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Determination of the levels of Selected Essential and Toxic Metals in Canned Tomato Paste

By

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**DETERMINATION OF THE LEVELS OF
SELECTED ESSENTIAL AND TOXIC METALS
IN CANNED TOMATO PASTE**

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LIST OF ABBREVIATIONS

AAS:	Atomic Absorption Spectrometry
FAAS:	Flame Atomic Absorption Spectrometry
CCPFV:	Codex Standard for Processed Fruits and Vegetables
CIT:	Crystal International Trading
DPP:	Differential pulse polarography
EC:	Commission of the European Communities
EIA:	Ethiopian Investment Agency
ETFRUT:	Ethiopian Fruit and Vegetable Marketing Enterprise
FAO/WHO:	Food and Agriculture Organization/World Health Organization
MDL:	method detection limit
MoARD:	Ministry of Agriculture and Rural Development
MWS-2:	microwave digestion system
NTSS:	natural tomato soluble solids
PME:	pectin methylesterase
PG:	polygalacturonase
RDA:	recommended dietary allowance
% RSD:	percent relative standard deviation
UAAIE:	Upper Awash Agro – Industry Enterprise
USDA:	United States Department of Agriculture

ABSTRACT

In this study, a reliable digestion procedure for the analysis of tomato paste was optimized and levels of selected essential metals (Zn, Fe, Cu and Mn) and toxic metals (Pb and Cd) were determined in four tomato paste of local origins using Atomic Absorption Spectrometry. The results showed that the zinc concentration in the four tomato paste examined ranged between 2.54 – 6.63 mg Kg⁻¹, iron between 63.11 – 96.10 mg Kg⁻¹, copper 6.95 mg Kg⁻¹, manganese between 11.5 – 23.02 mg Kg⁻¹, and cadmium between 0.511 – 1.22 mg Kg⁻¹. The results are also compared with International Standards. The cadmium content obtained showed that the level is higher than the FAO/WHO limits.

Key words: Tomato paste, metals, Flame Atomic Absorption Spectrometry

1. INTRODUCTION

1.1. Background

Vegetables and Fruits have many similarities with respect to their compositions, methods of cultivation and harvesting, storage properties and processing. In the true botanical sense, many vegetables are considered fruits. Thus, tomatoes, cucumbers, egg plant, peppers, and others would be considered as fruits, since fruits are those portions of the plant that house seeds. However, the important distinction between fruits and vegetables is based on their use [1]. Vegetables are considered as plant items that are eaten with the main course of a meal, whereas fruits are generally eaten alone or as a dessert.

The commercial tomato belongs to the genus *Lycopersicon*. It is a relatively small genus within the large and diverse botanic family *Solanaceae*. The genus is currently thought to consist of the cultivated tomato a species most frequently referred to as, *Lycopersicon esculentum* Mill [2] and seven closely related wild *Lycopersicon* species. All cultivated tomato varieties belong to the specie *Lycopersicon esculentum* Mill.

The most likely region where the tomato was first domesticated is the Pueblo–Vera Cruz area of Mexico, where the greatest varietals diversity of the cultivated form can be found today [3]. It is thought to have reached this area as a weedy cherry tomato, var. *cerasiforme*, and upon domestication, to have become the large-fruited *L. esculentum* Mill by selection.

Tomatoes contribute to a healthy, well-balanced diet. They are rich in vitamins, minerals, proteins [4], essential amino acids [5, 6], sugars and dietary fibers [7]. Tomatoes also contain Vitamin C, ascorbic acid, vitamin E and low amounts of the water-soluble type B vitamins, thiamin, niacin and riboflavin [8].

Tomatoes and tomato products are used as ingredients in many traditional dishes, because of the compatibility with other food ingredients and due to the concentration and availability of several nutrients in these products and to their wide spread consumption by humans all over the world [9]. However, heavy metals are one of a range of important types of contaminants that can be found on the surface and in the tissue of fresh tomatoes as well as processed tomato products. As a result heavy metal accumulation in tomato and processed tomato products may pose a direct threat to human health [10].

Heavy metals find their way into foods through a variety of routes. They may be taken up from soils through the roots of plants or be deposited on the surfaces of plant leaves from air born particulates or aerosols. Contaminated water may be used for irrigation, and food processing. Food processing machinery and food packaging materials may contain heavy metals that leach into foods. Food- processing operations may remove heavy metal contaminants from foods as well as add them. In addition, long-term use of farmland irrigated with waste water has resulted in the accumulation of heavy metals in the soil and their transfer to the various crops under cultivation, with levels of contamination that exceed permissible limits [11]. Furthermore, type of fertilizer, agricultural chemicals and contaminating dirt's [12], has been ascribed to presence of trace elements in fruits and vegetables. Other sources of heavy metals contamination of most foodstuffs may also include adaptation of mechanized farming, sprays, seed preservatives and components from global pollution [13].

In Ethiopia, Tomato Paste, which constitutes a significant volume among canned vegetable products, is supplied mainly by the only two domestic competitors ELFORA and Upper Awash Enterprise [14]. These processing plants are depending on their farmland, most of which is irrigated, for the supply of raw materials [15]. Both plants use fertilizer and agricultural chemicals for the production of row materials. In addition, the manufacturing machines used by ELFORA and Merti Fruits and Vegetables processing plants are relatively more obsolete than modern tomato processing lines, which might decrease metal contamination.

Hence, the need to determine and/or monitor the levels of essential and toxic heavy metals in domestic processed fruits and vegetables such as tomato paste become essential due to their beneficial or damaging effects. Moreover, it has become necessary to know about the concentration of essential and toxic heavy metals in canned tomato paste to ensure the quality of the product and to comply with quality and specification standards developed by the codex committee on processed fruits and vegetables (CCPFV).

Different techniques can be used in the determination of metals in tomato paste. Among them, atomic absorption spectrometry (AAS) based procedures, for instance flame atomic absorption spectrometry (FAAS) [16, 17, 18] is the most frequently used. Because of rapidity and reproducible determination at trace to ultra-trace concentrations an electroanalytical techniques such as Differential pulse polarography (DPP) [19] have been used for the measurement of elements (such as Cd, Pb, Cu, and Zn) in low levels, while cyclic voltammetry [7] has been applied when the elements are present at higher levels.

Several authors have proposed acid digestion, using different reagents, for the previous pre-treatment of tomato paste sample. David I. *et al.*, 2008 [17] proposed an acid digestion procedure in a MWS-2 Berghof mineralization and digestion system with nitric acid HNO_3 (65 %) in order to analyze tomato paste samples.

Türker and Yüksel [18] have studied the effectiveness of various acid digestions for tomato paste samples in kjeldahl digestion flask; they reached the conclusion that the wet ashing procedure involving 15 mL of HNO_3 and 2 mL of H_2O_2 (30 %) was the most suitable for digestion of tomato paste. Abdul Waheed *et al.* 2003 [16] in a study of selected essential and non-essential metals in various canned and raw food stuffs consumed in Pakistan, proposed an acid digestion procedure in an open beaker with 50.0 ml of HNO_3 (70 %) under controlled heating.

Taking all this in to account, the levels of essential and toxic metals in canned tomato paste produced by commercial canning plants in Ethiopia, which are available in local markets and consumed as a part of food, have been investigated.

1.2. Growth, Ripening and Composition of Tomatoes

1.2.1. Growth and Ripening

It takes about 6 to 7 weeks for tomatoes to reach their full size from flowering followed by about 12 days to ripen. During ripening, the tomato changes from green to red as chlorophyll is destroyed and lycopene is synthesized. There is also increased production of the volatile compounds which generate aroma. During growth, the plant forms insoluble protopectin that firmly binds cell walls together [20]. Pectin is also associated with cellulose to form plant cell walls. During ripening, protopectin is converted to soluble pectin by the enzyme protopectinase, and the pectin so formed binds the cells together but less firmly.

