2019-05-20

Determination of Zinc in Some Infants and Young Children Foods available in Supermarkets and Pharmacies Using Atomic Absorption Spectroscopy

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1. INTRODUCTION

Balanced nutrition is important especially during periods of infancy and child hood where there is a rapid growth and development.[1] For the first 4-6 months of a baby's life breast milk alone is enough for good growth.[2] However, complementary nutrient-dense foods are also required after 6 months of age to satisfy the need for additional energy requirement and to supply several micronutrients, notably iron, zinc, calcium, and vitamin A after 6 months of age.[3]

In addition to proteins, carbohydrates, and fats; minerals are also essential for health and growth of human kind. A well balanced diet with macro (such as Ca, K, Na, Mg, Cl) and micronutrients (such as Zn, Fe, Cu, I) provides adequate amounts of nutrients for normal health and development. Zinc is one of the essential micronutrients in human nutrition[4] and its existence in infant foods have been found to be critical during infancy and childhood, when the period of rapid growth takes place, as it plays a role in cell replication and synthesis of nucleic acid and proteins, such as growth hormone.[5] Zinc is also a critical nutrient for infants' healthy growth and development of the central nervous system, rapid wound healing and in many complex metabolic processes.[6] Zinc deficiency among children produces serious consequences for health such as retarded growth, an increase in infectious diseases, and the impairment of cognitive function. Supplementation studies have shown that zinc is related to height and weight increase in children.[5]

Zinc is therefore one of the essential nutrients to be obtained from infant formulations. The importance of a certain infant formulation depends on their content of minerals.[5] Many brands of infant formulae are designed to provide required nutrients as recommended diet intake (RDI) of minerals for infants and children. The composition of commercial infant formulae and baby foods can be very different from the recommended dietary intake for minerals and therefore information is needed on the levels of minerals in these food groups.[7] The aim of this study is to determine the zinc content of some infants and young children complementary foods available in supermarkets and pharmacies using atomic absorption spectroscopy.

2. LITERATURE REVIEW

2.1 Zinc: its role in human health

Zinc is a bluish-white metallic element (atomic number 30, atomic weight 65.4), which makes up about 0.02% of the earth's crust and is the twenty third most abundant element.[8] The electronic configuration of zinc(II) is $3d^{10}$ with two electrons per orbital.[9] Because of its nature as a transitional element in the periodic table; zinc possesses certain chemical properties that make it especially useful and important in biological systems.[8] Intrinsic acid properties of Zn^{II} pertinent to its biological functions.[10] Zinc is the most common Lewis acid in bioinorganic chemistry.[9] Zinc triggers redox reactions due to its electron-withdrawing properties.[11] Zinc is an essential trace element important for good health, proper development and functioning of the body. It is the most abundant trace element in all cells of the body, except in red blood cells.[12]

More than 300 enzymatic reactions are known to depend on the presence of zinc[13], with representatives known for each of the fundamental enzyme classes (oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases).[14] Zinc is not limited to a few functional roles like some elements such as iron and calcium.[12] The role of zinc can be catalytic, structural, synthesis and stability of them.[12,14,15] It plays a key role in several metabolic processes, synthesis of nucleic acids and specific proteins, such as growth hormones and their receptors.[8,12] For these reasons, zinc plays a central role in cellular growth, differentiation, and metabolism.[8] It is used to control the enzymes that operate and renew the cells in our bodies. The formation of DNA, the basis of all life on our planet, would not be possible without zinc.[16]

All dysfunctions in zinc deficiency depend on the key role of zinc in some biological reactions in the human metabolism. Table 1 shows the sources and functions of important endogenous zinc binding ligands.[13]

Table 1. Sources and functions of important endogenous zinc binding ligands.[13]

Name	Source	Function
Enzymes		
RNA polymerase	bacteria, viruses	promoter, recognition
Deoxythymidine kinase	eucaryonts,	activator
Deoxy-RNT transferase	viruses, thymus	activator
5-Nucleotidase	bacteria	activator
Alcohol dehydrogenase	vertebrates, plants	activator
Protein kinase C	mammals	activator
Superoxide dismutase	bovine erythrocytes	co-activator
Leucine aminopeptidase	bovine lens, pig kidney	co-activator
Phospholipase C	mammals	co-activator
Alkaline Phosphatase	mammals, bacteria	catalysis, co-activator
DNA polymerase	eucaryonts	catalysis
Reverse transcriptase	viruses	catalysis
tRNA synthetase	E. coli	catalysis
Nucleoside polymerase	calf thymus	catalysis
Carboxypeptidase A	vertebrates	catalysis
Carboxypeptidase B	mammals	catalysis
Collagenase	mammals, bacteria	catalysis
Endopeptidases	peptide hormones	hydrolysis
Hormones		
Insulin	pancreas	secretion, storage
Growth hormone	brain	activator
Thymulin	thymus	T cell differentiation
Steroid receptors	glucocorticoid, estrogen	genetic expression
Angiotensin-converting enzyme	mammals, bacteria	catalysis
Carrier and structure proteins		
Transferrin	liver	transport
Albumin	liver	transport
α-Macroglobulin	liver	transport
Metallothionein	tissue, liver	inducer, metal transfer

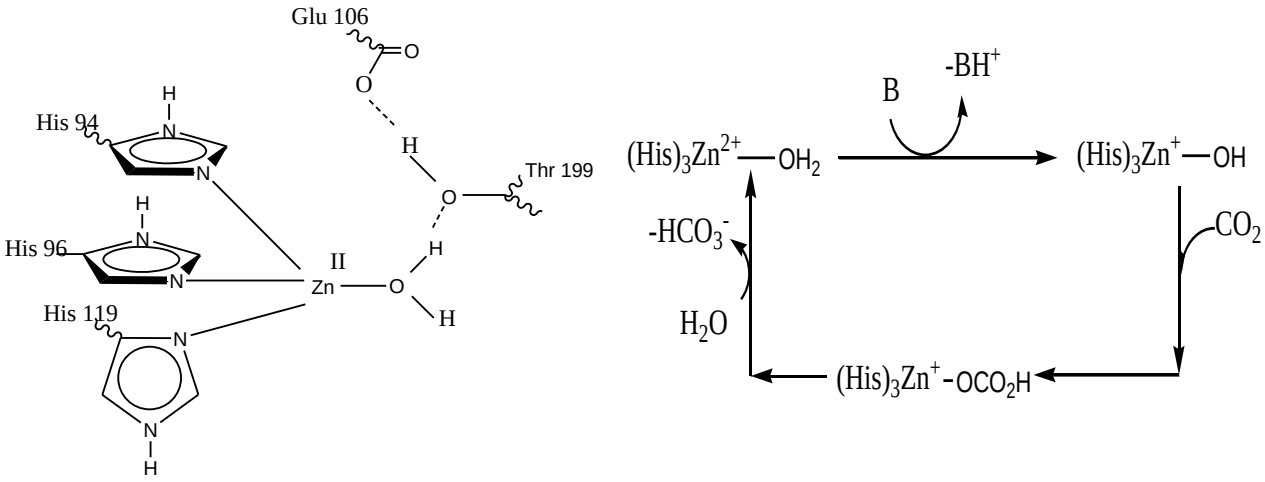
Unlike iron and copper, Zn does not undergo reduction or oxidation under physiological conditions, making it a stable component of protein complexes. This allows it to be transported in biological systems without inducing oxidant damage as can occur with other trace elements such as iron and copper. [12]

