Parameter | Wavelength (nm) | Slit width (nm) | Optimum working range (µg/ml) |
|---|---|---|---|
| Al | 309.2 | 0.5 | 0.03–250 |
| Fe | 248.3 | 0.2 | 0.06-15 |
| Ni | 232.0 | 0.2nm | 0.01-20 |
| Pb | 217 | 1 | 0.05-30 |

### 4.4.3. Determination of Level of Metals

The samples were each placed in a tagged plastic sampling bottle for the determination of the trace metals (Al, Fe, Pb and Ni) with atomic absorption spectrometry (Vario 6 spectrometer, Analytic Jena, Germany) in the Center for Environmental Science. Standards of these metals from stock solution were prepared at the concentration of 0, 1.25, 2.5, 5 and 10 mg/l and set for the calibration of the AAS reading and blank were run in each procedure for quality assurance. For the case of Al, Potassium Chloride (0.1%) was added to standards and samples to overcome interference from other alkali metals.

### 4.4.4. Moisture Content Determination

Moisture content of sauce was determined following the AOAC method (AOAC, 2005). Briefly, empty drying dishes made of porcelain were dried using a drying oven (Germany, Memmert) for 1 hour at 105 $^{0}$C. The dishes were cooled for 30 minutes in desiccators with granular silica gel and weighed using a digital analytical balance to the nearest milligram ($W_1$). Duplicates of about 5.000 g of fresh samples were weighed ($W_2$) in dried and pre-weighed drying dishes. The dishes and their contents were then placed in drying oven and dried for 5 hrs at $105^0$C. The dishes and their contents were cooled in desiccators to room temperature and weighed ($W_3$). The procedure was repeated until a constant weight was recorded. The results were calculated in fresh weight base except for leaching of metals in to the cooked food to minimize the moisture variability resulting in the preparation of the sauces.

**Moisture content is expressed as:**

$$\% \text{ Moisture Content} = (W_2 - W_3) / (W_2 - W_1) * 100$$

Where:

$W_1$=weight of the dish

$W_2$= weight of fresh sample and the dish

$W_3$= weight of dried sample and the dish

### 4.5. Statistical Analysis

Data analysis was computed using SPSS software version 16. Descriptive statistics like range, and arithmetic mean ± SD were used to express the level of each metal. One-way ANOVA was used to test the variability of the level of metals among the sauces and the buffer solutions. Two-way ANOVA was also used to identify the interaction of effect of pH and boiling duration. The level of significance was compared with tukeys' at $p < 0.001$. Calibration graphs and tables were drawn using Microsoft Office Excel 2010.

The different concentrations of metals obtained during analysis were converted in to mg/kg dry matter using the formula below.

Formula for conversion to mg/kg of dry matter (Osborne & Voogt, 1978)

$$\text{Mg/kg} = \frac{(b-a)*Vs}{Wt} + \frac{100}{100-c}$$

Where, b - Concentration of a sample

Vs - Volume of solution

Wt - weight of sample (fresh weight)

c – Moisture content

All values are expressed interms of dry bases except for results found in a buffer solution.

## 5. RESULTS AND DISCUSSION

To determine the elemental concentration using Atomic Absorption Spectrometry it is important to keep the linearity of concentration against absorbance curve to ensure the accuracy and reliability of the instrument (Martens, 1992). The calibration curves for Al, Fe, Ni and Pb were fairly linear (figure 2).