If the fruit is allowed to grow past maturity, the pectin itself is broken down into soluble compounds by the pectinolytic enzymes polygalacturonase (PG) and pectin methyl-esterase (PME), and the fruit becomes soft and mushy [20]. The enzyme endopolygalacturonase (PG) is synthesized during ripening, but in the whole fruit the action of PG is limited by a number of factors. These include the amount of calcium bound to pectin, the distribution of PG in the cell walls, and the extent of methyl esterification of polygalacturonate [21]. During the comminution of tomatoes to pulp for paste and puree manufacture, the enzymatic depolymerization of pectin by PG and PME can bring about a large reduction in the viscosity of the product [22] unless action is taken to heat denature the PG and PME enzymes by 'hot breaking'. The enzymes have a high optimum temperature of 60 to 66 °C and only become inactivated at a temperature of about 82 °C [20]. Thus, 'hot breaking' requires that the tomato pulp be heated as quickly as possible to 82 °C or above to denature PG and PME, retain pectic substances, and

preserve as much of the potential viscosity as possible. However, some loss of pectic substances is inevitable, even when the crushed tomato pulp is heated rapidly [23].

1.2.2. Composition of Whole Tomato

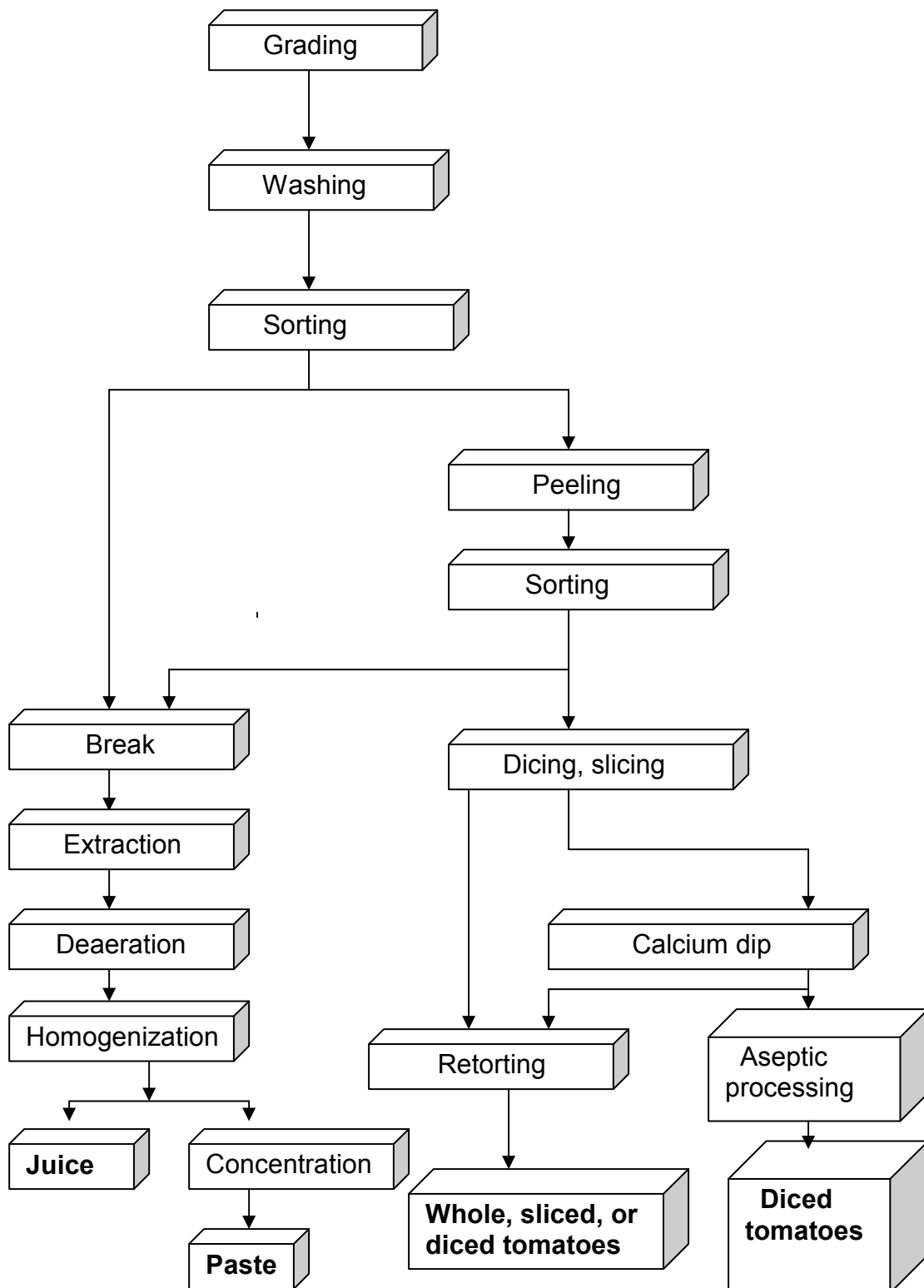
The tomato fruit comprises skin, pericarp, and locular contents. The locular cavities are filled with jelly-like parenchyma cells that surround the seeds. The cell walls are composed of α -cellulose, pectins, hemicellulose, and some protein [21]. Tomato-based products consist mainly of disintegrated cells of the pericarp suspended in a clear serum. Of the 5 to 10 % dry matter in the whole ripe fruit, about 75 % is soluble [24]. Such a wide variation in the dry matter content is due to tomato variety, the nature of the soil in which the tomato is grown, and the amount of rainfall during the growing and harvesting season [20].

Nearly half the total dry matter consists of the reducing sugars glucose and fructose, about 10 % is organic acids, principally citric and malic acids, about 1 % is skin and seeds, with the remainder being alcohol insoluble solids (cellulose, pectins, hemicelluloses, and proteins), minerals (mainly potassium), pigments, vitamins, and lipids [25]. The organic acid content is responsible for a pH between 4.2 and 4.6 [26]. Glutamic acid is the principal amino acid found in tomato.

1.3. Tomato Paste Production

The basic sequence of operations in the production of tomatoes into juice, paste, whole, sliced, or diced tomatoes are shown in figure 1.

Figure 1: Flowchart for tomato processing



1.3.1. Washing, Sorting, and Trimming

In modern plants, tomatoes are washed in water tanks agitated with compressed air, followed by rinsing with high-pressure water sprays to remove spray residues, microorganisms, dirt, mold, *Drosophila* (fruit fly) eggs, and larvae adhering to the fruit [20]. The wash water is chlorinated to 5 to 10 ppm to maintain sterility. Unfit whole fruit is picked out and discarded, while partly defective fruit is trimmed by hand. Sorters and trimmers remove off-color fruit or parts. They also trim rotten areas, mold portions, insect damage and sunscald [20]. This is the final control point for ensuring a low mold count in the final product.

1.3.2. Breaking

In the process described by Moresi and Liverotti [27], the washed tomatoes are chopped into small pieces by a rotary comb chopper, and the chopped tomatoes are pumped into a heat exchanger and preheated to either 60 °C for a 'cold break' or preheated to 90 to 95 °C and held for 1 to 2 min for a 'hot break'. A 'hot break' juice has a high consistency due to better extraction of pectic substances and due to retention of pectin by denaturation of the enzymes that would have caused its breakdown. A 'cold break' juice has a low consistency due to the activity of pectolytic enzymes and gives greater serum separation [28].

1.3.3. Juice Extraction

The heated tomato pulp is passed through two (or three) juice extractors to remove the skin and seeds, and to squeeze the juice out of the remaining pulp [29]. Juice extractors may be either of the screw type or paddle type; a screw-type extractor uses an expanding helical screw to subject the pulp to increasing pressure against a screen, whereas a paddle type extractor beats the pulp against a screen [29].

Moresi and Liverotti [27], describe two juice extractors of screen size 1.5 and 0.4 mm, respectively. In the terminology of the tomato processor the first juice extractor is known as a 'pulper' and the second juice extractor is known as a 'finisher' [30]. Moresi and Liverotti reported the yield of tomato juice from the juice extraction stage to be 95.0 %. A further screw press may be added to extract more juice from the residue leaving the juice extractors.