A. Carbonic anhydrase

Carbonic anhydrase has a role in the acid base homeostasis of living organisms by catalyzing the reversible dehydration of carbonic acid, a process which is critical to the transport and elimination of carbon dioxide.[17,18] Thus it plays an important role in respiration, transporting CO_2 between metabolizing tissues and the lungs, and in the intracellular CO_2/HCO_3^- equilibration.[14]

$$CO_2 + H_2O \xrightleftharpoons{CA} HCO_3^- + H^+ \qquad 1$$

Carbonic anhydrase uses the hydroxyl ion generated by ionization of the Zn^{2+} -OH_2 as the nucleophile for hydrolysis reaction. The Zn^{2+} ion is coordinated to the protein by three invariant His residues, with the remaining tetrahedral site occupied by a water molecule. The water molecule is involves in a hydrogen bond with a Thr residue, which in turn is hydrogen bonded to a Glu residue (fig. 1a).[17,19] The overall features of the mechanism of the action of carbonic anhydrase are illustrated in fig. 1b comprising the following steps: (i) deprotonation of the coordinated water to give the active zinc hydroxide derivative $[(His)_3Zn\text{-}OH]^+$, (ii) nucleophilic attack of the zinc bound hydroxide at the carbon dioxide substrate to give a bicarbonate (more correctly termed hydrogen carbonate) intermediate $[(His)_3Zn\text{-}OCO_2H]^+$, and (iii) displacement of the bicarbonate anion by H_2O to complete the catalytic cycle.[14,17,19]



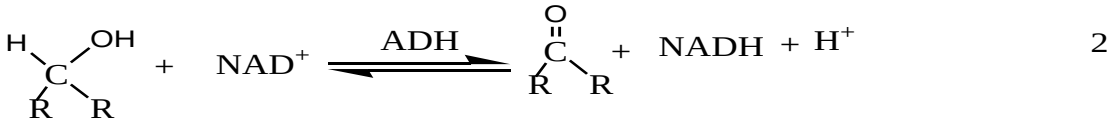
A B

Fig.1. (A) The active site of human carbonic anhydrase and (B) A simplified mechanism of action for the enzyme: B = general base, probably His 64.[17,19]

The key role of Zn^{2+} ion in the mechanism is via its charge makes the bound water molecule more acidic than free H_2O. This generates a source of nucleophilic zinc-bound OH.[17,19]

B. Alcohol dehydrogenase

Alcohol dehydrogenases are a class of enzymes, which catalyze the oxidation of primary and secondary alcohols to the corresponding aldehyde or ketone by the transfer of a hydride anion to NAD^+ with release of a proton.



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The structure and essential features of the catalytic cycle for Liver Alcohol Dehydrogenase enzyme are summarized in fig.2. The water molecule is displaced from the zinc atom by the incoming alcohol substrate. Deprotonation of the coordinated alcohol yields a zinc alkoxide intermediate, which then undergoes hydride transfer to NAD^+ to give the zinc-bound aldehyde and NADH. A water molecule then displaces the zinc bound aldehyde to give the original catalytic zinc center.

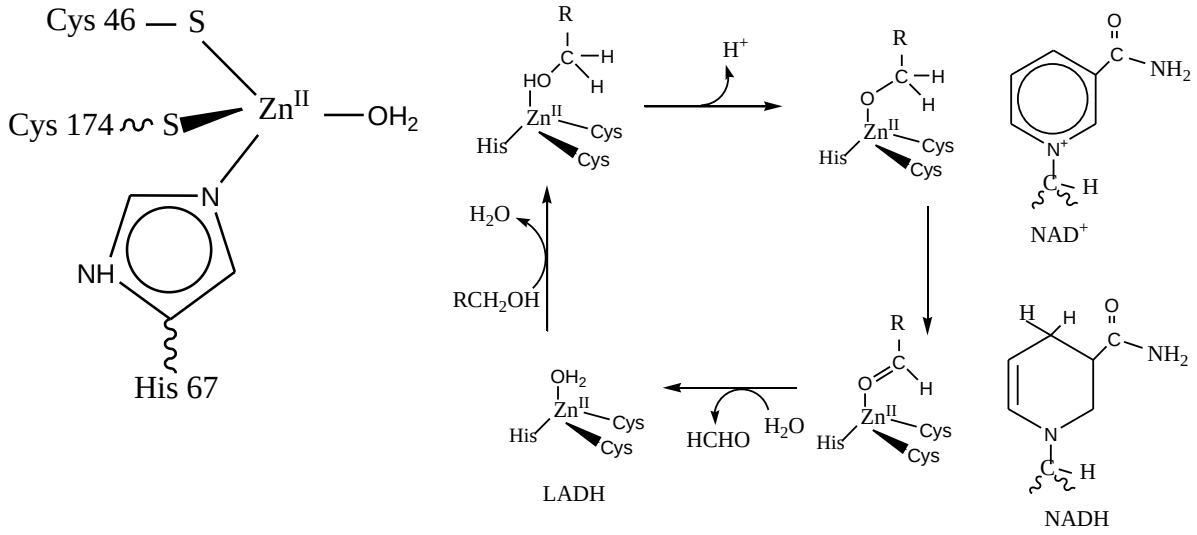

Fig. 2 (A) the catalytic site of liver alcohol dehydrogenase (LADH) (b) the essential features of its catalytic cycle. [14,17,18,19]

The role of zinc in the dehydrogenation reaction is to promote deprotonation of the alcohol, thereby enhancing hydride transfer from the zinc alkoxide intermediate. [14,17,18,19]

Infants and young children obtain zinc from breast milk; infant formula; meat; poultry; liver; egg yolks; cheese; yogurt; legumes; whole-grain breads; cereals; and other fortified or enriched grain products. Meat, liver, and egg yolks are good sources of available zinc, whereas whole-grain products contain the element in a less available form.[20] Pertaining to zinc intake, the richest dietary sources of zinc are the organs and flesh of mammals, fowls, fish and crustaceans, and zinc fortified foods. Organ, flesh meat and poultry do not contain any known specific anti-nutritional factors (e.g. phytates and fibers) that hinder zinc absorption. Eggs and dairy products are also rich in zinc and free of phytates, but they have slightly lower zinc content than can be found in organ and flesh foods.[21] Animal products are usually the best source of dietary zinc, in terms of both content and bioavailability,[22] e.g. pureed beef is high in zinc content, and previous studies have shown there is significantly higher zinc absorption in pureed beef compared to cereals.[23]

Starchy roots and tubers have much lower zinc content than legumes and cereals. In general, fruits and vegetables are not considered rich sources of zinc, although some green leafy vegetables like spinach have moderate amounts of zinc but with uncertain bioavailability. Vegetarian diets are often lower in zinc content and contain factors that hinder zinc absorption in contrast to meat based diets. Hence vegetarian families unable to meet their zinc intake through adequate inclusion of non-meat sources of bioavailable zinc in the diet (e.g. eggs and dairy products), zinc fortified foods or zinc supplements are at higher risk of zinc deficiency.[21]

Zinc is absorbed into the body through the small intestine, which also regulates whole-body homeostasis through changes in both the fractional absorption of dietary zinc and excretion of endogenous zinc in gastrointestinal secretions.[24] The upper small intestine is the site of absorption.[25] Absorption adapts to physiological needs, increasing during lactation and decreasing with aging. Zinc absorption is determined largely by its solubility in the intestinal lumen, which in turn affected by its chemical form and the presence of inhibitors of uptake.[24]

