LEVELS OF SOME NUTRIENTS IN COW'S WHOLE MILK AND COMMERCIAL INFANT FORMULAS DISTRIBUTED IN ADDIS ABABA, ETHIOPIA

A Thesis submitted to the School of Graduate Studies
Addis Ababa University

In partial Fulfillment of the Requirements for the Degree of Master of Science in Chemistry

By

Engdawork Admasu

June 2005

ADDIS ABABA UNIVERSITY

OFFICE OF RESEARCH AND GRADUATE PROGRAMMES

LEVELS OF SOME NUTRIENTS IN COW'S WHOLE MILK AND COMMERCIAL INFANT FORMULAS DISTRIBUTED IN ADDIS ABABA, ETHIOPIA

By

ENGDAWORK ADMASU

Department of Chemistry

Science Faculty

Approved by the Examining Board	**Signature**
Dr. Negussie Megersa **Chairman, Department Graduate Committee**	
Prof. B. S. Chandravanshi **Advisor**	B S Chandravanshi
Dr. Taddese Wondimu **Advisor**	Taddese
Dr. Girma Akalu **Advisor**	Girmas
Prof. M. Subbaiyan **Examiner**	
Dr. Mesfin Redi **Examiner**	

DEDICATION

My father Admasu Engda:

Loved in life, honored in departing, treasured in memory one of my best.

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my advisors Prof. B.S. Chandravanshi, Dr. Taddese Wondimu and Dr. Girma Akalu who have shouldered the onerous task of advising me to bring this study into effect. I appreciate all the efforts they made, valuable comments they gave and precious time they expended towards the completion of this paper. A grateful acknowledgement are hereby made to the Department of Chemistry, Addis Ababa University, Ethiopian Health and Nutrition Research Institute (EHNRI) and their staff members for providing me with all the necessary resource materials while I was carrying out this project. My sincere thanks are to Dr. Yonas Chebude and Ato Mengistu G/Tsadik for their unlimited cooperation in my work.

Special thanks are to Dairy Development Enterprise, Food Science and Nutrition Network for Africa (FOSNNA) regional coordinators, International Livestock Research Institute, ILRI (Kenya), Nutrition and food Science: University of La Laguna (Spain).

My special thanks also go to my mother W/ro Belaynesh W/Agegnehu, my sisters and brothers for their encouragement.

In the end I offer many thanks and glory to GOD, who is always the source of my strength and inspiration.

Engdawork Admasu

Table of contents

List of Tables

pages

List of Figures

Page

Abstract

Three brands of infant formulas (n = 3) and 618 cow's whole milk samples from four different farms, which supply milk to Addis Ababa, were analyzed for various essential (Ca, Mg Fe, and Zn) and non-essential (Cd, Ni, Pb, and Tl) nutrients, fat and protein levels. Known weights of powdered infant formulas or freeze dried cow's whole milk samples were digested with 3.5 mL HNO_3 and 2.0 mL $HClO_4$ on a hot plate for 4:30 h. The contents of nutrients in the digests were then analyzed employing standard methods. The following mean levels were recorded in cow's whole milk: Ca (mg/L) 1600; Mg (mg/L) 65; Fe (mg/L) 1.25; Zn (mg/L) 5.33; Cd (mg/L) 0.18; Ni (mg/L) 1.27; Pb (mg/L) 2.63; Tl (mg/L) 2.15; fat 3.7% (m/m); protein 3.2% (m/m). The data for the mineral concentrations in cow's whole milk were found within normal intervals described in the literature. The three infant formula brands analyzed in this study generally showed low nutritional contents when compared with the recommended dietary allowances (RDA) for use in North America. The observed values of the analyzed non-essential elements were below the drinking water maximum admissible concentrations for the infant formulas. However, significantly higher concentrations of these elements were observed in the cow's whole milk. An important contribution to the daily intake of calcium for Addis Ababa population due to the consumption of cow's whole milk was observed. The accuracy of analytical results was checked by analyzing the NIST reference material SRM 8435 (whole milk powder) and good agreement was achieved with certified values.

Key words: Milk, infant formula, nutrients, non-essential elements, flame atomic absorption spectroscopy, Ethiopia.

CHAPTER 1

INTRODUCTION

We who have access to the vast array of existing modern technology have the power to use it to build a better, healthier society; or, we can use it for destructive purposes thereby hurting/injuring others. Nowhere is this more evident than in food and health technology. Livestock production is a major contributor to economic development, both driving economic growth and benefiting from it. As an engine of growth, it provides increased income, employment, food and foreign exchange earnings, as well as better nutrition [1]. The differences in consumption levels between developing and developed countries reflect the widely varying nutritional status that exists between them. Animal products contain important nutrients in bioavailable form, essential for growth and proper physical and mental development [1, 2].

Milk and milk products are the major source of essential nutrients in diet. Thus, the knowledge of the minerals concentrations in milk samples and milk products is of particular interest. The mineral content of milk varies widely due to numerous factors, such as lactation (composition of milk varies considerably during lactation, with the major changes usually occurring soon after the start of lactation period), the breed of animal, climate, season, dietary composition of animal feed and soil contamination. Also, the mineral content may vary because of its handling by humans [3].

An estimated total of about 1.4 million metric tons of milk is produced in Ethiopia from an estimated population of 4.6 million cows, 9.3 million goats, 3 million ewes and 0.15 million camels. On a national scale the per capita milk consumption is estimated at 19 to 30 kg per annum [4].

A recent survey undertaken by the Addis Ababa Agricultural Bureau shows that there are a total of 5,167 small, medium and large dairy farms in and around Addis Ababa city. The total milk production from these dairy farms amounts to 34,649,450 liters per annum. Of this, 73% is sold, 10% is left for household consumption, 9.4% goes to calves and 7.6% is processed mainly into butter and cheese. The total amount of milk available to Addis Ababa is 43,849,675 liters per annum [5]. This figure is higher than the total dairy production from the dairy farms, the difference accounts for the informal milk supplied by the individual herds.

Reliable data on the nutrient composition of foods for human consumption are critical for many areas of endeavor including health assessment, the formulation of appropriate institutional and therapeutic diets, nutrition education, food and nutrition training, epidemiological research on relationships between diet and disease, plant breeding, nutrition labeling, food regulation and consumer protection, as well as for a variety of applications in agriculture, trade, research, development and assistance.

The analysis of cow's milk, goat milk, camel milk, etc. has been investigated in different countries [6-9]. The effect of season and locality was studied to determine the nutrient composition of South African milk. The purpose of the study was to compile food composition tables for South Africa, containing accurate, reliable data based on South African milk as consumed by the average population [6].

The determination of Se, Fe, Cu, Zn, Na, K, Ca, and Mg concentrations in raw and sterilized cow's milk in Canary Island has been made and the data have been compared with literature data. The contribution of the daily consumption of milk to the mineral intake of the Canarian population was established. Also, the seasonal changes in the elements concentrations of cow's milk were studied [3].

The determination of the levels of basic nutrients, major and trace minerals and cholesterol in commercial goat fluid, evaporated and powdered milk products manufactured in the United States and differences in the nutrient contents between different types of commercial manufactured goat milk products have been studied [7].

Investigations on raw bulk cow's milk and cheese samples were undertaken in Italy in order to measure the composition ranges to be considered as 'normal' for elements Al, Ba, Cd, Co, Cu, Fe, Mg, Mn, Ni, Pb, Pt, Sr and Zn as well as to elucidate any possible relationship between animal feeding, environmental conditions, time of year of sample collection, manufacturing processes and distribution of elements in cow's milk and cheese [8]. Along similar lines, the investigatins on milk and cheese of sheep and goat origin have been done [9].

Some studies are also carried in Ethiopian cow's milk. From the nutritional composition of whole milk in Ethiopia fat, protein, ash and total solid data were reported in 1987 [10]. The existing pasteurized milk producing companies analyze some of the nutrients in their laboratories. In general economic analysis [11], veterinary [12, 13], feeding and management [14] and breed traits [15] of the dairy sector in Ethiopia are investigated but laboratories do not participate in national monitoring programmes for contaminants such as pesticide residues, heavy metals and mycotoxins [16].

This research project is designed in response to bridge the above mentioned gap, in the analysis of essential and non-essential metals and other nutrients in imported infant formula and in cow's whole milk collected in Addis Ababa, which is the most consumed [5] in the country as compared to the pasteurized milk.

The findings of the present research undertaking will be useful in establishing baseline data on the levels and temporal variation of some nutrients in whole milk. This research is expected to show the levels of nutrients in whole cow's milk from Jersey breed, Holstein and crossbreeds and of toxic heavy metals, which can help to monitor pollution in the urban and peri-urban areas around Addis Ababa in 150 km radius. To the same extent the imported commercial infant formulas are analyzed for their nutritional values and toxic heavy metals. It is also expected to indicate future research directions in dairy development in Ethiopia.

In Ethiopia hunger and malnutrition have been recurrent problems, particularly for the poor and unprivileged. According to the study by the Ethiopian Ministry of Economic Development and Cooperation, 50 percent of the Ethiopian population are living below the food poverty line and cannot meet their daily minimum nutritional requirement of 2200 calories [17]. Women in the reproductive age group and children are most vulnerable to malnutrition due to low dietary intakes, inequitable distribution of food within the household, improper food storage and preparation, dietary taboos, infectious diseases, and care. Particularly for women, the high nutritional costs of pregnancy and lactation also contribute significantly to their poor nutritional status. Investing in women's and children's nutrition will have both short-term and long term effects on the social and economic well-being of not only the individual but the community and the nation (ACC/SCN, 1992) [18].

1.1. Importance of milk

Milk is an important foodstuff in human diet, both in its original form and as various dairy products. It provides energy and a wide variety of nutrients essential for the growth and mental development of children [2]. Although milk can be obtained from animals such as camel, goat, and sheep, humans largely use the milk from cows. The basic ingredient in most baby milk preparations is cow's milk, which has been modified to make it as similar to mother's milk as possible.

As an excretion of the mammary gland, milk can carry numerous exogenous chemicals such as pesticides, disinfectants, drugs, and metals, which constitute risk factor for the health of consumer. Metals found in milk can play either beneficial or inimical roles depending on their nature and/or concentration; for instance, copper is an essential element because its deficiency affects iron metabolism in human infants whereas its excess concentration causes acrodynia [19]. Zinc is an integral part of enzymes and hypogondalism and dwarfism resulting from its deficiency could be controlled if up to 25% of the daily intake constitutes dairy products. With the creation of growing awareness, the consumer becomes more demanding than in the past and expects healthy milk rich in nutrients, but without health risks. From the commercial point of view, milk products that didn't pass stringent quality requirements may suffer from market constraints [9].

Birth weight, child growth, and adolescent growth determine nutritional status before and during pregnancy (maternal nutrition). Maternal nutrition also influences fetal growth and birth weight

(ACC/SCN, 1992) [17]. The presence of an intergenerational link between maternal and child nutrition means a small mother will have small babies who in turn grow to become small mothers. Some findings on the relationship between maternal and child nutrition [20, 21] showed that a high proportion of low-birth-weight and stunted children were observed among malnourished mothers.

Infant formulas are liquids or reconstituted powders fed to infants and young children. They serve as substitutes for human milk. Apart from breast milk, infant formulas have a special role to play in the diets of infants because they are the major source of nutrients for infants and a unique source of food during the first months of life [22]. They are handy for urban women [23]. Despite the strong endorsement of breastfeeding by the American Academy of Pediatrics (AAP), current breastfeeding rates worldwide are far from optimal, particularly among low-income women, despite mounting evidence about the health, psychosocial and societal benefits derived from breastfeeding [24]. Mothers are sometimes worried about or have been discouraged from breastfeeding because of concerns regarding toxins found in the body and often transferred to the baby through lactation [25]. Other reasons for mothers not to breastfeed their babies may include the demands of work or school, life circumstances, such as difficult home situations, and social problems with breastfeeding in public [24].

There has been a general interest worldwide, to substitute infant formulas for breast-feeding, despite increasing evidence about the benefits from breastfeeding. Some of the reasons for mothers to substitute infant formulas for breast milk include convenience for urban women [23], the demands of work or school, and life circumstances. The growing HIV prevalence among women of childbearing age in some countries has also raised concern relating to the risk of mother to-child-transmission of the virus through breastfeeding. HIV-positive childbearing women are compelled to formula feed their children so as to minimize the risk of transmission of the virus. The basic ingredient in most baby milk preparations is cow's milk, which has been modified to make it as similar to mother's milk as possible.

1.2. Role of infant formulas in child nutrition

Despite the benefits of infant formulas as a major source of food for infants, the presence of contaminants, such as heavy metals, pesticides and polychlorinated biphenyls (PCBs) in infant formula may pose health risks to children. It has been reported that children are more susceptible to exposure [23] because of their greater intestinal absorption than adults, and a lower threshold for adverse effects [26]. These pollutants may arise from the raw materials used in production, poor quality production processes, adulteration of infant foods and bad practices by mothers as regards to infant formulation preparation and handling [27].

The American Academy of Pediatrics' Committee on Nutrition, since 1969, recommended ''the early use of fortified formula which results in augmentation of iron stores which help prevent later development of iron deficiency''. Infant formulas have therefore been classified as low-iron or iron-fortified, based on whether they contain less or more than 6.7 mg/L of iron [28]. Iron fortified formulas in the USA are up to 12.7 mg/L and within the range 0.2–0.5 mg/L in Europe [8].

Most infant formulas are derived from animals or plants and therefore are mostly milk-based or soy-based formulations. Because of the nutritional properties of soybean, dairy-like products, or infant formulas based on soybean, are proposed as one of the most interesting alternatives for adults or children who are allergic to animal proteins [29].