1.3.4. Concentration

Tomato juice is concentrated by evaporation under partial vacuum either in a batch or continuous process. In the traditional batch process, the evaporation may be entirely carried out in steam-jacketed vacuum pans (known as 'boules') fitted with agitators, or the juice may be preconcentrated in a tubular evaporator to about 12 % solids before transfer to the boules [29]. Evaporation at low pressure reduces the boiling point of the juice so that the resulting paste retains most of its color and flavor [20]. Continuous processes tend to produce a more consistent paste than batch processes [29].

1.3.5. Pasteurization

Continuous pasteurization of tomato paste at 90 to 92 °C, before it is canned, prevents subsequent spoilage by lactobacilli [27]. A gear pump transfers viscous tomato paste from the evaporator to filler. The filler usually comprises a receiving tank for the tomato paste, a tubular heat exchanger for pasteurization, and a recirculation tube. The recirculation tube returns the hot paste to the receiving tank if flow through the filling nozzles is restricted, to prevent 'burn-on' fouling of the heat exchanger and loss of product quality [29].

1.3.6. Filling, Closing, and Cooling

The pasteurized paste is automatically hot-filled into lacquered tin cans that have been pre-sterilized with steam [27]. The cans are immediately seamed, inverted to sterilize the lids, and held for about 3 min prior to cooling [29].

Canned tomato paste may be described as a conduction pack and unless the cans are cooled as quickly as possible the retention of heat leads to deterioration of flavor and color. Cans may be air cooled or water cooled. Air cooling simply requires that the cans be stacked in rows with air spaces in between to allow the passage of an air current [29]. Water cooling involves agitating the cans for about 2 hours under a spray of atomized water that has been chlorinated to 15 ppm residual chlorine [20].

1.4. The Current Status of Fruits and Vegetables Processing in Ethiopia

1.4.1. Processing of selected enterprises

In Ethiopia, the number of fruits and vegetables processing industries is limited. Currently, there are only five fruits and vegetables processing plants in the country [31]. These plants presently process a limited variety of products: tomato paste, orange marmalade, vegetable soup, frozen vegetables and wine. Currently most processed products are geared to domestic markets (EIA, 2006).

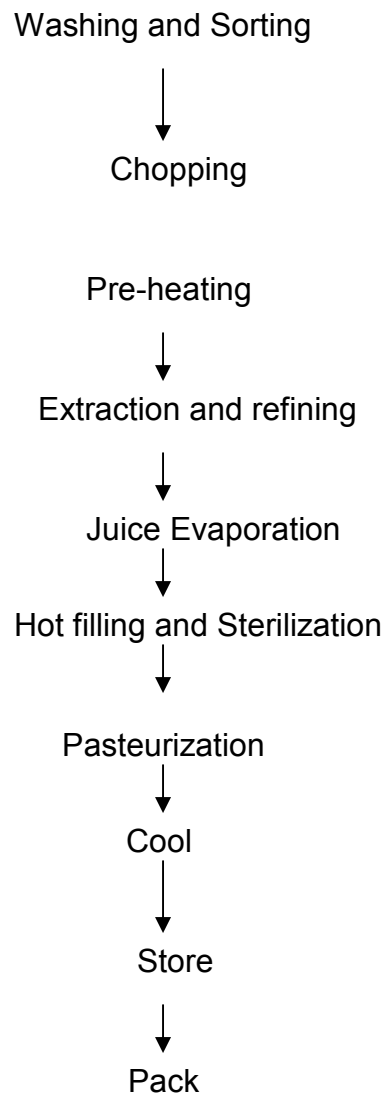
1.4.2. Description of the process

Tomato Paste

The processing plants of tomato paste in Ethiopia are: Merti and ELFORA (Melge Wondo, Kombolcha, and Gonder). Both Merti and ELFORA produce products [14] in consumer packaging of cans containing similar weights of 850 g, 410 g, and 70g.

The fundamental processing procedures pursued in the four plants are more or less the same [15] except that the manufacturing machines used by ELFORA are more obsolete than the ones used by Merti which are relatively more of recent times.

The basic processing procedures of tomato paste in the plants include performance of the following fundamental functions:



2. OBJECTIVES

2.1. General Objectives

Taking all the above into account, the main goal of this study is to determine the levels of essential (Zn, Fe, Cu and Mn) and toxic (Pb and Cd) metals in canned tomato paste produced by commercial canning plants in Ethiopia, which are available in local markets and consumed as a part of food.

2.2. Specific Objectives

- ✚ To determine the metal contents of canned tomato paste available in local markets which is produced by commercial canning plants in Ethiopian.
- ✚ To develop a reliable digestion procedure for analysis of canned tomato paste.
- ✚ To compare the levels of metals in tomato paste with international standards.

3. EXPERIMENTAL

3.1. Reagents and Chemicals

A stock standard solution of 1000 mg/L, in 2 % HNO_3 of the metals Zn, Fe, Cu, Mn, Pb, and Cd, (Buck Scientific Puro- Graphic) were used for preparation of intermediate standards.

Working standard solutions for the construction of calibration curves were prepared by appropriate dilution of intermediate standard solutions of 1000 mg/L, in 2 % HNO_3 of the metals Zn, Fe, Cu, Mn, Pb, and Cd, (Buck Scientific Puro- Graphic) with deionized water. Nitric acid (69 - 72 %) and Hydrogen peroxide (30 %) were used for digestion of samples. Deionized water was used throughout this work.

3.2. Instrumentation and Apparatus

Analytical digital balance (Sciencetech, SA120, and U.S.A.) was used to weigh tomato paste sample. Micropipette (Dragonmed) was used for measuring different amounts of acid mixtures and standard solutions. A 100 mL round bottom flask were used to keep the sample for digestion. A Kjeldahl digestion apparatus was used to heat the sample. A 50 mL volumetric flask was used to dilute digested sample solutions and for preparation of standard solutions. A refrigerator (Hitachi, Tokyo, Japan) was used to keep the digested sample until analysis. A flame atomic absorption spectrophotometer (Buck Scientific Model 210VGP AAS, Este Norwaik, U.S.A.), was used for measuring the concentration of metals in the sample solutions using air-acetylene flame. Concentration mode of FAAS was used for all measurements. Single element hollow cathode lamps of each element of interest were used as radiation sources.

3.3. Sample Collection and Preparation

In the present study four different brands of commercially available tomato paste were used. The four types of tomato paste samples used in the study were Merti, Melge, Kombolcha, and Gonder. Samples of 410 g of Merti, and 410 g Melge, 410 g Kombolcha and 70 g of Gonder were purchased from ETFRUT and ELFORA Mini Supermarket respectively located at different location in Addis Ababa.

For the preparation of bulk samples, three cans of each brand were opened with a can opener and the contents were transferred to an electric blender for homogenization.

Table 1: Characteristics of tomato paste purchased from ETFRUT and ELFORA mini Supermarket in Addis Ababa.

Brand name	Ingredient	Dry matter (%)	Quantity (gram)	Manufacturing date	Best before
Merti	Tomato (100 % natural)	22-24	410	October 2008	November 2010
Melge	Tomato	24	410	March 2008	February 2011
Kombolcha	Tomato	28	410	May 2008	April 2011
Gonder	Tomato	28	70	May 2008	April 2011

All tomato paste samples used were packaged in metallic cans.

3.4. Procedure

3.4.1. Cleaning Apparatus

Apparatus such as volumetric flask, pipettes, and digestion flasks were washed with detergents and tap water, and then rinsed with deionised water, soaked in dilute HNO_3 for 24 hrs and again rinsed with deionised water.

3.4.2. Digestion of Tomato Paste Samples

About 0.50 g of tomato paste sample was taken from the bulk samples and weigh in a plastic cup and placed in a 100 ml and 4 ml nitric acid was added to a round bottom flask containing samples and allowed to stand for 10 min. Then the round bottom flask containing samples was fitted with a condenser and heated in a Kjeldahl heating apparatus by setting the temperature dial first at 4 (120°C) for 10 min., then at 9 (270°C) for 50 min., and continued heating at a temperature of 10 (300°C) for 1 hr.