Among the several factors that interfere with digestion and absorption of dietary zinc, the phytate content of the diet is the most important factor.[21] Stress conditions such as infectious disease may also alter absorption efficiency.[24] Although most cereals and legumes have relatively high amounts of zinc, they contain high concentrations of phytates, which reduce the amount of absorbable zinc from these foods, and therefore they are relatively poor sources of zinc. It is clear from this discussion that cereal or legume based diet without an adequate inclusion of animal source foods or an external source of zinc often will not allow individuals to meet their daily zinc requirements.[21]

Zinc is stored in a wide variety of locations throughout the body. These locations include: eyes, prostate gland, bones, kidneys, pancreas, voluntary muscles, skin, fingernails, hair, and liver. Zinc is mainly excreted in the feces[25] and some amount is also loss from the body through urine, menstrual flow, semen, sloughed skin, nails, and hair, although quantitatively these other routes of zinc loss are relatively small compared with gastrointestinal excretion.[8]

Individual zinc status is influenced by intake of zinc from the diet, its absorption (or bioavailability) and the loss of it from the body.[21] To meet the needs of this important nutrient, experts recommend introducing complementary foods around 4 to 6 months of age. Fortified infant cereal is the most commonly recommended first complementary food. However, most cereals do not account for the infant's zinc needs.[23]

The fact that zinc plays a central role in gene replication, protein synthesis and cellular division makes it extremely important during periods of rapid growth and development in both pre- and postnatally.[12] Infants and young children have higher zinc requirements than adults because of the essential role of zinc in growth.[23,26] Inadequate consumption of this nutrient can have long-lasting negative effects on their growth, learning and behavioral development.[23]

2.2 Pathophysiology of zinc deficiency

2.2.1 Zinc deficiency disorders

Zinc deficiency has been defined as low total body zinc mass, calculated with zinc tracer studies.[27] It was in 1869 Raulin who showed for the first time that zinc is essential in biological systems.[28] Because of many wide ranges of zinc-dependent metabolic processes (such as the acid base homeostasis, oxidation of primary and secondary alcohols to aldehydes and ketones) are required by cells, the consequences of zinc deficiency are so many and varied and also they are nonspecific.[8]

The main causes of zinc deficiency are low intake, increased requirements, malabsorption (due to components that complex zinc, making it less available for absorption), increased losses.[24, 29] When intake of absorbable zinc is inadequate, diet-induced zinc deficiency occurs. Low intakes are exacerbated by life stage (infants, children, adolescents, and pregnant and lactating women) or pathological conditions (preterm birth, low birth weight, and diarrheal disorders) that increase zinc losses or enhance needs.[24] The greater prevalence of zinc deficiency in infants is associated with low birth weight, early weaning, feeding with only

whole cow's milk–based formula, and complementary feeding with low in meat products and high in phytate content.[30]

Infants and children, individuals with alcoholic liver; inflammatory bowl; and severe or persistent diarrhea diseases, pregnant and lactating women, patients receiving total parentrals nutrition, and older adults (65 years and older) are at a high risk for zinc deficiency.[16]

Due to the requirements of wide range of zinc-dependent metabolic processes by cells, zinc deficiency results in adverse consequences on the body. The magnitude of which depends on whether the resulting deficit is marginal or severe. Marginal zinc deficiency is the most common and, although asymptomatic, it is associated with impaired immune function (increased susceptibility to infections or decrease ability to clear them), anorexia, dysfunction of smell and taste, irritability, depression, anger, sleepiness, reduced sperm production in men and decreased mental ability. Severe zinc deficiency, which rarely occurs, is characterized by severely impaired immune function, dermatitis, growth impairment, pregnancy outcome, alopecia, lethargy, and recurrent infections such as diarrhea.[21]

2.2.2 Symptoms of zinc deficiency Disorders

The human body does not store zinc unlike iron. Zinc deficiency symptoms are non specific in their nature and it causes a block in protein and nucleic acid synthesis. The immune system, the skin and the gastro-intestinal tract are the tissues of the body with the highest rate of protein synthesis, and they are the main targets for deficiency signs to appear.[31]

The clinical features of zinc deficiency disorders (ZDD) are growth retardation, stunting, delayed sexual and bone maturation, dermatitis, loss of hair, diarrhea, defects in the immune system, impairment of taste acuity and appetite, low plasma zinc concentrations and behavioral changes. Supplying zinc rapidly reverses the external signs of deficiency and improves the other characteristics. However, none of these signs of zinc deficiency is sufficiently specific or sensitive on its own to detect marginal or subclinical zinc deficiency.[8]

2.3 Epidemiology of zinc deficiency disorders

2.3.1 Global perspective of zinc deficiency disorders

Micronutrient deficiency of zinc, in addition to previously recognized three major micronutrient deficiencies namely vitamin A deficiency (VAD), iron deficiency disorders, and iodine deficiency disorders (IDD); has recently received global attention as ZDD contributes low birth weight, growth failure, impaired immunity, and to infant mortality and morbidity. Data on global mortality in children under 5 years of age due to different micronutrient deficiencies in 2004 are given in Table 2. Zinc deficiency was responsible for the maximum number of deaths after vitamin A followed by iron and iodine deficiencies.[32]

Table 2. Global mortality in children under five years of age in 2004[32]

Deficiency	Deaths
Vitamin	666,771
Zinc	453,207
Iron	20,854
Iodine	3,619

Zinc was shown to be an essential nutrient in the mid−1930s. It was not until 30 years later, however, that widespread zinc deficiency was identified as the underlying cause of stunted growth and delayed sexual maturation in adolescent boys in western Asia and the Middle East.[33] Zinc deficiency is now known to occur in many population groups in developing countries, and is increasingly felt to be an important public health problem associated with poor child growth delayed maturation, poor appetite, and serious infectious diseases such as diarrhea, pneumonia, and malaria due to impaired immune function.[33,34] Below fig. 3 shows the national risk of zinc deficiency in children under 5 years.

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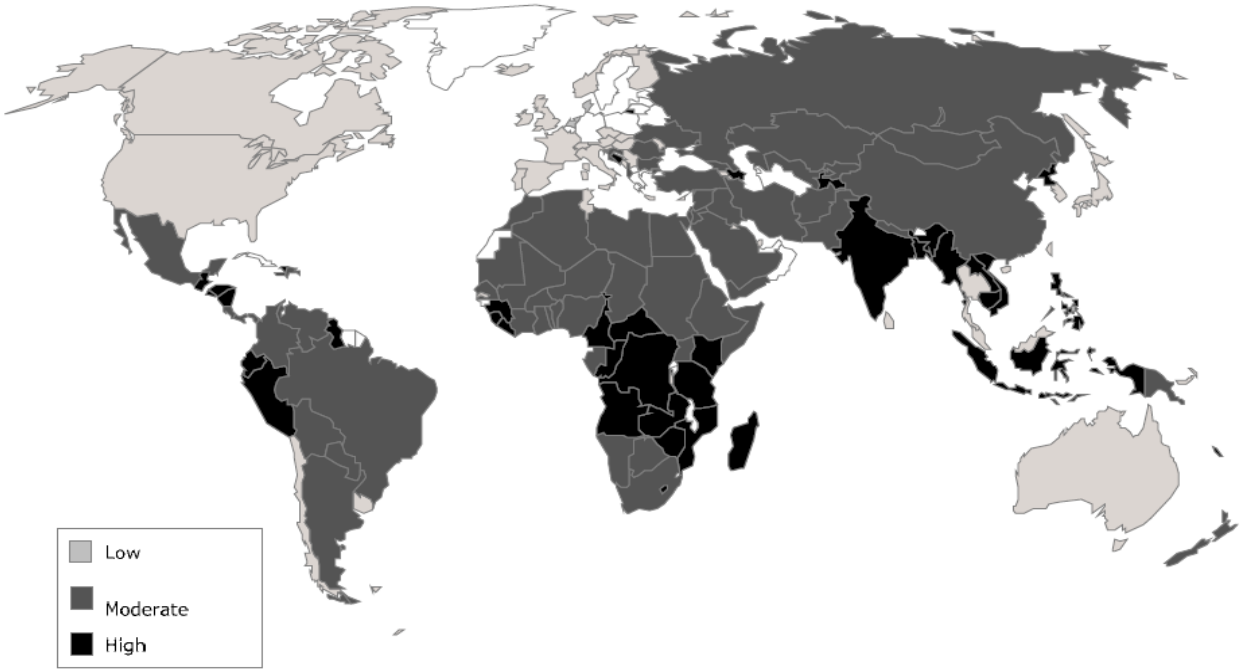