Recently, and in the past, many authors have worked on infant formulations because of the need to maintain the good health of infants. Some research findings on infant formula report high aluminum content [30, 31], inadequate daily intakes of copper and zinc from infant formula consumption [23] and low selenium content in infant formulations [32].

1.3. Objectives

1.3.1. General objectives

The main objective of this project was to collect baseline data on the levels of nutrients (trace metals, fat, and protein) in cow's whole milk and infant formula from the outlets of different suppliers to Addis Ababa, major dairy farms and supermarkets, respectively. The data may assist in monitoring trends in pollution through cow's milk and ensuring the importation of safe formulas.

1.3.2. Specific objectives

The specific objectives of this project were:

1 To collect samples of cow's whole milk from selected farms in and around Addis Ababa and infant formula from major supermarkets. To compare nutrients in pure Jersey breed milk (found only in one farm in the country), Holstein breed and the cross breed cow's milk.

2 To determine the levels of both essential (Ca, Fe, Mg, and Zn) and non-essential (Cd, Ni, Pb, and Tl) elements in infant formula products from three brands and to estimate the daily intakes of these elements from infant formula consumption.

3 To compare the metal content in infant formula brands derived using feeding tables supplied by infant formula manufacturers with some international standards in attempts to justify analytical findings wherever appropriate.

4 To determine fat and protein content of milk samples.

5. To compare the level of nutrients in the cow's whole milk in Addis Ababa with literature data for essential (Ca, Fe, Mg, and Zn) and non-essential (Cd, Ni, Pb, and Tl) elements [22].

CHAPTER 2

EXPERIMENTAL

2.1. Instrumentation

BUCK SCIENTIFIC MODEL 210VGP (USA) atomic absorption spectrometer equipped with deuterium arc background corrector was used for analysis of the analyte metals; Kejeltec Auto 1003, Tecator, Hoganas (Sweden) was utilized for collecting ammonia from the digested sample in the analysis of protein; Tecator/Udy, Ra, Fa Tec Extraction Unit, Hoganas (Sweden) was used for fat extraction, Selecta model 2001241 (Spain) drying oven was used for drying glassware, FREEZE DRY-3, LABCONCO (USA) was used to freeze dry the whole milk samples; and Kjeldahl apparatus was used as a hot plate during digestion of milk samples.

2.2. Chemicals and reagents

All solutions were prepared using analytically pure reagents: nitric acid (70% trace metal grade; Spectrosol® BDH England), perchloric acid (70% Analar® BDH, England), lanthanum nitrate hydrate (99.9% Aldrich, Muwaukee, USA). A stock standard solution of 1000 mg/L (Buck scientific PURO-Graphic^{tm} calibration standards, prepared as nitrates for each element in 2% HNO_3) was used in preparing calibration standards. The calibration solutions were made from the stock solutions using distilled and deionized water immediately before analysis.

Standard reference material whole milk powder SRM 8435 was purchased from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). Potassium sulphate (Analar® BDH, Poole, England), sulphuric acid (98% BDH, Poole, England), hydrogen peroxide (30% BDH, Poole, England), sodium hydroxide (98% Analar® BDH, Poole, England), boric acid (Anhydrous, 99%, Sigma grade, ST Louis, USA), bromo cresol (Merck, Germany), methyl red (Fluka, Switzerland), ethanol (99% Absolute), and diethyl ether (Analar® BDH, Poole, England) were used as received.

2.3. Samples

2.3.1. Cow's whole milk

Cow's whole milk samples were collected from four different dairy farms in Ethiopia; selected on the basis of their breed type, supply amount, and geographical location. Samples at all four farms were collected in two different periods of the year (December 2004 and February 2005) 2004/2005 to better evaluate the influence of different feeding patterns on the levels of trace elements in milk for the cow's whole milk. A total of 618 samples (four farms x two times x 78 Jersey; 125 Holstein; 59 Holstein; 47 Cross breeds; were collected according to Colorado public health and environment milk sampling and testing manual 2000 and the International Dairy Federation (IDF) standard methods of sampling for bulk milk (tankers, silos, etc.). As recommended in these manuals, in the case of small retail samples, the whole container represents the sample, but must not be less than 100 mL [31]. The sample characteristics of cow's whole milk are described in Table 1.Cow's whole milk samples were collected in low-density polyethylene (LPDE) plastic bottles at four farms and each milk sample was frozen at –20°C and then freeze-dried using Labconco-3 freeze dryer.

Table 1. Background information on cow's whole milk sampling sites and number of cows sampled.

Farm	No. of cows sampled	Farm location*		Altitude (m)	Breed
Adaberga	78	38°22` E, 9°18` N	Inchini area	2635	Jersey
Ayele	59	39°07` E, 8°36` N	Mukaturi	1781	Holstein
Selale No. II	125	39° 00`E, 8° 45` N	Mojo	2631	Holstein
Genesis	47	38°52` E, 9°34` N	Debrezeyt	1995	Cross breed

*source: The Ethiopian Government's Ministry of Land Reform and Administration, Survey and Mapping Department, 2004.

In each farm from each cow a minimum of 100 mL (up to 150 mL) of milk samples were collected and mixed in the milking metal bucket and homogenized using vortex. The sample (750 mL) from each farm were transferred separately to low density poly ethylene plastic bottles to 75 % of the volume of the bottle, i.e. four samples (one representative from each dairy farm were brought) to the laboratory in less than 24 h after milking. Then triplicate analysis was done on each sample. Thus a total of 4 x 3 = 12 samples were analyzed for the first period of sampling. Similarly 4 x 3 = 12 samples were analyzed from the second round samples.

The relative locations of the farms are indicated in the map of Ethiopia below.

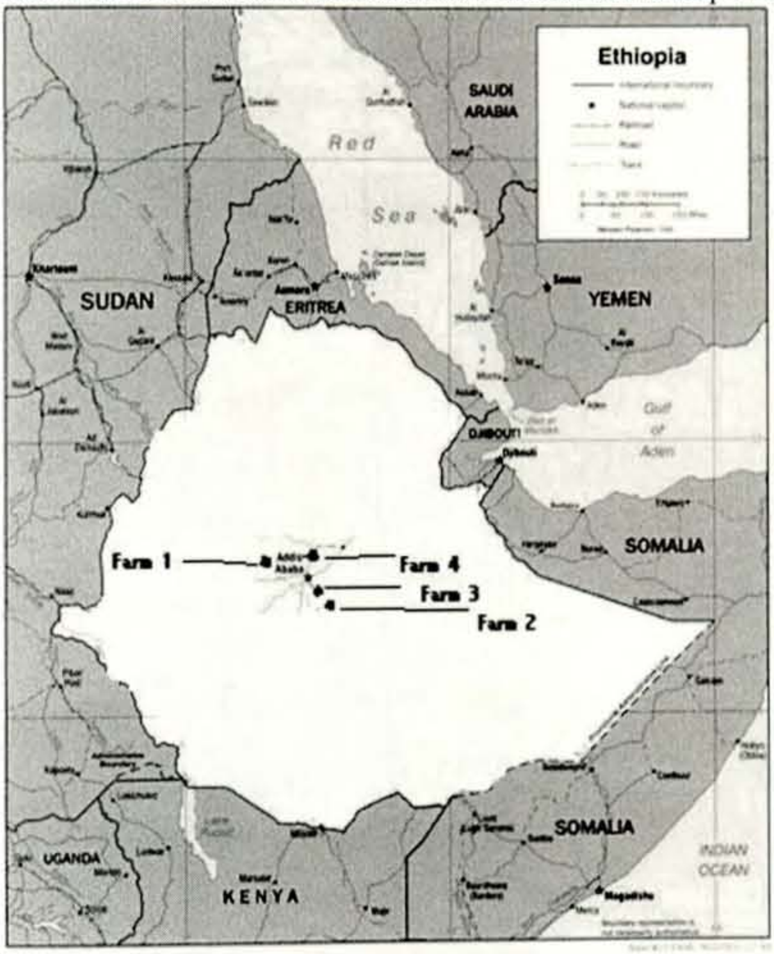

Figure 1. Map of Ethiopia indicating the relative locations of sampling dairy farms.
Farm 1: Adaberga Farm; Farm 2: Ayele Farm; Farm 3: Genesis Farm; Farm 4: Selale No. II.

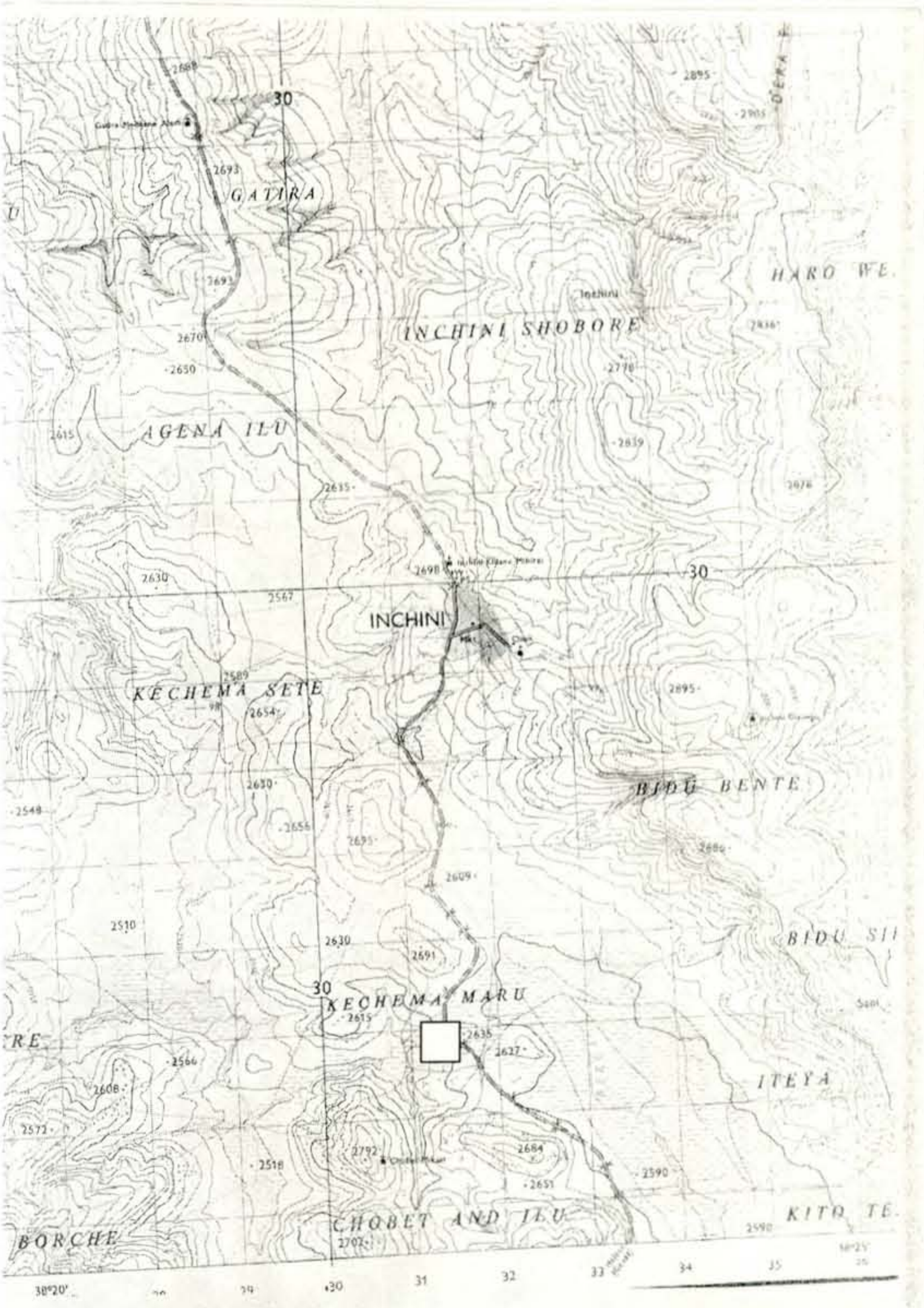

Figure 2. Adaberga Farm: located in Inchini area used for collecting cow's whole milk samples. (□ Indicates the farm site).

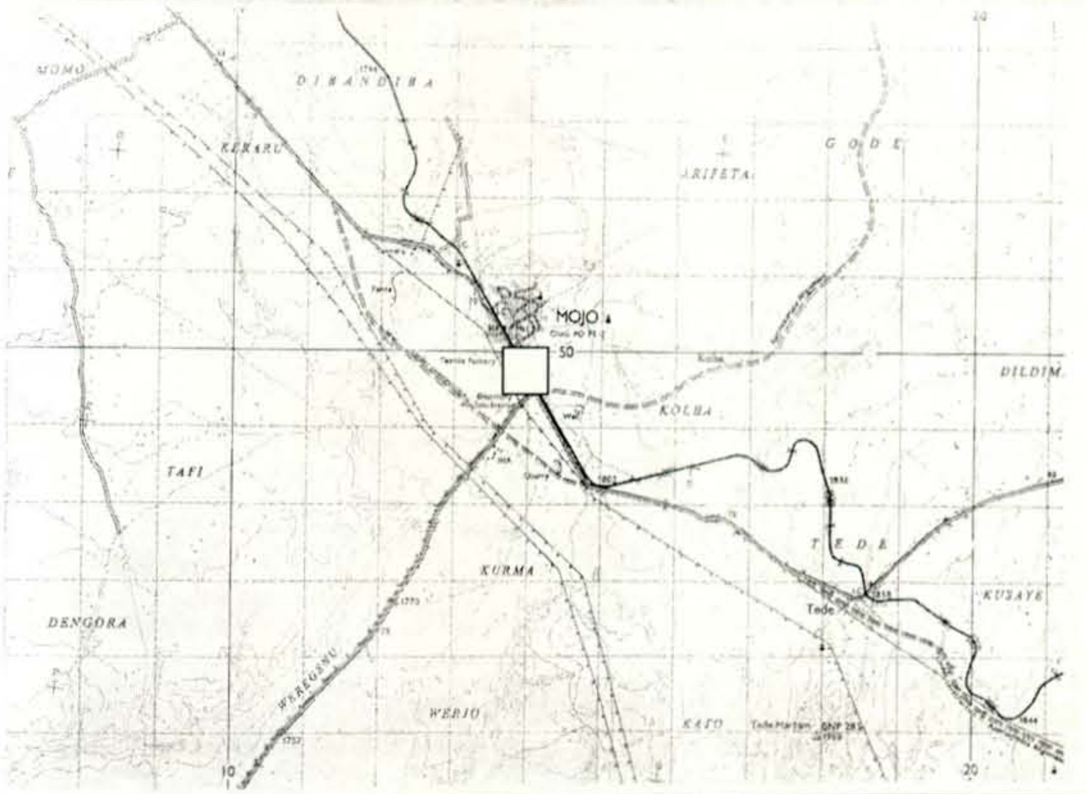

Figure 3. Ayele Farm: located in Mojo area used for collecting cow's whole milk. (□ Indicates the farm site).