After a total of 2 hr digestion, the sample was cooled for 10 min., and 2 ml of Hydrogen peroxide was added and then continued heating at a temperature of 10 (300°C) for 1:30 hr.

After a total of 3:30 hr digestion, the digested sample was allowed to cool for 10 min., and the contents of the flask were rinsed into a 50 ml volumetric flask and the volume was made up to the mark with deionized water.

Each tomato paste sample was digested in triplicate and hence a total of twelve digests were made for the tomato paste samples. Three reagent blank solutions were prepared similarly. All the digested samples were stored in refrigerator, until the levels of all the metals in the sample solutions were determined by FAAS.

3.4.3. Determination of Metals in Tomato Paste Samples

For the determination of metals in tomato paste samples, four series of working standard solutions were prepared from the 10 mg/L intermediate standard solutions of their respective metals, which were prepared by diluting the stock standard solutions of the metals with deionized water. Optimum acetylene and air flow rates were chosen to obtain suitable flame conditions. Other conditions such as slit width, wave length, and lamp current were selected for each hollow cathode lamp according to the manufacturer's recommendation.

Four point calibrations were established by introducing the prepared working standard solutions in flame atomic absorption spectrophotometer. Immediately after calibration of the instrument, the reagent blank and the sample solutions were aspirated in to the atomic absorption spectrophotometer consecutively and a minimum of three readings were taken for each sample solution and reagent blank solution and the mean value of the concentration signal was used for subsequent calculations. The concentration signals were evaluated by subtracting the value of blank from the signal of the sample.

The signals of reagent blank and samples were measured by aspirating the solutions into the atomizer using instrumental parameters given in Table 2.

Table 2: Instrumental operating conditions for the determination of metals in tomato paste samples using atomic absorption spectrophotometer.

Element	parameters			
	Wavelength (nm)	Slit width (nm)	Lamp current (mA)	Instrumental detection limit(ppm)
Zn	213.9	0.2	7	0.005
Fe	248.3	0.7	2	0.03
Cu	324.7	0.7	1.5	0.02
Mn	279.5	0.7	3	0.01
Pb	283.2	0.7	2	0.1
<i>Cd</i>	228.9	0.7	2	0.005

3.4.4. Recovery Experiment

In order to determine the reliability of the method for the analysis of the samples for Zn, Fe, Cu, Mn, Pb, and Cd, Certified Standard Reference Materials(CSRM) were not available for use, instead spiking method was adopted using digestion method.

3.4.5. Spiking Experiment

75 μ L of Zn, 500 μ L of Fe, 50 μ L of Cu and 25 μ L of Mn were drawn with graduated pipette and used to spike 0.5 g of Gondar tomato paste sample at once in a round bottomed flask and then the sample were digested as used for original sample. Similarly, 25 μ L of Cd standard solution were used to spike 0.5 g of Merti tomato paste sample at once in a round bottomed flask and then the sample were digested as used

for original sample. Then the digestate were transferred in to a 50 mL volumetric flask and diluted up to the mark with deionized water. Finally the solutions were analyzed for each element with atomic absorption spectrophotometer. As used for original samples triplicate spiked samples were prepared and triplicate readings were recorded.

3.4.6. Method Detection Limit

Limit of detection is the smallest mass of analyte that can be distinguished from statistical fluctuations in a blank, which usually corresponds to the standards of the blank solution times a constant. The limit of detection is most commonly defined as the mass of analyte that gives a signal equal to three times the standard deviation of the blank [32]. Three reagent blank (HNO_3 , H_2O_2) samples were digested following the same procedure as the samples and each of the samples were determined for the elements of interest (Zn, Fe, Cu, Mn, Pb, and Cd,) by the atomic absorption spectrophotometer. The pooled standard deviation for each element was calculated from the three reagent blank measurements to determine method detection limit.

4. RESULTS AND DISCUSSION

4.1. Optimization of the Digestion Procedure

For tomato paste samples different digestion procedures using HNO_3 and H_2O_2 acid mixtures were assessed by varying volume of the acid mixtures, digestion time and temperature and the amount of sample. Attempted digestion procedure for tomato paste samples is shown in table 3.

Table 3: Attempted digestion procedure for tomato paste samples.

Amount of sample	Reagent volume	Temperature and Digestion time	Color of digestate
1.0 g	6 ml HNO_3 ; 2 ml H_2O_2	120 $^\circ\text{C}$ / 10 min, 270 $^\circ\text{C}$ /50 min, 300 $^\circ\text{C}$ /1hr, and 300 $^\circ\text{C}$ /1:30 hr.	Yellow
	4ml HNO_3 ; 2 ml H_2O_2	120 $^\circ\text{C}$ / 10 min, 270 $^\circ\text{C}$ /50 min, 300 $^\circ\text{C}$ /1hr, and 300 $^\circ\text{C}$ /1:30 hr.	Light yellow
	3 ml HNO_3 ; 1 ml H_2O_2	120 $^\circ\text{C}$ / 10 min, 270 $^\circ\text{C}$ /50 min, 300 $^\circ\text{C}$ /1hr, and 300 $^\circ\text{C}$ /1:30 hr.	Light yellow
0.50 g	6 ml HNO_3 ; 2 ml H_2O_2	120 $^\circ\text{C}$ / 10 min, 270 $^\circ\text{C}$ /50 min, 300 $^\circ\text{C}$ /1hr, and 300 $^\circ\text{C}$ /1 hr.	Light yellow
	4ml HNO_3 ; 2 ml H_2O_2	120 $^\circ\text{C}$ / 10 min, 270 $^\circ\text{C}$ /50 min, 300 $^\circ\text{C}$ /1hr, and 300 $^\circ\text{C}$ /1 hr.	Very light yellow
	3 ml HNO_3 ; 1 ml H_2O_2	120 $^\circ\text{C}$ / 10 min, 270 $^\circ\text{C}$ /50 min, 300 $^\circ\text{C}$ /1hr, and 300 $^\circ\text{C}$ /1 hr.	Light yellow
0.50 g	6 ml HNO_3 ; 2 ml H_2O_2	120 $^\circ\text{C}$ / 10 min, 270 $^\circ\text{C}$ /50 min, 300 $^\circ\text{C}$ /1hr, and 300 $^\circ\text{C}$ /1:30 hr.	Clear solution
	4ml HNO_3 ; 2 ml H_2O_2	120 $^\circ\text{C}$ / 10 min, 270 $^\circ\text{C}$ /50 min, 300 $^\circ\text{C}$ /1hr, and 300 $^\circ\text{C}$ /1:30 hr.	Clear solution
	3 ml HNO_3 ; 1 ml H_2O_2	120 $^\circ\text{C}$ / 10 min, 270 $^\circ\text{C}$ /50 min, 300 $^\circ\text{C}$ /1hr, and 300 $^\circ\text{C}$ /1:30 hr.	Very light yellow

The optimum procedure was selected depending on minimal reagent volume consumption; shorter digestion time and obtaining clear solution. The optimal procedure chosen based on these criteria required a total of 3.30 hour for the complete digestion of 0.50 g tomato paste sample with 4 ml HNO₃ and 2 ml H₂O₂. The digestion gave a clear solution which is suitable for the analysis of metals by FAAS. This digestion procedure of tomato paste samples were developed with modification of literature procedure [18].

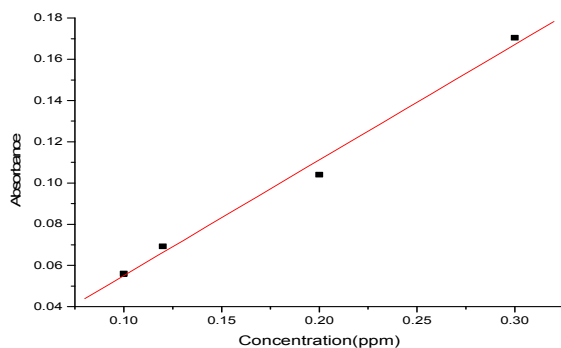
4.2. Calibration of the Instrument

Calibration curves were prepared to determine the concentration of metals in the sample solution. A series of working standard solutions were prepared from the 10 mg/L intermediate standard solutions of their respective metals and the solutions were aspirated into the atomizer and absorbance was recorded. The calibration graphs and correlation coefficients of each of the elements were determined by plotting working standards concentration versus their corresponding absorbance. The working standard solutions and the correlation coefficient of the calibration curve for each of the metals are shown in table 4.