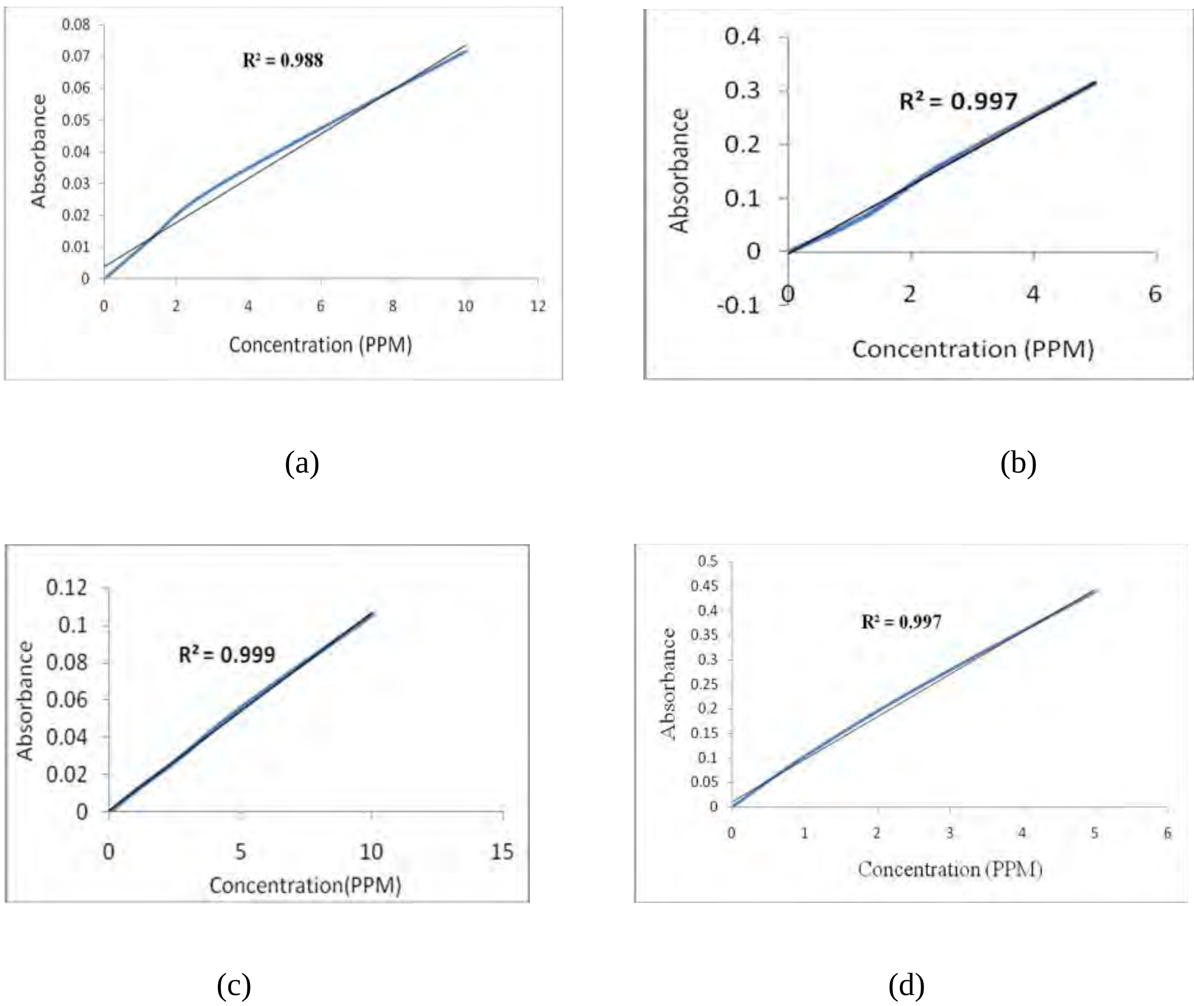



Figure 2. Calibration curves: Al (a), Fe (b), Pb (c) and Ni (d)

### 5.1. Metallic Content of Various Food Ingredients

The level of metals in different food ingredients used to make both *shiro wot* and tomato sauce is summarized in Table 3. As clearly indicated, in the table below Pb were found to be below the instrumental detection limit.

Table 3. The level of metals in various food Ingredients

| Food Type | Aluminium (mg/Kg) | Iron (mg/Kg) | Nickel (mg/Kg) | Lead (mg/Kg) |
|---|---|---|---|---|
| *Shiro* Powder | 39.15±0.3* | 30.51±0.4 | 3.42±0.7 | ND |
| Tomato | 19.81±0.1 | 15.2±0.2 | 0.92±0.1 | ND |
| Onion | 9.82±0.2 | 18.43±0.4 | 0.64±0.2 | ND |
| *Berbere* | 18.80±0.4 | 38.9±0.8 | 2.8±1.6 | ND |

* All values are means of Triplicate ± SD.

ND= Not Detectable

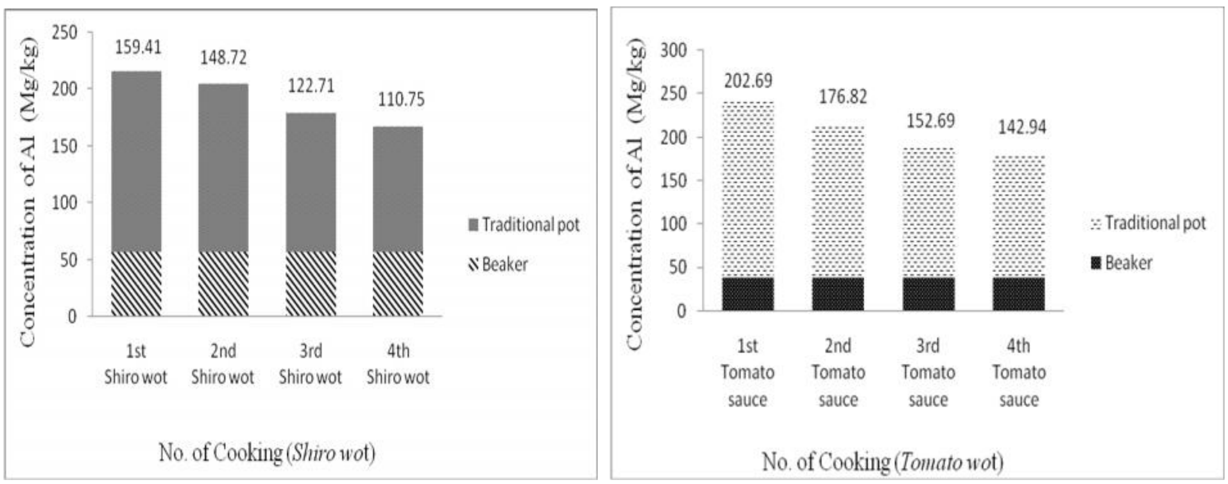
### 5.2. Leaching of Metals from the Traditional Cooking Pot into the Food

#### 5.2.1. Aluminium

Results on leaching of Al during the preparation of various foods in a traditional cooking pot and their repeated usage are presented in Figure 3. The Al level of *shrio wot* prepared in control (56.60±0.9 mg/kg) was significantly ($p<0.001$) different from first time (159.40±1.1 mg/kg), second time (148.72±1.5 mg/kg), third time (122.71±1.1 mg/kg) and fourth time

(110.75±1.9 mg/kg) cooking. *Shiro* samples from the third time and fourth time cooking were not significantly ($p>0.05$) different in their Al concentration (Appendix 3).

The control tomato sauce sample (prepared in a beaker) had a mean Al content of 58.38±0.3 mg/kg which was significantly ($p<0.001$) different from the mean Al content of tomato sauce prepared in the traditional cooking pot. The mean level of Al in tomato sauce decreased from the first cooking (203.68±2.03) to the fourth (142.94±2.7 mg/kg) (Appendix 4).




