DISTRIBUTION OF ESSENTIAL/NON-ESSENTIAL HEAVY METALS IN EDIBLE LIVER

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BY

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DEDICATED TO

MY ADORABLE FATHER

WALI UR REHMAN

Who gave me the sheen of confidence and encouraged me to achieve success in every sphere of life and led me to the destination.

DECLARATION

This is to certify that this dissertation submitted by ***NAVEED ZAFAR ALI*** is accepted in its present form by the Department of Chemistry, Quaid-i-Azam University, Islamabad, as satisfying the dissertation requirements for the degree of Master of Science in Chemistry.

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ABSTRACT

Two selected essential metals (Fe & Zn), four non-essential metals (Ni, Cr, Cd, & Pb), and four macro-nutrients (Na. K. Mg, & Ca) were determined in edible liver samples of various animals by using FAAS, wet digestion technique, based on the use of HNO_3-$HCLO_4$ methods. Optimum operating analytical conditions were established for each metal separately on a Schimadzu AAS system, model 607-A. the edible liver samples pertaining to lamb, calf, chicken, goat and buffalo, employed for metal analysis were obtained from local, meat vendors in Islamabad and Rawalpindi. The

results are reported as X±SD, on wet weight basis. The accuracy determined experimentally ranged between ±1.0----±1.5% for the triplicate runs of sub-samples. In case of essential metals, the concentration of Zn was found to range from 2.99 mg/kg to 5.53 mg/kg in buffalo and lamb liver samples respectively, while the concentration range of Fe was from 121.0mg/kg to 398.1mg/kg in chicken and calf liver samples respectively. In case of non-essential metals the concentration of Ni ranged between 0.40----39.30mg/kg in buffalo and lamb liver sample. The concentration of Car was found table between 04.64----13.14mg/kg in goat and lamb liver, while that of Cd between 0.03----0.19mg/kg in lamb and buffalo liver samples respectively. Similarly, Pb levels ranged between 0.19----1.95mg/kg in lamb and goat liver samples.

In case of macronutrients, the Na levels were found to be between 728.1 and 1327.7mg/kg in goat and buffalo liver samples; K between 15.01----33.37mg/kg in the chicken and goat liver samples; Ca between 261.4----435.0mg/kg in goat and chicken liver samples respectively.

From the viewpoint of enhanced levels of non-essentials metals in the liver of various animals, the present study brings forth a serious caution against the use of liver as regular food item, since the toxic metal levels are well above the stipulated safe limit of 1mg/kg laid down by W.H.O. The daily dietary allowance (DDA) must be accordingly fixed for the ingestion of these metals through liver.

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CHAPTER 1

INTRODUCTION

1.1 GENERAL BACKGROUND

Man's body is made up of the nutrients he eats. In addition to providing the substance for building and maintaining the body, the energy for all of the body's functions comes from the food consumed.

The food scientist must consider the nutritive aspects of food from two broad points of view: first, what nutrients do foods contain and what are man's requirements for these. The nutrients of food, which must be supplied by the diet t produce and maintain optimum health, belong to the broad groups of carbohydrates, proteins, fats, vitamins and minerals.

❖ FUNCTIONS OF FOOD AND ITS COMPONENTS

In order to perform vital functions of the body, animals need sufficient energy and chemical constituents for use in many different ways in the body. Food fulfills the following requirements of animals.

1. Provides energy, which is released from the food by oxidation in cellular reparation.
2. Provides constituents for repair and growth of cells, tissues and organs.
3. Provides essential materials to make enzymes, which are biological catalysts.
4. Provides materials to maintain various processes of life including reproduction.

The food which animals, including humans, take consists of the following basic components, also called nutrients.

1. **Carbohydrates**
2. **Fats**
3. **Proteins**
4. **Vitamins**
5. **Minerals**
6. **Water**

We now consider the role of minerals.

- **MINERALS**

Animals also require in their food certain inorganic substances which we cell minerals. See Table 1 for daily need of some minerals. The most important on them are Carbon, Hydrogen, Nitrogen, Oxygen and Sulphur.

Bones and teeth need calcium. It is very important for growing children. The main sources of calcium are milk, cheese, eggs, fruits, green vegetables and almonds. Phosphorous is a constituent of body cells and serves to regulate the metabolism of proteins. It is present as calcium phosphate in bones and teeth. The foods rich on phosphorous are fish, oysters, eggs, milk and cheese.

Iron is essential for the formation of haemoglobin, the red pigment of the blood.

Fluoride helps fight tooth decay. Iodine is part of thyroid hormone and its absence on our diet causes deficiency of the hormone. Seafood are rich in iodine.

Zinc, Manganese, Molybdenum and Chromium are required by a variety of enzymes.

Table 1 Recommended daily amount of minerals in diet.

NUTRIENT	RECOMMENDED DAILY AMOUNT
Calcium	0.8-1.2g
Fluoride	0.0015-0.004g
Iodine	0.150g
Phosphorus	0.8-1.2g
Potassium	1.9-5.6g
Sodium	1.1-3.3g
Iron	0.0100-0.0175g

1.2 MEAT AND ITS COMPOSITION

Man has sought after meat and fish, because of their fine flavour, since the beginning of history. There is no doubt that they play a most important port on our nutrition. The principal nutrient supplied is protein, although from the quantity standpoint many meat cuts contain a higher percentage of fat. In general, the proteins of meat and of fist are of good biological value and especially important as supplements to the proteins of grains many of which, by themselves are rather poor biologically.

Muscled meat, in itself, is not a complete food: it is seriously deficient in calcium, and the high phosphorus content makes the calcium phosphorus ration out of balance. Ascorbic acid, the fat-soluble vitamins and certain B vitamins are likewise deficient. In general there is practically no carbohydrate present.

COMPOSITION OF MEAT

The basic composition of meat varies considerably between different types and cuts. The fat-to-protein ration for instance may vary from 0-13:1 up to 100:1 or even higher. The average nitrogen in the fat-free (N/FF) figure, which, in view of its approximate constancy, is used for assessing the meat content of products.

Nutritionally meat contains some thiamin, riboflavin and nicotinic acid, but, but apart from offal's (particularly liver) is almost devoid of vitamins A, C and D. for the purpose of calculating the proportion of liver on products the following figures for mg Fe/100g may be of value: beef 4.0, pork 1.5 and liver 13.2.