Fig. 3 National risk of zinc deficiency in children under 5 years.[35]

2.3.2 Zinc deficiency disorders in Ethiopia

Zinc deficiency is common in infants and young children of developing countries and leads to stunted growth, increased risk of infection, and possibly poor neurodevelopment.[36] Of the nearly 1.9 billion children in the developing world, 31% are stunted. Despite the continued progress in all the developing countries, it is still predicted that there will be 128-155 million underweight children by the year 2020 with 35% of these children to be from sub-Saharan Africa. The most important documented forms of malnutrition in Ethiopia are protein energy malnutrition and zinc, iodine, iron and vitamin A deficiencies.[37]

Prevalence of stunting

Growth faltering in Ethiopia is largely caused by high rates of infection, limited household food availability, and poor infant feeding practices leading to inadequate energy and nutrient intakes.[37] In rural Ethiopia, the national prevalence of stunting was reported to be 64%, which is the highest in the world. Furthermore, the prevalence of stunting increases from 57% during infancy to 73 % in the second year of life. Regional variations within the country have also been observed with prevalence's varying from 74% in the northwest to 49% to the south

(omo). More surprising and difficult to explain, however, is the fact that a very high prevalence of stunting (73%) was observed in a food surplus region of the country, such as west gojam.[12]

Inadequate intakes of dietary zinc, arising from low intakes and/or poor bioavailability are a major factor of zinc deficiency in developing countries.[38] Furthermore, the diets are predominantly plant based and contain high amounts of phytate- a potent inhibitor of zinc absorption- that forms insoluble complexes with zinc in the gastrointestinal tract and are low in animal products that are good sources of highly bioavailable zinc.[12,38] Above fig. 3 shows the prevalence of moderate zinc deficiency in Ethiopia. In Southern Ethiopia two of the major dietary staples consumed—starchy foods based on enset (*Enset ventricosum*) and enjera prepared from teff (*Eragrotis teff*) —are fermented. A project has been made to quantify the zinc intakes of pregnant Ethiopian subsistence farmers. The project showed that high prevalence of Zn inadequacy: 99-100% for inadequate intakes.[38]

Thus zinc deficiency may have far reaching consequences for infant, child and maternal health in Ethiopia but has long been overlooked. In recent years, however, zinc deficiency has been acknowledged to be an important public health problem. Stunting is highly prevalent in children of Ethiopia. Its aetiology has been poorly understood but it has been hypothesized that zinc deficiency may play a role.[12]

Prevalence of diarrhea

Diarrheal disease is one of the major causes of morbidity and-mortality in under five year children in the world. Worldwide, there are about 1.3 million under five year children deaths attributable to diarrhea. Health status in Ethiopia is one of the lowest in the world with estimated health service coverage of 60%. Various reports on health status in Ethiopia have shown that diarrheal disease is one of the major causes of mortality and morbidity in under five children next to lower respiratory tract infection.[39]

2.4 Assessment and diagnosis of zinc deficiency Disorders

Zinc deficiency is thought to be widespread in most developing countries but the magnitude is not well known because of lack of specific clinical signs and/or biochemical indicators associated with mild zinc deficiency. Cases of severe zinc deficiency are rare while mild to moderate zinc deficiency is common but not easily recognizable. Dietary and serum zinc assessment have been proposed to assess zinc status in the population. At present, the most reliable method for diagnosing zinc status is to monitor the impact of zinc on zinc dependent variables such as growth and cellular immunity in blind, randomized, placebo controlled intervention studies. Such an approach is however time consuming, expensive and necessitates good compliance and follow-up.[12]

Assessment of dietary zinc status in a population requires several steps, consisting of the measurement of food intake distributions in the population; the analysis of local staple foods, from which zinc intake distributions can be determined, and the comparison of zinc intakes with requirement estimates to determine the risk of inadequate intakes.[40] The concentration of zinc in serum or plasma is the most common parameter used as an indicator of zinc status although it is influenced by a variety of factors such as infection, stress and physiological and pathological conditions which limit its diagnostic value.[12]

2.5 Treatment and prevention of zinc deficiency disorders

Approaches to increase zinc status is either through dietary diversification, supplementation, food fortification or reducing the intake of inhibitors of zinc absorption through processing techniques, and biofortification.[21,27,41]

2.5.1 Dietary diversification

Dietary diversification is to include more micronutrient rich foods in the diet is and generally considered to be a sustainable approach in addressing most micronutrient deficiencies.[21] However, advocating the increased inclusion of foods rich in bioavailable zinc in the diet

(increase intakes of foods with a high content and bioavailability of zinc) will be difficult to achieve since zinc rich foods are usually of animal origin and the relatively high cost of animal source foods make less accessible to most poor families.[21,41]

2.5.2 Zinc supplementation

Supplementation with micronutrients has been employed in numerous countries to address various micronutrient deficiencies. Supplementation involves the provision of an additional amount of the nutrient, usually in a chemical form instead of food, to a targeted group of a given population to address a specific micronutrient deficiency. Most of the evidence supporting the utility of zinc supplementation has derived from clinical trials with very few attempts to deliver zinc supplements in large-scale interventions.[21]

There is no body store for zinc, thus bioavailable zinc from food or supplements must supply zinc on a regular basis. Supplementation trials assessing the benefits of zinc on the treatment and prevention of infectious diseases have been conducted in populations with likely zinc deficiency since 1988. Although understanding of the role of zinc in immune function has increased, specific mechanisms by which zinc acts for the prevention and treatment of infectious diseases in humans are still not clear.[27]

During periods of rapid growth the requirements for zinc is high.[29] As a consequence, zinc deficiency affects children's physical growth.[42] There are also indications that maximal growth potential can be limited by zinc deficiency in infants, children, and adolescents.[43] A number of studies indicate that preventive zinc supplementation increases linear growth and weight gain in previously stunted or underweight children.[42] Although not universally observed, both weight gain and linear growth velocity have been significantly greater in the zinc-supplemented children than in those who received a placebo.[8] Supplementation program indicates that the growth of male infants fed a zinc supplemented formulae was greater than that of control infant fed the same formula without a zinc supplement.[43]

A test has been done that whether zinc deficiency is responsible for the low rate of growth of stunted children in Ethiopia. A randomized placebo-controlled study in which stunted and non-stunted infants aged 6-12 months were supplemented with zinc(10mg/day) or a placebo, (six days a week) for six months. Length or other anthropometric factors were measured monthly and the concentration of zinc in hair and serum was measured at the end of the intervention.