Table 4: Concentration of the standard solutions used to establish calibration graph and their correlation coefficients.

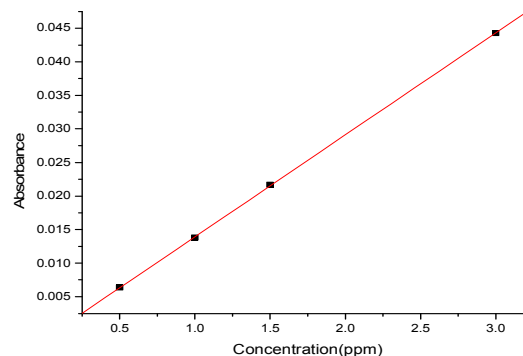
Element	Concentration of standard (mg/L)	Correlation coefficient
Zn	0.1, 0.12, 0.2, 0.3	0.99551
Fe	0.25, 0.5, 1.0, 2.0	0.99997
Cu	0.1, 0.2, 0.4, 0.8	0.9993
Mn	0.1, 0.25, 0.5	0.99996
Pb	0.2, 0.4, 0.8, 1.6	0.99877
Cd	0.2, 0.4, 0.8, 1.6	1

The calibration graphs of each of standard solution of metals of interest obtained are shown in figure 2.



$$Y = -9.83306 + 0.56061X$$

$$R = 0.99551$$

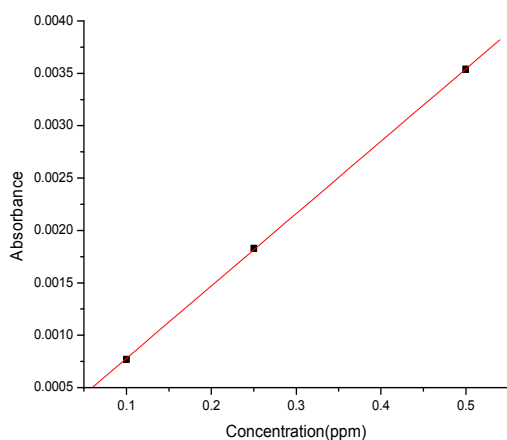


$$Y = -0.00125 + 0.01519X$$

$$R = 0.99997$$

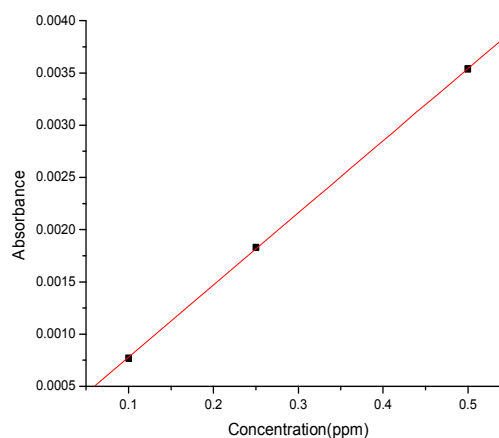
Figure 2a: Calibration graph of zinc standard solution

Figure 2b: Calibration graph of iron standard solution



$$Y = -6.34391 + 0.05044X$$

$$R = 0.9993$$

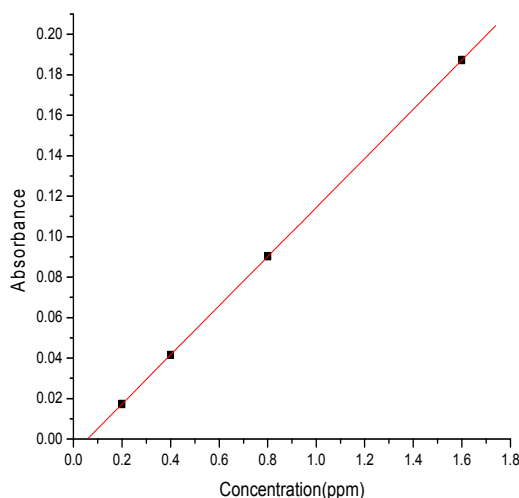


$$Y = 8.82959 + 0.00691X$$

$$R = 0.99996$$

Figure 2c: Calibration graph of copper standard solution

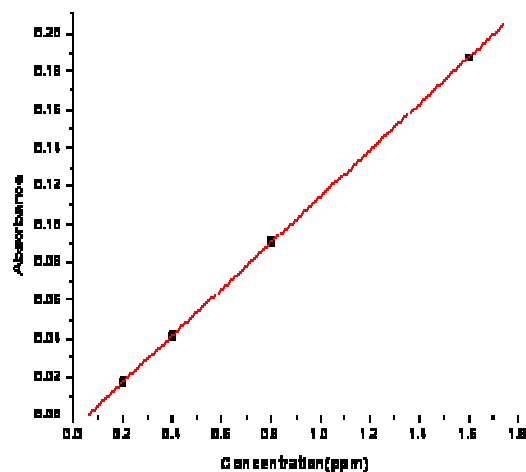
Figure 2d: Calibration graph of manganese standard solution



$$Y = 4.65565 + 0.00511X$$

$$R = 0.99877$$

Figure 2e: Calibration graph of lead standard solution



$$Y = -0.00693 + 0.1214X$$

$$R = 1$$

Figure 2f: Calibration graph of cadmium standard solution

Since R was greater than 0.995 for all metals, calibration curves were linear within the analytical range.

4.3. Method validation

4.3.1. Precision

The precision of an analytical procedure expresses the closeness of agreement between a set of results. The precision of an analytical procedure is expressed as the variance, standard deviation, coefficient of variation, and relative standard deviation of a series of measurements [33, 34]. In this study the precision of the results were

evaluated by the pooled standard deviation, and percentage relative standard deviation of the results of three samples (N = 3) and triplicate readings for each sample giving a total of nine measurements for a given bulk sample.

These parameters are useful in estimating and reporting the probable size of indeterminate error. In the precision test, the average % RSD for all selected trace metals are in the range of 1 to 10 %, except for cadmium. The % RSD of Cd found in Melge and Merti tomato paste sample were 15.2 % and 11.04 % respectively. Therefore, the result shows the precision of the results obtained by this method is good. The mean value and % RSD of each metal in each tomato paste sample is shown in Table 10.

4.3.2. Method Detection Limit

Method detection limit is defined as the minimum concentration of analyte that can be measured by the analytical method with a given confidence limit [32, 35]. In this work, after digestion of three blank solutions, three reading was obtained for each blank. Then the pooled standard deviation of the three blank reagents was calculated. The method detection limit of each element was obtained by multiplying the pooled standard deviation of the reagent blank by three ($3\sigma_{\text{blank}}$, n = 9), which is summarized in table 5.

Table 5: Method detection limit of elements assessed in tomato paste samples (n = 9, and in mg/L).

Metal	Method detection limit(mg/L)	Instrument Detection limit (mg/L)
Zn	0.01	0.005
Fe	0.09	0.03
Cu	0.03	0.02
Mn	0.6	0.01
Pb	0.145	0.1
Cd	0.006	0.005

Table 8 suggested that the method detection limit of each element is above the instrumental detection limit.

4.3.3. Validation of the Optimized Procedure

A recovery test of the total analytical procedure was performed for all of the selected metals in selected samples (Gonder and Merti) by spiking analyzed samples with aliquots of metal standards and then reanalyzing them. As can be seen from Table 6, acceptable recoveries (>95 %) were obtained for the analyzed metals.

Table 6: Analytical results obtained for spiked Tomato Paste Samples.