(a) (b)

Figure 3. Al concentration (mg/kg) in *shiro wot* (a) and tomato sauce (b)

The contribution of Al in different food ingredients to prepare both *shiro wot* and tomato sauce is summerized in Table 4.

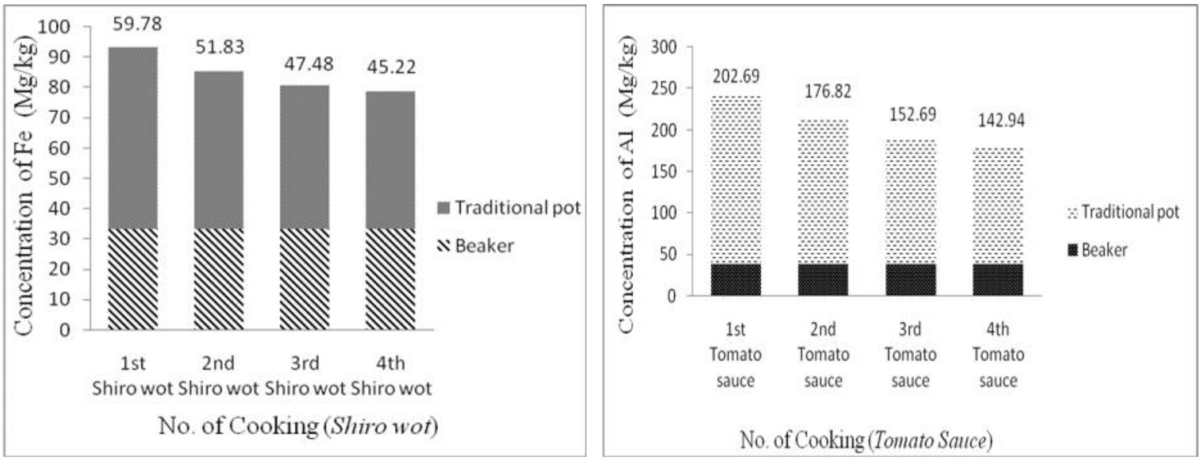
Table 4. Al concentration (mg/kg) in the ingredients of *Shiro wot* and tomato Sauce

| Ingredients | *Shiro Wot* | Tomato sauce |
|---|---|---|
| *Shiro* powder | 39.15±0.3 | - |
| Onion | 9.82±0.2 | 9.82±0.2 |
| Tomato | - | 19.81±0.1 |
| *Berbere* | - | 18.80±0.4 |

- Not used

#### 5.2.2. Iron

Results on leaching of Fe during the preparation of various foods in a traditional pot and their repeated usage are presented in the Figure 4. The Fe level of *Shiro wot* cooked in a control (33.44±0.2 mg/kg) was significantly lower ($p<0.001$) than first time (59.79±0.3), second time (51.83±0.5 mg/kg), third time (47.48±0.3 mg/kg) and fourth time (45.22±0.2 mg/kg) preparations. However, there was no significant ($p>0.05$) variation between the third and fourth time of cooking in a traditional cooking pot. Fe content in tomato sauce prepared in a beaker (55.07±2.1 mg/kg) were also significantly ($p<0.016$) different from that of tomato sauce prepared in a traditional pot at the first time (112.62±1.1 mg/kg), second time (109.79±3.4 mg/kg), third time (84.76±1.2 mg/kg) and fourth time (67.20±0.6 mg/kg) cooking. Nonetheless, there was no significant varation ($p>0.05$) in the third time (84.76± 1.2 mg/kg) and fourth time (67.20±0.6 mg/kg) cooking of tomato sauce in a traditional cooking pot (Appendix 5 & 6).




(a) (b)

Figure 4. Fe concentration (mg/kg) in *shiro wot* (a) and tomato sauce (b)

The contribution of Fe in different food ingredients to prepare both *shiro wot* and tomato sauce is summerized in Table 5.

Table 5. Fe concentration (mg/kg) in the ingredients of *Shiro wot* and tomato Sauce

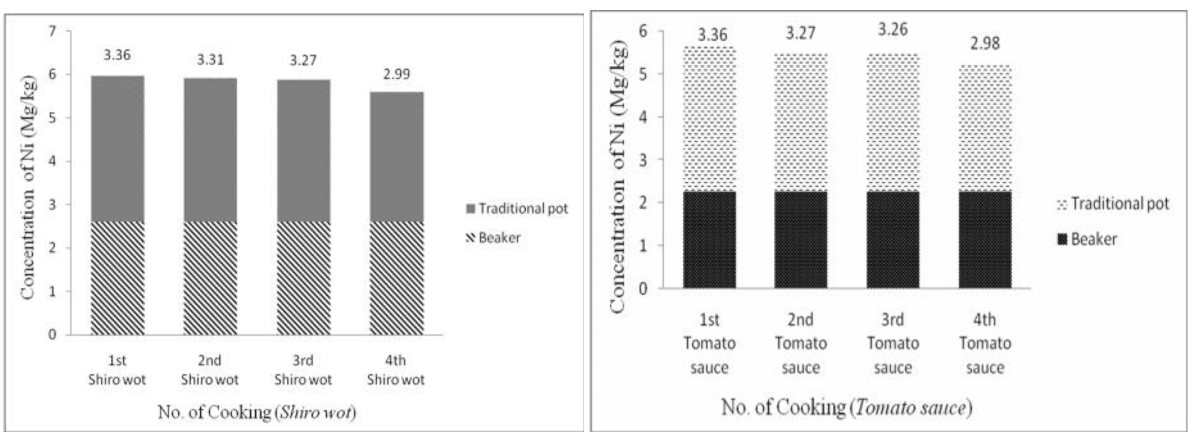
| Ingredients | *Shiro Wot* | Tomato sauce |
|---|---|---|
| *Shiro* powder | 30.51±0.4 | - |
| Onion | 18.43±0.4 | 18.43±0.4 |
| Tomato | - | 15.2±0.2 |
| *Berbere* | - | 38.9±0.8 |