The study showed the length of stunted infants increased significantly more when supplemented with zinc than with placebo; and the effect was greater than in non stunted infants. Zinc supplementation also increased the weight of stunted children and of non stunted children. Zinc supplementation resulted in a markedly lower incidence of anorexia and morbidity from cough, diarrhea, fever and vomiting in the stunted children. Finally, the study identified that combating zinc deficiency can increase the growth rate of stunted children to that of non stunted infants in rural Ethiopia.[12]

Diarrhea and fever, especially in infants and young children, may increase intestinal losses of zinc and thus may exacerbate zinc deficiency.[12] Therapeutic effects of zinc was first studied on diarrhea and then observed to decrease the incidence of additional diarrhea episodes[27] and it is currently included as the standard care for this.[44]

Zinc nutritive is important and clinical trials in acute diarrhea indicate that zinc supplementation holds substantial promise.[45] WHO (world health organization) and the United Nations Children's Fund (UNICEF) now recommend that zinc supplementation should be included as a component in diarrhea treatment regimens, and efforts are under way in a number of countries to scale up zinc supplementation during diarrhea.[42]

In Ethiopia a nationwide rural survey, which is appeared on the J. of Ethiopian Health Development, 12.5% of 6-59 months of children had diarrhea in the two weeks period prior to the survey. It is estimated that 230,000 under five children die due to diarrhea. Since the introduction of ORS, many lives have been saved by oral rehydration therapy.[39]

Zinc for the treatment of the common cold was first investigated in the early 1980s with the use of zinc gluconate lozenges. Prasad et al. found zinc acetate lozenges to shorten the mean overall duration of cold symptoms and a decreased overall severity of symptoms based on patient scoring. Cross-sectional studies have also shown relationships between low zinc status and increased incidence of malaria. However, which may not be the best measure of clinical episodes of malaria.[27]

Zinc was first used for skin healing and wound repair by ancient Egyptians in the form of calamine. Zinc deficiency is now recognized to have adverse effects on the healing process and may increase the time for tissue repair. Wounds have also been shown to heal faster with both oral zinc supplementation and topical treatment. Pories et al. randomized 20 patients to oral zinc sulfate or placebo and observed the duration of wound healing. Zinc-supplemented patients healed in 45.8 days compare to 80.1 days for placebo recipients.[27]

HIV-positive persons are at risk for micronutrient deficiency due to decreased consumption of food, increased malabsorption, and increased losses of zinc from an increased incidence of diarrheal infection. Zinc is a necessary component of basic immune function, thus a deficiency may lead to faster progression to more advanced stages of disease. Supplementation trials of HIV-positive patients have shown zinc supplementation to decrease opportunistic infections and improve weight gain and CD_4 cell count.[27]

The global effects of zinc are in part due to the essential role that zinc plays in basic protein biochemistry and in cell replication. Zinc has a direct effect on brain growth and morphology through its role in enzymes that mediate protein and nucleic acid synthesis.[6] Experiments have shown that accident victims who are given zinc supplements respond with improved cognitive function. Zinc is diverted to the healing tissues following injury or surgery and becomes less available for other essential functions.[16]

During infancy, there is some implication that zinc supplementation improves motor development and promotes activity in the most severe cases of zinc deficiency. Six-to nine-year-old first graders with low zinc status were assessed biochemically and

neuropsychologically before and after treatment. Zinc supplementation for 10 weeks resulted in significantly better zinc status and improvement in this particular neuropsychological assessments.[6]

2.5.3 Food fortification

Improving intake of dietary zinc can also be achieved through food fortification, which involves increasing the zinc content of foods through the addition of the mineral to food after processing.[21]

Fortification of staple foods with zinc is an option for increasing daily zinc intake. Before fortification can be realized, target foods and a target population must be identified. The food to serve as the fortifying vehicle must be digested in a predictable quantity to ensure the micronutrients are reaching the target population in an adequate quantity and quality. In addition, the process of fortifying must not change or alter the food itself or increase the price such that consumers are not willing to buy it.[27]

2.5.4 Biofortification

Increasing the concentration of micronutrients (especially Zn) in food crop plants is a growing global challenge, with potentially great implications for both crop production and human health. It is believed that Zn deficiency is the most widespread micronutrient deficiency in crop plants and human beings.[46] Cereal crops are inherently very low in grain Zn concentration and about a half of the cereal-growing areas in the world contain low levels of plant-available Zn in the soil, hence the plants grown in such areas suffer from Zn deficiency stress and contain low levels of Zn in the grain.[46,47] To meet the daily Zn requirement of humans, the amounts of Zn in the seed need to exceed the optimum level for the crop itself.[46] Currently, improving the grain Zn concentration of cereal crops using biofortification of cereals crops with zinc is a high-priority global issue.[46,47] Among the strategies discussed in the literature, agricultural approaches (e.g. breeding, application of fertilizers) seem to be the most promising and sustainable solution to the Zn deficiency problem.[46]

Breeding new plant genotypes for high grain concentration of Zn (genetic biofortification) is the most sustainable solution to the Problem. Although developing new micronutrient-rich plant genotypes is a long term process and its effectiveness can be limited by the low amount of readily available pools of micronutrients in soil solution. Application of Zn-containing fertilizers (i.e. agronomic biofortification) is a short-term solution and represents a complementary approach to breeding.[47]

2.6 Zinc toxicity

For many years most of the nutrition research on zinc has focused on its essential roles in the body. Many studies have examined the consequences of a deficient state on growth, development, and health and the prevalence of deficiency in various population groups. Relatively little attention has been directed toward toxic properties of zinc other than where there has been a clear industrialized hazard, such as with metal- fume fever due to inhalation of zinc oxide fumes or as a consequence of severe pollution of a localized environment. Indeed, most review texts indicate that zinc is relatively nontoxic and those animals, including humans, exhibit considerable tolerance to high intakes of zinc. Although it is true that overt symptoms of toxicity require ingestion of relatively large amounts of zinc, there is increasing evidence that use of zinc supplements by humans, even at fairly modest concentrations, may have adverse consequences under certain circumstances.[48]

Zinc has a low potential of toxicity. Acute toxicity symptoms, such as nausea, vomiting, diarrhea, fever, and lethargy, are observed only after ingestion of gram doses. When zinc intake is increased, homeostasis is maintained through increased urinary fecal excretion. Long-term zinc intakes higher than requirement could, however, interact with the metabolism of other trace elements, especially copper and iron, can be impaired. At higher doses of zinc, impairments of copper absorption status and changes in immune response have been observed.[8] Decreased copper status may also inhibit the transport of iron and result in anemia. Although zinc-induced copper deficiency and the resulting anemia are serious, it occurs only after excessive zinc intake over a long period of time and is easily corrected by adjusting the intake levels of zinc and copper accordingly.[49]

3. STATEMENT OF THE PROBLEM

Although many questions remain concerning the precise mechanism and magnitude of malnutrition effects on health, there is now considerable evidence that malnutrition has effects on physical growth, morbidity, mortality, cognitive development, reproduction, physical work capacity and risks for several adulthood chronic diseases.[37]

Zinc is an essential micronutrient particularly important for children growth.[50] The metal is an essential trace element required for the activity of over 300 enzymes and involves in most major metabolic pathways. Zinc participates not only in catalytic processes, but also in the structure, synthesis and stability of some regulatory proteins. Zinc also with important hormones involves in bones growth and development.[5,15]

Adequate zinc in infants' foods is especially essential for the normal growth and development. The deficiency of zinc in nutrition causes a serious of health problems in children, many of which can become chronic, such as weight loss, stunted growth, weakened resistance to infections, and even early death.[5] Unfortunately, zinc does not have any functional tissue reserves that can be released in deficient states like iron or vitamin A and thus dietary zinc is crucial to meet the body's daily demand.[4]

Severe negative effects of zinc deficiency on human health in developing countries have been recognized by the United Nations.[5] Poor infant feeding practices leading to inadequate energy and nutrient intake, such as zinc, is one of the factors that affect nutritional status of children in Ethiopia.[37] Knowing the content of zinc in infants' diet would therefore be helpful in making dietary choices for a better nutrition policy for children.[5]

General objective

The main goal of this study is:
To determine the content of zinc in baby foods available in supermarkets and pharmacies.