1.3 TRACE ELEMENTS AND FOOD

From the viewpoint of the food analyst the term 'trace element' refers to the inorganic elements (mostly metals) which may be present in foods in amounts usually well below 50ppm and have some toxicological or nutritional significance. Calvery broadly classified trace elements according to their effect on life and placed them into three classes: (1) The essential nutritive elements, e.g Co, Cu, Fe, I, Mn, Zn (2) The non-nutritive, non-toxic elements, e.g Al, B, Cr, Ni, Sn which are not known to have produced harmful effects when present in quantities non exceeding 100ppm and (3) The non-nutritive toxic elements, e.g. as Sb, Cd, F, Pb, Hg, Se which are known to have deleterious effects even when the diet contains less than 100ppm.

A complicating factor, however, is that element such as copper and zinc, although essential for life processes when present tin traces, have an emetic action when ingested in higher amounts. Cumulative poisoning due to ingestion of food containing elements such as lead or arsenic over a long period is probably rare. In addition to the action on the

body, however, a few elements tend to have a detrimental effect on the quality or nutritive value of the food, e.g. copper causes off-flavours in mild and dairy products and tends to destroy vitamin C in fruit products.

1.4 SOURCES OF TRACE ELEMENTS

The presence of the more undesirable trace elements in foods can usually be attributed to one of the following causes: -

I. Natural occurrence, e.g in shellfish due to ingestion of estuary water contaminated by industrial effluents, deposition in the lover of animals

II. Spray and dust used as insecticides during cultivation, e.g. leas arsenate.

III. Use of impure chemicals for the manufacture of raw materials, e.g. the use of arsenic-contaminated sulphuric acid for the manufacture of acid phosphates from fluorine-contaminated rock phosphate.

IV. Accidental contamination due to confusion of materials of similar appearance, e.g. white arsenic for corn fl9ur (for dusting sugar confectionery), tartar emetic for cream of tartar (in self-raising flour).

V. Foods (especially acid foods and those containing salt or alcohol) may dissolve metals from the equipment, e.g. from tinplate, foils, solders, galvanized iron and cheap enamels and glazes.

1.5 THE LIVER

The liver is a chemical warehouse in human body. The basic function of the liver can be divided into:-

I. Its vascular functions for storage and filtration of blood.

II. Its metabolic functions concerned with the majority of the metabolic systems of the body.

III. Its secretary and excretory functions that are responsible for forming the bile that flows through the bile ducts into the gastrointestinal tract.

1.5.1 **METABOLIC FUNCTIONS OF THE LIVER are very complicated and they** incorporate various metals. The liver cells altogether are a large chemically reactant pool having a very high rate of metabolism, sharing substrates and energy from one transported to other areas of the body.

Various other functions of the liver are:

A . CARBOHYDRATE METABOLISM

In carbohydrate metabolism the liver performs the following specific functions:

1. Storage of glycogen
2. Conversion of glacotose and fructose to glucose
3. Gluconeogenesis
4. Formation of many important chemical compounds from the intermediate products of carbohydrate metabolism.

The liver especially important for maintaining a normal blood glucose concentration. For instance, storage of glycogen allows the liver to remove excess glucose from the blood, store it and then return it to the blood when the blood glucose concentration begins to fall too low. This is called the glucose buffer function of the liver. As an example, immediately after a meal containing large amounts of carbohydrates, the blood glucose concentration rises about three times as much in a person with a non-functional liver as in a person with non-functional liver as in a person with normal liver.

Gluconeogensis in the liver is also concerned with maintaining a normal blood glucose concentration, for gluconeogenisis occurs to a significant extent only when the glucose

concentration begins to fall below normal. In such a case, large amounts of amino acids are maintaining a relatively normal blood glucose concentration.

B.: FAT, METABOLISM

Although some fat metabolism can taken place in almost all cells of the body, certain aspects of fat metabolism occur mainly in the liver. Specific functions of the liver in fat metabolism are (1) a very high rate of oxidation of fatty acids to supply energy for other bodily functions, (2) formation of most of the lipoprotein s, (3) synthesis of large quantities of cholesterol and phopsholipids, and (4) conversion of large quantities carbohydrates and proteins to fat.

To derive energy from neutral fats, the fat is fist split into glycerol and fatty acids; then the fatty acids are split by beta oxidation into two-carbon acetyl radicals and then from acetyl coenzyme A (acetyl-CoA). This in turn can enter the citric acid cycle and be oxidized to liberate tremendous amounts of energy. Beta-oxidation can take place in all cells of the body, but it occurs especially rapidly in the hepatic cells. Yet the liver itself cannot utilize all the acetyle-CoA into the acetoacetic acid, which is a highly soluble acid that passes from the liver cells into the extracellular fluids and then is transported throughout the body to be absorbed by the other tissues. These tissues in turn reconvert the acetoacetic acid into acetyle-CoA and then oxidize it in the usual manner. In these ways the liver is responsible for a major part of the metabolism of fats.

About 80% of the cholesterol synthesized in the liver is converted into bile salts, which in turn are secreted into the bile; the remainder is transported in the lipoproteins, which are carried by the blood to the tissue cells everywhere in the body. Phospholopids are used by

the cells to form membranes, intracellular structures, and multiple derived chemical substances that are important to cellular function.

Most of the fat synthesis in the body from carbohydrates and proteins also occurs in the liver. After fat is synthesized in the liver it is transported in the lipoproteins to the adipose tissue to be stored.

C. PROTEIN METABOLISM

Even though a large proportion of the processes for carbohydrate and fat metabolism occurs in the liver, the body could probably dispense with many of three functions of the liver and still survive. On the other hand, the body cannot dispense with the services of the liver in protein metabolism for more than a few days without death ensuing. The most important functions for the liver in protein metabolism are (10) deamination of amino acids, (2) formation of urea for removal of ammonia from the body fluids (3) formation of plasma proteins, and (4) inter conversions among the different amino acids and other compounds important to the metabolism processes of the body.