- Not used

### 5.2.3. Nickel

The mean levels of Ni in *shiro wot* as well as tomato sauce samples are indicated in the Figure 5 below. There was no significant ($p>0.05$) variation between the mean Ni level of *shiro wot samples* cooked in traditional cooking pot (3.24±0.3 mg/kg) and the beaker (control)(

2.61±0.1 mg/kg). There was also no significant ($p>0.05$) difference in the mean level of Ni among *shiro wot* samples using the cooking pot for the first time (2.98±0.3 mg/kg), second time (3.27±0.6 mg/kg), third (3.33±0.2 mg/kg) and fourth time (3.36±0.2 mg/kg). About 0.76 and 0.39 mg/kg of Ni was contributed by the traditional cooking pot. From preparation of tomato sauce the highest concentration was recorded in the first cooking (3.36 ± 0.24 mg/kg) whereas, the lowest was at the fourth cooking (2.98 ± 0.25 mg/kg) accounting the inherent 2.26±0.1 mg/kg of Ni in the tomato sauce. The contribution of Ni from the cooking pot was 1.10±0.2 and 0.72±0.2 mg/kg from first cooking and fourth cooking, respectively (Appendix 7).




(a) (b)

Figure 5. Ni concentration (mg/kg) in *shiro wot* (a) and tomato sauce (b)

The contribution of Ni in different food ingredients to prepare both *shiro wot* and tomato sauce is summerized in Table 6.

Table 6. Ni concentration (mg/kg) in the ingredients of *Shiro wot* and tomato Sauce

| Ingredients | *Shiro Wot* | Tomato sauce |
|---|---|---|
| *Shiro* powder | 3.42±0.7 | - |
| Onion | 0.64±0.2 | 0.64±0.2 |
| Tomato | - | 0.92±0.1 |
| *Berbere* | - | 2.8±1.6 |

- Not used

### 5.3. Influence of pH on Metal Leaching

The leaching of metals with series of eight extractions, considering the pH and boiling duration, from the traditional cooking pot were analyzed for their Al, Fe, Ni and Pb level. Ni and Pb were below the instrumental detection limit.

It can be observed from Table 7 and Table 8 the pH medium (at pH 7 & 9) showed a statistical ($p<0.001$) difference in the level of both Al and Fe leaching from the traditional cooking pot. The pH=4 buffer solution has the highest ($p<0.001$) leaching of Al, within the levels of 6.63±1.7 to 32.4±2.7 mg/kg followed by pH=9 within the ranges of 3.82±1.4 to 23.6±2.1 mg/kg. At neutral pH, the mean leaching levels of Al were negligible.

Table 7. The effect of pH on Al (mg/kg) leaching from the cooking pot

| Duration of boiling (Min.) | pH – 4 | pH – 7 | pH – 9 | P-value |
|---|---|---|---|---|
| 12 | 6.6±1.7[a] | ND | 3.8± 1.4[a] | P>0.05 |
| 24 | 7.3±2.1[a] | ND | 5.4± 2.8[a] | P>0.05 |
| 36 | 9.8±2.1[a] | ND | 8.3± 3.5[a] | P>0.05 |
| 48 | 14.3±1.8[a] | ND | 10.7± 4.3[a] | P>0.05 |
| 60 | 17.2±1.6[a] | 1.58±1.5[b] | 14.7± 2.6[a] | P<0.001 |
| 75 | 23.3±1.6[a] | 1.83±1.3[b] | 16.8± 2.5[c] | P<0.001 |
| 90 | 28.3±1.4[a] | 2.53±1.2[b] | 17.8± 1.9[c] | P<0.001 |
| 120 | 32.4±2.7[a] | 3.55±0.9[b] | 23.6± 2.1[a] | P<0.001 |

Means not sharing a common superscript letter in the same row are significantly different. All values are means of Triplicate ±SD.

ND= Not Detectable

Table 8. The effect of pH on Fe (mg/kg) leaching from the cooking pot

| Duration of boiling (Min.) | pH – 4 | pH – 7 | pH – 9 | P value |
|---|---|---|---|---|
| 12 | 0.41±0.1 | ND | ND | |
| 24 | 0.80±0.5 | ND | ND | |
| 36 | 2.23±0.3$^{a}$ | ND | 0.26±0.1$^{b}$ | P=0.001 |
| 48 | 3.26±0.3 $^{a}$ | ND | 0.37±0.2$^{b}$ | P<0.001 |
| 60 | 3.76±0.7$^{a}$ | 0.80±0.1 $^{b}$ | 0.77±0.2$^{b}$ | P<0.001 |
| 75 | 3.81±0.8$^{a}$ | 0.4±0.3$^{b}$ | 1.6±0.2$^{b}$ | P<0.001 |
| 90 | 4.11±0.8$^{a}$ | 0.8±0.1$^{b}$ | 2.2±0.2$^{c}$ | P<0.001 |
| 120 | 4.25±0.8$^{a}$ | 1.43±0.4$^{b}$ | 2.80±0.4$^{c}$ | P<0.002 |

Means not sharing a common superscript letter in the same row are significantly different. All values are means of Triplicate±SD.