Element	^a Amount before addition (mg Kg ⁻¹)	Amount added (mg Kg ⁻¹)	^b Amount found (mg Kg ⁻¹)	(%)Recovery
Zn	6.63 ± 0.33	1.5	6.79 ± 0.4	106.4
Fe	96.1 ± 3.04	10	106.02 ± 2.24	99.2
Cu	6.95 ± 0.89	1.0	7.05 ± 0.02	104
Mn	16.88 ± 1.45	0.5	16.94 ± 0.03	110
Cd	0.511 ± 0.06	0.5	0.772 ± 0.03	96.7

^aValues are average value of triplicate samples and three readings in mg Kg⁻¹

^bValues are mean ± SD of triplicate readings of triplicate analysis in mg Kg⁻¹

Thus, on the average good recoveries were obtained for all elements in the sample validating that the optimized procedure has good accuracy.

4.3.4. Determination of Metals

The concentration (mg Kg^{-1}) of metals in the aliquot digested was calculated using the following equation:

$$C = \frac{A}{W} \times V$$

C = total metal concentration (mg Kg^{-1})

Where A = mg/ml of metal in digested sample

V = final volume of the digested sample solution (ml)

W = weight of digested sample (gm)

The levels of selected essential (Zn, Fe, Cu, and Mn) and toxic (Pb and Cd) metals determined in canned tomato paste samples are reported as mean of nine measurements with corresponding total standard deviation and percent relative standard deviation for each metal in a given sample. The metal levels determined were based on fresh weight. The results of the concentrations of metals found in the sample analyzed are shown in table 7.

Table 7a: The levels of metal concentration (mg Kg⁻¹, wet weight) in tomato paste samples.

Metal	Gonder		Melge	
	Mean ± SD	%RSD	Mean ± SD	%RSD
Zn	6.63 ± 0.33	4.99	3.13 ± 0.63	10.56
Fe	96.10 ± 3.04	3.16	63.11 ± 6.26	9.91
Cu	6.95 ± 0.89	12.87	ND	-
Mn	16.88 ± 1.45	8.59	23.02 ± 1.55	6.72
Pb	ND	-	ND	-
Cd	ND	-	1.22 ± 0.19	15.2

ND = Not detected

Table 7b: The levels of metal concentrations (mg Kg⁻¹, wet weight) in tomato paste samples.

Metal	Merti		Kombolcha	
	Mean ± SD	% RSD	Mean ± SD	% RSD
Zn	3.52 ± 0.33	9.39	2.54 ± 0.93	12.9
Fe	91.24 ± 6.82	7.47	81.20 ± 9.64	11.87
Cu	ND	-	ND	-
Mn	19.71 ± 2.05	10.39	11.5 ± 0.45	3.9
Pb	ND	-	ND	-
Cd	0.511 ± 0.06	11.04	ND	-

ND = Not detected

The mean concentration (Table 7) of all metals in tomato paste samples is shown in figure 3.

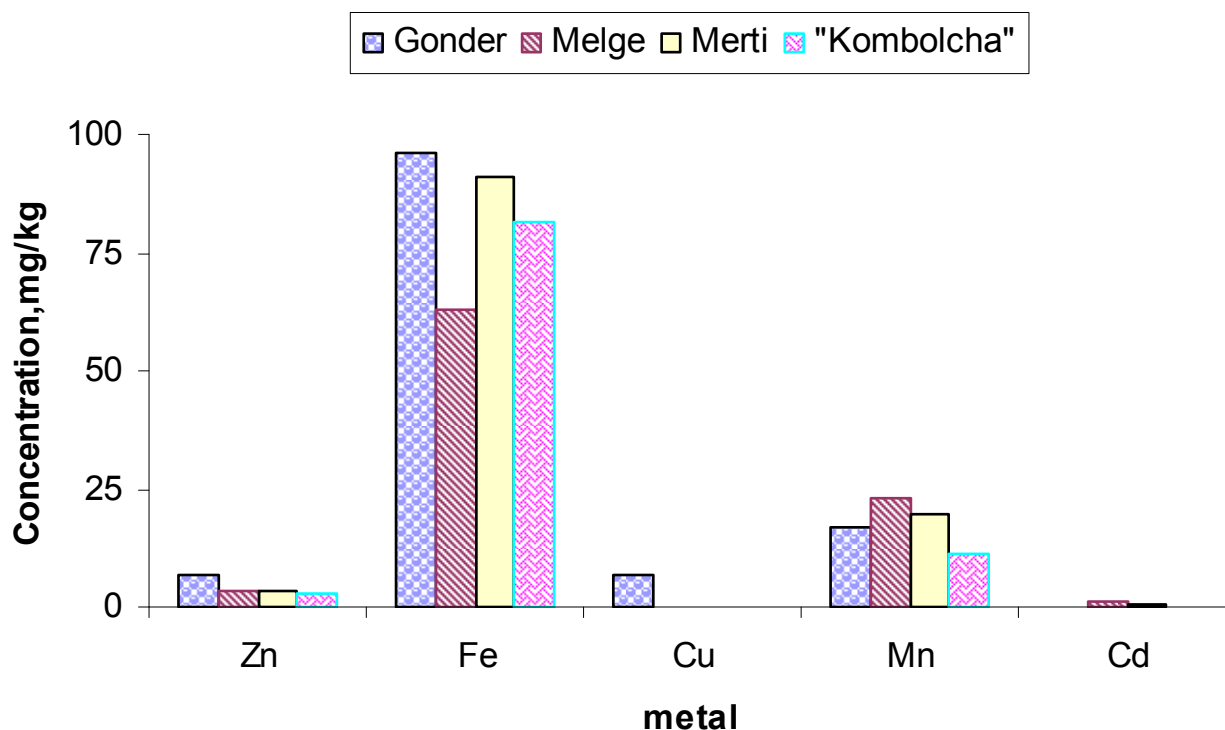


Figure 3: The mean concentration of all metals in tomato paste samples.

Zinc is one of the important metals for normal growth and development in human beings. Deficiency of zinc can result from inadequate dietary intake and results in impaired absorption, excessive excretion or inherited defects in zinc metabolism [36, 37]. Zinc in tomato paste samples was determined in the range of 2.54 to 6.63 mg Kg⁻¹. While the highest level of zinc was determined in Gondar tomato paste sample, while the lowest level was in Kombolcha tomato paste sample.

Iron plays many key roles in biological systems, including oxygen transport (hemoglobin and myoglobin), respiration and energy metabolism (cytochromes and iron-sulfure proteins), destruction of hydrogen peroxides (hydrogen peroxidase and catalase), and DNA synthesis (ribonucleotide reductase).

The deficiency of iron is one of the leading risk factors for disability and death worldwide. It results in anemia which is recognized by its symptom such as low blood iron level, small red blood cells and low blood hemoglobin values [38]. The iron contents determined in tomato paste samples were in the range of 63.11 to 96.1 mg Kg⁻¹; highest in Gondar tomato paste and lowest in Melge tomato paste. Overall, the analyzed tomato paste samples contained, relative to the other trace metals, higher concentrations of iron.

Copper is required as an essential dietary trace element. It is required for cellular metabolism in enzymatic and non-enzymatic systems. Copper acts as important metallic- activators of several enzymes. Deficiency of copper causes low white blood cell count and poor growth. Excess intake of copper can cause vomiting, nervous system disorder and Wilson's diseases [39]. Copper was determined only in Gondar tomato paste (6.95 mg Kg⁻¹).

Manganese activates numerous essential enzymes [38]. In the present study manganese were determined in the range of 11.5 to 23.02 mg Kg⁻¹. The highest concentrations of manganese were found in Melge (23.02 mg Kg⁻¹) tomato paste samples, while the lowest level of manganese was found in Kombolcha tomato paste (11.5 mg Kg⁻¹).

Lead being a serious cumulative body poison, enters into the body system through air, water, and food, and can not be removed by washing the fruits and vegetables [40, 41]. Lead was not detected in all of the samples analyzed.