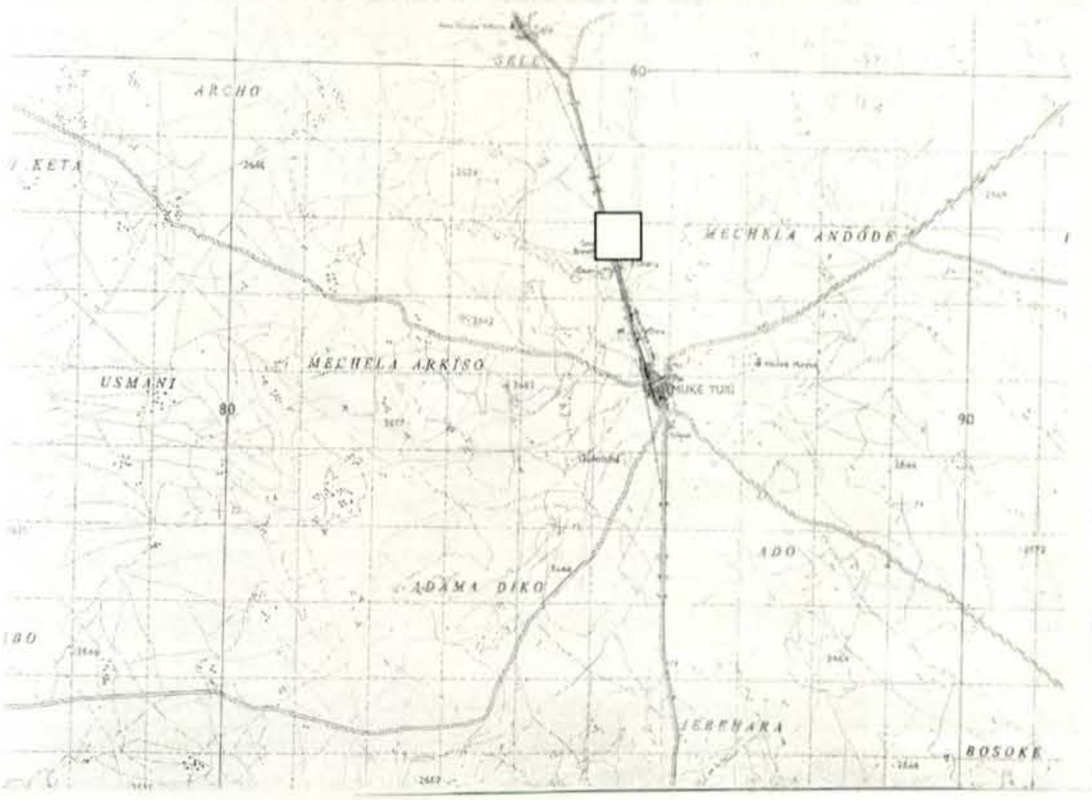

Figure 4. Selale No. II Farm: located in Mukaturi area used for collecting cow's whole milk. (□ Indicates the farm site).

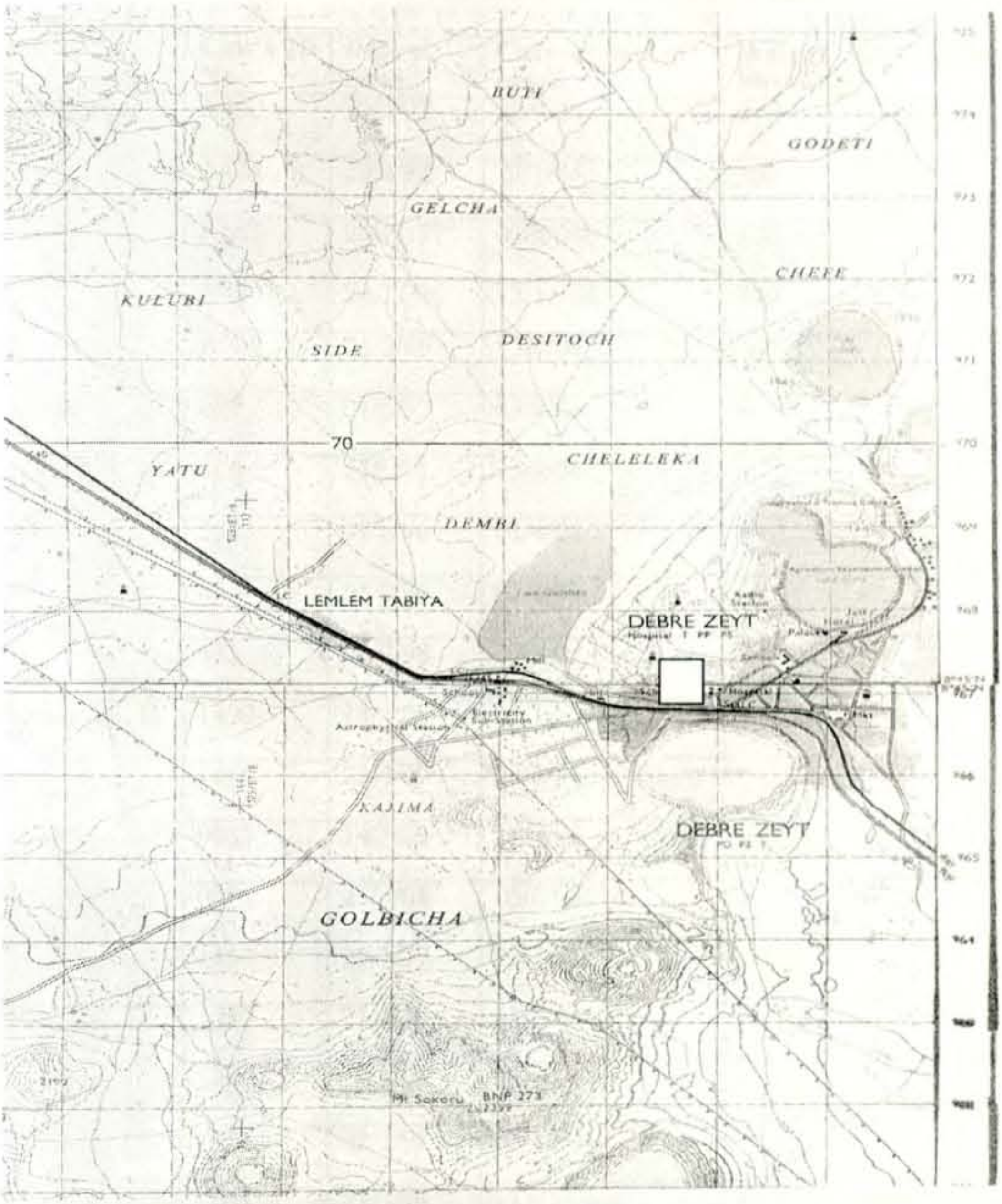

Figure 5. Genesis Farm: located in Debrezeyt area used for collecting cow's whole milk.
(Indicates the farm site).

The representative milk samples collected from each farm, the breed, lactation period, and age of each cow were considered and collected from the individual lactation and breed records as shown in Table 2.

Table 2. Partial individual lactation and breed record of sampled farms.

Farm	Cow's ID No.	Date of birth	Current date of caved	Weight after giving birth (kg)
Adaberga	A460	29/4/92	29/9/04	370
	A474	29/4/92	27/1/04	360
	A687	24/11/97	02/6/04	340
	A690	24/11/96	12/11/04	340
	A708	06/2/96	13/9/04	360
Ayele	689	07/6/97	13/9/04	---
	686	15/5/01	15/9/04	---__
	233	12/6/96	01/4/04	---__
	436	05/9/96	01/4/04	---__
	533	06/9/98	30/9/03	---__
Selale No. II	1490	13/3/00	02/12/04	---__
	1456	23/4/99	27/3/04	---__
	0055	14/7/01	18/9/04	---__
	007	25/6/01	05/8/04	---__
	1437	29/7/97	23/7/04	---__
Genesis	1387	23/7/90	13/7/04	---__
	1467	18/6/93	23/10/04	---__
	1736	22/1/95	23/10/04	---__
	1961	23/3/99	11/02/04	---__
	1781	03/9/95	17/9/04	---__

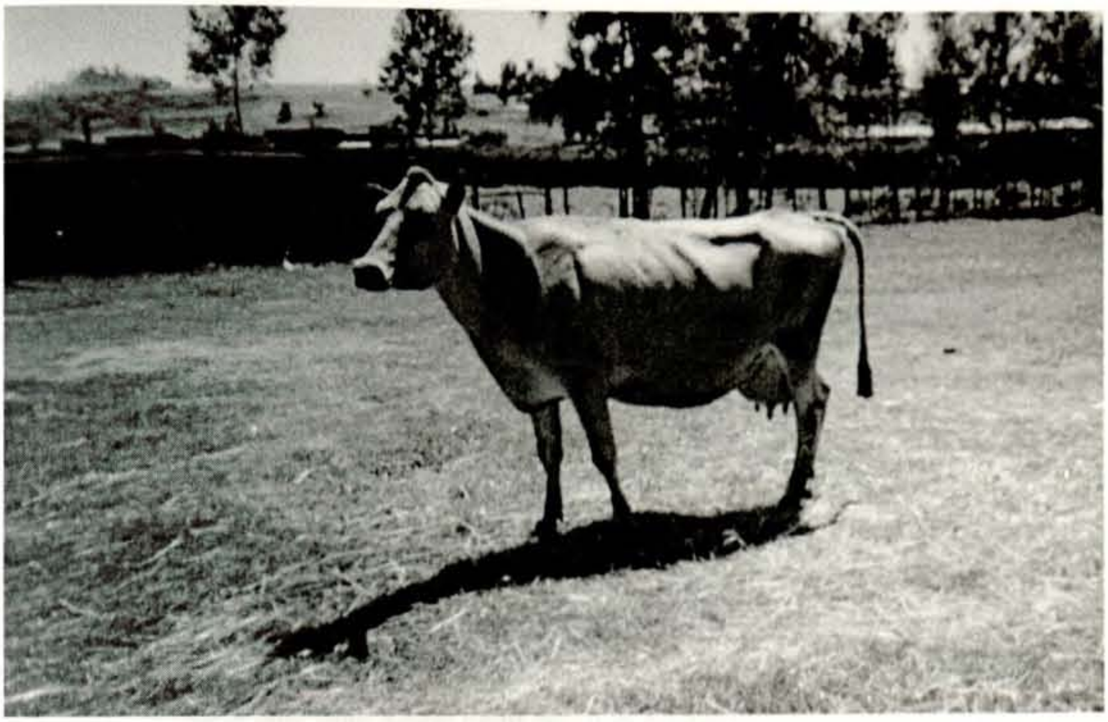

Figure 6. Jersey breed cow from Adaberga Farm, Inchini area .

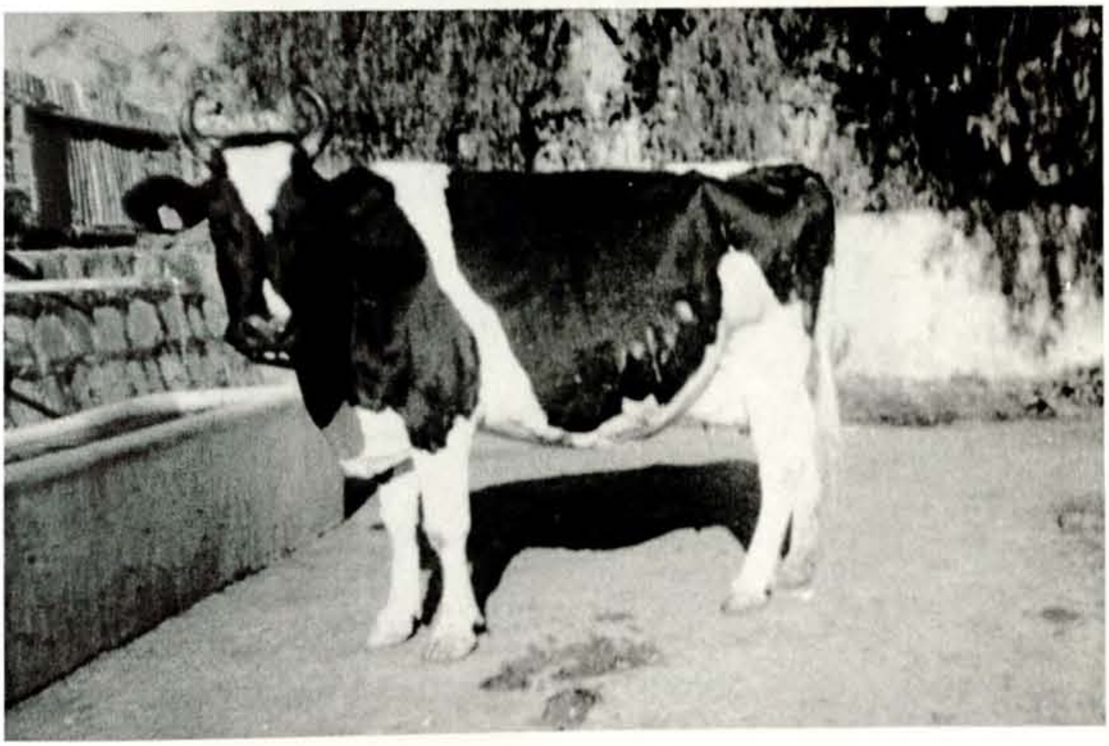

Figure 7. Holstein cow from Ayele Farm in Mojo.