Deamination of the amino acids is required before these can be used for energy or before they can be converted into carbohydrates or fats. A small amount of deamination can occur in the other tissues of the body, especially in the kidney, but the percentage of damination occurring extrahepatically is so small that it is almost completely unimportant.

Formation of urea by the liver removes ammonia from the body fluids large amounts of ammonia are formed by the deamination process, and still additional blood. Therefore, in the absence of this function of the liver to form urea, the plasma ammonia concentration

rises rapidly and results in hepatic coma and death. Indeed, even greatly decreased blood flow through the liver as occurs occasionally when a shunt develops between the portal vein and the vena cava can also cause excessive ammonia in the blood, an exceedingly toxic condition.

Essentially all the plasma proteins, with the exception of part of the gamma globulin, are formed by the hepatic cells. This accounts for about 90 per cent of all of the plasma proteins. The remaining gamma globulins are the antibodies form plasma proteins at a maximum rate of 15 to 50 grams a day. Therefore, after loss as much as half the plasma proteins from the body, these can be replenished in a week or two. It is particularly interesting that plasma proteins depletion causes rapid mitosis of the hepatic cells and actual growth of the liver to a larger size; these effects are coupled with rapid output of plasma protein untill the plasma concentration returns to normal. Among the most important functions of the liver is its ability to synthesize other important chemical compounds from amino acids. For instance, the so-called non-essential amino acids can all be synthesized in the liver. To do this, a keto acid having the same chemical composition (except at the keto oxygen) as that of the amino acid to be formed is first synthesized. Then an amino radical is transferred through several stages of transamination from an available amino acid to the keto acid to take the place of the keto oxygen.

D. STORAGE OF IRON.

Except for the iron in the haemoglobin of the blood, by far the greater proportion of the iron in the body is usually stored in the liver in the form of ferritun. When the iron in the circulation body fluids reaches a low level, the ferritin releases the iron. Thus, the apoferritin system of the liver acts as a blood iron buffer and also as an iron storage medium.

E. MINERAL METABOLISM

The functions of many of the minerals, such as sodium, potassium, chloride and so forth have been reported.

The daily requirements of these are given in Table 1.

Magnesium. Magnesium is approximately one sixth as plentiful in cells as potassium. Magnesium is especially required as a catalyst for many intracellular enzymatic reactions, particularly those relating to carbohydrate metabolism.

Table 1. REQUIRED DAILY AMOUNTS OF MINERAL

Na	**3.0g**
K	**1.0g**
Cl	**3.5g**
Ca	**1.2g**
P	**1.2mg**
Fe	**18.0mg**
I	**150.0μg**
Mg	**0.4g**
Co	**Unknown**
Cu	**Unknown**
Mn	**Unknown**
Zn	**15.0mg**

Calcium. Calcium is present in the body mainly in the form of calcium phosphate in the bone. Excess quantities of calcium ions in the extra cellular fluids can cause the heart to stop in systole and can act as a mental depressant.

Phosphorus. Phosphate is the major anion of intracellular fluids, Phosphates have the ability to combine reversibly with many coenzyme system and also with multiple other compounds that are necessary for operation of the metabolic processes.

Iron. Two thirds of the iron in the body is in the body is in the from of hemoglobin, through smaller quantities are present in other forms, especially in the liver and in the bone marrow. Electron carriers containing iron (especially the cytochromes) are present in the mitochondria of all cells of the body and are essential both for transport of oxygen to the tissues ad for operation of oxidative systems within the tissue cells, without which life would cease within a few seconds.

Important trace elements in the body. A few elements are present in the body in such small quantities that they are called trace elements. Usually, the amounts of this in the foods are also minute. Yet without any one of the them a specific deficiency syndrome is likely to develop. Two of the most important of these are:

Iodine. The best known of the trace elements is iodine. The entire body contains an average of only 14 milligrams. Iodine is essential for the formation of thyroxin and triiodothyronine, the two thyroid hormones that are essential for maintenance of normal metabolic rates in all of the cells.

Zinc. Zinc is an integral pat of many enzymes, one of the most important of which is carbonic a hydrate, present in especially high concentration in the red blood cells. This enzyme is responsible for rapid combination of carbon dioxide with water in the red

blood cells of the peripheral capillary blood and for rapid release of carbon dioxide from the pulmonary capillary blood into the alveoli.

1.5.2 THE MINERAL REQUIREMENTS

Sodium, potassium, magnesium and phosphorus are present in adequate amounts in a diet, which is ample in other respects, and as a rule, no special attention need be paid to them. Sodium chloride, for example, besides that which is present naturally in many foodstuffs is added in cooking and as table salt in quantities determined by individual taste. Protein foods constitute the chief source of the phosphorus of the diet and when the allowance of protein is adequate, the phosphorus intake takes care of itself. Potassium and Magnesium are derived from cereals and vegetables and are also present in sufficient amounts in an ordinary diet.

The minerals in which the diet is most likely to be deficient are calcium, iron and iodine. The intake of calcium, which enters so largely into the composition of bones especially likely to be inadequate in the diets of children. Children require at least a gram of calcium per day. The adult requirement is about 0.8 gram daily. As a result of the deposition of mineral in the bones of the fetus, the demand for calcium increases during pregnancy when the allowance should be from 1.5 to 2 grams daily.

Only a small part of the calcium in cereals and vegetables is utilized by the body. Meat contains minimal amounts. Mild is therefore, especially for children, the best source of calcium.

Iron is an indispensable constituent of the diet, since it is necessary for the synthesis of haemoglobin the daily requirement is from 15to20 mg. The chief sources of food iron

are meats (especially liver), eggs and such vegetables and cereals as spinach, beans and peas whole wheat and oatmeal. Milk is very poor in iron.

Iodine is an essential constitute of the thyroid hormone; goitre results when the diet is deficient in this element. Seafood are the chief natural courses of iodine, though many brands of table salt (iodised salt) contain small quantities (1 part in 100,000) which have been added by the manufacture. The daily requirement of iodine is placed at about 100 micrograms.

There are a number of other mineral elements, which are required to be taken in minute quantities for special purposes e.g. the elaboration of certain hormones and enzymes or for the incorporation into particular tissues. Among these so-called 'trace elements' are: cobalt (in vitamin B) zinc (in insulin) manganese (in tissue enzymes) and copper (required in Hemopoiesis). No attention need be paid to these elements in planning a diet since the small amounts required are present in a diet adequate in other respects.