Cadmium is a toxic element in foods and natural waters. It accumulates principally in the kidney and liver. Cadmium in foods is mostly derived from various sources of environmental contamination [42]. Concentration of cadmium was found in two of the samples analyzed; in Merti (0.511 mg Kg⁻¹) tomato paste samples and in Melge (1.22 mg Kg⁻¹) tomato paste samples.

The mean concentration graph of all metals for each brand of tomato paste is shown in figure 4.

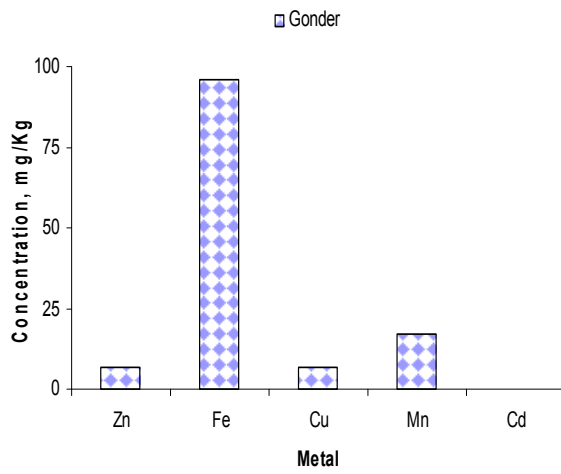


Figure 4a: Mean graph of metals in Gondar tomato paste

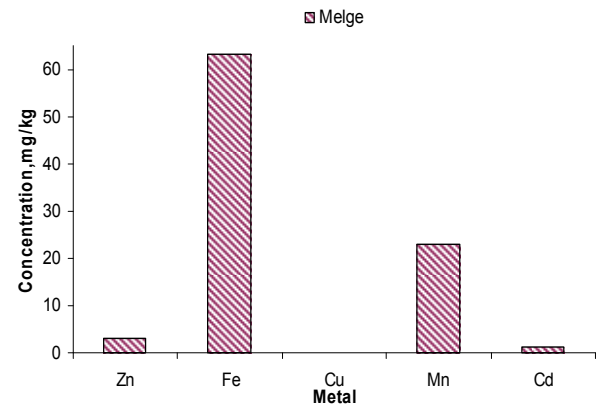


Figure 4b: Mean graph of metals in Melge tomato paste

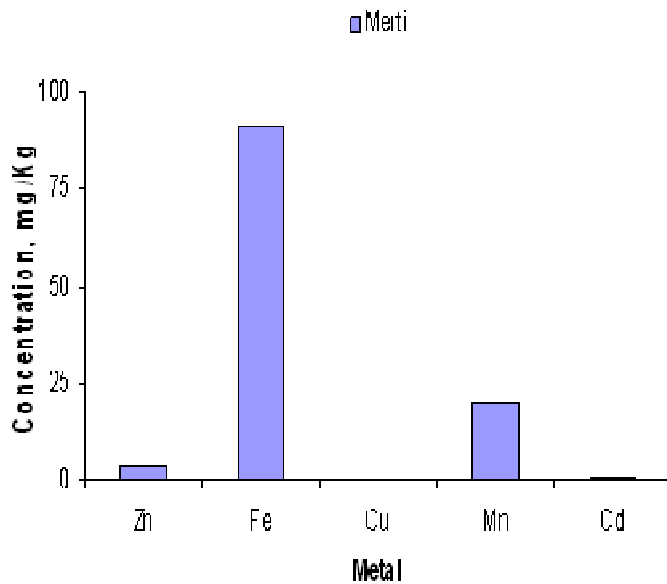


Figure 4c: Mean graph of metal in Merti tomato paste

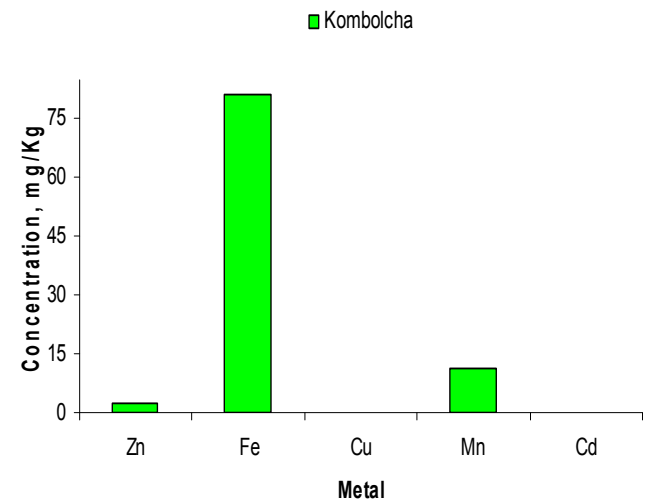


Figure 4d: Mean graph of Kombolcha tomato paste

From the mean graph it can be seen that the trend of occurrence of the metal concentrations in each tomato paste samples analyzed is in the order of Fe > Mn > Cu > Zn in Gondar brand, Fe > Mn > Zn > Cd in Melge brand, Fe > Mn > Zn > Cd in Merti brand, Fe > Mn > Zn in Kombolcha brand. This trend suggests that local tomato pastes have higher concentrations of iron, zinc, and manganese than copper and cadmium.

4.3.4.1. Comparison of Observed Metal Concentrations with International Standards

Limits have been recommended for the levels of trace metals (Zn, Cu, Pb, and Cd) in foods by different official bodies. The codex standard for canned tomato paste set a limit of 1.0 mg Kg⁻¹ for lead. The CIT Company tomato paste specifications recommended a maximum value for lead of 1.5 mg Kg⁻¹, a maximum copper content of 10.0 mg Kg⁻¹, and a maximum zinc content of 19.0 mg Kg⁻¹.

The Kenya standard for tomato product specifications recommended a maximum value for lead of 1.0 mg Kg⁻¹, a maximum copper content of 10.0 mg Kg⁻¹ and a maximum zinc content of 50.0 mg Kg⁻¹.

Venezuelan standard recommend a maximum limit of 1.0 mg.Kg⁻¹ for lead, 5.0 mg Kg⁻¹ for copper and 5.0 mg Kg⁻¹ for zinc. FAO/WHO [43] set a limit of 0.02 to 0.2 mg Kg⁻¹ for cadmium and 0.5 to 1.0 mg Kg⁻¹ for lead in tomatoes. ICRCL [44] set a limit of 0 to 1 mg Kg⁻¹ for cadmium and 1 to 50 mg Kg⁻¹ for lead in tomatoes. WHO/EU [45] limit 0.01 mg Kg⁻¹ for cadmium and 5.0 mg Kg⁻¹ for lead.

The levels of Zn in the tomato paste samples studied were lower than the CIT Company, Kenya standard, and Venezuelan standard for canned tomato paste maximum limit given above.

The levels of Cu also below the maximum limit set by CIT Company, Kenya standard, and Venezuelan standard for canned tomato paste. There are no available recommended standard values for iron and manganese for canned tomato paste.

In all canned tomato paste studied lead was below the detection limit (0.1 mg Kg^{-1}) of the instrument. So that levels of lead were below the maximum limits set by the above official bodies.

The levels of cadmium in Melge tomato paste sample studied were higher than the FAO/WHO, WHO/EU and ICRCL limits, while in Merti tomato paste samples was higher than the FAO/WHO and WHO/EU limits, but lower than the ICRCL limits.

In general, the concentration of Cd metals was found at elevated levels as compared to international standards. On the other hand, the concentration of zinc, copper, and lead was found to be below the maximum limits set by the above official bodies.

The high concentrations of cadmium in canned tomato paste examined in the present study might be taken up from soils through the roots of tomato plants or be deposited on the surfaces of tomato plant leaves from air born particulates or contaminated water might be used for irrigation and food processing or Food processing operations might add heavy metal contaminants in to tomato paste. As a result, the present study might be a springboard for further investigation (research).

5. CONCLUSION

The optimized digestion procedure provides an easy method for the digestion of tomato paste samples and percent recovery obtained was above 95 %.

The average concentration of zinc, copper and lead in local tomato paste was found lower than limits set by international Standards, while the concentration of Cd metal was found at elevated levels as compared to international standards.