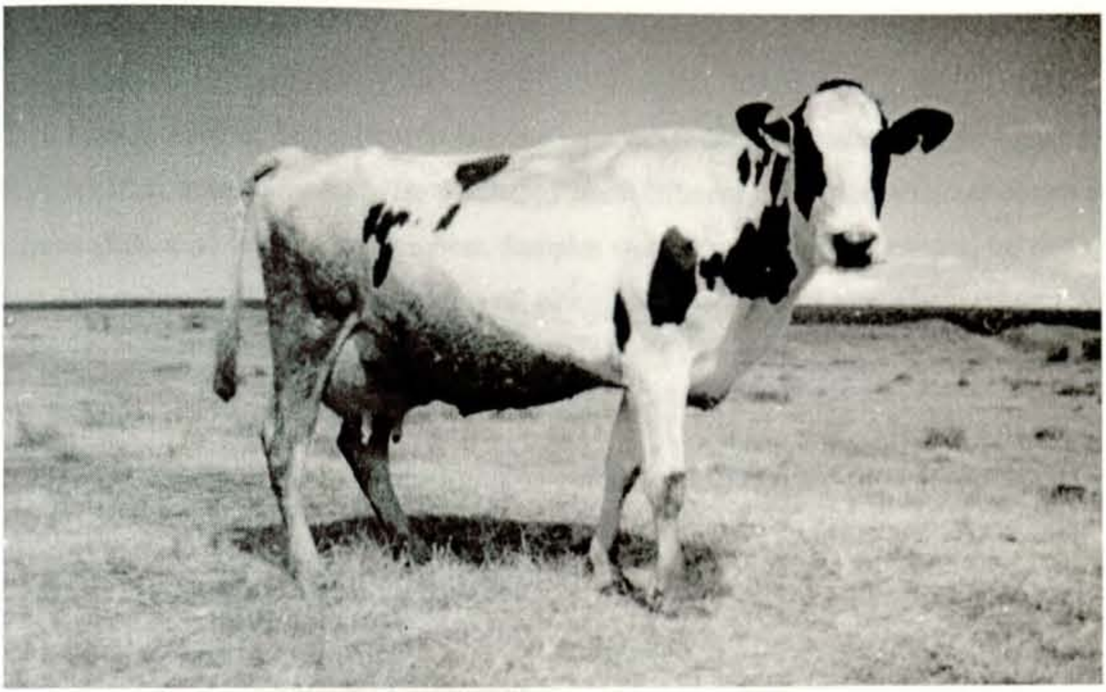

Figure 8. Holstein cow from Selale Farm No. II in Mukaturi.

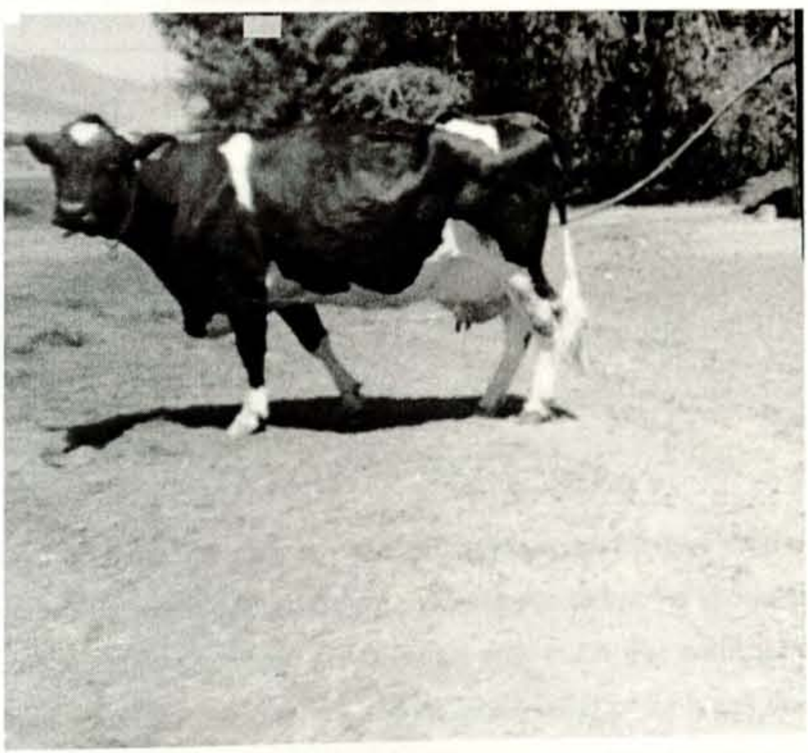

Figure 9. Cross breed cow from Genesis Farm in Debrezyt.

2.3.2. Infant formulas

Two brands, each of first milk iron fortified powdered infant formulas and one brand follow-on iron fortified infant formula were purchased from different major supermarket chains in Addis Ababa (Ethiopia) to allow randomness. Samples were bought at various times, between October and December 2004. Three containers of 400 g (NAN and S-26) and 450 g (Guigoz) capacity were purchased and analyzed in triplicates. A total of 27 samples 3 x 3 x 3 were analyzed.

Table 3. Characteristics of infant formulas purchased in triplicate from supermarkets in Addis Ababa. All samples were packaged in metal containers.

Brand	Sample information
NAN (Netherlands)	Powder; iron-fortified; milk-based; 0–12 months or from birth
S-26 (Ireland)	Powder; iron-fortified; milk-based; 0–12 months or from birth
Guigoz (Netherlands)	Powder; iron-fortified; milk-based; follow-on or above 12 months

2.4. Procedure

2.4.1. Contamination control

Washing procedures for sample containers, the digestion vessels, glassware for standards and sample tubes for metal determinations followed recommended procedures [33]. In brief, sample containers and other glassware were cleaned with metal-free nonionic detergent solution, rinsed many times with tap water, soaked in 50% HNO_3 acid for 24 h and then rinsed three times with metal-free deionized water. All sample containers were rinsed again with deionized water prior to use. Blanks, consisting of deionized water and reagents as well as SRM 8435 were subjected to a similar sample preparation and analytical procedure.

Tips for micropipette (model No. MT II, capacity 1000 μL) were subjected to the same decontamination. All the analytical procedures were carried out under an exhaust hood to avoid dust contamination [34].

2.4.2. Determination of moisture content of whole milk

The determination of moisture content of cow's whole milk was done by freeze drying the samples followed by oven drying according to Drying method IDF standard 26A (ODM) [35].

To avoid foaming 20 mL of whole milk was added to a 100 mL freeze dry bottle or in similar proportion in triplicate and solidified in refrigerator then freeze dried using Labconco freeze dry-3. From the freeze dried samples 1-3 g of sample was dried by the oven drying method described above.

2.4.3. Digestion of milk samples

A number of potential digestion procedures were tested during the initial stages of procedure development by varying volume and composition of reagents and duration of digestion times. Such procedures are summarized in Table 4 below. However, only one procedure yielded clear solutions in which metals could be determined with atomic absorption spectrophotometer as described in detail below.

Aliquots ranging between 0.5 g and 1.0 g of freeze dried whole milk or powdered infant formula samples were placed in each of micro Kjeldahl vessels. The contents were digested on a micro Kjeldahl flask digestion unit (setting the temperature dial at 8) for 2.30 h after addition of 2.5 mL of conc. HNO_3 and 1.0 mL of conc. $HClO_4$. The contents were then further digested for additional 2 h after introducing 1.0 mL of conc. HNO_3 into the digestion vessels until the solution was clear. After adding a solution of $La(NO_3)_3.xH_2O$ to make the solution about 10 g/L expressed as La to release calcium and magnesium from their respective refractory phosphates to the digest, the sample was transferred to 25 mL volumetric flask and diluted with distilled and deionized water to its mark. Levels of various metals in these solutions were determined using flame atomic absorption spectrometer.

Table 4. Procedures tested for digesting cow's whole milk and infant formula samples.

Sample	Method	Reagents	Condition of digest	Time, h	Remark
Freeze dried cow's whole milk and Powdered infant formula (iron fortified)	***I***	***3.5 mL*** ***HNO_3*** ***2.0 mL*** ***$HClO_4$***	***Clear solution***	***4.30***	***Optimal***
	II	7.0 mL HNO_3 4.0 mL $HClO_4$	Clear solution	4.30	Optimal
	III	5 mL HNO_3 2 mL H_2O_2	Oil suspension	2.00	Yellow in color
	IV	1 mL H_2O (deionized) 0.5 mL H_2O_2 2 mL HNO_3	Oil suspension	2.00	Yellow in color
	V	3 mL (4:1) HNO_3-$HClO_4$	Crystal developed	2.00	Pale yellow in color
	VI	3 mL HNO_3 1 mL HCl	Precipitate	2.00	Yellow in color
Cow's whole milk liquid form	VII	3 mL (4:1) HNO_3-$HClO_4$	Clear solution	1.30	Yellowish-white
	VIII	10 mL (9:1 v/v) HNO_3-$HClO_4$	Clear solution	1.30 (After keeping the solution overnight)	Yellowish

2.4.4. Determination of fat content of milk

Fat content of the powdered milk was assayed by ether extraction method using Soxlet apparatus for the extraction of fat in cow whole milk and Mojonnier extraction for infant formulas. In brief samples in triplicate were heated with concentrated hydrochloric acid to release fat. A mixture of ether and petroleum ether were used for extraction. After extraction, the aliquots of the lipid extract in triplicate were volatilized under the exhaust hood, dried in a laboratory oven at 105°C for 2 h, and fat contents of the powdered milk samples were quantified by determining percent dried weight relative to original milk weight [7].

2.4.5. Determination of protein content of milk

Total nitrogen was assayed by micro-Kjeldahl procedure, using micro-Kjeldahl analyzer. Freeze dried milk (0.5 - 1 g) was digested in conc. H_2SO_4 (98 %), using 1 mL of $CuSO_4.5H_2O$ (0.05 g/mL) as catalyst with K_2SO_4 (12 g) as boiling point elevator, to release nitrogen from protein and retain as ammonium salt. 40% (w/v) NaOH was added to release NH_3, which was distilled, collected in 40 g H_3BO_3 diluted to 1 L in water and titrated with 0.1 N H_2SO_4 prepared as in Association of Official Analytical Chemists method number 936.15 and using methyl red / bromocresol green mixture as an indicator [36].

Total protein = % total N x 6.38 [7].

2.4.6. Determination of major and trace metals

For the determination of analytes in freeze dried cow's whole milk and commercial infant formula samples BUCK SCIENTIFIC MODEL 210VGP atomic absorption spectrometer equipped with deuterium arc background corrector and standard air-acetylene flame system was used. A hollow-cathode lamp for each metal (Ca, Mg, Zn, Ni, Cd, Pb, and Tl) operated at the manufacturer's recommended conditions were used at it's respective primary source line. The acetylene and airflow rates were managed to ensure suitable flame conditions. The burner height was adjusted for optimum sensitivity and the nebulizer uptake rate was optimized (6-7 mL/min) to provide optimum absorbance signal in conventional sample aspiration. The spectrometer was operated with the time constant of 0.2 s [37]. The operating conditions and detection limits provided by the instrument manufacturer for the flame atomic absorption spectrometer are given in Table 5. External calibration curves were traced the standard aqueous solutions listed in Table 6.

Table 5. Detection limits and instrument operating conditions for the determination of metals by atomic absorption spectrometer.

Metal	**λ (nm)**	**Slit width (nm)**	**Lamp current (mA)**	**Detection limit (mg/L)**
Ca	422.7	0.7	2.0	0.01
Mg	285.2	0.7	1.0	0.001
Zn	213.9	0.7	2.0	0.005
Fe	248.3	0.2	7.0	0.03
Ni	341.0	0.2	3.0	0.04
Cd	228.9	0.7	2.0	0.005
Tl	276.8	0.7	7.0	0.1
Pb	283.3	0.7	2.0	0.1

Table 6. Concentrations of standard solutions used to establish calibration graphs for the determination of metals in infant formulas and cow's whole milk samples.

Concentration of standard (mg/L)	**Element**
0.02, 0.05, 0.2, 0.5, 1.0	Mg, Zn
0.1, 0.5, 1.0, 5.0, 10.0	Pb, Tl
0.01, 0.05, 0.10, 0.50, 1.0	Cd
0.5, 1.0, 2.0, 4.0, 8.0	Ca
0.05, 0.2, 0.5, 2.0, 4.0	Fe, Ni

CAHPTER 3

RESULTS AND DISCUSSION

3.1. The Digestion Procedure

For practical reasons all whole milk samples under examination were first lyophilized. This greatly facilitated the subsequent digestion of organic compounds, while at the same time allowing dilution to be minimized.

Wet and dry organic matrix destruction was compared for each type of samples and wet digestion was selected after a systematic comparison of their respective performances, a wet digestion at relatively low temperature, but high efficiency of combustion and low risk of contamination, was chosen. Evaluating the analyte concentration in the milk samples made the comparison. The dry digestion showed significantly lower levels of the analytes analyzed.