*ND= Not Detectable*

### 5.4. Influence of Boiling Duration on Metal Leaching

It can be observed from Table 9 the lowest (p<0.001) concentration of Al was recorded in the first 12 min. of boiling at pH value of 4 and 9. Whereas, the highest (p<0.001) concentration is recorded in the last time of boiling (120 min.).

The migration of Al from the cooking pot was approximately 5 times higher at 120 min. of boiling when it's compared with 12 min. of boiling. However, there was no leaching was observed for 48 mins at neutral pH and then negligible for even 2 hrs of boiling.

Table 9.The effect of boiling duration on Al (mg/kg) leaching from the cooking pot

| Duration of boiling (Min.) | pH – 4 | pH – 7 | pH – 9 |
|---|---|---|---|
| 12 | 6.6±1.7[a] | ND | 3.8±1.4[a] |
| 24 | 7.3±2.1[a] | ND | 5.4±2.8[a] |
| 36 | 9.8±2.1[a] | ND | 8.3± 3.5[ab] |
| 48 | 14.3±1.8[b] | ND | 10.7± 4.1[abc] |
| 60 | 17.2±1.6[b] | 1.6± 1.5[a] | 14.7± 2.6[bc] |
| 75 | 23.3±1.6[c] | 1.8± 1.3[ab] | 16.8± 2.5[bcd] |
| 90 | 28.3±1.4[d] | 2.5± 1.2[ab] | 17.8± 1.9[cd] |
| 120 | 32.4±2.7[d] | 3.6± 0.9[ab] | 23.6± 2.1[d] |

Means not sharing a common superscript letter in the same column are significantly different at ($P<0.05$). All values are means of Triplicate ±SD

ND = Not Detectable

It can be observed from Table 10 the highest content of Fe (4.25 ± 0.81 mg/kg) was recorded at the pH value of 4 for a boiling period of 120 mins. However, leaching of Fe (at PH 7 & 9) was below the instrumental detection limit for 48 min then showed increased trend afterwards.

The migration of Fe from the cooking pot was approximately 6 fold higher at 120 min. of boiling when it's compared with 12 min. of boiling. However, there was no leaching was observed for 24 min. in case of pH 9 and 48 mins. at neutral pH (Appendices 8-10).

Table 10. The effect of boiling duration on Fe (mg/kg) leaching from the cooking pot

| Duration of boiling (Min.) | pH – 4 | pH – 7 | pH – 9 |
|---|---|---|---|
| 12 | 0.41±0.1[a] | ND | ND |
| 24 | 0.80±0.5[a] | ND | ND |
| 36 | 2.23±0.3[b] | ND | 0.26±0.1[a] |
| 48 | 3.26±0.3[bc] | ND | 0.37±0.2[a] |
| 60 | 3.8±0.7[c] | 0.80±0.1[a] | 0.77±0.2[b] |
| 75 | 3.81±0.8[c] | 0.41±0.3[ab] | 1.56±0.2[c] |
| 90 | 4.11±0.8[c] | 0.77±0.1[b] | 2.2±0.2[d] |
| 120 | 4.3±0.81[c] | 1.43±0.4[c] | 2.80±0.4[d] |

Means not sharing a common superscript letter in the same column are significantly different at ($P<0.05$). All values are means of Triplicate ±SD

ND= Not Detectable

Results of two way analysis of variance (two way ANOVA) indicates that in addition to the main factors such as, pH ($P<0.001$) and boiling duration ($p<0.001$) the interaction of pH and boiling duration had significant ($p<0.001$) effect on leaching of Al and Fe (Appendixes 15&16).

Besides to the natural metallic content of the foodstuffs, additional contamination can occur through articles that come into contact with food that contain metals (Semwal *et al*., 2006). Metals and alloys can come in contact with food materials mainly in processing equipment, containers, household utensil and also in foils for wrapping foodstuffs. They are often covered by a surface coating, which reduces the migration in foodstuffs. When they are not properly coated these metals can give rise to migration into the foodstuffs and therefore could either endanger human health or bring about an unacceptable change in the composition of the foodstuffs (Marsh & Bogus, 2007).

The use of metallic utensils for cooking and storage of foods is known to contribute metals into the food, and leaching of these metals from cooking pots is influenced by various factors, such as pH, temperature, frequency of usage, duration of contact or heating and the presence of various food ingredients (Semwal *et al*., 2006).

The present study confirmed that cooking duration, pH, and frequency of usage can affect the migration of metals into the food. The specific type of metal in the raw material for the manufacturing of the present study cooking pot is not known. This is because based on the informal interview with the *Senegal Dest* producers, the raw materials used for this purpose were usually scrap aluminum alloys like block engines, carburetors and wheels obtained from automobiles as well as building materials like the roofing sheets and furniture besides several others. In addition, some other metals like Fe and Ni in the form of stainless steel, and metals like Pb can also be used to make cookwares.

The type of food is an important factor during the study of leaching of metals from the food into the pot as migration of metals into the food is higher in acidic foods followed by alkaline (Semwal *et al*., 2006). The study by, Semwal *et al*., (2006) reported that leaching of metals under the preparation of lower pH (4.25) food in an aluminium cooking pot increase the concentration of Al metal by 20.3 mg/kg. Another study by Valadez-Vega, (2011) showed that boiling of acetic acid for 45 hr lead to the migration of 127.52 ppm for Cd, 70.38 ppm for

Co, and 188.08 ppm for Pb. Similarly, the finding of the present study showed Preparation of tomato sauce with a pH value of about 4.5 had a greater leaching than preparations of *shiro wot* with a pH value of about 5.7. There was also an increase in metal leaching in case of pH 4.0 followed by pH 9.0, whereas for neutral pH the increase in metal leaching was negligible. Due to the fact that metals are attacked by most dilute mineral acids and solubility increases markedly at pH below 4.5 and above 8.5. However, at neutral pH solubility becomes negligible (Elinderand Sjögren, 1986). This is due to the formation of non-oxidative leaching involves in the chemical dissolution process using acid or alkali as reagent (Singer & Stumm, 1970). It is well established that Al dissolution is highly dependent on pH. Al exhibits a passive behavior in aqueous solutions due to the protective compact $Al_2O_3$ film on its surface. However, the solubility of this protective film increases in acidic and alkaline medium (Beliles, 1994).