In order to identify the cause of excessive cadmium content in canned tomato paste examined, more comprehensive studies are required to investigate the cause of the elevated levels of cadmium metal in local tomato paste.

REFERENCES

1. FAO. Fruits and Vegetables Processing: Agricultural Services Bulletin, **1995**, NO. 119. Rome: FAO
2. Atherton J. G. and J Rudich. 1986. The Tomato Crops: A scientific basis for improvement. Chapman and Hall, London.
3. Jenkins, J.A. The origin of the cultivated tomato, Economic Botany, **1948**; vol., 2: 379 - 392.
4. Attia E.A., Hamed H.S. and Hemmat I.M., Production of protein isolate from tomato wastes, Egyptian J of Agri Research, **2000**; vol. 78(5), pp:2085 - 2096.
5. Goose, P. and B Raymond, Tomato Puree, Juice and Powder, Food Trade Press, **1964**.
6. Gould W.A., Tomato Production, Processing & Technology. 3rd Ed. Baltimore, Md.: CTI publishing Co., **1992**.
7. Davies JN, GE Hobson, The constituents of tomato fruit: The influence of environment, nutrition and genotype. Crit Reviews Food Sci, Nutr, **1981**; vol. 15(3), Pp: 205 - 280.
8. Beecher G.R., Nutrient content of tomatoes and tomato products, Proc. of the Soc. for Exp. Biol. and Med., **1998**; vol. 218 (2), pp: 98 - 100.
9. Consensus Document on Compositional Considerations for New Varieties of Tomato: Key Food and Feed Nutrients, Anti-nutrients and Toxicants, **2008**.

10. Türkdogan MK *et al.*, Heavy metals in soils vegetables and fruits in the endemic upper gastrointestinal cancer region of Turkey, *Environ Toxicol. Pharmacol*, vol. 13(3), pp: 175 - 179.
11. Nrgholi B., Investtigation of the Firozabad wastewater quality and quantity variation for agricultural use, Final report Iranian Agril. Eng. Res. Institute, **2007**.
12. Banu E, N Preda, S.S Vasu, Food products and their toxicity: Technical. Publishing House, Bucharest, **1985**.
13. Jones J.M., Concentration of Cu and Zn in some fruits and vegetables commonly available in North-Central Zone of Nigeria. *Cereal Foods World*, **1987**; Vol. 22, pp: 573 – 579.
14. Getinet Hailu, Marketing and Sales Strategies of ELFORA Agro-Industries Plc AddisAbaba University, **2007**: <http://hdl.handle.net/123456789/1155>
15. Dendena *et al.*, The Juicy of the economy? Export Potential of Ethiopian Processed Fruits and Vegetables: Addis Ababa, **2005**.
16. Abdul Waheed *et al.*, Comparative study of selected Essential and Non-essential metals in various canned and raw food stuffs. *J Nutr & Food Sci*, **2003**; vol. 33(6), pp: 261-267.
17. David *et al.*, The heavy metals analyses in canned tomato paste. *Journal of Agro alimentary Processes and Technologies*, **2008**; Vol. 14, pp: 341 - 345.
18. Türker A.R. and M Yüksel, Digestion method for flame AAS determination of transition metals in Canned Tomato Paste. *J Atomic Spectro*, **1997**, Vol. 18(4).

19. Koçak *et al.*, Some heavy metal and trace essential element detection in canned vegetable food stuffs by pulse polarography (DPP). Electron J. Environ. Agricultural food chemistry: ISSN 1579-4377.
20. Gould, W. A., Tomato Production, Processing and Quality Evaluation, 2nd ed., Westport, Connecticut: AVI Publishing, **1983**.
21. McGlasson, B., In: Macrae, R., Robinson, R. K., and Sadler, M. J., Eds., Encyclopaedia of Food Science, Food Technology and Nutrition, London: Academic Press, **1993**; pp: 4579 – 4584.
22. Thakur, B. R., Singh, R. K., and Nelson, P. E., Quality attributes of processed tomato products: a review, Food Reviews Int., **1996**; vol. 12, pp: 375 – 401.
23. McColloch, R. J. and Kertesz, Z. I., Recent developments of practical significance in the field of pectic enzymes, Food Technology, **1949**; vol. 3, pp: 94.
24. Hewitt J. D. and Garvey, T. C., In: Nevins, D. J. and Jones, R. A, Eds., Tomato Biotechnology, New York: Liss, **1987**.
25. Petro-Turza, M., Flavor of tomato and tomato products, Food Reviews Int., **1987**; vol. 2, pp: 309 – 351.
26. Lamb, F. C., Tomato products, Bulletin 27, Washington: National Canners Association, **1977**.
27. Moresi, M. and Liverotti, C., Economic study of tomato paste production, J. Food Technology, **1982**; vol. 17, pp: 177 – 192.
28. Shomer, I. *et al.*, Mechanism which enables the cell wall to retain homogeneous appearance of tomato juice, J. Food Sci., **1984**; vol. 49, pp: 628–633.

29. Goose, P. and Binstead, R., Tomato Paste and other Tomato Products, 2nd ed., London: Food Trade Press Ltd., **1973**.
30. Morris, C. E., New tomato plant processes 90 tons per hour, Food Engineering, **1991**; vol. 63(4), pp: 136 and 139 – 140.
31. Eliase Abebe, Technical Assessment on Viability of Integrated Fruits Processing in Ethiopia, **2007**: <http://hdl.handle.net/123456789/1611>
32. Butcher D.J. and J Sneddon, A practical guide to graphite furnace atomicabsorption spectroscopy, John Wiley and Sons,**1998**; vol. 147, pp: 32-184.
33. Matusiewicz H and Kopras M., Methods for Improving the Sensitivity in Atom Trapping FAAS. Journal of Analytical Atomic Spectrometry, **1997**; vol. 12, pp: 1287- 1291.
34. The cooperation on International Traceability in Analytical Chemistry and EURACHEM, Guide to Quality in Analytical Chemistry an Aid to Accreditation, **2002**; vol., 25.
35. Gazso L.; G Giorgi., Determination of trace metal pollution of various environmental samples using atomic absorption spectrometric methods; **2001**.
36. Colak, H., Soylak, M. & Turkoglu, O. Determination of trace metal content of various herbal and fruit teas produced and marketed from Turkey. Trace Elements and Electrolytes, **2005**; vol., 22, pp: 192– 195.
37. Narin, I., Tuzen, M., Sari, H. & Soylak, M. Heavy metal content of potato and corn chips from Turkey. Bulletin of Environmental Contamination and Toxicology, **2005**; vol., 74, pp: 1072–1077.

38. Underwood, E.J, Trace elements in human and animal nutrition. 4th ed., New York: Academic Press Inc., 1977.

32. Lewis, A.G., The Biological Importance of copper, a review, **1995**; vol. 60.

40. De Leonardis, A., Macciola, V. & De Felice, M. Copper and iron determination in edible vegetable oils by graphite furnace atomic absorption spectrometry after extraction with diluted nitric acid. International Journal of Food Science and Technology, **2000**; vol., 35, pp: 371– 375.

41. Divrikli, U., Saracoglu, S., Soylak, M. & Elci, L. Determination of trace heavy metal contents of green vegetable samples from Kayseri-Turkey by flame atomic absorption spectrometry. Fresenius Environmental Bulletin, **2003**; vol., 12, pp: 1123–1125.

42. Adriano, D.C. Trace Metals in the Terrestrial Environment. New York: Verlag Spiegler, **1984**.

43. FAO/WHO. Food Additives and Contaminants, Joint FAO/WHO Food Standards Programme, **2001**. ALINORM 01/12A: 1 – 289.

44. ICRCL: Interdepartmental Committee for the Redevelopment of Contaminated Land, Guidance on the Assessment and Redevelopment of Contaminated Land, **1987**, Paper 59/83 2nd Ed.

45. WHO/EU: WHO and EU Drinking Water Quality Guidelines for Heavy Metals and Threshold Values Leading to Crop Damage (mg/L), **1983**.