A series of digestion procedures involving minor changes in reagents volumes, reagent compositions, order of reagent additions, and digestion times, were tested during the initial stages of digestion. Accordingly, six procedures are tested for freeze-dried samples and two for liquid milk samples (Table 4).

The optimum procedure was selected depending on:

- Minimal reagent volume consumption
- Reduced reflux time/digestion time
- Obtaining clear solution/minimum residue
- Easy of complexity and
- Simplicity.

The optimal procedure chosen on the basis of these criteria required 4.3 h for the complete digestion of 0.5 – 1.0 g of milk (freeze-dried or infant formula) with 3.5 mL HNO_3 and 2.0 mL $HClO_4$, as given in Table 4. This digestion procedure of milk and infant formula samples were developed with some modification of literature procedure, which was used to measure 26 elements in infant formula by ICP-OES [19] and cow's milk from the Canary Island [3].

The common drawbacks of the other tested procedures were their higher chemical composition, longer duration for complete digestion and precipitate formation in the digest of some of the tested procedures. Digesting liquid milk was simpler but required fresh samples, at times which may be difficult to attain in light of the relative distance involved in transporting samples from the dairy farms.

3.2. Moisture Determination

The water content has a strong influence on the physico-chemical stability of milk powder during storage and distribution. Additionally technological functionalities like dissolution and wettability can also be affected by the water content [38]. The oxidative stability of milk powder varies with the water content [39]. Legislation imposes strict limits as milk powder or dried milk is an important commodity in international trade. Up to now international standards for moisture content are most commonly based on methods using loss of weight upon drying under controlled conditions in an oven [35].

According to most definitions, water can be present in food in at least three forms: free water, adsorbed water and bound water. Free water is present in the void volume or in the pores of the food. It can serve as a dispersing agent, as a solvent for crystalline compounds or for microbiological growth. Adsorbed water is located on the surface of the macromolecules in the food matrices. Bound water is defined as the water of hydration bound to the product by strong hydrogen bonds. Bound water relates to the monolayer of water molecules whereas adsorbed water is present in the form of multilayers in the matrices. This explanation follows the theory of the BET [35].

Water determination in infant formula powder and freeze dried whole milk powder can be performed using either direct or indirect methods. The most common direct techniques are either desiccation or oven drying based [35]. In this research work freeze drying followed by the oven drying method was used.

3.2.1. Drying method IDF standard 26A (ODM)

This is standard gravimetric method which consists of drying 1-3 g of a test sample at 102.0 ± 2.0 °C under atmospheric pressure for 2 h. Constancy of mass was tested by additional drying steps of 1 h until the difference in mass does not exceed 0.5 mg. The number of samples in the oven was limited to six to provide comparable amounts of released volatiles during all measurement series [35]. The results are shown in Figure 10.

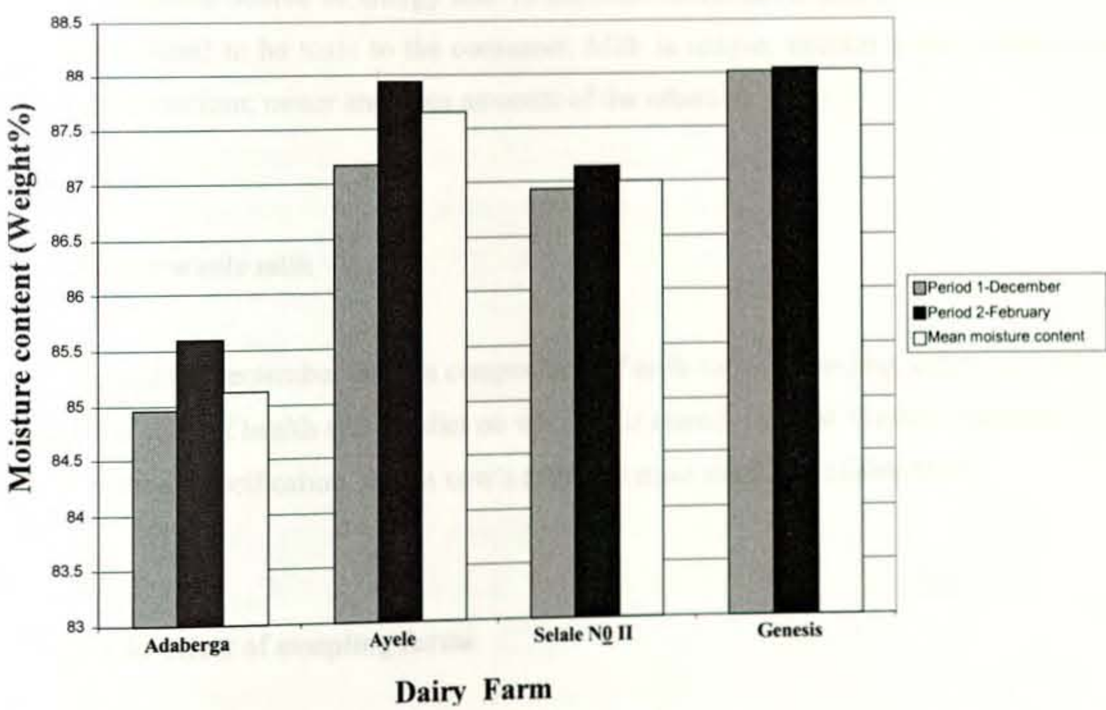

Figure 10. Variations of moisture content determined using drying method IDF standard 26A in cow's whole milk collected from four dairy farms.

When the moisture content of the two periods of sampling was compared it showed higher moisture content in period 2: February samples for farms (Adaberga, Ayele, and Selale No. II). This was most likely due to the feeding habit. During this periods the feeds were dry and so the cow's were supposed to take more water however the moisture content from the Genesis farms showed constant this is because their feed throughout is green feed.

When the moisture content was compared between farms the variation was pronounced in their breed type. The Jersey breed from Adaberga farm was with lower moisture content, the Holsteins from Selale No. II and Ayele farms were intermediate and the cross breeds from the Genesis farm were with higher water content.

3.3. Determination of Major, Minor, and Trace Elements

Milk is a natural source of energy and 15 essential nutrients. It also contains compounds that have been found to be toxic to the consumer. Milk is unique, nutritious and a major source of protein and calcium; minor and trace amounts of the others.

3.3.1. Cow's whole milk

It is important to remember that the composition of milk varies according to the type and breed of animal, its state of health and the diet on which it is reared. In most Western countries, the word 'milk' without specification, means cow's milk, the most readily available kind.

3.3.1.1. The effect of sampling farms

The four sampling farms (Adaberga, Ayele, Selale No. II and Genesis) were statistically evaluated to find out whether the observed differences in analytical results of samples collected from these farms were significant. For this purpose, the statistical tool "Analysis of Variance" was applied.

In general the analysis of variance for whole milk showed significant differences between the sampled farms. At $p \leq 0.001$ level of the analyzed components of whole cow's milk evaluated (Tables 7, 8, 9, 12, and 14) protein was not significantly affected ($p > 0.05$) for Adaberga, Ayele and Selale No. II Farms and moisture content was not significantly affected for Ayele, Selale No. II and Genesis Farms.

Whole milk obtained in Adaberga Farm contained significantly more fat ($p \leq 0.05$) compared to the other three farms; dominantly because the cows were Jersey breed.

Whole milk obtained in Selale No. II Farm contained more fat ($p \leq 0.001$) compared to Ayele and Selale No. II Farms. Although differences were found, some of these differences could be related to the random error of this type of analysis. Also, the processes involved in the milking, transport and storage are factors that can affect the mineral concentrations in milk for human consumption.

It is important to note that the milking was done manually on a metal bucket and one person was assigned to milk at least 10 cow's one after the other. While the assignment of a single person to milk at least 10 cows a day may have negligible role in the levels of metals in the milk, collection in a metallic bucket obviously contribute metals to the milk.

3.3.1.2. Effect of sampling period

In general when the effect of the sampling periods was investigated, the analysis of variance results for whole milk (Tables 7, 10 and 12) showed highly significant differences between the two periods, at $p \leq 0.001$ level. This is similar to literature results [6], who reported higher calcium, and magnesium values with significantly lower contents of zinc but unlike to this report some higher levels of iron was observed in the present study. The higher levels of iron is most likely associated with contamination during milking, the nature of their feeds; which is most likely associated with contamination of the area soil by fertilizer remains.

For non-essential metals (Table 8) the analysis of variance results for cow's whole milk showed significant differences between the two periods of sampling (December and February) at $p \leq 0.05$ except for cadmium which showed similarity throughout. The temporal variation in the levels of the non-essential elements is entirely related to the feeding culture.

Table 7. Mean concentrations of essential elements determined in cow's whole milk samples collected in two sampling periods (n = 3).

Farms	Sampling period	Concentration of element (mg/L)			
		Ca	Mg	Fe	Zn
Adaberga	December	2237 ± 310	71.9 ± 1.1	1.5 ± 0.1	6.0 ± 0.3
	February	1606 ± 376	70.5 ± 0.7	1.3 ± 0.0	6.5 ± 0.3
Ayele	December	1711 ± 297	64.3 ± 1.7	1.3 ± 0.0	3.0 ± 0.2
	February	1285 ± 171	60.2 ± 0.8	1.4 ± 0.0	4.4 ± 0.2
Selale No. II	December	1900 ± 239	72.8 ± 1.3	1.3 ± 0.1	3.8 ± 0.2
	February	1110 ± 30	66.0 ± 0.2	1.6 ± 0.0	6.1 ± 0.2
Genesis	December	1766 ± 259	61.5 ± 0.4	1.7 ± 0.1	3.8 ± 0.3
	February	1187 ± 149	52.9 ± 2.2	1.5 ± 0.0	5.2 ± 0.3

Period 1: December 2004; **Period 2:** February 2005. n: number of samples.

The proximate composition of whole milk differed significantly between the two periods. Total moisture percentages were higher in milk obtained in period 2 (February) compared to that collected in period 1 (December) which is similar to the report from South Africa (Table 20).

In period 1 (December 2004) whole milk had a higher protein ($P \leq 0.001$) than period 2 (February) in the four sampled farms but the fat content showed variation from farm to farm (Table 14). Variations of milk composition, especially in the fat content are dependent on cow breed. Also the milking intervals significantly influence the fat content, for example 2 h interval fat equals 6% compared with 12 h interval of milking fat equals 3.6% [40].

Table 8. Mean concentration of non-essential elements in cow's whole milk.

Farms	**Sampling period**	**Concentration of element (mg/L)**			
		Cd	**Ni**	**Pb**	**Tl**
Adaberga	December	0.08 ± 0.0	2.2 ± 0.1	4.1 ± 0.1	1.3 ± 0.1
	February	0.12 ± 0.0	1.5 ±0.1	1.9 ± 0.1	2.3±0.0
Ayele	December	0.02 ± 0.0	1.7 ± 0.0	3.6 ± 0.1	3.1 ± 0.1
	February	0.04 ± 0.0	1.0 ± 0.2	2.4 ± 0.1	2.1 ± 0.0
Selale No. II	December	0.04 ± 0.0	1.7 ± 0.2	1.1 ± 0.1	2.3 ± 0.1
	February	0.2 ± 0.0	0.8 ± 0.3	1.8 ± 0.1	1.4 ± 0.1
Genesis	December	0.04 ± 0.0	1.4 ± 0.1	3.4 ± 0.1	2.5 ± 0.0
	February	0.04 ± 0.0	0.9 ± 0.1	2.6 ± 0.1	2.2±0.0

Period 1: December 2004; **Period 2:** February 2005.

3.3.2. Commercial infant formulas

Infant formulas are liquids or reconstituted powders fed to infants and young children. They serve as substitutes for human milk. Infant formulas have a special role to play in the diets of infants because they are often the only source of nutrients for infants. For this reason, the composition of commercial formulas is carefully controlled and Food and Drug Administration (FDA) requires that these products meet very strict standards.

Great care must be given to the decision to make infant formulas at home, and safety should be of prime concern. The potential problems associated with errors in selecting and combining the ingredients for the formula are very serious and range from severe nutritional imbalances to unsafe products that can harm infants. Because of these potentially very serious health concerns, FDA does not recommend that consumers make infant formulas at home.

Table 9. Mean concentration of essential and non-essential elements in commercial infant formulas.

Concentration (mg metal/L)	NAN	S-26	Guigoz
Ca	400 ± 40	342 ± 35	440 ± 27
Mg	30.7 ± 1.7	37.4 ± 0.9	53.5 ± 0.4
Fe	6.9 ± 0.3	8.1 ± 0.4	11.3 ± 0.5
Zn	2.8± 0.2	4.7 ± 0.5	4.35± 0.4
Cd	0.00019±0.00001	0.00009± 0.00001	0.00002 ± 0.00001
Ni	0.0009 ± 0.0002	0.0012 ± 0.0002	0.00137 ± 0.00002
Pb	0.0007 ± 0.0002	0.0018 ±0.0002	rf
Tl	rf	rf	rf

rf: result found, with a statement of its uncertainty; the same status has been described as not detected in other literatures.
NAN, Netherlands; S-26, Ireland; Guigoz, Netherlands.

For proper comparison of the element levels in infant formula with drinking water standards, the element levels in all the three powder formulas analyzed were converted to mg/L for both the essential elements and non-essential elements, using the specified feeding tables supplied by the infant formula manufacturers. Table 9 suggests that the average levels of essential (calcium, magnesium, and iron) elements in Guigoz brand were higher than the average levels obtained from NAN and S-26 brands.

Table 10. Average daily intakes of elements from commercial infant formulas.

Intake (µg metal/day)	Infant formula brand		
	NAN	**S-26**	**Guigoz**
Ca	93210	102620	73440
Mg	7150	11200	8910
Fe	16000	2440	24850
Zn	350	1410	730
Cd	0.04	0.03	0.002
Ni	0.01	0.36	0.23
Pb	0.17	0.54	rf
Tl	rf	rf	rf

rf: result found, with a statement of its uncertainty; the same status has been described as not detected in other literatures.