1.6 THE PRESENT STATUS OF METAL ANALYSIS IN MEAT IN PAKISTAN

The problem of food contamination is growing from serious to more serious during the recent years. This is specifically true of meat & its products used extensively by vast population segment of Pakistan. The country is going through a rapid process of Industrialization & fast urbanization & therefore environmental pollution going up the ladder day by day. In big cities the industrial metal are being released into the open atmosphere through the chimneys of industrial units & this in turn generates dispersion of the toxic pollutants to far-flung towns and villages. Inter city roads and major highways are today the worst culprits of motor vehicular exhaust damaging the back ground level of many toxic trace metals existing previously. Therefore, not only human beings but

animals as well as are exposed to the ill effects of toxic metals. Food grains vegetables and fodder is not accepted by the pollution and consequently every living organism is at a grave health risk with respect to the absorption of toxic metals via air, water & food.

No viable attempt has been made in Pakistan to assess and study the impact of the metal pollutant in food items. Although a consulted effort was required to undertake such work to safeguard the heath of people, no coordinated work to this aspect has ever appeared in literature. Only scanty reports not really relevant to a program study are available, but the are to nave to warrant a consolidated study on the issue.

As has been brought about in the previous sections, the situation of metal pollution of meat is very serious. Of the various organs of animal body used inedible dishes, the liver profoundly occupies a central place. A large population segment in Pakistan is accustomed to the delicious taste of roasted liver in the breakfast. On religious festivities this again is the most popular dish. Not withstanding the fact that liver in itself is a chemical warehouse in the body of an animal, its use on a very extensive scale must be legally controlled since it contains the whole gamut of toxic trace metals.

During the present study an attempt was made to first evolve base line information regarding the concentration levels of some selected metals in the liver. This study would certainly add to the awareness of the general masses regarding the adverse effect of these metals and controlled strategy towards implementing proper food habits among the people.

This study warrant as safe use of liver as a food item through a proper check on ADI, permissible as per legislation made by health authorities around the world.

1.7 OBJECTIVES OF PRESENT STUDY

The broad objectives of the present study are as follows.

1. To evolve a base live information on the levels of selected essential & non-essential metals in the edible liver of various animals.

2. To examine the data against those laid down for safe human ingestion by world organization such as W.H.O.

3. To assess the present toxic and non-toxic impacts of heavy toxic trace metals.

4. To analyse and examine the data for safe implementation of human health grounds.

5. To study a possible inter relationship between pairs of metals so that their level control for future work could be initiated.

CHAPTER 2

HISTORIC PERSPECTIVE

2.1 EARLIER WORK

In the field of analysis of trace metal in liver a serious concern has arisen in recent years regarding trace metal content and its implications towards human health. Cadmium and lead levels in cattle liver have been determined by atomic absorption spectroscopy in different liver samples in various studies, and it turned out that the metal contents were within permissible level. Similarly, in other studies, the level of cadmium in water and fish were determined to be above the toxic limit. Once exposed, the Cd levels increased progressively in all the selected fish tissues, especially in the liver. (1,2). This study generated a deep interest in liver analysis.

The trace element concentration in the liver and spleen of the animal body were determined by freezing, drying pulverizing the sample. The samples were run through AAS and were found that the toxic metal level was above the safe limit. Similarly, the study on different elements such as Selenium as a chemo preventive agent in human primary hepatocellular characinoid show that it is quite effective in the prevention of primary liver cancer. (2)

In large-scale studi3es, the trace element concentrations were determined liver tissues archived in the National Marine Mammal Tissues Bank. Tissues were collected from 139 animals representing 13 species of marine mammals from around the U.S. It was found that the toxic elements were above the permissible level. (3-4)

The concentrations of certain heavy metals in the muscle, liver and kidney of mountain goats are also reported in literature. Accordingly, the levels of Cd were 10 times higher in the liver as compared with other organs. The toxic metal levels so determined were above the permissible level again (6).

Later studies in this field of metal analysis in liver revealed that the cadmium and lead in edible meat and liver pastes had concentrations above the green limit. So that use of such pastes was not fit for human health.

The average concentration levels measured were 19 ng/g for Cd and 15 ng/g for Pb in duck liver pastes. Later on, it was observed that the metals in the container are also responsible for such a high level of toxic elements in these pastes (8).

The contents of Pb, Hg in the liver of small animals were determined also and the findings supported the hypothesis that an environment toxin such as mercury can enter and damage neurons. Another relevant study on the detection of toxic metals in rat liver revealed that the chronic Cd chronicle in rats caused considerable decrease of reduced gluathoine and the ascorbic peroxide rate also increased. High red meat diets have been linked with risks of colorectal cancer; but their effect on mutation which occurs in the cancer are unknown (9). Diet is supposed t influence the colorectal cancer etiology. Most studies showed an increased such to develop a colorectal cancer for those eating higher amounts of meat (10).

In the field of meat analysis, different meat samples were analysed for some trace metal by employing AAS. Data revealed that Pb can tent in cattle liver was 37 μg/kg which was above permissible level (11). The effect of Cd with and without selenium, on breeding

hens, cocks and chicks was investigated in a study (12). Cadmium chloride was orally administered to the hens. The result showed variability in Cd levels in hens. The levels were high in liver and lower in muscles and other organ (13). Similarly work on the toxicity in the liver due to heavy metals provides evidence to the fact that if the level of toxic element in the liver of different animals (including human beings) is above the permissible level then it would cause ill-effects on health, and could prove to be fatal subsequent upon constant ingestion.

2.2 ANALYTICAL METHODOLOGY

COMMON EXPERMENTAL TECHNIQUES

In the detection of toxic metals in meat samples different analytical techniques have been adopted, but no single technique fully satisfies all the requirements. Generally atomic and nuclear spectrometry has been frequently applied for trace metal determination. Atomic absorption sepctrophotmetry (AAS), inductively couple plasma/atomic emission spectrometry (ICP/AES), X-ray fluorescence spectrometry (XRP), Neutron Activation Analysis (NAA) and Anodic Stripping Voltammetry (ASV) are the major technique that have been successfully applied for the determination for trace metals in meat and body organ analysis. Nowadays, the development of new methods like gas chromatography/mass spectroscopy and dynamic ion-exchange chromatography has helped greatly in the determination of toxic metals in liver and meat samples. We now review some of the silent features of these techniques.