Specific objectives

The specific objectives of this study are:

- To know the differences between baby foods available in supermarkets and pharmacies based on their zinc content.
- To assess the nutritional value of infant foods available in supermarkets and pharmacies in terms of their zinc content.
- To compare the zinc content of infant foods available in supermarkets and pharmacies with the recommended level set by Food Consumption and Nutrition Division (FCND) discussion paper and with breast milk.
- To compare the zinc content of infant foods available in supermarkets and pharmacies with Indian manufactured and used infant formula.

4. EXPERIMENTAL METHOD

4.1 Materials, Reagents and Chemicals

4.1.1 Sample collection

Ten types of baby food samples, which are available in supermarkets, Ethiopia, were collected. The samples collection method was based on observations of the recommended age, photo of baby or texts that recommends for baby on the foods and by interviewing the sellers that which foods are mostly bought by mothers for their babies. The samples names, their ingredients, the recommended ages, manufacturer country with the company, their price and photographs are described below in table 3.

Table 3.Samples with their ingredients, recommended ages, manufacturer country, price and photographs

No.	Name of samples	Ingredients on the label	Recommended ages	Manufacturer country, company	Price (Birr /g)	photograph
1	S-26, stage-1	***milk:** Skimmed milk, reduced minerals whey,(47 mg/kg)[a]	From birth onwards	Ireland, Askeaton, Co. Limeric	0.36	
2	Promil, stage-2	***milk:** Skimmed milk, demineralized whey, (36 mg/kg)[a]	6 months onwards	Ireland, Askeaton, Co. Limeric	0.36	
3	CERELAC, stage-1	***Wheat and Milk:** Wheat flour, skimmed milk powder, caramel sugar,	From 6 months	Egypt, Nestle Egypt S.A.E	0.25	
4	CERELAC, stage-2	***Fruits, Wheat and Milk:** Wheat flour, skimmed milk powder, Guava, banana, apple, mango,	From 6 months	Egypt, Nestle Egypt S.A.E	0.28	

5	Cerifam	Wheat flour, dry milk, soya flour,	From 4 months	Ethiopia, ISO 22000: 2005	0.042	
6	BABY KING	***Apple, Rice and Milk:** Precooked rice, skimmed milk, apple puree, (35 mg/kg)[a]	From 6 months	Egypt, Lemako-Misr	0.28	
7	Safa	***Corn starch**		Dubai-UAE, Hassani food industries	0.05	
8	Couscous	***Durum wheat**		Tunisia,Societes industry	0.075	
9	Mother's choice	***Baby cereal with Milk Rice + Carrot:** Rice flour, skimmed milk powder, real carrot,	6 months onwards	Oman, Omani Euro food industries S.A.O.G	0.20	
10	SUN(BUBER SUSU PENUH GIZI	***Pisang Banana:** Rice, corn, skimmed milk powder, banana powder,	From 6 month onwards	Indonesia ,PT. Gizindo primanusant 40553	0.25	

*The main composition of the foods;

[a] The content of zinc on the label.

4.1.2 Reagents and Chemicals

HNO_3 (69%, Maidenhead-Berkshire-United Kingdom) and hydrogen peroxide solution (35% w/v, Blulux, Laboratories Pvt. Ltd. India) were used for sample digestion. Zn $(NO_3)_2$ (Blulux, Laboratories Pvt. Ltd. India), was used for standard solution preparation. Distilled water was used for solution preparation throughout the experiment.

4.1.3 Sample digestion

The sample was digested by wet ashing method.[51, 52] A 1 g sample of ready-made powdered infant formula was accurately weighed into a 100 ml flat bottomed pyrex flask, 12 ml of concentrated oxi-acidic mixture of HNO_3/H_2O_2 (2:1) was added, and the mixture was heated on a hot plate at 60 ^{0}C for 4 hr to complete dryness with occasional stirring and then, diluted to 10 ml volume with distilled water.[52] Appropriate dilutions of the final solution were made in order for the absorption to fall within the absorption values of standard solutions of zinc.[53] The blank digests was carried out in the same way.[52]
Finally, the level of zinc in the clear solution was measured using flame atomic absorption spectrophotometer.

4.2 Measurements

4.2.1 Instruments and Apparatus

Oven (J.P.Select, Spain), Digital analytical balance (Mettler Toledo, Model AT250, Switzerland) were used for drying the washed apparatus and weighing the samples respectively. In addition, flat bottom pyrex flasks and Phillip Home: Model-C- MAG HS 4 S25, IKA hot plate were used for digestion of the samples. Finally, model of NOM AAS 300, (Analytic jena, Germany) was used for analysis of zinc.

4.2.2 Measurements of zinc in the foods sample

A concentrated stock solution of 1000 mg/L was prepared from its nitrate salt.[51] A series of five working standard solutions, 1, 2, 3, 4 and 5 ppm were prepared from the stock solution by diluting with distilled water to setup the standard curve. The calibration curve was obtained by running the working standard solutions using atomic absorption spectrophotometer.

Soon after calibration, except Safa and Couscous, the eight sample solutions were diluted using dilution factor of 12.5 and then all samples aspirated into the AAS instrument. Three

replicate measurements were carried out for all foods. The operating conditions of AAS employed for zinc is given in Table 4.

Table 4. Instrumental operating parameters for determination of zinc in the samples using FAAS

parameter	Element, Zinc
Fuel	Acetylene gas
Wavelength (nm)	213.9
Detection limits (mg/L)	0.012
Slit width (nm)	0.5
Lamp current (mA)	2.0
Energy (mV)	2.71

5. RESULTS AND DISCUSSIONS

5.1 Calibration

In addition to the absorbance the instrument was also directly read the concentration simultaneously. In one aspiration the instrument reads the sample four times in terms of both absorbance and concentration, gives four absorbance values with their corresponding four concentration values in one aspiration. However the calibration curve equation was used to check the concentrations of the foods which are obtained by dilution factor. Below fig. 4 shows the calibration curve of zinc standard solutions.