The result found from thallium analysis was with its limit of uncertainty in all the brands analyzed. Though lead was detected in NAN and S-26 brands, non of the lead levels exceeded the 15 µg/L lead stipulated European Union (EU) directive for lead in drinking water. However, the presence of lead in infant milk is of great concern since infants are very sensitive to its toxic effects. Childhood exposure to lead may induce suppression of mental capacity or retardation [22] aggressive behaviors [41] and there is a high negative association between lead exposure and children's intelligence quotient [42].

Nickel was detectable in all the three brands. However, the nickel levels in these brands were all below the action level of 50 µg/L nickel for drinking water.

Cadmium was detectable in all the brands but none of these samples exceeded the 5 µg/L Cd stipulated limit for drinking water [22]. Exposure to cadmium can lead to kidney dysfunction [42].

The maximum and minimum iron levels were 6.86 mg/L (NAN brand) and 11.31 mg/L (Guigoz brand) respectively. Adequate iron intake is necessary for growth and development. It is also vital for transporting oxygen in the blood stream and for the prevention of anemia.

Estimates of the daily intakes of both essential and non-essential elements in infant formula were calculated, using the feeding tables specified by the manufacturers of the different brands. For first milk powder infant formula NAN brand, for a baby weighing 7.5 kg and 6 months 30.1 g were specified per day. Similarly for the above age and weight 42.0 g were specified per day for S-26 and for follow on powder infant formula Guigoz 24.0 g were specified per day.

This indicates that various brands of commercial infant formula may have different nutrient values and may, as well, cause potentially different levels of exposures to toxic elements.

A 6 – 12 months old baby would require approximately 5 kg of powdered infant formula every month and in a year 60 kg. Thus, the daily consumable weight of infant powdered formula would be 164.4 g of infant formula powder [23]. As a result the amounts of formula recommended by the manufacturers were lower in all the analyzed brands.

3.3.3. Method detection limit (MDL)

A method detection limit (MDL) followed was the USEPA's definition and procedures codified in 40 CFR 136, Appendix B, i.e., the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero. The determinative procedures involve spiking seven replicate aliquots of reagent water or sample matrix with the analytes of interest at a concentration within one to five times the estimated MDL. The seven aliquots are then carried through the entire analytical process, and standard deviation of the seven replicate determinations is calculated. The standard deviation is multiplied by one-tailed t-statistic factor of 3.143 to give MDL [43].

Table 11. Method detection limits of elements ($3.143\delta_{blk}$, n = 7) determined using the blank solution.

Element	Ca	Cd	Fe	Mg	Ni	Pb	Tl	Zn
Detection limit (mg/L)	0.42	0.01	1.05	0.02	0.03	0.29	0.10	0.08

The MDL defined in 40 CFR 136 is based on 1% false positive, i.e., there is only 1% probability that a sample with no analyte will produce a concentration greater than or equal to the MDL. In this regard, MDL described in Table 11 were good results with respect to the analyte results in the samples and were comparable to the instrument detection limit.

3.4. Determination of protein content

Nitrogen in foods not only comes from amino acids in protein but also exists in additional forms that may or may not be used as a part of the total nitrogen economy of humans and animals. For certain food staffs, total nitrogen values have sometimes been partitioned in to "true protein" and "non true protein".

There are three kinds of proteins in milk: caseins, lactalbumins, and lactoglobulins. All the three are globular proteins, which tend to fold back on themselves into compact, nearly spheroidal units and are more easily solubilized in water as colloidal suspensions than fibrous proteins are. They are "complete proteins", so-called because they contain all the amino acids essential for building blood and tissue, and they can sustain life and provide normal growth even if they are the only proteins in the diet. These proteins not only contain more amino acids than plant proteins, but they contain greater amounts of amino acids than the proteins in eggs and meats.

Measurement of total nitrogen by kjeldahl analysis is the historical reference method for determination of the protein content of dairy products and is used for both calibration and validation of alternative methods for protein determination.

3.4.1. Cow's whole milk

The results are given in Table 12 and shown in Figure 11. The observed data on protein level showed between Adaberga, Ayele and Selale No. II Farms there were no significant difference when compared with the difference observed with Genesis Farm. This most likely because the three farms share identical feeding culture as compared to the Genesis Farm which feeds its own green feed throughout the year regardless of breed type.

The influence of nutrition on milk protein have been variable, but increasing energy intake and dietary crude protein content. Improving the supply of methionine and lysine have resulted in an increase in milk protein content. Dietary fat has consistently been associated with reductions in milk protein content and increases in milk protein yield. Thus, as milk yield increases is small, but we would expect protein in milk at 70 lb to be 2.92 % and at6 80 lb to be 2.89 % [44]. Accordingly, the milk yield in Genesis Farm as compared to the other studied farms were significantly high and so it showed lower percentage in the protein level as compared to the other studied farms. The high milk yield in Genesis Farm can be noted from the milking frequency (three times in 24 h). Therefore milk protein content is negatively correlated with milk yield.

Table 12. Percent (% m/m*) protein content of cow's whole milk.

Dairy Farm	Adaberga	Ayele	Selale No. II	Genesis
Period 1:December	3.75	3.21	3.41	2.84
Period 2:February	3.43	3.07	3.12	2.73
Mean protein content	3.59±0.20	3.14±0.10	3.26±0.20	2.78±0.10

*mass/mass

3.4.2. Commercial infant formulas

Table 13. Mean Protein content (%m/m*) determined in three brands of commercial infant formulas

Brand	NAN	S-26	Guigoz
Mean Protein content (% m/m)	9.63±0.20	11.44±0.02	17.50±0.10

*mass/mass

The protein levels for first milk brands (NAN and S-26) were nearly similar and significantly lower than the follow on infant formula (Guigoz). As a result, the first milk formulas may have less risk of protein allergy on infants as compared to the follow on formula (Guigoz). And the risk of such allergy should be minimized as the physiology of the baby grows. The results are given in Table 13 and shown in Figure 11.

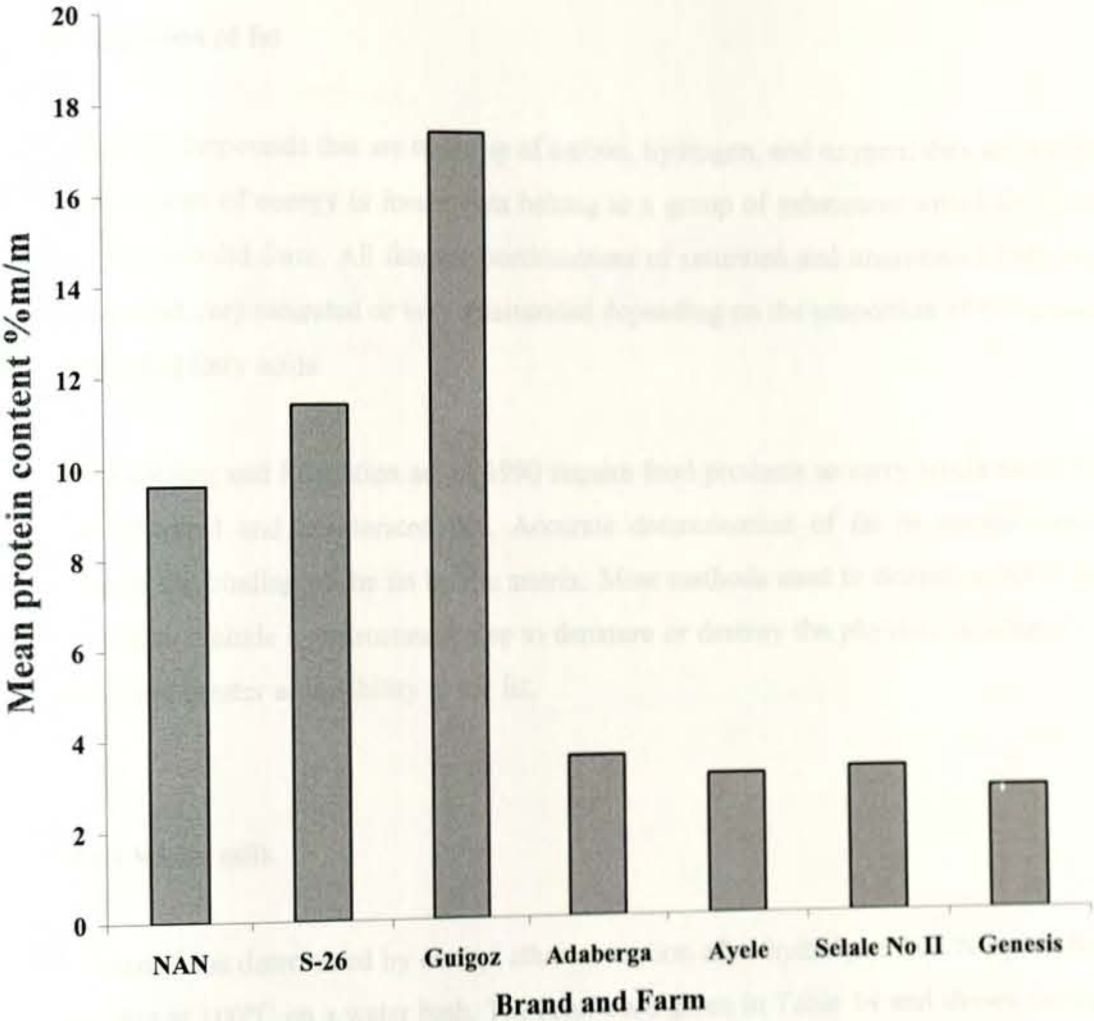

Figure 11. Variation of protein content in cows whole milk and commercial infant formulas

From Figure 11 the mean protein content (%m/m) increases from significantly across the three brands (Guigoz significantly higher than S-26 and was higher than NAN) and the cow's whole milk from the different farms showed lower levels from each of the infant formulas.

3.5. Determination of fat

Fats are organic compounds that are made up of carbon, hydrogen, and oxygen; they are the most concentrated source of energy in foods. Fats belong to a group of substances called lipids. Fats come in liquid or solid form. All fats are combinations of saturated and unsaturated fatty acids. Fats can be called very saturated or very unsaturated depending on the proportion of the saturated or the unsaturated fatty acids.

Nutritional Labeling and Education act of 1990 require food products to carry labels that list the content of saturated and unsaturated fats. Accurate determination of fat in certain foods is difficult due to the binding of the fat by the matrix. Most methods used to determine fat in these difficult matrices include a pretreatment step to denature or destroy the physical structure of the matrix and allow greater accessibility to the fat.

3.5.1. Cow's whole milk

Total fat content was determined by diethyl ether extraction after hydrolysis in 3 N hydrochloric acid for 60 min at 100°C on a water bath. The results are given in Table 14 and shown in Figure 12. The fat content of Adaberga Farm was significantly higher than the fat content from the other three farms. The observed high fat content from the Adaberga Farm may be due to the breed characteristic nature of Jersey cows.

Table 14. Percent fat content (%m/m*) determined in whole milk samples obtained from four dairy farms.

Sampling period	Dairy Farm			
	Adaberga	Ayele	Selale No. II	Genesis
Period 1: December 2004	5.08±0.01	3.28±0.02	3.43±0.08	3.26±0.00
Period 2: February 2005	5.05±0.00	3.15±0.00	3.66±0.07	3.20±0.04
Mean fat content (% m/m)	5.06±0.01	3.22±0.07	3.54±0.14	3.23±0.04

*mass/mass

3.5.2. Commercial infant formulas

Table 15. Mean fat content (%m/m*) determined in three brands of infant formulas.

Infant formula brand	NAN	S-26	Guigoz
Mean fat content (%m/m)	24.41±2.20	12.63±1.40	3.79±0.20

*mass/mass

The results in Table 15 show high fat content in NAN brand and moderate amount of fat in S-26 and lower amount of fat in Guigoz.

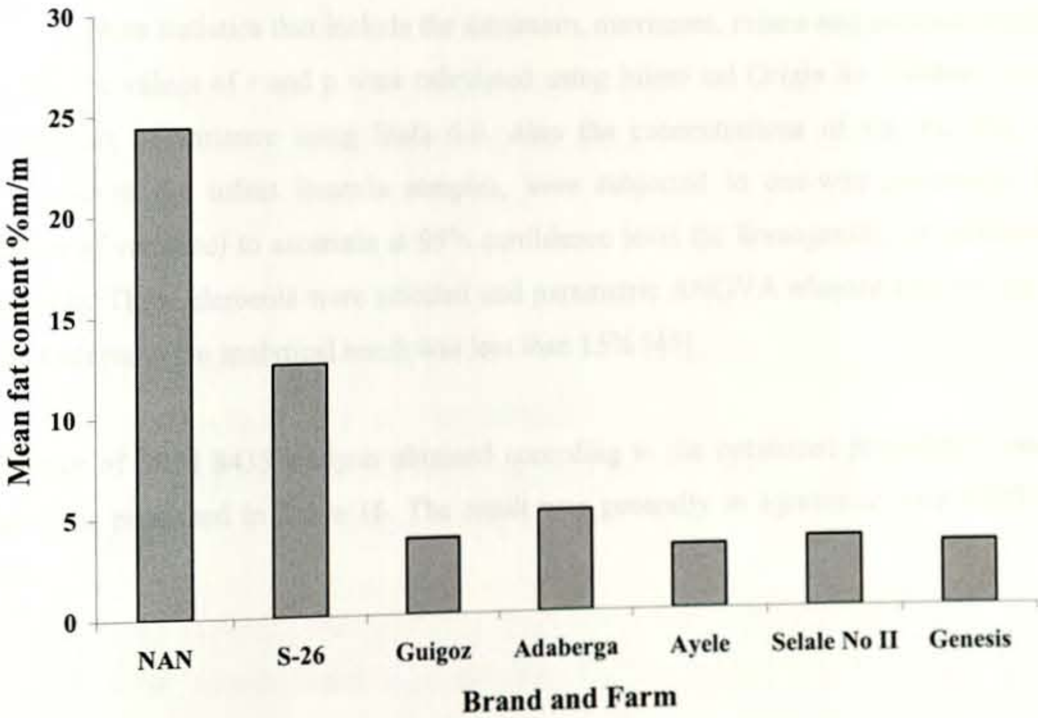

Figure 12. Variation of fat content in cows whole milk and commercial infant formulas

From Figure 12 the two brands (NAN and S-26) have significantly high levels of fat compared to the third brand Guigoz and the cow`s whole milk from the different farms.