2.2.1 ATOMIC ABSORPTION SPECTROSCOPY (AAS)

Until recently flame atomic absorption spectroscopy was the most widely used of all atomic spectral methods because of its simplicity, effectiveness and relatively low cost of operation. Atomic absorption spectroscopy is the most widely used technique for the analysis of trace metals in meat. For this, the samples are treated with nitric acid followed by treatment with perchloric acid until digestion is complete. The resulting solution so obtained is directly aspirated into flame via a nebulizer. Electro-thermal atomisation technique has greatly increased the sensitivity of AAS. The element so present in the flame absorbs that characteristic radiation coming out from hollow cathode lamp. The absorption is noted and finally the unknown trace element is detected by calibration against standard solutions.

2.2.2 NEUTRON ACTIVATION ANALYSIS (NAA)

Among the techniques used to determine the trace element in liver, NAA has retained a favoured position. The technique usually required minimum sample preparation, and allows several trace elements to be determined simultaneously. NAA is usually carried out through at least two neutron radiation-decay-measurement cycles for multi element determinations. The gamma spectra of the short lived nuclides are recorded for a few minutes after irradiation and decay period of few minutes, each are allowed to lapse. The spectra of the long-lived nuclides are recorded for an hour after several days or weeks. Infact it is possible to increase the sensitivity and selectivity of NAA by chemically isolating the element or elements of interest from the irradiated sample prior to recording its gamma spectrum.

2.2.3 ATOMIC EMISSION SPECTROSCOPY (AES)

Emission spectroscopy is based on the principle that an excited atom emits radiation characteristic of the atom itself. Hence by knowing the wavelength of emitted radiation for a particular atom and by measuring its intensity, the analyst can identify an element, presence and its concentration. Earlier photographic instruments were used for recording the emitted intensities. Later, by the 1960's direct reading spark emission spectrometers were widely introduced for elemental analysis. Flame emission spectroscopy is most widely used for elemental determination. Flame photometers are simple in design and operation but in applications, being excellent for the determination of sodium and potassium, but quite inadequate for most other elements in complex matrix. The excitation source can be mixtures of acetylene and air, acetylene and oxygen, acetylene and nitrous oxide or argon hydrogen entrained air. Matrix characteristics can introduce problems, which are difficult to control.

Plasma radiation sources are considered best, for the purpose. The most common plasma from is the inductively coupled plasma referred to as to as ICP. Temperature within the plasma itself ranges form 6000 to 10,000K, which minimizes the effects due to self-absorption and other interference. Since the ICP has a fairly large dynamic analytical range, thus the samples with a wide range of elemental concentrations can be handled without further dilution or further concentration. Until now as many a seventy elements have been identified by this technique. In emission spectroscopy the sample itself is emitter so there is no radiation.

2.2.4 X-RAY FLUORESCENCE SPECTROSCOPY (XFS)

In this technique, the atoms are bombarded with radiation of distinct energy, electrons are removed from an inner shell, resulting in excited atoms. This causes an electronic rearrangement in which electron from outer shells fall into holes or vacancies by ejected electrons according to definite transition rules. The electromagnetic radiation thus emitted presents in characteristics, simple and readily predictable spectrum for each excited element. This radiation after an appropriate wavelength or energy separation can be used for qualitative detection and quantitative determination of most elements. In comparison with optical atomic spectroscopy, where mainly photoelectrons of the outer shell are involved, the transition of outer electrons into inner shell vacancies leads to a comparably high-energy radiation with ranger from about 0.6 up to 120 Kev.

Characteristic X-rays can be generated in the classical manner with X-ray tubes, with X-rays or gamma rays from radioactive sources, and with particles such as electrons, protons, particles or even heavier ions from appropriate accelerations. The use of X-rays as exciting radiation is term X-ray fluorescence. In particle Induced X-ray emission (PIXE), high X-ray intensity is generated by incoming particles passing almost all of their energy inadvantgeous for very thing samples and the excitation of elements of low atomic number. Conventional techniques in X-ray analysis achieve absolute detection limit in ug rang with under optimal conditions, a precision of 2%. The detection power is however is not sufficient for direct ultra trace determination in numerous biological materials.

2.2.5 COLORIMETRY

Colorimetry is one of the most frequently used methods of analysis. It is based upon absorption of radiation by a coloured solution. The amount of radiant energy absorbed is proportional to the concentration of the absorbing material in solutions. By measuring the absorption of light it is to determine quantitatively the amount for absorbing substance present. Visual calorimetric methods rely on the comparison with one or more coloured solutions of known concentration. This method is used to determine relatively small amounts (from trace up to amounts of one or two percent) but not frequently used for this analysis of large quantities. The procedures for such estimations used are quite involved and require separation steps to eliminate interfering substances if any.

2.2.6 ELETROCHEMCAL METHODS.

The electrochemical techniques are polarographic, both direct (DC) and alternating (AC) current and Anodic or catholic stripping votammetry and polarogrphy. The polargraphic techniques are suitable for dilute solutions with sensitive of 10 – 10 mol/litter, with some degree of improved sensitivity by inverse polarography and voltammetry involving the additional concentration step where the lower omit or sensitivity is 10 mol/litre.

A good number of elements can be determined by cathodic or anodic stripping voltammetery. Accuracy is less than in usual polarography and voltammetry, but good sensitivity combined with relatively low instrument costs makes the technique attractive.

For the determination of bismuth, cadmium, copper, lead, thallium and zinc in several biological tissue types, samples are prepared by wet oxidation with perchloric acid. These

elements can be determined at subnanogram concentration with precisions of less than 10%. However, about 100 determinations per day can be made, Therefore, a major drawback is the lack for multi-element capability, making the technique slow if determinations for several elements are to be made.

2.2.7 OTHER ANALYTICAL TECHNIQUES.

There are other analytical techniques that have application for elemental analysis in biological matrices, such as spark source mass spectrometry, laser microprobe spectrometry, gas chromatography, fluorometry and nephelometry, and the use of specific electrodes. Although these instrumental techniques, and there are others are applicable for elemental analysis, they are not widely used for assay work with biological substances.