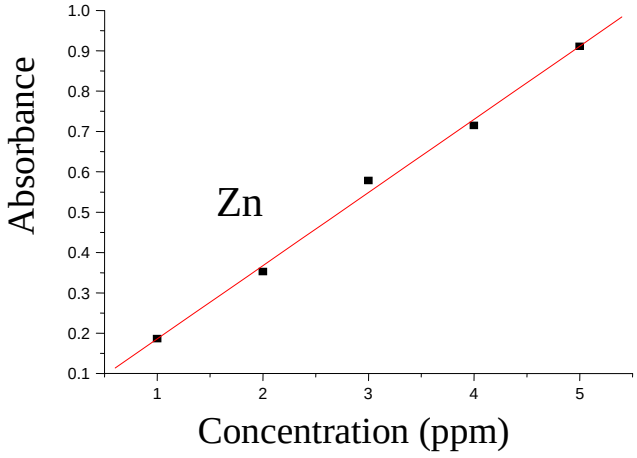

Fig. 4 Calibration curve for zinc standard solutions

$$Y = A + B\,X$$

Parameter	Value	Error
A	0.00492	0.02225
B	0.18124	0.00671

R	SD	N	P
0.99795	0.02121	5	1.11275E-4

5.2 Determination of zinc in the foods

Infant formulas are the main complementary foods for infants and young children in accessing additional essential metals when breast milk alone is inadequate.[3] Zinc is one of these essential metals that can be obtained from infant formula and required for their well-healthy growth and development. This is shown in table 4 which summarizes the results of the present work. The values for the eight foods were obtained by multiplying each replication concentration with the dilution factor, 12.5, and checked with the equation of the calibration curve. The values of couscous have also checked with the calibration curve equation. However its absorbance, correspondingly the concentration, values directly read bythe instrument fall in the absorbance, correspondingly the concentration, ranges of the standard solutions. Though the absorbance of Safa is below the absorbance ranges of the standard solutions, the four values of each absorbance, correspondingly the concentration, were stable within the one replication measurement, unlike the others eight foods before dilution and its concentrations also reproduced when the calibration curve equation is used. Below table 5 shows the Atomic absorption spectrophotometer results of zinc contents of foods which are available in supermarkets and pharmacies

Table 5. Atomic absorption spectrophotometer results of zinc contents of foods which are available in supermarkets and pharmacies

No.	**Foods**	**Zinc content, mg/kg ***
1	S-26	$36.439^{a} \pm 0.657$
2	SUN	$26.446^{b} \pm 0.525$
3	BABY KING	$20.940^{c} \pm 0.315$
4	CERELAC-1	$27.448^{b} \pm 0.899$
5	CERELAC-2	$30.941^{d} \pm 0.896$
6	Cerifam	$42.743^{e} \pm 0.816$
7	Safa	$0.768^{f} \pm 0.043$
8	Couscous	$4.725^{g} \pm 0.049$
9	Mother's choice	$24.918^{h} \pm 0.918$
10	Promil	$23.377^{i} \pm 0.794$

*values are means ± 95% confidence interval level (N=3); values which are not connected by the same letter are significantly different (p=0.00 one way –ANOVA); values with same letter are also significantly different but with p = 0.006

5.3 Distribution of zinc in the foods

The distribution of zinc in the studied foods is shown below using histogram.

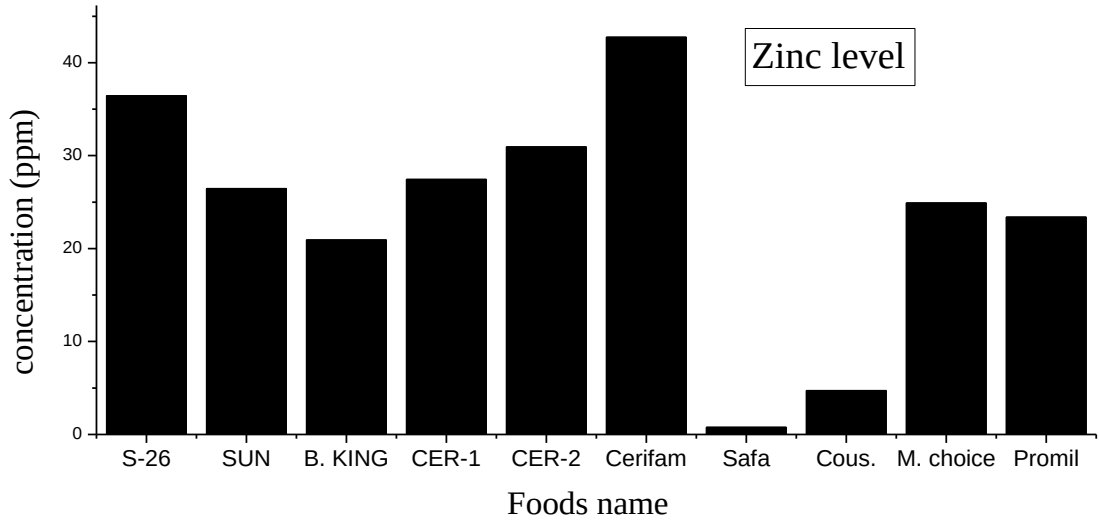

Fig.5 distribution pattern of zinc in the foods

5.4 Discussion

The concentration of zinc was highest (42.743 ± 0.816) in Cerifam and lowest (0.768 ± 0.043) in corn starch based Safa.

Animal products are usually the best source of dietary zinc, in terms of both content and bioavailability.[22] However wheat, soy, and dry milk based- Cerifam has higher zinc content than milk based S-26 and Promil foods (42.743 ± 0.816 compared with 36.439 ± 0.657 and 23.377 ± 0.794, respectively; p = 0.00). This may be due to S-26 contains reduced minerals-whey and the skimmed milk has less zinc content than dry milk and wheat flour according to USDA National Nutrient Database for Standard Reference values of zinc in selected foods.[54] The promil has already demineralized whey. Therefore, it seems that zinc is likely to occur in higher amount in Cerifam than S-26 and Promil.

Fruits, wheat and skimmed milk based Cerelac-2 food has lower zinc content than S-26 (30.941 ± 0.896 compared with 36.439 ± 0.657; p = 0.00). This is may be due to the fruits,

which are not considered as rich sources of zinc,[21] may be in high amount. It has no also a second milk product part in addition to the skimmed milk, like whey, i.e. part of the milk product that contains higher zinc content, in case of S-26.[54] However its zinc concentration was greater than wheat and milk based Cerelac-1 food (30.941 ± 0.896 compared with 27.448 ± 0.899; p = 0.00). This is due to the fact that it's additional fruit ingredients.

The zinc concentration of wheat and milk based Cerelac-1 than banana based SUN food (27.448 ± 0.899 compared with 26.446 ± 0.525; p = 0.006). In addition to wheat and milk, Cerelac-1 has caramel sugar and SUN has rice and corn in addition to banana. The concentration of zinc in wheat flour is 29.33 mg/kg, in caramel sugar is 3.96 mg/kg, in rice is 10.91 mg/kg, and in corn is 18.19 mg/kg.[54] Therefore it is fact that Cerelac-1 to have a little bit higher zinc content than SUN.

Starchy roots and tubers have much lower zinc content than cereals and legumes.[21] SUN has higher zinc content than rice, milk and carrot based Mother's choice food (26.446 ± 0.525 compared with 24.918 ± 0.918; p = 0.00). This is due to the fact that SUN is comprised of collective cereals, rice and corn, but Mother's choice is only of one cereal type, rice, and roots, carrot.

Even though animal products are usually the best source of dietary zinc, in terms of both content and bioavailability,[22] Zinc content of milk based Promil has lower zinc content than Mother's choice food (23.377 ± 0.794 compared with 24.918 ± 0.918; p = 0.00). This is may be due to that Promil contains skimmed milk and demineralized whey and the Mother's choice incorporates rice and carrots in addition to skimmed milk.