3.6. Accuracy and precision

For checking the precision and accuracy of the method, a standard reference material (SRM 8435: whole milk powder) was analyzed. The calibration curves were with $R > 0.999$ and $p < 0.0001$ for all the analyzed elements.

Statistical analysis

The descriptive statistics that include the minimum, maximum, means and standard deviations of the data, the values of r and p were calculated using Micro cal Origin for windows version 6.0 and analysis of variance using Stata 6.0. Also the concentrations of Ca, Fe, Mg, and Zn, determined in the infant formula samples, were subjected to one-way parametric ANOVA (analysis of variance) to ascertain at 95% confidence level the homogeneity of variances across the brands. These elements were selected and parametric ANOVA adopted because the number of non-detects in the analytical result was less than 15% [45].

The result of SRM 8435 analysis obtained according to the optimized procedure (described in Table 4) is presented in Table 16. The result was generally in agreement with NIST certified values.

Table 16. Results for the analysis of whole milk reference material SRM 8435.

Analyte	Observed (mg/Kg)	Certified (mg/kg)
Calcium	0.85±0.124*	0.92 ± 0.049*
Cadmium	rf	0.0002
Iron	2.10±0.02	1.8±1.1
Magnesium	784±2.3	814 ± 76
Nickel	rf	0.01
Lead	0.10±0.01	0.11±0.05
Zinc	31.20±0.01	28.00±3.1
Protein	24.88±0.7*	25.86± 0.7*
Fat	18.10±2.6*	21.30 ± 2.4*

rf: result found, with a statement of its uncertainty.
*results expressed in mass/mass, % m/m.

In reporting low concentrations in the absence of a definitive answer, most of us have settled for reporting results below a detection limit, c_L in one of the following possible ways.

- Not detected
- Less than c_L
- A value of zero
- An arbitrary fraction of c_L , e.g., $c_L/2$
- The result found, with a statement of its uncertainty.

Reporting the value found, accompanied by its uncertainty, is clearly the best method of reporting because it provides the most information [46] and this is used in this thesis.

The observed mean results were generally lower but in tolerable range than the certified values except for Fe that showed higher value typically. This might be due to the effect of background corrector as Fe was analyzed in the presence of Ni where the, SRM digested with HNO_3. Generally, the results observed were in good agreement with the certified values.

3.7. Contributions of nutrients in milk to daily dietary intakes

To see the nutritional status of Ethiopians evaluation of the contribution of milk to daily dietary intakes is compared to the recommended dietary allowance set by US National Research Institute (Table 17).

Table 17. Contribution to daily dietary intake of Ca, Mg, Fe, and Zn from the consumption of cow's milk in adult population of Ethiopia.

Element	RDA	Contribution of 60 mL of cow`s milk[2]	
		Amount	**%RDA**
Ca (mg)	800	96.03	12.00
Mg (mg)	350 (300)[1]	3.90	1.11 (1.30)
Fe (mg)	10 (15)[1]	0.33	3.30 (2.20)
Zn (mg)	15 (12)[1]	0.31	2.06 (2.58)

[1]The recommended values for females are reported between parentheses.
[2]Source: Ethiopia Fact sheet: UNDP Human Development Report, 2002; Calculated from milk consumption 22 kg/capita.

The milk consumption in Ethiopia is reported to be 22 kg/capita [47], from this one can draw a conclusion that 22 L of milk will be consumed per 365 per person (which is equivalent to 60 mL per day per person).

Table 18. Concentrations of Fe and Zn from different countries.

Country	Fe (mg/L)	Zn (mg/L)	n	Description	Reference
Ethiopia	1.25 ± 0.03	5.33 ± 0.34	618	Whole (two periods)	This work
Italy	0.65 [1]	3.82 [1]	—	Raw	[48]
	—	4.80 [1]	—	—	[49]
	—	3.58 [1]	—	—	[50]

Spain	–	3.0±0.2	9	Bottled cow milk	[51]
	—	3.1 [1]	70	Raw	[52]
	1.36± 1.65 [1]	3.78± 0.76 [1]	129	Raw	[53]
	0.14 [1]	3.82 [1]	300	Pasteurized	[54]
	0.44± 0.11 [1]	4.21± 0.41 [1]	120	Raw (12 months)	[55]
	0.54± 0.44 [1]	3.31± 0.17 [1]	190	Sterilized	[56]
	0.441± 0.115 [1]	4.206± 0.407 [1]	120	Raw (12 months)	[57]
	0.46± 0.10 [1] 0.30± 0.03 [1]	3.70± 0.22 [1] 3.63± 0.06 [1]	10 10	Raw Pasteurized	[58]
	0.290±0.112	3.419± 0.019	27	Raw	[59]
	0.52± 0.18 0.17±0.03	4.41±0.67 3.06± .14	151 18	Raw (12 months) Sterilized	[3]
Turkey	2.68± 0.24	3.64± 0.16	18	—	[60]
U.S.A.	—	(3 - 5)	—	Mature	[61]
	0.506	4.23	36	—	[62]
	0.200± 0.400 [1]	3.7± 0.7 [1]	—	—	[63]
	0.200± 0.300 [1]	—	—	—	[64]
Cuba	2.9 ±0.5	—	16	Pasteurized	[65]
Japan	0.27 [1]	—	—	Raw	[66]
Pakistan	0.60 [1]	4.20 [1]	—	Raw	[67]
Burundi	—	4.4 ± 0.3 4.1 ± 0.3	19 2	Raw Processed	[68]
Egypt	0.15 [2]	3.46 [2]	5	Raw	[69]

[1] (mg/kg).
[2] (% total solids, TS = 11.541%).

Table 19. Concentration of Ca and Mg from different countries.

Country	Ca (mg/L)	Mg (mg/L)	n	Description	Reference
Ethiopia	1600±315	65.00±6.77	618	Whole (two periods)	This work
Spain	1060± 50 [1]	—	89	Raw (10 months)	[70]
	1089± 243	146 ± 20	129	Raw (12 months)	[53]
	1280 [1]	110 [1]	300	Pasteurized	[54]
	1280± 80 [1]	107 ± 8 [1]	190	Sterilized	[56]
	1199 ± 106 [1]	105± 7.5 [1]	120	Raw (12 months)	[57]
	1251 ± 48 1289± 60 [1]	116 ± 3 [1] 115 ± 5 [1]	10 10	Raw Pasteurized	[58]
	1653 ± 207 1309± 62	114± 19 5 121± 14	1306 6	Raw (12 months) Sterilized	[3]
Italy	1673	106	—	Whole	[71]
Turkey	—	92.3 ± 2.2	18	—	[60]
U.S.A.	1060± 280 [1]	98 ± 19 [1]	57	Whole	[72]
	1010 ± 200 [1]	100 ± 10 [1]	—	Whole	[60]
	0.128 [2]	61	36	>3 months	[61]
Egypt	—	9.8± 0.3 [3]	5	—	[69]
South Africa	121.0 [2]	11.43 [2]	-	Whole (Summer)	[6]
	119.4 [2]	11.80 [2]	-	Whole (Winter)	

[1] (mg/kg).
[2] %.
[3] mg/g ash
n: number of samples

Table 18, 19, and 20 show a selection of literature data describing concentrations of cow's milk published in other countries. The Ca concentration is similar to report from Spain [3] and Italy [71] but significantly higher from other countries reports described in Table 19. The results of Fe obtained in this study were similar to data published in Spain [54], and USA [62], significantly lower than reports from Turkey [60] and Cuba [65] but higher than other countries repots in Table 18.

The concentration of Zn obtained in this study was higher than most reports in Table 18. Which is most likely associated with the level of copper in their feeding culture. As the content of copper increases the content of zinc decreases.

The moisture content, protein (total) and total fat contents described in Table 20 are similar to the reports in South Africa in different localities [6]. But reports from Portugal show significantly lower total fat from Barrosã breed [40] because of their milking duration compared in Table 20.

Table 21 shows literature data describing levels of Pb and Cd in different countries. In general significantly higher concentrations of Pb and Cd were observed in this report compared to reports from Germany [73] and India [23].

The average consumption of cow`s milk in Ethiopia is 22kg/capita [47]. From this calculated 60 mL/day/person is the average consumption per day. The average contributions each element from consumption of 60 mL cow's milk have been calculated and presented in Table 17, these mineral intakes were compared with the recommended dietary allowances (RDA) for adults established by U.S. National Research Council. One can deduce that cow's milk is an excellent source for Ca. Ethiopian mean consumption of 60 mL of cow's milk supplies 12% of the RDM requirements and smaller amount of Zn and Fe to the RDA values (Table 16) confirming that cow's milk is a very poor source of these elements [3].

Table 20. The mean proximate composition of whole milk obtained in different countries.

Country	Moisture (weight %)	Protein (weight %)	Total Fat (weight %)	Breed	n	Reference
Ethiopia	85.11±0.34	3.59±0.22	5.06±0.01	Jersey	156	This work
	87.65±0.38	3.14±0.09	3.22±0.07	Holstein	250	
	87.01±0.11	3.26±0.20	3.54±0.14	Holstein	118	
	88.03±0.27	2.78±0.07	3.23±0.04	Cross breed	94	
South Africa	87.98	3.27	3.45	Locality	-	[6]
	87.69	3.28	3.56	Locality	-	
	87.95	3.20	3.46	Locality	-	
	88.31	3.19	3.29	Locality	-	
	87.96	3.30	3.38	Locality	-	
Portugal	-	3.27±0.47	4.94±1.44	Frísia	5	[40]
	-	3.96±0.50	2.76±0.43	Barrosã	5	

n : number of samples

Table 21. Concentration of Pb and Cd from different countries.

Country	Pb	Cd	n	Description	Reference
Ethiopia (mg/L)	2.63	0.18	618	Whole milk	This work
Germany (µg/L)	1.8	0.1	_	Cow milk	[73]
India (µg/L)	1.7	0.07	75	Cow milk	[23]

CHAPTER 4

CONCLUSION

Essential mineral contents (Ca, Mg, Fe and Zn), protein and fat contents of cow's whole milk distributed in Addis Ababa fall into normal intervals described in the literature though the non-essential elements (Cd, Ni, Pb, and Tl) show significantly higher levels. The average consumption of cow's milk distributed in Addis Ababa supplies 12% of the RDA requirements of Calcium and small amounts of magnesium and zinc. Cow's milk sampled in December presented higher concentrations of calcium, magnesium and iron, indicating higher contributions of these elements to the consumer, than cow's milk sampled in February. This could probably be ascribed to brief precipitation taking place in January which may have rendered these nutrients available in the cows' feedstuff.

In this study, the levels of eight elements (Ca, Mg, Fe, Zn, Cd, Ni, Pb, Tl), fat and protein were also determined in powder infant formula samples distributed in Addis Ababa. The ANOVA results suggest that there were significant variations in the levels of some elements, fat and protein across the infant formula brands, which could be attributed to the different manufacturing practices, variations in quality of raw materials, finished products, and packaging containers used by the infant formula manufacturers.

The nutritional contents of the analyzed infant formula brands were found to be lower than the RDA or DRI values for the essential elements calcium, magnesium, iron and zinc. Bottle-fed infants consuming formulations with low levels of essential elements may suffer nutritional deficiencies and consequently health problems. The concentration of non- essential elements cadmium, nickel, lead and thallium were below their respective stipulated drinking water standards. Also the estimated daily intakes of lead and cadmium from infant formula were below the FAO/WHO Joint Committee on food additives recommended provisional tolerable weekly intake (PTWI) of 25 and 70 μg/kg body weight, respectively. These findings reveal safety of the investigated formula brands for consumption relative to toxic elements.

To assess the nutritional status and environmental pollution through cow's whole milk, we examined milk sampled from the major farms around Addis Ababa, which is the most important for residents of Addis Ababa. However, to develop comprehensive data bank on the same sampling dairy farms by analyzing the remaining element levels may be equally important for evaluating trends in pollution and nutritional status. Further more, future studies should focus on the identification of local breed cow's in different areas of the country.

REFERENCES

1. Yishak, M.; Ehui, S.; Jabbar, M.; Shapiro, B. *Handbook of Livestock Statistics for Developing Countries*, ILRI: Addis Ababa, Ethiopia, **1999.**
2. Charlotte, Neumann; Diane, M. Harris; Lisa, M. Rogers *Nutr. Research*, **2002**, 22,193-220.
3. Rodríguez Rodríguez, E. M.; Sanz Alaejos, M.; Díaz Romero, C. *J. Food Comps. Anal.*, **2001**, 14, 419-430.
4. Nutritional importance of traditional milk products in the national diet. 1. Southern and eastern Africa general. Available at: http://www.fao.org/docrep/003/t0251e/T0251E01.htm
5. Azage, T.; Million, T.; Yoseph, M.; Alemu, Y. *Market-oriented Urban and Peri-Urban Dairy Systems*, ILRI: Debre Zeit Research Station, **1998**.
6. Louwrens, E. Smit; Hettie C. SchÖnfeldt; Willie, H. J. de Beer; Marie F. Smith *J. Food Comps. Anal*, **2000**, 13, 345-365.
7. Park, Y. W. *Small Ruminant Research*, **2000**, 37, 115-124.
8. Coni, E.; Bocca, A.; coppolelli, P.; Camroli, S.; Cavallucci, C.; Trabalza, m.; *Food Chem.***1996**, 57, 253-260.
9. Coni, E.; Bocca, A.; Ianni, D.; Caroli, S. Preliminary evaluation of the factors influencing the trace element content of milk and dairy products, *Food Chem.*, **1995**, 52, 123-130.