2.3 SAMPLE DIGESTION METHOD.

Representative samples of edible liver were obtained randomly from local meat market. In a 150mL beaker an accurately weighed (5.000g) portion of liver sample was taken and 15mL of 65% Nitric acid was added. It was then heated over a hot plate at about 60-70C for an hour or until a homogenous solution was formed. It was allowed to cool to room temperature. Subsequently 5mL of 60% perchloric acid was added to the sample solution and it was again heated up to 60-70C until no white fumes were coming out of the solution. Afterwards the solution was placed to cool down to room temperature. In a neat and clean 50mL measuring flask the solution was poured and diluted up to the mark with doubly distilled water. The suspended particles if present were filtered before proceeding further and at that point again there was no need to dilute the solution again up to mark.

Thus finally a transparent and clear solution was obtained, ready for aspiration on to the AAS system.

2.4 PREPARATION OF STANDARDS.

The standards of desired concentrations were prepared using either the ready stock solutions (BDH or E. Merck) or pure soluble salts of the relevant metals with guaranteed purity of 99.9% were used. Subsequent dilutions from the stock solutions, usually 100 ppm, were made using double distilled water. The final finished volume of each standard was 50.0mL in a flat-bottomed measuring flask. In cases where soluble salts were taken for the preparation of standards, nitrates were preferably selected. For the stabilization of standards about 5mL of dilute nitric acid (5% v/v) was added to each sample solution. The standards thus prepared were intended for use within a week after which time fresh standards were prepared following the procedure outlined above.

2.5 QUANTIFICATION OF RESULTS.

To convert the measured absorption values into concentration of the metal being determined it is necessary either to make use of a calibration curve or to carry out the standard addition procedure. We describe here the calibration curve procedure since it was used for the quantification.

2.6 CALIBRATION CURVE PROCEDURE.

A calibration curve for use in atomic absorption was plotted by aspirating into the flame samples of solutions containing known concentration of the element to be determined measuring the absorption of each solution, and then constructing a graph in which the

measured absorption is plotted against the concentration of solution. At least four standard solutions were used covering the optimum absorbance range of 0.1 to 0.4 A, and if the calibration curve was found to be non-linear the measurements with additional standard solutions should be carried out. In common with all absorbance measurements, the readings were taken after the instrument ‘zero’ has been adjusted against a blank which may be either distilled water, or a solution of similar composition to the test solution but without the component to be determined. It was usual to examine the standard solutions in order of increasing concentration, and after making the measurements with one solution, distilled water is aspirated into the flame to remove all traces of solution before proceeding toe the next solution. At least two, and preferably three, separate absorption readings were made with each solution, and an average value taken.

Using calibration curve it is a simpler matter to interpolate from the measured absorbance of the test solution the concentration by the relevant element in the solution. The working graph was checked occasionally by making measurements with the standard solution and if necessary a new calibration curve was drawn.

CHAPTER-3

RESULTS AND DISCUSSION

3.1 THE DATA

The data on selected trace metals, essential and non-essential, macronutrients in various liver samples are given in tables 3 through 5.

The selected essential metals were Zn and Fe, while the non-essential metals were Ni, Cr, Cd and Pb. The macro-nutrients included in the study were Na, K, Ca and Mg. All metal estimations were conducted using the acid digestion, FAA method, under optimum operating conditions, established for each metal separately. The variables for this optimisation process are reported in table 1, where wavelength, HCL current, optical slit width and fuel rate are shown to have specific optimal magnitudes. In case, where lines other than reported one in the tables were found not to yield better absorptions, only a single line giving maximum resonance energy was selected for analytical operations. The sensitivity of detection is reported for each metal separately as function of detection limit, expressed as mg/L of the analytic, compatible at 1% absorption condition.

The standard calibration method was used for the quantification of results. Standard reference material was used for ascertaining the accuracy of the measured values. The two results normally agreed within 1-2%, and in cases where disparity was beyond that limit, procedural adjustments were made, both in sample digestion and optimisation of controlling parameters.

The reported results appear in the Table as mg/L±SD, where SD refers to the standard deviation pertaining to triplicate sub-samples of a given sample. The magnitude of SD fell normally within ± 1-1.5% of measured estimate; however, in cases where the metal concentrations were very low, SD was found to be considerably high. Therefore, higher dispersion in data was observed for such cases.

The table don't carry the BDL entry followed by NA for any of the estimated metals, evidencing a 100% incidence of metal occurrence in the liver. Thus, no single metal was found below detection limit, and, therefore, all liver samples were found to be associated with finite amounts of the selected metals.

3.2 SAMPLE SELECTION

The liver samples collected for the present study were obtained from local markets in Islamabad and Rawalpindi. They come from lamb, cow, calf, chicken, goat and buffalo. The term edible, was used as a pre-number to the livers of these animals in the text of chapter 2 for the simple reason that liver of these animals constitutes a very popular dish amongst a vast segment of local population of Pakistan. The selection of liver samples was therefore logically based on these six animals, without a regard as to the gender or age of the animals, this could not be confirmed since the animals are slaughtered at the Municipal Corporation's slaughter houses, dispatching the carcase, and the organs, to the vendor point without a prior labelling as to the type and size of the animal. It was, therefore, safely assumed that the animals were usually young, falling in age group of 1.5y to 2.5y for the goat/lamb category, 3y-5y for the cow/buffalo category and about 1-2y for the calf. No post or prior treatment was either expected or given to the liver samples, except for washing with distilled water, and soaking the sample under folds of

filter paper, what man No.41. The procured samples were coded as “liver sample” LS, in order. The relevant information on the samples is provided in Table 2.

3.3 NON ESSENTIAL METAL DISTRIBUTION

The contents of Ni, Cr, Cd and Pb in various liver samples are given in Table 3, on wet-weight basis, as mg/kg liver. Nickel concentrations regard from 0.4mg/kg to 39.30mg/kg correspondingly in buffalo liver and lamb liver. This is a very substantial enhancement of Ni concentration in the liver of two animals that are apposed to each other in terms of their body weights. These levels indicate a very low retention of the metal in the liver of buffalo compared with that of lamb. The latter animal appears to retain much of the Ni in its chemical storehouses, and thus possess apparent threat to consumer.