The processing (refining, milling, or polishing) of cereals removes some of the essential minerals. The content of minerals in rice depends on the availability of minerals in the soil.[53] The concentration of zinc in rice, milk, and apple based-BABY KING is lower than in Promil food (20.940 ± 0.315 compared with 23.377 ± 0.794; p = 0.00). This is may be due to the zinc content of the soil where the rice grown may be low, or the processing of the rice and apple may wastes zinc.

The concentration of zinc in Couscous and corn starch is 8.32 and 0.00 mg/kg respectively.[54] The concentration of zinc is higher in BABY KING than in durum wheat based-Couscous and

corn starch-based Safa food (20.940 ± 0.315 compared with 4.725 ± 0.049 and 0.768 ± 0.043; p = 0.00). This is due to the fact that the concentration of zinc in couscous and corn starch is lower than in rice. In addition, both Couscous and the Safa have no any kind of dairy products.

The zinc content of wheat based couscous is higher than corn starch based Safa (4.725 ± 0.049 compared with 0.768 ± 0.043; p = 0.00). From the above discussion it is because of their ingredients, the concentration of zinc in Couscous is 8.32 mg/kg and in corn starch is 0.00 mg/kg.

Even though, the Safa constitutes from corn starch whose zinc content is 0.00 mg/kg, the processing quality may played a role for the existence of zinc in the flour, or during the food processing procedure it could enter to the food due to environmental contamination.[52]

It can be seen that the zinc content in all, except in Safa, foods is higher than the average breast milk (ranged from 0.5-4.7 mg/L)[55] and they are appeared to be adequate for the infants and young children (up to 23 months) daily recommended dietary zinc intake, by Food Consumption and Nutrition Division (FCND) discussion paper. Table 6 shows the daily recommended dietary zinc intake, by FCND discussion paper[56] and the upper limits of infants and young children, by WHO.[57]

Table 6. Daily recommended dietary zinc intake and the upper limit of infants and young children.

Recommended zinc intake per day by age group[a] (months)		**Upper limits of zinc intake by age (months)**	
Months	Zn intake, mg/day	Months	Zn intake, mg/day
6-8	4.1	6-12	13
9-11	4.1	13-72	23
12-23	4.1		

[a] Estimates assume average breast milk intake.

The table below shows how much gram from each of food a baby should take in response to table 6, recommended dietary zinc intake of infants and young children.

Table 7. The expected amount of food a baby should take from each food in response to table 6, recommended dietary zinc intake of infants and young children.

No.	**Foods**	**The expected amount of zinc demand, in gram**
1	S-26	112.51
2	SUN	155.03
3	BABY KING	195.797
4	CERELAC-1	149.373
5	CERELAC-2	132.51
6	Cerifam	95.922
7	Safa	5338.54
8	Couscous	867.724
9	Mother's choice	164.539
10	Promil	175.386

The above table shows the amount of zinc demand ranges from 95.922-5338.54 gram. According to Kersting et al., 1998 study on Measured consumption of commercial infant food products in Germany infants which is appeared on the journal of European Food Safety Authority (EFSA) paper, the highest daily total food intake (solids and fluids, including water) of infants with 3-12 months ranges from 154-136 g/kg body weight (B. wt) respectively and for young children (1-3 years) is 104 g/kg B. wt.[58] Assuming the average B.wt of infants with 3-12 month who takes the foods available in supermarkets and

pharmacies are ranges from 4-9 kg respectively and 12 kg for young child with 12-23 month, the values correspond to the highest total food intake of 616-1224 and 1248 gram respectively. According to this directive all, except Safa, foods are enough to satisfy the infants and young children (up to 23 month) daily dietary zinc requirement (by FCND discussion paper) and each, except Safa, foods cannot be toxic in terms of zinc if they are taken up to the infants and young children (up to 3 years) highest consumption level, by WHO.

Below table 8 shows the foods that have zinc content description on the label is greater than this work results. This is may be due to the quality of sample digestion process or from the instrument sensitivity.

Table 8. Contents of zinc on the food label and the experimental value.

Food items	**Zn content on the label, mg/kg**	**Zn content of experimental value, mg/kg**
S-26	47	36.439 ± 0.657
Promil	36	23.377 ± 0.794
BABY KING	35	20.940 ± 0.315

The zinc content of the cereal based baby foods analyzed in the present study was compared to infant formula used in India.[53] The reported values, except Nestrum, were significantly higher than the findings of the present study; below table 9 shows the value of both foods.

Table 9. The content of zinc in both Indian and current studied foods.

Indian manufactured baby foods		**Current studied foods**	
Food items	Zn content, mg/kg	**Food items**	Zinc content, mg/kg
Nestrum (Rice flour based)	28	BABY KING	20.940
Cerelac (wheat flour and skimmed milk based)	85	CERELAC-1	27.448
Cerelac (wheat flour, orange, and skimmed milk)	82	CERELAC-2	30.941
Farex (wheat, mung bean, and milk solids)	62.7	Cerifam	42.743
Dexolac (extruded rice flour and skimmed milk)	45	Safa	0.768
		Couscous	4.725
		Mother's choice	24.918
		SUN	26.446

The zinc content in Indian ranged from 28 – 85 mg/kg, and in the present study ranged from 0.768 – 42.743 mg/kg. The higher values of zinc obtained in their study may be due to the zinc content of the soil where the cereals grown may be higher than the soil where the studied food grown; or it may be from the instrument sensitivity or from using of quite different sample digestion procedure.

6. CONCLUSION AND RECOMMENDATION

Conclusion

The study showed that the level of zinc in ten infant formulas available in supermarkets and pharmacies was variable and all, except Safa, have higher zinc content than the average amount of zinc available in breast milk. The study also have displayed comparison of zinc level of the ten foods with other five foods manufactured and sold in India and the level of recommended dietary zinc intake which is announced by Food Consumption and Nutrition Division (FCND) discussion paper.

The content of zinc in infant foods follows the order: cerifam > S-26 > cerelac-2 > cerelac-1 > SUN > mother's choice > promil > baby king > couscous > Safa. Cerifam which is cost effective and manufactured by Ethiopia has highest nutritional value in terms of zinc than the others while Safa which is manufactured by Dubai-UAE has lowest zinc content. The results of this study also indicated that the ten foods have lower zinc contents than those of Indian manufactured and used infant foods. According to assumption, in reference to EFSA, the zinc content of all, except Safa, foods available in supermarkets and pharmacies are appeared to be adequate for the daily recommended dietary zinc intake of infants and young children (up to 23 month).

Recommendation

Since zinc is very important nutrient for infants' growth, this mineral should be highly available in their formula. However, there is not much information available on the levels of zinc in many of the ten foods available in supermarkets and pharmacies. The result showed that all, except Safa, foods are appeared to be adequate for infants and young children dietary zinc requirement. Thus it is promoted that parents to use these, except Safa, foods to their babies after 6 months of age, since mothers breast milk alone does not provide adequate dietary zinc for infants after 6 months of age. However, this does not mean all the zinc content of the foods is absorbed, due to some anti nutritional factors such as phytates and fibers.

Further studies on Nationwide ZDD in Ethiopia and strategies to control and eliminate zinc deficiency are highly needed in general. The government bodies should also try to develop

regulations on the standard zinc level in infants formulation to control zinc deficiency in infants and children.

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