Frequency of usage is the other factor in the migration of metals into the food. Study by Odularu *et al*., (2013) showed that old metallic pots had the lowest concentration (Al- 289 mg/kg) of leaching while new steel pots had the highest leaching of Al (295 mg/kg). Similarly in this study, leaching in both food types was found to be higher ($p<0.001$) during first time preparation (new cooking pot) than in the second time, subsequent preparations. Similar observations have also been reported earlier (Semwal *et al.*, 2006).

The increasing migration of metals into the foodstuffs can be caused by longer cooking duration (Kamerud *et al*., 2013; Semwal *et al.*, 2006; Rajwanshi *et al.,* 1999). Study by Kamerud *et al.,* (2013) showed that Ni concentrations increased 26-fold and Cr increased approximately 7-fold after six hrs of cooking. Similarly, the finding of the present study shows that for 12 min. of cooking the concentration of metals (Al, 6.63±1.7 mg/kg; Fe, 0.41±0.1mg/kg) increased by 6-fold and 5-fold, respectively compared to the concentration of Al (28.30±1.4 mg/kg) and Fe (4.25±0.8 mg/kg) for 120 min of cooking. This is may be due to the longer time contact of cooking pot with the food, the higher the formation of non-oxidative leaching and then chemical dissolution process occur (Singer & Stumm, 1970).

According to the study conducted by Pennington and Jones (1989), cooking in aluminium vessels increases the content of Al in the foodstuffs examined. Nonetheless, these cookwares had greater leaching and can contribute a lot to the daily consumption. The Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1989) established a provisional tolerable weekly intake (PTWI) at 7 mg/kg bodyweight for the total intake, which means that for an average 50-kg person, a daily intake of 50 mg of Al is tolerable/permissible. *Shiro wot* is the most commonly consumed stew as a family food as well as complementary food in many parts of Ethiopia (Baye *et al.,* 2013), according to WHO weight for age chart, 2 years of a child weigh 15kg which means the contribution of Al from the consumption of 50gm (assuming theoretical gastric capacity of 30 g/kg body weight per meal) *shiro wot* cooked in a new traditional pot contributes 34% of daily consumption limit of 15mg (1mg/Kg/of body weight per day). Therefore, these utensils are a real source of Al contamination and contributes high amount of Al in daily basis.

In addition, Fe concentrations in the first cooking of *shiro wot* and tomato sauce cooked in a traditional cooking pot increased by 79.0% and 95.7%, respectively. The results indicate that the use of this pot contributes significantly to the total daily intake of iron through foods, especially those acidic in nature. Fe leaching also depends on the chemical composition of the raw food materials (the nature of the food) and the various ingredients. High acidity food preparations such as tomato sauce resulted in greater leaching of Fe into the food from the pot. This is in agremment with Kuligowski & Halperin, (1992).

The soluble Ni released from contact material is more readily absorbed than complex-bound Ni from food. Therefore, the contamination of Ni containing food materials, e.g. kitchen utensils and electric kettles should be reduced (guidelines on metals and alloys used as food contact materials, 2002). WHO (1997) has given a TDI of 0.005 mg/kg bodyweight. Nevertheless, the migration of Ni to foodstuffs should be as low as reasonably achievable and no more than: 0.1 mg/kg as a general limit. However, the level of metals obtained from this study is much higher than the recommended limit.

# 6. CONCLUSIONS AND RECOMMENDATIONS

Cottage industries can contribute significantly to the economic boom of developing countries like Ethiopia. However, goods produced by these small holder industries can pose a health risk due to the fact that they are far reach from the control and regulation.

Cooking utensils are currently produced traditionally using metallic raw materials of various sources this includes scrap aluminum alloys like block engines, carburetors and wheels obtained from automobiles as well as building materials like the roofing sheets and so on. Among the products *Senegal dest* has good acceptance by the population to the existent of reaching consumers in the largest open market in Africa (*Merkato*).

In the present study, leaching of Al and Fe to the food was very significant and can pose a health risk. Strong acidic foods like tomato increase the amount of metallic contaminants into the food. The study shows further that the longer time the cooking takes, the greater the amount of Al, Fe and Ni that leache into the foods from the cooking pots. In addition, frequency of cooking had a greater effect on the migration of metals into the food. The concentration of metals leached in new utensils (first time cooking) was higher than that of the subsequent time of cooking.

Unfortunately, toxicological control does not exist Ethiopia exert over the cooking pots; thus, the final products of the cooking pot can show various levels of toxic metals. The high metal content observed in this study indicates the absence of quality control and makes the need evident for the government to exercise preventive measures in this situation by enforcing the quality control of raw materials. There is a certain need to create awareness in the community on the danger evoked by high levels of metals on human health.

Producers of these utensils should have guidance regarding the use of their product with high acidic or alkaline foodstuff. An appropriate guidance could be: “information for the User: Do not use this utensil to store acidic food like tomato. Use for foods only that take no long period of time for cooking” or “To be used for neutral foods only”. Manufactures should comply with the good manufacturing practice (GMP) for products intended to come into contact with foodstuff.