The case of Ni distribution in liver of lamb is only a scattered case for the rest of the metals, and for the rest of the animals. In no other case, any other metal as well, was found to have such an elevated level of a given element in liver. Following lamb, the next lower metal level, at 9.40mg/kg, was exhibited by chicken and then by calf, goat and cow. No wide dispersion were observed in these cases, as the Ni concentrations ranged between 3.90-9.40mg/kg, as against the range of 0.40-39.3mg/kg in the case of buffalo and lamb.

In the case of distribution of Cr in the liver of various animals, the observed range varied between 4.64mg/kg and 13.14mg/kg, respectively for goat and lamb. Hence, lamb once more surpasses other animals in favour of a higher Cr content, as that for Ni. Similarly, for other animals, the metal content did not represent a big marginal difference, in that

the observed range was from 5.74mg/kg to 12.20mg/kg, respectively for cow and calf. On a comparative scale, chicken and buffalo lives were found to contain comparable levels of Cr, closely standing at 10.89mg/kg and 10.79mg/kg respectively.

Cadmium presents a meagre margin of concentration in the liver of various animals, compared with other metals. Its range was found to vary between 0.03mg/kg and 0.19mgk/kg, for lamb and buffalo respectively. Other animals exhibited only a small concentration level of the metal in their livers, the observed range being from 0.05mg/kg to 0.17mg/kg. Although this reflects a 3-fold ratio, as one goes from the goat liver to the calf liver, the fact remains that the corresponding ratio for Cr stood at 30. This is a striking difference between the distribution of Cr and Cd in the liver of goat and calf.

As for the lead distribution, a relatively uniform pattern of distribution emerged. Although this toxic heavy trace metal is taken up by the animals through air, water and food cycle, it is not clear as to why consistent levels of the metal are retained in the liver of animals, and that too, at a closely matched level. The observed levels of the metal were from 0.19mg/kg to 1.95mg/kg, giving an overall 9-fold enhancement of the metal in the liver of goat compared with that in lamb. The levels of the metal in the liver of cow, calf, chicken and buffalo were almost comparable at 1.21, 1.63, 1.86 and 1.62mg/kg.

Of all the non-essential metals investigated, Cr exhibited the lightest levels, followed by Ni, Pb and Cd in that order. The only exception found was the isolated case of Ni in lamb liver, a case study rechecked and verified. On the whole, nicked and chromium levels exceed lead and cadmium levels.

3.4 ESSENTIAL METAL DISTRIBUTION

Table 4 summarized the observed concentrations of zinc and iron in the liver of various animals. The zinc levels ranged from 2.99mg/kg to 5.53mg/kg, respectively in the liver of buffalo and lamb. The metal in the liver of cow and goat were comparable at 5.32mg/kg and 5.33mg/kg. Unlike non-essential metals, here in the case of essential metal zinc the relative enhancement is not beyond a factor of 2, at the most. The goat, cow and lamb livers are a good source of the metal, zinc, which is required for most enzymes catalysed reactions in human body. No animal has shown a specific retention attitude towards the metal.

The case of iron distribution, however, is not truly matched with that of zinc. Here the levels in general are 30-40 times as higher, with a range of 121.0mg/kg to 398.1mg/kg, in the lever of chicken and calf. The amazing thing about this finding is that a red-meat source is better in iron content than a white-meat source, which in the context of supply of essential metal is considered more competitive.

On the average, all livers are a good source of both zinc and iron, especially lamb, cow and calf livers afford better nutritive values of these metals compared with other animals. However, the chicken is only a moderate source of both zinc and iron. Calf liver qualifies well for iron, and lamb, cow and goat livers for zinc. Even if stipulated daily intakes are kept in view, the liver is a good source of these metals.

3.5 MACRO-NUTRIENT DISTRIBUTION

The data on the distribution of macronutrients in the liver of various animals are given in table 5. The sodium content was found to be quite divergent, spreading from a minimum of 728.1mg/kg, i.e., by an almost 2-fold. These levels were found in the goat liver and buffalo liver respectively. On to the lower side, only lamb and cow come next to goat and buffalo, with substantial sodium content of around 900mg/kg. Of all the liver samples analysed, thus the buffalo liver exceeds in sodium content, followed immediately by calf and chicken livers.

The sodium content of liver of the selected animals is considerable, especially when seen from the viewpoint of body weight of adult animal. For reason of comparison, a 2kg-body weight of a chicken would contain about 2g sodium in the liver, a statistics that remains unmatched in respect of other animals. This clearly demonstrates independence of macronutrient levels on animal body weight. The cases of K, Ca and Mg may also be reviewed on the same pretext.

For potassium, the highest level (33.37mg/kg/) was met in the case of goat liver, while the minimum (15.01mg/kg) in the case of chicken. So, goat liver has surplus potassium, while chicken is a humble resource, with 50% as small potassium content. Comparable to the goat liver, was the case of buffalo liver, that contained as much potassium, only to be followed by lamb and calf, at about 21-22mg/kg.

The calcium content of the liver sample was not found to be wide spread; it varied within 40.7mg/kg and 98.6mg/kg for cow and lamb. The remainder level for calf and chicken

stood close to 80mg/kg, while for goat and buffalo they stood close to 60mg/kg. In that respect, lamb liver was the richest source of Ca. As in case of sodium, here the Ca content of liver was independent of body weight of the animal.

The magnesium levels ranged between 261.4mg/kg and 435.0mg/kg, in favour of goat and chicken. For the livers of other animals, the magnesium content was a close competition for lamb, cow and calf, as the individual levels were around 350mg/kg. The buffalo liver was found to have Mg close to the minimum level exhibited by the liver of the goat. In that respect, lamb and chicken exceed other animals in respect of their Mg content of liver.

For the class of the macronutrients, the elemental concentrations were highest for Na, followed by Mg, Ca and K in that order. Buffalo liver contained highest Na, and hence must be taken continuously, especially by those who suffer from hypertension, even of mild degree. The Na intake of course, is dictated by the amount of liver in the serving, which by international standards is set at 100g for about person per-day. That amounts to an intake of about 133mg Na in one serving, well within the safe range of about 1-2g Na per day. However the cumulative impacts of the macronutrients should be properly taken care of, as their excursive amounts take transform their role from safe to toxic limits.