10. Nutritional importance of traditional milk products in the national diet. 1. Southern and eastern Africa general. Available at: Available at: http://www.fao.org/docrep/003/t0251e/T0251E07.htm

11. Mulat D., Agricultral technology, economic viability and poverty alleviation in Ethiopia. Available at: http://www.aec.msu.edu/agecon/fs2/ag_transformation/atw_demeke.PDF.

12. Kerro Dego O., Tareke F., *Trop. Ani. Health Prod.*, **2003**, 35, 197-205.

13. Woldemeskel, M. *Dtsch. Tierärztl. Wschr.*, **2000**, 107: 144-146.

14. Million, T.; Tadelle, D. *LRRD*, **2003**, 15, 3.

15. Demeke, S.; Neser, F.W.C.; Scheman, S.J. *J. Anim. Breed Genet.* **2004**, 121, 57-65.

16. Ethiopia, participation of laboratories in national monitoring programmes for contaminants.
Available at: http://www.afro.who.int/des/fos/country profiles/ethiopia.pdf

17. Ministry of Planning Economic Development (MOPED), *Poverty situation in Ethiopia*. Welfare Monitoring Unit, Addis Ababa, Ethiopia, **1999**.

18. Woldemariam, Girma; Timotiows, Genebo; Determinants of Nutritional Status of Women and Children in Ethiopia, *Ethiopia Health and Nutrition Research Institute,* Addis Ababa, Ethiopia; ORC Macro Calverton, Maryland USA, **2002.**

19. Buldini, P.L.; Cavalli, S.; Sharma, J.L. Matrix removal for the ion chromatographic determination of some trace elements in milk, *Microchem. J.*, **2002**, 72, 277-284.

20. Loaiza, Edilberto DHS Comparative Studies No. 24, Calverton, Maryland, USA: Macro International Inc., *Maternal nutritional status*, **1997.**

21. Teller, H.; Yimar, G. Levels and determinants of malnutrition in adolescent and adult women in southern Ethiopia. *Ethiopian Journal of Health Development*, **2000,** 14, 57-66.

22. Abua, Ikem; Alwell, Nwankwoala; Seyi, Odueyungbo; Kafui, Nyavor; Nose, Egiebor *Food Chem.*, **2002**, 77, 439-447.

23. Tripathi, R.M.; Raghunath,R.; Sastry,V.N.; Krishnamoorthy,T.M. Daily intake of heavy metals by infants through milk and milk products. *Sci. Total Environ.*,**1999**, 227, 229-235.

24. Guttman, N.; Zimmerman, D.R. Low-income mothers' views on breastfeeding. *Social Science &Medicine*, **2000**, 50 , 1457-1473.

25. Walker, M Known contaminants found in infant formula. *Mothering*, **2000**, 100 ,67-70.

26. Cambra, K.; Alonso, E.; Blood lead levels in 2-to 3-year-old children in the Greater Bilbao area (Basque county, Spain): relation to dust and water levels. *Archives of Environmental Health*, **1995**, 50, 362 -366.

27. Fein, S.B.; Falci, C.D. Infant formula preparation, handling, and related practices in the United States. *J. Am. Diet. Assoc.*, **1999**, 99, 1234-1234.

28. American Academy of Pediatrics, Committee on nutrition, iron fortification of infant formulas. American Academy of Pediatrics (AAP) on infant formulas in the United States. *Pediatrics*, **1999b**, 104, 119-124.

29. Ramos, L.; Torre, M.; Laborda, F.; Marina, M.L. Determination of polychlorinated biphenyls in soybean infant formulas by gas chromatography. *J. Chromatogra. A*, **1998**, 823, 365-372.

30. Fernandez-Lorenzo, J.R.; Cocho, J.A.; Rey-Goldar, M.L.; Couce, M.; Fraga, J.M. Aluminum contents of human milk, cow's milk, and infant formulas., *J Pediatr Gastroenterol Nutr.* , **1999**, 28 , 270-275.

31. Sahin, G.; Aydin, A.; Isimer, A.; Ozalp, I.; Duru, S. Aluminum content of infant formulas used in Turkey. *Biological Trace Element Research*, **1995**, 50, 87-88.

32. Torres, M. A.; Verdoy, J.; Alegría, A.; Barberá ,R., Farré ,R.; Lagarda, M.J. Selenium contents of human milk and infant formulas in Spain. *Sci. Total Environ*, **1999**, 228 , 185-192.

33. American Public Health Association, Standard methods for the examination of water and wastewater, 20th ed., New York: **1998**.

34. Maria, Plessi; Davide, Bertelli; Agar, Monzani *J. Food Comps. Anal*, **1997**, 10, 36-42.

35. Christoph, Reh; Shinivasa, N. Bhat; Stephane, Berrut *Food Chem.*, **2004**, 86, 457-464.

36. Barbano, D. M.; Clark, J. L.; dunham, C. E.; Fleming, J. R. Kjeldahl method for determination of total nitrogen content of milk: collaborative study *Journal of official Analytical chemists*, **1990**, 73, 849-859.

37. Carlos, G. Bruhn; Victor, H. Campos; Victor, P. Díaz; Hernán , J. Cid; Joaquuim A. Nóbrega, *Bol. Soc. Quim.*,**2002**, 47, 123-135.

38. Mathlouthi, M. *Food control*, **2001**, 12, 409-417.

39. Stapelfeldt, H.; Nielsen, B.; Skibsted, L. *International Dairy Journal*, **1997**, 7, 331-339.

40. Preciosa, pires; Élia, Fernandes; Manuela, Vilarinho; Máario, Barros; Rita, Ferreira; Luísa, Carneiro; Gonçalo, Almeida; Manuela, vaz Velho *Electron. J. Environ. Agric. Food Chem.*, **2003**, 2.

41. Schwartz, J. Low-level lead exposure and children' s IQ: a meta-analysis and search for a threshold. *Environmental Research*, **1994**, 65, 42–55.

42. World Health Organization, International programme on food safety. *Environmental health criteria 135*. In: Cadmium-environmental aspects. Geneva: **1992**.

43. Data Quality Indicators I: Method Detection Limits, Published by the U.S. Army Corps of Engineers Hazardous, Toxic and Radioactive Waste Center of Expertise, Available at http://www. environmental. usace.army. mil/library/ newlett/ vol110598/quality110598.html

44. James, D. F. Milk protein Center for Animal Health and Productivity, School of Veterinary Medicine, University of Pennsylvania. Available at: http://cahpwww.vet.upenn.edu/mun/milk_protein.html

45. McBean, E.D.; Rovers, F.A.; Statistical procedures for analysis of environmental monitoring data & risk assessment. Prentice Hall: New Jersey; **1998**.

46. Analytical Methods committee, *Analyst*, **1997**, 122, 495–497.

47. Ethiopia fact sheet: UNDP Human Development Report, **2002**.

48. Del Petre, V.; Di Stanislao, F. Trace elements in cow milk sold in the Marche region. *Nuovi Ann.Ig. Microbiol.*, **1984**, 35, 313-323.

49. Baldini, M.; Bocca, A.; Mosca, M. Investigation of microelement content of bulk milk from different regions of Italy. *Food Addi. Contam.*, **1988**, 5, 45-50.

50. Franco, M. A.; Balestrieri, F.; Sabbatini, M.; Serra, A.; Valutazione della concentrazione di ioni metallici presenti nel latte prodotto in Sardegna. *Riv. Soc. Ital. Sci. Aliment*, **1981**,10, 35-40.

51. Campillo, N.; Viñas, P.; López-García, I.; Hernández-Córdoba, M.; *Talanta*, **1998**, 48, 615-622.

52. Barreira Rodriguez, J. E.; Arribas Jimeno, S. Simultaneous voltametric determination of zinc, lead, copper in cow milk. *Anal. Quim.*, **1990**, 86, 252-256.

53. LÓpez Mahía, P.; Paseiro Losada, P.; Simal Lozano, J. Elementos traza en leche natural de vaca. *Aliment.*, **1991**, 226, 45-47.

54. Moreno-Rojas, R.; Amaro-LÓpez, M. A.; Zurera-Cosano, G. Mineral content of pasteurized milk produced in Andalucia. *Rev. Esp. Cienc. Tecnol. Aliment.*, **1993b**, 33, 435-444.

55. Moreno-Rojas, R.; Amaro-LÓpez, M. A.; Zurera-Cosano, G. Micronutrients in natural cow, ewe and goat milk. *Int. J. Food Sci. Nutr.*, **1993a**, 44, 37-46.

56. Moreno Rojas, R.; Amaro LÓpez, M.; Cañal Ruiz, C.; García Gimeno, R.; Zurera Cosano, G., Mineral content in Spanish sterilized milk. *Rev. Esp. Cienc. Tecnol. Aliment.*, **1994**, 34, 323-332.

57. RincÓn, F.; Moreno, R.; Zurera, G.; Amaro, M. Mineral composition as a characteristic for the identification of animal origin of milk. *J. Dairy Res.*, **1994**, 61, 151-154.

58. Zurera-Cosano, G.; Moreno-Rojas, R.; Amaro-LÓpez, M. Effect of processing on contents and relationships of mineral elements of milk. *Food Chem.*, **1994**, 51, 75-78.

59. Martín-Hernández, C.; Amigo, L.; Martín-Alvarez, P. J.; Júarez, M. Differentiation of milks and cheeses according to species based on the mineral content. *Z. Lebensm. Unters. Forsch.*, **1992**, 194, 541-544.

60. Yenigün, A.; Taneli, B.; Oksel, F. Trace Toxic Elements in Nutrition and Health. *In Proceeding International Conference on Health and Diseases: Effects of Essential Toxic Trace Elements* (M. Abdulla, S. B. Vohora, M. Athar, Eds.), 4th, ed. Wiley Eastern: New Delhi, India., **1995**; pp 339-340.

61. LÖnnerdal, B.; Keen, C. L.; Hurley, L. S. Iron, copper, zinc, and manganese in milk. *Ann. Rev. Nutr.*, **1981**, 1, 149-174.

62. Salih, Y.; McDowell, L. R., Hentges, J. F.; Mason, R. M.; Wilcox, C. J. Mineral content of milk, colostrum, and serum as affected supplementation. *J. Dairy Sci.*, **1987**, 70, 608-612.

63. Pennington, J. A. T.; Schoen, S. A.; Salmon, G. D.; Young, B.; Johnson, R. D.; Marts, R. W. J. E. *Composition of core foods of the U.S. food supply*, **1982-1991II**. Calcium, magnesium, iron and zinc. *J. Food. Comp. Anal.*, **1995b**, 8, 129-169.

64. Pennington, J. A. T.; Young, B. E. Iron, zinc, copper, manganese, selenium and iodine in foods from United States total diet study. *J. Food Comp. Anal.*, **1990**, 3, 166-184.

65. Batilde Lima, L.; Banguela Perez, S.; De Ortega Bolaños, M. J.; Torricella, R.; Camejo Corrales, J.; Leche fuida y yogur natural enriquecidos con hierro. *Aliment.*, **1995**, 259, 63-66.

66. Gunshin, H.; Yoshikawa, M.; Doudou, T.; Kato, N. Trace elements in human milk, cow's milk, and infant formula. *Agric. Biol. Chem.*, **1985**, 49, 21-26.

67. Bano, N.; Naeem, M.; Khan, H.Trace metal studies in milk, milk products and eggs. *J. Nat. Sci. Math.*, **1985**, 25, 67-74.

68. Benemariya, H.; Robberecht, H.; Deelstra, H. Zinc, copper, and selenium in milk and organs of cow and goat from Burundi, Africa. *Sci. Total Environ.*, **1993**, 128, 83-98.

69. Rashed, M. N. Determination of trace elements in milk of some animals from Aswan (Egypt). *Int. J. Environ. Anal. Chem.*, **1992**, 48, 41-50.

70. Fernández De La Campa, M. R.; Andrés García, E.; Moro García, R.; Alvarez Bartolomé, M. L.; Arribas Jimeno, S. *Aliment*, **1986,** 23, 33-34.

71. Mazzotta, D.; Brandolini, V.; Vecchiati, D.; Menziani, E.; Mallea Angles; A., Pansini, F. S.; Abbasciano, V., Investigation of zinc and other cations content of milk and its derivatives. *Riv. Sci. Alimen.*, **1993**, 22, 287-291.

72. Pennington, J. A. T.; Wilson, D. B.; Young, B. E.; Johnson, R. D.; Vanderveen, J. E. Mineral content of market samples of fluid whole milk. *J. Am. Diet. Assoc.*, **1987**, 87, 1036-1042.

73. Ostapczuk, P.; Valenta, R.; Rutzel, H.; Nurnberg, H,W. Application of differential pulse anodic stripping voltammetry to the determination of heavy metals in environmental samples. *Sci. Total Environ.* **1987**, 60, 1-16.

DECLARATION

I, the undersigned, declare that this is my original work and has not been submitted for a degree in any other university and that all sources of material used for the thesis have been duly acknowledged.

Name: Engdawork Admasu

Signature ________________

This thesis has been submitted for examination with our approval as University advisors.

Name: 1. Prof. B. S. Chandravanshi

Signature ________________

2. Dr. Taddese Wondimu

Signature ________________

3. Dr. Girma Akalu

Signature ________________

Place and date of submission: School of Graduate Studies,
Addis Ababa University
June 2005