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**APPENDICES:**

**APPENDIXES 1: The moisture content of Ingredients, food cooked in a beaker and traditional pot.**

| Sample type | Wt. of Crucible | Wt. of fresh Sample | Wt. Cru. + Sample | Wt. Cru. + Dry Sample | wt. Dry | % of moisture | Mean of mois. | dry matter=100-%moisture |
|---|---|---|---|---|---|---|---|---|
| **shi.P 1** | 13.908 | 5.004 | 18.906 | 18.588 | 0.318 | 6.35491607 | 6.405521 | 93.64508393 |
| **shi.P 2** | 13.354 | 5.003 | 18.362 | 18.039 | 0.323 | 6.45612632 | | |
| **Berb 1** | 13.708 | 5 | 18.708 | 18.203 | 0.505 | 10.1 | 10.29741 | 89.9 |
| **Berb 2** | 13.883 | 5.012 | 18.895 | 18.369 | 0.526 | 10.4948125 | | |
| **Oni 1** | 13.973 | 5.076 | 19.024 | 14.631 | 4.393 | 86.5445232 | 86.89855 | 13.45547675 |
| **Oni 2** | 13.943 | 5.052 | 19.004 | 14.596 | 4.408 | 87.2525732 | | |
| **Shiro Wot 1** | 13.564 | 5.001 | 18.571 | 14.273 | 4.298 | 85.9428114 | 86.336 | 14.05718856 |
| **Shiro Wot 2** | 13.558 | 5.011 | 18.591 | 14.245 | 4.346 | 86.7291958 | | |
| **Shiro Sa (Beaker) 1** | 13.24 | 5.009 | 18.421 | 14.193 | 4.228 | 84.4080655 | 85.76682 | 15.59193452 |
| **Shiro Sa (Beaker) 2** | 13.64 | 5.041 | 18.691 | 14.299 | 4.392 | 87.1255703 | | |
| **Tom Sau. 1** | 13.322 | 5.001 | 18.343 | 14.711 | 3.632 | 72.6254749 | 83.91748 | 16.08251623 |
| **Tom Sau. 2** | 13.818 | 5.111 | 18.929 | 14.475 | 4.454 | 87.1453727 | | |
| **Tom. Sa (Beaker) 1** | 13.534 | 5.004 | 18.621 | 14.492 | 4.129 | 82.5139888 | 82.70581 | 17.48601119 |
| **Tom. Sa 2** | 13.233 | 5.011 | 18.599 | 14.445 | 4.154 | 82.8976252 | | |

**APPENDIX 2: pH value of the *Shiro wot* and tomato Sauce**

| No. of cooking | *Shiro* Sauce | Tomato Sauce |
|---|---|---|
| First cooking | 5.72 ± 0.07 | 4.53 ± 0.05 |
| Second cooking | 5.76 ±. 0.57 | 4.51 ± 0.01 |
| Third cooking | 5.78 ± 0.07 | 4.53 ± 0.25 |
| Forth cooking | 5.68 ± .051 | 4.35 ± 0.05 |

**APPENDIX 3: Ducans Test for significance Al content of shrio sauce samples**

**Value**

Duncan

| Method | N | Subset for alpha = 0.05 | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| 1 | 3 | 5.6640 | | |
| 5 | 3 | | 11.0753 | |
| 4 | 3 | | 12.2713 | |
| 3 | 3 | | | 14.8721 |
| 2 | 3 | | | 15.9400 |
| Sig. | | 1.000 | .066 | .096 |

Means for groups in homogeneous subsets are displayed.

**APPENDIX 4:Ducans Test for significance Al content of Tommato sauce samples**

**Value**

Duncan

| Method | N | Subset for alpha = 0.05 | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| Beaker | 3 | 3.8388 | | |
| 4th cooking | 3 | | 14.2935 | |
| 3rd cooking | 3 | | 15.2689 | |
| 2nd cooking | 3 | | 17.6822 | 17.6822 |

| | | | | |
|---|---|---|---|---|
| 1St cooking | 3 | | | 20.3676 |
| Sig. | | 1.000 | .131 | .202 |

Means for groups in homogeneous subsets are displayed.

**APPENDIX 5:** Ducans Test for significance Fe content of shrio sauce samples

Value

Duncan

| Method | N | Subset for alpha = 0.05 | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 |
| Beaker | 3 | 3.3442 | | | |
| 4th cooking | 3 | | 4.5223 | | |
| 3rd cooking | 3 | | 4.7482 | | |
| 2nd cooking | 3 | | | 5.1829 | |
| 1st cooking | 3 | | | | 5.9788 |
| Sig. | | 1.000 | .256 | 1.000 | 1.000 |

Means for groups in homogeneous subsets are displayed.

**APPENDIX 6:** Ducans Test for significance Fe content of Tomato sauce samples

Value

Duncan

| Method | N | Subset for alpha = 0.05 | |
|---|---|---|---|
| | | 1 | 2 |
| Beaker | 3 | 5.5078 | |
| 4th cooking | 3 | 6.7195 | |
| 3rd cooking | 3 | 8.4761 | 8.4761 |
| 2nd cooking | 3 | | 10.9793 |
| 1st cooking | 3 | | 11.2619 |
| Sig. | | .102 | .122 |

Means for groups in homogeneous subsets are displayed.

**APPENDIX 7: Multiple test for significance Ni content of shrio sauce samples**

**ANOVA**

Value

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | .024 | 4 | .006 | 2.019 | .168 |
| Within Groups | .030 | 10 | .003 | | |
| Total | .054 | 14 | | | |

**APPENDIX 8: Multiple test for significance Al among samples in a pH Buffer 4**

**Value**

Tukey B

| PH Value 4 | N | Subset for alpha = 0.05 | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 |
| 12 | 3 | 6.6383 | | | |
| 24 | 3 | 7.2900 | | | |
| 36 | 3 | 9.7625 | | | |
| 48 | 3 | | 14.3062 | | |
| 60 | 3 | | 17.2312 | | |
| 75 | 3 | | | 23.3250 | |
| 90 | 3 | | | | 28.3062 |
| 120 | 3 | | | | 32.3688 |

Means for groups in homogeneous subsets are displayed.