3.6 CONCLUSIONS

From the forgoing discussion, following conclusions emerge out:-

1. Lamb liver contains highest levels of the toxic metals nickel and Chromium. (LS-01)

2. Goat liver contains maximum lead. (LS-05)
3. The buffalo liver contains maximum cadmium. (LS-06)

Hence, on the basis of this study the lamb, goat and buffalo livers are not suitable food items as they contain elevated levels of toxic metals.

In case of essential metals: -

1. The lamb liver is a rich source of Zn. (LS-01)
2. The calf liver is a rich source of Fe. (LS-03)

Hence, for supplementing Zn & Fe deficiencies, the lamb and calf liver may be added to regular food,

For selected macro-nutrients:-

1. The buffalo liver contains enriched Na content. (LS-06)
2. The goat liver is a good source of K. (LS-05)
3. The lamb liver is a rich source of Ca. (LS-01)
4. The chicken liver is excellent for Mg supply. (LS-04)

In conclusion, the present study forbids the use of liver of the animals as a dietary intake food item on regular basis. The liver may, however, be taken as a food supplement where essential metal deficiencies are obvious in a person, but that too at the risk of building up toxic metal levels in body. When possible, the upper safe limit of 1mg/kg of any heavy metal pollutant in food items must be exercised strictly for all adults on daily basis, as laid down by W.H.O.

Table 1 Standard optimum analytical conditions for various metals.

ELEMENTS	WAVE LENGTH (nm)	HC CURRENT (mA)	SLIT WIDTH (nm)	FLAME	FUEL RATE (L/min)	❖ DETECTION LIMIT (ppm)
Ni	232.0	4.0	0.15	AIR-ACETYLENE	1.7	0.10
Cr	357.9	5.0	0.50	AIR-ACETYLENE	2.6	0.09
Cd	328.8	4.0	0.30	AIR-ACETYLENE	1.8	0.02
Zn	213.9	4.0	0.50	AIR-ACETYLENE	2.0	0.02
Fe	248.3	8.0	0.20	AIR-ACETYLENE	2.0	0.10
Pb	217.0	7.0	0.3	AIR-ACETYLENE	1.8	0.20
Ca	422.7	6.0	0.3	AIR-ACETYLENE	6.6	0.04
Mg	285.2	4.0	0.5	AIR-ACETYLENE	1.6	0.007
Na	589.0	6.0	0.5	AIR-ACETYLENE	1.6	0.02
K	755.5	5.0	0.5	AIR-ACETYLENE	1.9	0.04

❖ **at 1% absorption**

Table 2 Relevant information on samples

S.No.	SAMPLE CODE	ANIMAL	DESCRIPTION
1	LS-01	Lamb	Lamb Liver; Source-local market, Rawalpindi.
2	LS-02	Cow	Cow Liver; Source local market, Islamabad.
3	LS-03	Calf	Calf Liver; Source-local vendor, Islamabad.
4	LS-04	Chicken	Chicken Liver; Source-local shop, Islamabad.
5	LS-05	Goat	Goat Sample; Source-local market, Islamabad.
6	LS-06	Buffalo	Buffalo Sample; Source-local market, Islamabad.

Table 3 Levels (mg/kg, wet weight) of non-essential metals in various liver samples.

S.No	SAMPLE CODE	ANIMAL	METAL CONCENTRATION (mg/kg)			
			Ni	Cr	Cd	Pb
1	LS-01	Lamb	39.30 ± 0.32	13.14 ± 0.14	0.03 ± 0.001	0.19 ± 0.01
2	LS-02	Cow	3.90 ± 0.04	05.74 ± 0.06	0.09 ± 0.003	1.21 ± 0.02
3	LS-03	Calf	6.30 ± 0.06	12.20 ± 0.12	0.17 ± 0.01	1.63 ± 0.01
4	LS-04	Chicken	9.40 ± 0.08	10.89 ± 0.11	0.06 ± 0.001	1.86 ± 0.02
5	LS-05	Goat	4.10 ± 0.14	04.64 ± 0.05	0.05 ± 0.001	1.95 ± 0.02
6	LS-06	Buffalo	0.40 ± 0.01	10.79 ± 0.11	0.19 ± 0.01	1.62 ± 0.02

Table 4 Levels (mg/kg, wet weight) of essential metals in various liver samples.

Sr.No.	SAMPLE CODS	ANIMAL	METAL CONCENTRATION (mg/kg)	
			Zn	Fe
1	LS-01	Lamb	5.53 ± 0.06	287.5 ± 0.8
2.	LS-02	Cow	5.33 ± 0.06	155.7 ± 0.5
3	LS-03	Calf	3.95 ± 0.04	398.1 ± 0.4
4	LS-04	Chicken	4.63 ± 0.04	121.0 ± 0.7
5	LS-05	Goat	5.32 ± 0.05	189.2 ± 0.2
6	LS-06	Buffalo	2.99 ± 0.03	141.2 ± 0.6

Table 5 Levels (mg/kg, wet weight) of selected macronutrients in various liver samples

S.No.	SAMPLE CODE	ANIMAL	METAL CONCENTRATION (mg/kg)			
			Na	K	Ca	Mg
1	LS-01	Lamb	908.8 ± 1.0	22.01 ±0.20	98.6 ±0.9	354.2 ±1.0
2	LS-02	Cow	896.5 ±1.0	15.95 ±0.15	40.7 ±0.4	343.4 0.8
3	LS-03	Calf	1079.7 ±5.0	21.68 ±0.21	84.1 ±0.8	340.4 ±0.7
4	LS-04	Chicken	1065.7 ±5.0	15.01 ±0.15	81.4 ±0.7	435.0 ±0.6
5	LS-05	Goat	782.1 ±1.2	33.37 ±0.30	62.9 ±0.6	261.4 ± 0.7
6	LS-06	Buffalo	1327.7 ±1.4	32.8 ±0.32	59.0 ±0.5	275.1 ± 0.6

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