**APPENDIX 9: Multiple test for significance Al among pH 7 Buffer samples**

**Concentration**

Tukey B

| PH Value 7 | N | Subset for alpha = 0.05 | |
|---|---|---|---|
| | | 1 | 2 |
| 12 | 3 | .0000 | |
| 24 | 3 | .0000 | |
| 36 | 3 | .0000 | |
| 48 | 3 | .0000 | |
| 60 | 3 | 1.5800 | 1.5800 |
| 75 | 3 | 1.8290 | 1.8290 |
| 90 | 3 | | 2.5300 |
| 120 | 3 | | 3.5500 |

Means for groups in homogeneous subsets are displayed.

**APPENDIX 10:** Multiple test for significance Al among pH 9 Buffer samples

**Concentration**

Tukey B

| PH Value 9 | N | Subset for alpha = 0.05 | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 |
| 12 | 3 | 3.8250 | | | |
| 24 | 3 | 5.4250 | | | |
| 36 | 3 | 8.3438 | 8.3438 | | |
| 48 | 3 | 10.6812 | 10.6812 | 10.6812 | |
| 60 | 3 | | 14.7063 | 14.7063 | |
| 75 | 3 | | 16.8062 | 16.8062 | 16.8062 |
| 90 | 3 | | | 17.8188 | 17.8188 |
| 120 | 3 | | | | 23.5938 |

Means for groups in homogeneous subsets are displayed.

**APPENDIX 11: Multiple test for significance Fe among pH 4 Buffer samples**

**Concetration**

Tukey B

| PH4 | N | Subset for alpha = 0.05 | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| 12 | 3 | .1405 | | |
| 24 | 3 | .8452 | | |
| 36 | 3 | | 2.2345 | |
| 48 | 3 | | 3.2582 | 3.2582 |
| 60 | 3 | | | 3.7652 |
| 75 | 3 | | | 3.8077 |
| 90 | 3 | | | 4.0955 |
| 120 | 3 | | | 4.2464 |

Means for groups in homogeneous subsets are displayed.

**APPENDIX 12: Multiple test for significance of Fe among samples in pH 7**

**Concentration**

Tukey B

| PH 7 Fe | N | Subset for alpha = 0.05 | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| 12 | 3 | .0000 | | |
| 24 | 3 | .0000 | | |
| 48 | 3 | .0000 | | |
| 36 | 3 | .0000 | | |
| 60 | 3 | .0802 | | |
| 75 | 3 | .4136 | .4136 | |
| 90 | 3 | | .7768 | |
| 120 | 3 | | | 1.4300 |

**APPENDIX 13: Multiple test for siginificance of results among pH Buffer**

**Concentration**

Tukey B

| pH | N | Subset for alpha = 0.05 | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| 2 | 24 | 1.1861 | | |
| 3 | 24 | | 12.6500 | |
| 1 | 24 | | | 17.4035 |

Means for groups in homogeneous subsets are displayed.

**APPENDIX 14: Multiple test for significance of Fe among samples in pH 9**

**concentration**

Tukey B

| PH value of Fe | N | Subset for alpha = 0.05 | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 |
| 12 | 3 | .0000 | | | |
| 24 | 3 | .0000 | | | |
| 36 | 3 | .2631 | .2631 | | |
| 48 | 3 | .3733 | .3733 | | |
| 60 | 3 | | .7733 | | |
| 75 | 3 | | | 1.5570 | |
| 90 | 3 | | | | 2.2120 |
| 120 | 3 | | | | 2.8021 |

Means for groups in homogeneous subsets are displayed.

Means for groups in homogeneous subsets are displayed.

**APPENDIX 15: Interaction of pH and Boiling Duration in Al Concentration**

**Tests of Between-Subjects Effects**

Dependent Variable:VALUE

| Source | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Corrected Model | 6330.611[a] | 23 | 275.244 | 53.045 | .000 |
| Intercept | 7807.334 | 1 | 7807.334 | 1.505E3 | .000 |
| PH | 3336.170 | 2 | 1668.085 | 321.473 | .000 |
| DURATION | 2209.162 | 7 | 315.595 | 60.821 | .000 |
| PH * DURATION | 785.280 | 14 | 56.091 | 10.810 | .000 |
| Error | 249.066 | 48 | 5.189 | | |
| Total | 14387.012 | 72 | | | |
| Corrected Total | 6579.677 | 71 | | | |

a. R Squared = .962 (Adjusted R Squared = .944)

**APPENDIX 16: Interaction of pH and Boiling Duration In Fe Concentration**

Dependent Variable:VALUE

| Source | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Corrected Model | 159.103[a] | 23 | 6.918 | 47.316 | .000 |
| Intercept | 136.769 | 1 | 136.769 | 935.511 | .000 |
| PH | 77.903 | 2 | 38.951 | 266.430 | .000 |
| DURATION | 60.200 | 7 | 8.600 | 58.825 | .000 |
| PH * DURATION | 21.000 | 14 | 1.500 | 10.260 | .000 |
| Error | 7.017 | 48 | .146 | | |
| Total | 302.890 | 72 | | | |
| Corrected Total | 166.121 | 71 | | | |

a. R Squared = .958 (Adjusted R Squared = .938)